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The role of different dietary patterns on irritable bowel syndrome (IBS) symptoms

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INTRODUCTION

The irritable bowel syndrome (IBS) is a very common gastrointestinal disorder with an unclear pathophysiology. It affects quality of life through gastrointestinal symptoms such as abdominal pain, bloating, altered bowel habits and meteorism and can be managed with pharmacological and non-pharmacological approaches. Among the non-pharmacological approaches there is the diet. In fact, more than 70% of patients report that their symptoms are exacerbated after ingesting food and choose to exclude certain foods from their diet themselves.

After the presentation of IBS in the first chapter, where the different subtypes of IBS are explained, the thesis proceeds with the description of a healthy gut microbiota compared to the variations in microbiota composition usually observed in IBS, focusing on the different subtypes.

Chapter 3 presents the different nutritional approaches suggested in the literature to manage IBS symptoms, while from chapter 4 the results of three experimental protocols tested during my PhD course are presented. The experimental protocols concern: 1) the effects of the administration of high amylose products included in a protocol based on the Mediterranean Diet on symptoms and microbiota in subjects with IBS - C (FRUMILOSIO project), 2) the evaluation of a Mediterranean nutritional protocol on symptoms and microbiota in patients with IBS - D and 3) a lowering-FODMAPs diet on symptoms in different subtypes of IBS (DOMINO study).

As studies evaluating dietary treatments stratifying for the different IBS subtypes are lacking in the literature, the aim of this PhD thesis is to evaluate the effects of different nutritional approaches on symptoms and microbiota composition in IBS subtypes.

CHAPTER 1. The irritable bowel syndrome

1.1. Classification and diagnosis

The irritable bowel syndrome (IBS) is defined as a functional disorder of the gut – brain interaction [1] characterized by symptoms like abdominal pain, bloating, urgency, incomplete evacuation and altered bowel habits without organic, biochemical or structural causes [1,2,3]. The IBS negatively impact on quality of life [1-4] and on daily activities and work [1-3].

Its diagnosis is based on Rome IV criteria: recurrent abdominal pain on average at least 1 day/week in the last 3 months, associated with two or more of the following criteria [2,5,6].

- Related to defecation
- Associated with a change in the frequency of stool
- Associated with a change in the form (appearance) of stool

These criteria have to be fulfilled for the last 3 months with symptoms onset at least 6 months before the diagnosis [6].

The Bristol Stool Scale (BSS) is a useful tool to evaluate the prevalent stool type and consistency, that ranges from hard lumps to watery stools (*Figure 1*) [7].

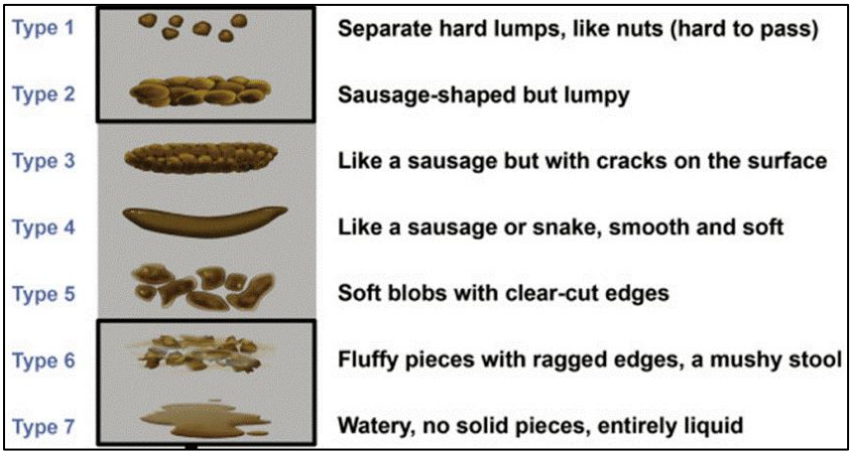

Figure 1 Bristol Stool Scale [7]

The shape and consistency of stools depend on the time they spend in the colon. Stool types 1 and 2 define a higher or lower degree of constipation due to excessive retention in the intestine and difficulty in expulsion. Type 3 is similar to an ideal condition that implies a need for the body to consume more fibre and/or water. Type 4 represents the ideal condition, with easily expelled stools followed by the feeling of complete emptying; type 5 is also considered acceptable. Types 6 and 7 are associated with a different degree of diarrhoea [8].

Through the BSS, four different IBS subtypes could be recognized according to stool types (*Figure 2*) [2,6]:

- IBS with predominant constipation (IBS-C): more than 25% of bowel movements with Bristol stool types 1 or 2 and less than 25% of bowel movements with Bristol Stool type 6 or 7.
- IBS with predominant diarrhea (IBS-D): more than 25% of bowel movements with Bristol stool types 6 or 7 and less than 25% of bowel movements with Bristol Stool type 1 or 2.
- IBS with mixed bowel habits (IBS-M): more than 25% of bowel movements with Bristol stool types 1 or 2 and more than 25% of bowel movements with Bristol Stool type 6 or 7
- IBS unclassified (IBS – U): patients who meet the diagnostic criteria for IBS, but whose bowel habits cannot be classified into one of the three classifications mentioned above.

Patients could experience a change from one IBS subtype to another, it is more common from IBS-C or IBS-D to IBS-M than from IBS-C to IBS-D and vice versa [9,10].

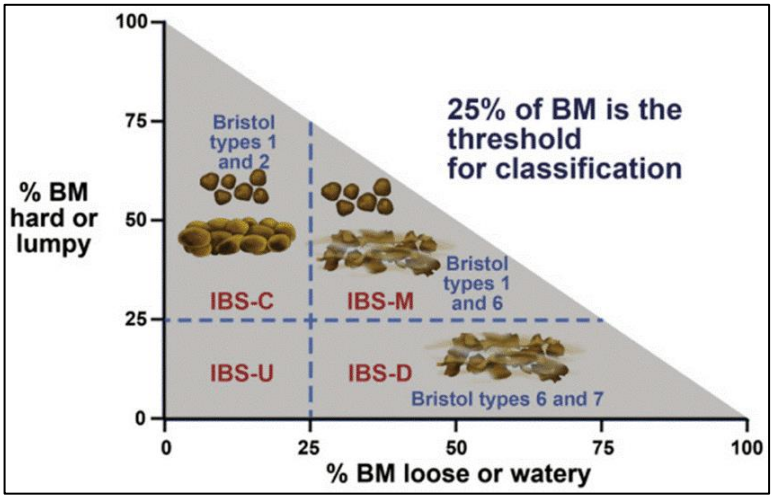

Figure 2 IBS subtypes according to the Bristol Stool Scale [2]

The diagnosis occurs through symptoms excluding other organic diseases. Among symptoms, we can distinguish between gastrointestinal and extra-gastrointestinal ones. To the first category belong symptoms such as abdominal pain, bloating, meteorism and alternate bowel habits while to the second belong headaches, fatigue, anxiety, depression and sleep disturbances [2]. In women, symptoms may be exacerbated during menstruation [11].

The clinical evaluation of IBS should be based on four features: clinical history, physical examination, minimal laboratory test and, if indicated, a colonoscopy or other appropriates tests [2]:

1) Clinical history: abdominal pain must be present, and a history of bowel habits alteration should be identified in association with abdominal pain. Abdominal bloating may be present but it is not mandatory to diagnose IBS [2].
2) Physical examination: it helps to exclude organic causes [2].
3) Laboratory tests: a complete blood count to evaluate anaemia or elevated white blood cells should be investigated [2]. In addition, C-reactive protein (PCR) and fecal calprotectin [2] should be measured, with a moderate quality of evidence [1] even if fecal calprotectin is not predictive in constipated patients. A low – evidence has been seen for fecal lactoferrin evaluation [1]. Serological tests for celiac disease should be performed in patients with IBS-D and IBS-M [2], with a moderate grade of evidence [1].
4) Colonoscopy: it is indicated in patients older than 50 years old, especially in case of alarming symptoms or signs, a history of colorectal cancer and diarrhea [2]. Colonoscopy is not recommended under 45 years old [1].

During evaluation of symptoms it is important to ask about some "red flags" that are not linked to IBS and that are needed of further evaluations such as: 1) age older than 50; 2) symptoms (especially diarrhea) during the night; 3) blood in the stools; 4) fever and raised inflammatory markers; 5) unintentional and unexplained weight loss; 6) anaemia or elevated inflammatory markers in clinical analysis; 7) family history of Inflammatory Bowel Diseases (IBD) or colon/ovarian cancer; 8) rectal or abdominal masses; 9) a change in bowel habits to loose and/or more frequent for more than 6 weeks in elderly subjects [3,10].

1.2. Epidemiology

IBS is one of the most prevalent gastrointestinal (GI) disorders in the world. According to Rome III criteria, the global prevalence was estimated as 11.2% [2,3] but with new Rome IV criteria (that exclude the discomfort from symptoms), the prevalence decreased to 4.6% [12]. In particular, a recent study conducted on more than 73.000 subjects, showed that this functional disorder affects about 4% of the global population [13]. It is estimated that IBS hits between 5% and 15% of the Western population [1,3]. This disorder is mainly prevalent in women (female to male ratio 2:1) maybe due to the effects of sexual hormones on gastrointestinal tract [1-3,13] with an overall prevalence of 11.2% under 50 years old but it can occur at any age [1-3]. The global IBS subtypes prevalence is: 32.4% IBS – C, 32.4% IBS – M 28.7% IBS – D and 6.5% IBS – U [13]. Among women, the IBS-C rate is higher than IBS-D while a contrary situation has been seen among men [13].

1.3. Physiopathology and etiopathogenesis

The physiopathology and etiology of IBS is still unclear [3,10]. It is a multifactorial disease in which many pathogenic factors are involved, including 1) genetic predisposition, 2) altered intestinal motility, 3) visceral hypersensitivity, 4) psychological factors, anxiety and depression, 5) infections and altered gut barrier permeability, 6) diet and food hypersensitivity (*Figure 3*) [2,3,9,10,14]. Currently, also an alteration in the gut microbiota composition could be recognized among the factors involved in IBS symptoms [2,3].

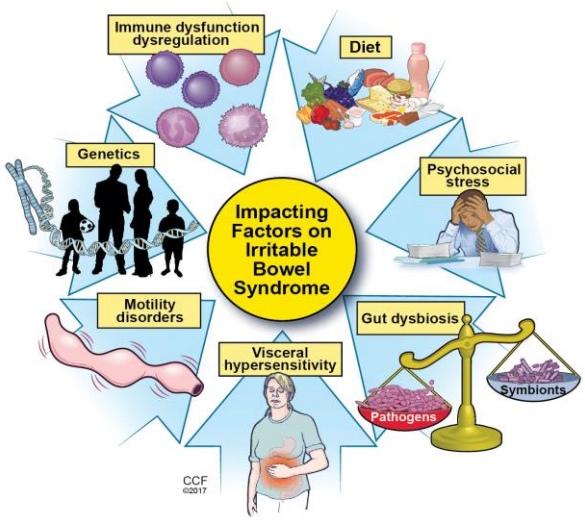

Figure 3 Factors involved in IBS [14]

To date, it is not clear if these factors represent a cause or a consequence of this syndrome [3,10]. For this reason, IBS is now considered as a gut – brain interaction with a bidirectional relationship [15].

However, IBS is a heterogeneous condition and all patients affected could show different symptoms and physiopathological factors [9]:

- **Genetic predisposition:** 33% of affected patients has a positive family history for IBS [10]. Having a parent with IBS seems to be a positive predictor for this condition compared to a twin with IBS, underlying that environmental factors may play a potentially greater role than genetic factors [10]. Numerous studies have investigated the possible role of genetic polymorphisms encoding for anti-inflammatory and pro-inflammatory interleukins, α-2 adrenergic, serotonin (SERT) and cholecystokinin (CCK) receptors [10]. A significant correlation between SERT receptor polymorphisms and the severity of IBS symptoms has been observed, although their possible direct role in IBS has still to be clarified [10].

- **Alterations in gastrointestinal motility:** Psychological and physical stress, can determine dysmotility in IBS patients. Some motor disturbances have also been documented in the small intestine, and it seems to be associated to an accelerated meal transit in patients with prevalent diarrhea or in a delayed meal transit in constipated subjects [10]. This alteration has not been observed in all patients affected by IBS. In fact, only 25% of IBS-C patients show a decreased intestinal transit time while between 15 and 45% of IBS – D patients show an increased one after a psychosocial stress [3]. Also a modification in the release of serotonin could be associated to the gastrointestinal motility. In fact, in IBS-C patients serotonin postprandial concentration is reduced, while it is increased in IBS - D subjects. Indeed, serotonin, produced by enterochromaffin cells, is implicated also in the transmission of visceral sensory stimuli [16].
- **Visceral hypersensitivity:** the perception of pain following the distension of the rectum through the use of a rectal balloon is felt for significantly smaller distention volumes in IBS subjects than in healthy ones with normal bowel functions [3]. This alteration has been documented at different GI levels such as ileum, recto – sigmoid and anorectal region. Nevertheless, recent studies show that a variable percentage from 20 to 70% of IBS patients suffer for visceral hypersensitivity. This hypersensitivity could be the result of the stimulation of various receptors of the visceral nerve fibres afferent to the intestinal wall triggered by intestinal distention or bloating [10].
- **Psychological disorders**: epidemiological studies showed that individuals with IBS could have faced traumatic situation through their life and it could represent a predisposing factor for symptoms [15]. About 75% of IBS patients show also higher levels of anxiety and depression than healthy controls. After the rectal distention through the balloon, it was seen a greater activation of brain regions associated to pain and emotionality. Moreover, IBS subjects show a reduced inhibitory feedback for emotivity and a greater activity after visceral stimuli [17], thus they seem to be more vulnerable to external stimuli for a dysregulation in the dorsolateral prefrontal cortex [18]. Psychological disturbances could influence gut-brain axis promoting the release of cortisol, adrenocorticotropic hormone and corticotropin that can influence mood, digestive motility, visceral sensitivity and inflammatory pathways [10].
- **Inflammation and altered gut barrier permeability:** the altered gut barrier permeability has been seen especially in IBS – D patients. It determines an immune activation [3] with inflammatory cells infiltration that generate the formation of localized oedema and the cytokine recall that could determine IBS symptoms and an alteration in peristalsis [18].

Inflammation seems to be associated to altered visceral sensitivity and permeability. An increased concentration of pro – inflammatory cytokines (IL-10, IL-6, TNF-alfa) has been seen in IBS patients and it is associated to the frequency and the intensity of abdominal pain [3]. Also the alteration of tight junction [18] and an increase in the mast-cells concentration [3] in the intestinal mucosa seems to be involved in IBS symptoms.

- **Food hypersensitivity:** in IBS a food sensitivity could be recognized, that is different from a food allergy because it is not Ig-E mediated [3]. Usually, the most investigated foods are diary products, legumes, vegetables, lactose – containing foods, fruits, artificial sweeteners and fatty - meals [10,19-21]. These last, have been seen to aggravate abdominal pain especially 30 and 60 minutes after the ingestion [3]. Also gluten ingestion could exacerbate symptoms also without gluten sensitivity. Maybe, gluten is responsible for inflammation in case of altered intestinal permeability and it could determine the onset of symptoms [19]. Also fructose, present in fruit and vegetables, may be involved in IBS symptoms. In fact, in some IBS cases, fructose malabsorption may be present in IBS patients, thus it could determine a higher fermentation with consequently gas production and water influx into the lumen [3].

1.4. Impact on quality of life (QoL)

Regarding the impact on quality of life, it has also been observed that patients with IBS have a higher morbidity directly or indirectly associated with the syndrome. This includes not only a higher incidence of anxiety disorders and depressive episodes, but also a twice as high incidence of surgery compared to healthy controls. The quality of life of patients with IBS is compromised not only by the clinical condition, but also by the patients' exaggerated perception of it. The complaints are usually of long duration and recurrent. Moreover, around 90% of patients usually go to general practitioner (GP) for symptoms severity and also the impact on secondary care (gastroenterologists or surgeons) is high (29%) [4].

Although there is no excess of mortality associated with IBS, the condition has a significant negative impact on the quality of life (QoL) and can affect both the work and daily activities of patients (in terms of increased absenteeism from school or work and reduced health-related quality of life). The IBS also has a high socio-medical cost and is one of the main reasons for gastroenterological visits [2,4]. Approximately 30% of people with IBS symptoms consult doctors not only for severe abdominal symptoms, but mainly because they have higher levels of anxiety and

a lower quality of life. Patients diagnosed with IBS have a high probability of having other functional diseases and usually undergo more surgery than the general population [22].

1.5. Treatment

The IBS management requires a multidisciplinary step by step treatment especially because symptoms are different, thus a personalized approach is necessary [12,23]. Moreover, also a careful follow up is necessary for this syndrome [2]. Before starting any treatments, it is necessary to consider seven key goals of IBS therapy [5]:

1) The correct diagnosis
2) Reassurance on the validity of symptoms
3) Education on the disorder treatment and which options are available
4) Relief on individual and global symptoms
5) Maximization of patient quality of life
6) Minimization of treatment risk – associated
7) Reduction of the negative impact of IBS on socioeconomic factors (e.g. prescription of unnecessary tests).

Pharmacological and non – pharmacological treatments are usually suggested, but the first-line intervention involves non pharmacological strategies [12].

Non – pharmacological approach

Prior to drug therapy, it is crucial to encourage patients to undertake lifestyle modification starting with a proper diet and increased daily physical activity. Moreover, it is also important to encourage relaxing activities and to improve sleep quality [2, 24].

A moderate physical activity could improve the intestinal gas clearance, bloating and constipation. Among mechanisms of physical activity that may improve symptoms in IBS patients there are increased GI motility, mechanical bouncing during activity, neuroimmune-endocrine alterations, effects on psychological factors. Physical activity, in fact, has positive effects on quality of life, fatigue, anxiety and depression. IBS patients should be encouraged to increase their physical activity level, for example they may take a 20 – minute walking every day [9,10] or also 30 minutes of cycling, yoga or swimming 5 days per week or more. A vigorous activity should be avoided because it could worsen symptoms [25].

Foods has a double role in IBS treatment because it represents both a trigger for symptoms and a tool for therapy [12]. More than 70% of patients declare that their symptoms exacerbate after food ingestion [21,26], especially dairy products, legumes, spicy foods, fried or fatty foods, fiber rich foods, some kind of fruits and vegetables [10,20,21]. The first- line approach for IBS consists in healthy eating and lifestyle changes [15,27] according to the National Institute for Health and Care Excellence (NICE) guidelines [28] and the British Dietetic Association (BDA) [29]. Foods effects depend on the type of IBS [10].

A more restrictive nutritional approach suggested in case of IBS is the low – FODMAP diet. It is a diet that exclude fermentable oligosaccharides, disaccharides, monosaccharides and polyols. In fact, FODMAPs determine gas production and liquid accumulation leading to bloating and IBS symptoms such as abdominal pain and diarrhea [12] thus their exclusion from the diet could improve symptoms. Unfortunately, the exclusion of these dietary components leads to a reduction in beneficial bacteria [3]. Although it demonstrated to have beneficial on IBS symptoms it has a low compliance due to its restrictive approach.

A debated issue in the IBS nutritional treatment is the role of the dietary fibers. They may accelerate intestinal transit increasing fecal mass but they could also exacerbate abdominal pain, like bloating and meteorism [24]. It is important to distinguish the type of fibers that are suggested. In fact, soluble fiber (psyllium and ispaghula husk) seems to be more beneficial on IBS symptoms (especially in IBS-C) than insoluble one (bran) that could worsen symptoms [2,9] both in the short and in the long term [24]. The amount of fiber suggested should be increased gradually to reach 20-30 g daily [9].

In the last years, also other nutritional approaches have been tested (e.g Mediterranean diet, gluten-free diet, lactose – free diet, fructose – free diet, low – carbohydrate diet). The dietary approach plays an extremely important key role in improving symptoms in IBS patients as will be discussed in more detail in Chapter 3.

Moreover, among the non – pharmacological approach there are also the psychological therapy or the alternative medicine. Regarding the psychological therapy, the so – called cognitive behavioural therapy (CBT), relaxation therapy, multi – component psychological therapy, hypnotherapy and dynamic psycho-therapy have been found to be more effective than control therapy. On the contrary, mindfulness, stress management, self – administered CBT, meditation training and CBT – internet delivered showed no significant improvements. It is still difficult, however, to define the real effectiveness of these psychological therapies for the heterogeneity of studies in literature and also

because the application is limited due to costs, prolonged duration, a lack of expert therapists and low adherence of patients [30, 31].

Among alternative medicine approaches, there are acupuncture, the utilization of supplements such as aloe vera, berberine, melatonin and ginger, or also the Chinese herbal medicine. Actually, none of them has showed greater benefits than placebo [30].

Finally, also **probiotics** may be suggested in case of IBS. Probiotics are live bacteria that, when consumed in sufficient quantity, confer a health benefit to the host [9]. They should be taken every day in order to increase the richness and biodiversity of beneficial species in the gut and to protect against the pathogens improving the gut barrier integrity [5]. On IBS symptoms, both in constipation or diarrhea subtypes, they may be useful to improve bloating, pain, flatulence and bowel movement habits [10,12] but they have also a beneficial action on visceral hypersensitivity, epithelial permeability and the reduction of immune mucosal activation [12]. As in IBS *Bifidobacteria* and *Lactobacilli* are reduced, supplements containing both of these strains and also *Sacccharomices sp*. have beneficial effects on symptoms because they reduce low-grade inflammation [12]. Moreover, *Bifidobacterium infantis* has been seen to determine significant improvements in symptoms like abdominal pain, bloating and also bowel movements compared to placebo in 2 randomized control trials (RCTs) [2,12]. To date, there is not a consensus on which type and dose of probiotic (or symbiotic, derived by a combination of probiotics and prebiotics) should be suggested [9,10]. An ACG meta – analysis found evidences to support using a combination of probiotics [32].

Pharmacological approach

Although the first line approach is a modification of dietary habits and lifestyle, the pharmacological approach could be taken in consideration depending on symptoms severity. Drugs are suggested depending on the prevalent symptom or stool type [18,23].

For abdominal pain:

- **Antispasmodics:** this kind of drugs are usually suggested to treat abdominal pain derived from the smooth muscle spasms, especially those of the gastrointestinal tract. There are two big categories of antispasmodics [2]:
    - anticholinergic/antimuscarinic agents (hysocyamine, cymetropium)
    - smooth-muscle relaxants (mebeverine, otilonium bromide).

In the literature there are few evidences regarding the first class of these drugs, while in the second class, otilonium bromide showed good results on IBS symptoms [2,33]. In general, this kind of drugs are the most suitable for postprandial abdominal pain and loose stools. Among side effects there are constipation, fatigue, dizziness, dry mouth and blurred vision [9].

Also peppermint oil is considered as an antispasmodic for its calcium channel blocking properties [9,31]. In fact, in a RCT on 72 IBS – D and IBS – M patients who received peppermint oil, the 40% of patients showed a reduction in symptoms after 4 weeks. Already after 24 h there was a significant difference between the group treated with peppermint oil and the one treated with placebo. Peppermint oil was responsible of the reduction of symptoms like abdominal pain, discomfort, bloating, pain during evacuation and urgency but not other symptoms such as constipation, diarrhea, and passage of gas. The meta – analysis of Khanna et al. on 726 patients, confirmed that peppermint oil is better than placebo in improving abdominal pain [34]. It is contraindicated for people who suffer for reflux [9].

- **Tricyclic Antidepressants (TCAs):** they are used at lower dosages than those required to treat depression, they help to modulate the activity of the neurotransmitters noradrenaline and serotonin, both of which are involved in pain perception. As a rule, tricyclic antidepressants (amitriptyline, desipramine) are used in IBS-D achieving an analgesic-like effect on visceral pain and positively impacting on patients' quality of life. Unfortunately, IBS patients suffering from anxiety are often reluctant to take antidepressants and prefer treatment with benzodiazepines instead [10,18].

For IBS-C:

- **Polyethylene glycol (PEG) laxatives:** they are first - level drugs useful for evacuation frequency and bowel function but not for abdominal pain and bloating [9]. They act drawing water into the lumen, through a process known as osmosis; in this way, stools become softer, and their transit through the intestine and therefore evacuation are accelerated. Laxatives are principally used in IBS-C or also in IBS – M [10,18]. The most common side effects linked to laxatives are abdominal pain and cramping [9].
- **Linaclotide:** it is a 14-amino acid peptide that acts as a guanylate cyclase C agonist [2,3,9]. The increase of the production of cyclic guanosine monophosphate determines an increment of luminal chloride and fluid secretion [2,9]. Linaclotide improves bowel and

abdominal symptoms in IBS – C patients increasing the number of complete spontaneous evacuation. Unfortunately, diarrhea may be an adverse event [2,3,9].

- **Lubiprostone:** it is a chloride channel activator that increase chloride and sodium secretion and fluid transit into the lumen. It may determine nausea but it overall determines an improvement in abdominal symptoms in IBS-C patients [3,9].
- **Plecanatide:** it is a novel treatment recently approved by Food and Drug administration to manage IBS-C symptoms. It promotes bowel transit and adds water to soften stools. It is useful for both pain and constipation [3].
- **Serotonin – receptor agonist:**
    - **5-HT4 agonists:** Activation of 5-HT4 receptors results in the release of excitatory neurotransmitters that modulate smooth muscle tone and the peristaltic reflex. Tegaserod is a 5-HT4 agonist initially approved by the FDA in female patients with IBS-C and in men and women under 65 years of age with chronic constipation. The main side effect is diarrhea, which is usually transient [35]. In March 2007, Tegaserod was removed from the market in the US and Canada due to clinical studies that showed a statistical increase of myocardial infarction and stroke but 2 months later, Tegaserod was reintroduced limited to IBS-C and chronic idiopathic constipation in women under 55 years of age.

For IBS-D:

- **Anti-diarrheal agents**: they are recommended for patients with IBS-D as these drugs act by reducing fecal volume [9] and prolonging the time stools remain in the intestinal tract - more precisely in the colon - by increasing anal pressure and reducing rectal pressure. Among them, Loperamide is the most commonly used as it reduces the intestinal transit and increases stool consistency [2,5,10,18].
- **Non - absorbable Antibiotics:** they do not have a systemic action, but they act only at the intestinal level. They are used to counteract bacterial contamination of the small intestine, which can often be the cause of symptoms such as bloating, pain and meteorism. Rifaximin is the non-absorbable antibiotic of first choice for the treatment of IBS-D. Actually, the exact mechanism of action of rifaximin for the treatment of IBS-D is not known, but it could be related to microbiota composition changes. The most common side effects in IBS-D patients treated with rifaximin include nausea and an increase in alanine

aminotransferase (ALT). Caution must be exercised when using rifaximin in patients with severe hepatic diseases or in combination with certain other drugs [2,10,18].

- **Eluxadoline:** it has been approved for IBS-D treatment and can reduce bowel movement frequency and improve stool consistency. It is contraindicated for subjects with liver diseases, pancreatitis, alcoholism and gallbladder [3].
- **Serotonin – receptor antagonists:**
    - **Alosetron:** It is a potent serotoninergic receptor antagonist indicated for the treatment of severe IBS-D in women with no anatomical or biochemical abnormalities of the gastrointestinal tract who have not responded satisfactorily to other treatments [3,9]. Compared to placebo, the use of this drug has been useful in reducing abdominal urgency and improving stool consistency as well as improving patients' quality of life overall [2,36,37]. Alosetron results in slowed intestinal transit, reduced fluid/ion intestinal secretions, reduced abdominal pain and increased stool consistency [5]. Constipation is among the side effects [9,36,37] but lower doses of alosetron resulted in a decreased constipation rate [36].
    - **Ondansetron:** it is less powerful than alosetron and may be useful to improve stool consistency, global IBS symptoms, urgency, stool frequency and bloating but not abdominal pain [9].

CHAPTER 2 The Gut Microbiota

2.1. Healthy gut microbiota

The gut microbiota represents the microbial community in the gastrointestinal tract and it is more concentrated in the colon. It is composed of a variety of bacteria, archaea, yeast and viruses and it includes between 10^{11} to 10^{12} different microorganisms [38]. The genes that constitute the microbiota formed the microbiome, which is 150 times larger than the human genome [39].

The microbe concentration is different from the upper to the lower GI tract. In fact, both lower pH and fast transit inhibit the bacterial growth in the upper GI tract, so, the density and diversity of microbes increase to approximately 10^{11} cells/ml in the colon [40]. The microbiota is a metabolically active community that exerts important influences on health and disease, and the host–microbiota relationship has been seen as a mutualistic ecosystem, and both benefit from this relationship [41].

Regarding taxonomy, bacteria are classified in phyla, class, order, family, genus and species [38]. The predominant bacterial phyla in the human gut are Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria (Figure 4), while among secondary phyla there are Fusobacteria and Verrucomicrobia [38]. Firmicutes and Bacteroidetes mainly dominate the large intestine (LI) representing around 90% [38]. Among these, Bacteroidetes are the most abundant ones; species from this family comprise about 30% of bacteria in the gut suggesting that it is particularly significant in the functions of the host's organism. Genus such as *Bacteroides* and *Prevotella* belong to Bacteroidetes [38]. The Firmicutes phylum is composed by 301 phylotypes and more than 200 different genera such as *Lactobacillus, Bacillus, Clostridium, Enterococcus* and *Ruminococcus* and most (95%) of its sequences belong to the *Clostridia* class [45,42-44]. Actinobacteria phyla is less abundant than Bacteroidetes and Firmicutes and it is principally represented by *Bifidobacteria* [38] (*Figure 4*). The human microbiota is established after birth and starts out as a dynamic ecosystem. At age 3 children's gut microbiota is comparable to that of an adult with predominant Firmicutes, Bacteroidetes and Actinobacteria [19,45]. During life the microbial composition increases in both diversity and richness and reaches highest complexity in the adulthood, while in the elderly there is a decrease of *Bifidobacteria* and an increase in *Clostridia* and Proteobacteria [45].

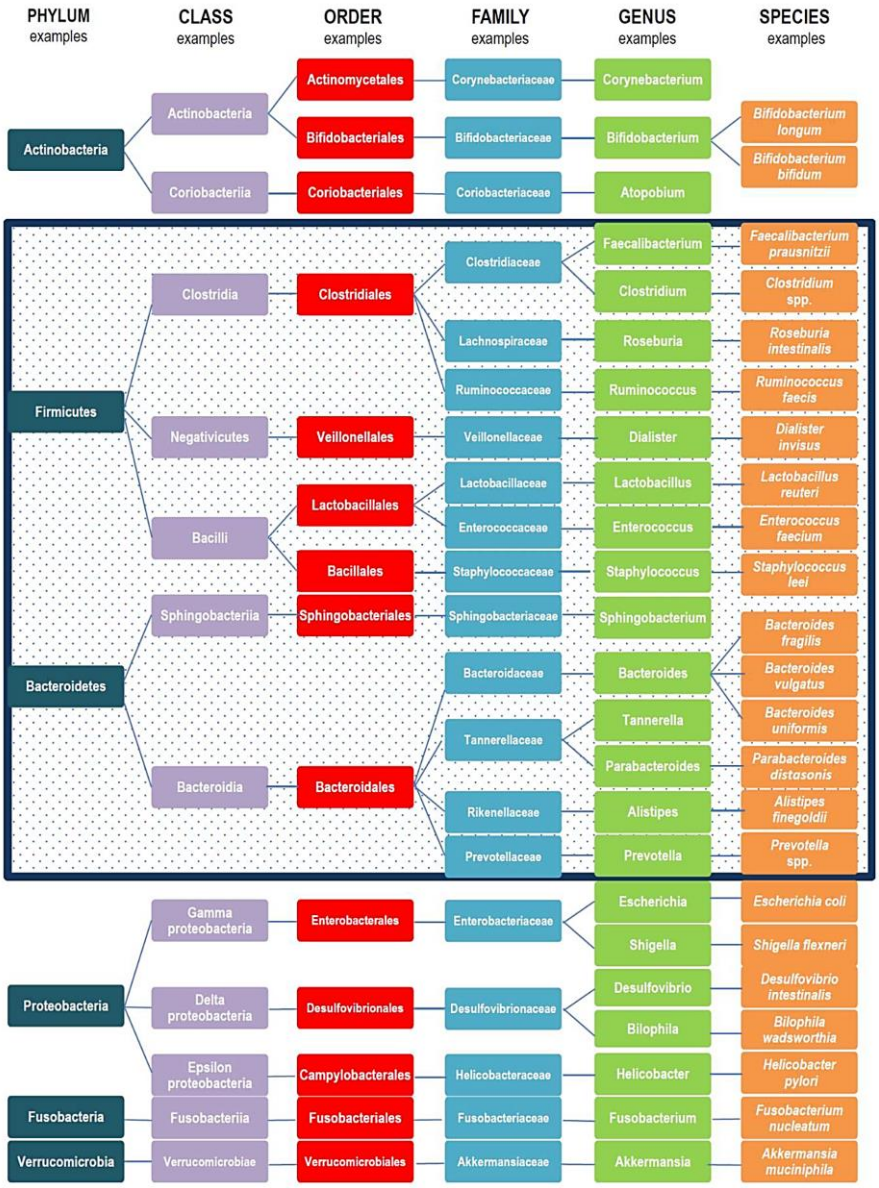

Figure 4 Gut microbiota composition (38)

Thus, also the ratio Firmicutes/Bacteroidetes changes through the life. Although it is considered as a predictive marker of health or disease (e.g. obesity, metabolic syndrome etc.), it can vary significantly among individuals [46] and can be altered during the life and in pathological conditions [47].

Researches on the human gut microbiota has predominantly been based on analyses of fecal samples, but two different GI microbiota populations exist [48]: 1) The one that is present in the colonic lumen (fecal microbiota), 2) and the other one that is specific of the intestinal mucosa (mucosal microbiota) [42,48]. The first one could be measured via stool sampling and is likely a combination of non-adherent luminal bacteria with a mix of shed mucosal ones. It is variable

depending on the diet and the environmental conditions [40]. On the other hand, the mucosal microbiota composition is highly stable in healthy subjects in different intestine tracts [49] suggesting that the host influence is stronger than the environmental factors. Moreover, the mucosal microbiota is involved in the 'crosstalk' between the lumen and the immune and enteroendocrine cells through nervous system. This may lead to IBS symptoms [50]. The faecal-associated microbiota differs from the mucosal one [48,51,44]. Particularly, Bacteroidetes are more abundant in the mucosal-associated microbiota while Firmicutes, Actinobacteria, *Bacilli* and Proteobacteria are more present in the faecal-associated microbiota [51]. Fecal – associated microbiota present a higher biodiversity than mucosal – associated one [51].

In the large intestine (LI) lumen (and then in the stools) there are: *Bacteroides, Bifidobacterium, Streptococcus, Enterobacteriaceae, Enterococcus, Clostridium, Lactobacillus* and *Ruminococcus*, while *Clostridum, Lactobacillus, Enterococcus* and *Akkermansia* are associated with the mucosa [44,51]. Bacteria are not uniformly distributed throughout the lumen [52]. The colic mucosa is covered by a mucus-stratified layer and there, mucin – degrading microorganisms such as *Akkermansia, Ruminococcus* and *Bacteroides* are more abundant [52,53]. The phylum Proteobacteria represents a small proportion of GI bacteria in healthy individuals [51]. Pathogens like *Campylobacter jejuni, Salmonella enterica, Vibrio cholera, E. coli, Bacteroides thetaiotaomicron* and *Bacteroides fragilis* may be present in LI but they are less abundant (0,1%) [44,].

The composition of the human microbiota is influenced by the host genotype, the environment, the diet but also by host factors such as gender, age, body weight or drug exposure [54-57]. The microbiota community is self-shaping as organisms 'assemble themselves according to available niches' [40] and compete for their position within the community, determined largely by the adaptability of the organism phenotype, the physical environmental condition of the GI tract (e.g. gastric acid, motility and GI secretions), genetic factors and colonization. There is an overall resilience of the healthy microbiome, which enables the system to return to an equilibrium after minor shifts, with only some temporal variability [58,56].

The gut microbiota is in a mutualistic relationship with the host and it produces a wide range of metabolites and chemicals, which influence host functions [59]. Thus, the intestinal microbiota must be considered as a real organ, with well-defined functions [59]:

- It enhances the immune system [44,56,60,61], protecting the host from pathogens

- It plays a crucial role in digestion and metabolism of dietary components harvesting additional energy and nutrients from the diet and it produces vitamin such as K [56,59,61],
- It controls gut barrier epithelial cell proliferation and differentiation [62],
- It influences brain–gut communication affecting the mental and neurological functions of the host [45,63].

Thus, gut microbiota plays a pivotal role in maintaining normal gut physiology and health. In addition, the gut microbiota is responsible for the fecal mass production decreasing the transit time and diluting the toxic substances that affect the health of the host [64]. Because of the complex interplay between diet and the gut, microbiota within the gut lumen environment contributes to the formation of short chain fatty acids (SCAFs): butyrate, propionate and acetate. These are the primary end products of fermentation of non-digestible carbohydrates (NDC) that become available as nourishment for the gut microbiota [65]. Metagenomic approaches have facilitated the characterization of SCFAs producing bacteria. Acetate production pathways are widely distributed among bacterial groups whereas pathways for propionate, butyrate and lactate production appear more highly conserved and substrate specific. For example, propionate production although distributed across a number of phyla is dominated by relatively few bacterial genera. Species such as *Akkermansia municiphila* have been identified as key propionate producing and mucin degrading organisms. Fermentation of resistant starch is thought to contribute significantly to butyrate production in the colon and is dominated by *Ruminococcus bromii*, so the absence of that microorganism significantly reduces resistant starch fermentation and butyrate production too. A surprisingly small number of microorganisms, dominated by *Faecalibacterium prausnitzii, Eubacterium rectale, Eubacterium hallii and Ruminococcus bromii*, appear to be responsible for the major fraction of butyrate production [43,65].

2.2. Eubiosis and dysbiosis

The stability and good composition of the intestinal microbiota is called eubiosis. It is the principal prerequisite for the proper functioning of the whole organism. It is characterized by:

- The ratio between beneficial and potentially harmful species
- The richness (taking into account the total number of species present)
- The biodiversity (taking into account the relative abundance of all species present).

An eubiotic intestinal microbiota is determined by the preponderance of potentially beneficial species, mainly belonging to Firmicutes and Bacteroidetes phyla, while potentially pathogenic

species, such as Proteobacteria *(Enterobacteriaceae)*, are present in a very low amount [80,95]. When the mutualistic relationship among microbiota members, metabolic products, and the host immune system is lost, a condition called dysbiosis occurs. Generally, in a dysbiotic ecosystem, potentially pathogenic microbes take over at the expense of potentially beneficial ones [23,66].

Dysbiosis is defined when there is an imbalance in the biological system of the microbiota that is when the type and number of intestinal microorganisms are reduced in their diversity and depleted of their beneficial microbes. When dysbiosis takes place, a loss of overall microbial diversity can be observed [66], and a parallel overgrowth of potentially harmful species occurs [67]. It can be one of the main factors responsible of the development of pathological conditions involving the whole organism, for example IBS. In fact, the dysbiosis in IBS has been associated to an increased visceral hypersensitivity, increased mucosal permeability due to a reduced function of the epithelial barrier, increased inflammation and immunity activation [68] (*Figure 5*).

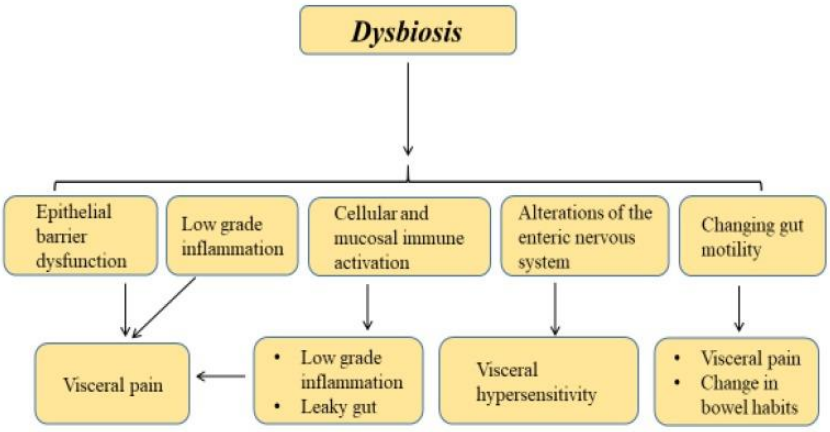

Figure 5 Dysbiosis in IBS [68]

The common dysbiotic findings in IBS show an increase in *Streptococcus spp* and pathogenic species, and a reduction of Bacteroides, *Bifidobacteria* and *Lactobacilli.* Also an increase in *Clostridium* may be seen. The study of Jalanka - Tuovinen et al. showed that IBS patients with abdominal pain had over five fold less B*ifidobacteria* compared to IBS subjects without pain [69].

2.3. Gut Microbiota in IBS patients

Several studies in the literature have shown an altered gut microbiota composition in IBS patients compared to healthy subjects [57] hence influencing the onset of IBS symptoms, such as abdominal pain, meteorism and the alteration of intestinal motility [70].

Emerging data support the existence of a gut dysbiosis, a reduced complexity and α diversity in microbial abundance and an alteration in its composition in IBS patients versus healthy controls

[30,71,72]. Despite inconsistences among the studies, a higher Fimicutes:Bacteroides ratio has been observed [72-74]. Moreover, IBS microbiota presents a higher proportion of pro-inflammatory bacteria such as *Shigella* species and lower proportion of anti – inflammatory species as *Faecalibacterium prausnitzii*, a butyrate producing bacterium [30]. Differences in the gut microbiota taxonomy of IBS patients compared to healthy subjects have been reported, such as a reduction in Bacteroidetes [38] and Actinobacteria [38, 75] and an increase in Firmicutes and Proteobacteria [72, 75,76]. These alterations negatively influence also on SCFAs production with an effect also on colonic inflammation and barrier defense [70, 75]. At the genus level, IBS patients generally have higher presence of *Ruminococcus* [72,73,77], *Lachnospiraceae* [78] *Clostridium* [72,73], *Dorea* [72], *Coprococcus* and *Blautia* and less *Faecalibacterium* [72,73] *and Bifibodacteria* [38,72]. Contrasting results have been seen for *Lactobacilli* [75]. In the meta-analysis of Pittayanon et al. they seem to be increased in IBS patients and, as they are associated to fermentation, it could explain symptoms like bloating and meteorism [75]. *Veillonella* and *Enterobacteriaceae* [38] resulted increased while *Prevotella* and *Desulfovibrionaceae* that include sulfur – reducing bacteria are decreased [70]. The study of Tap et al. showed that methanogen relative abundance and symptoms severity are negatively correlated with microbial richness suggesting that methane may contribute to a reduced gastrointestinal motility and constipation [74].

2.3.1. Gut microbiota in IBS-C patients

Gut microbiota dysbiosis has been shown to play a pivotal role in IBS-C [79]. IBS – C patients showed higher levels of **Firmicutes** (including *Clostridium* species) [73,80], in particular *Veillonella* [77] compared to healthy controls. *Lactobacilli* seem to be significantly higher in IBS – C compared to IBS – D subjects [77], but lower if compared to healthy controls [80]. On the contrary, another study showed that there were no significant differences between IBS – C patients and healthy controls not only for *Lactobacilli* but also for *Faecalibacterium prausnitzii* [80].

Regarding **Bacteroidetes** there are conflicting results in the literature. In fact, although, Rajilić-Stojanović et al. demonstrated that patients with IBS-C had a significantly lower level of Bacteroidetes [73], Durbán et al. and Parkes et al. found higher levels of Bacteroidetes in IBS – C subjects than healthy controls [81,82].

Also regarding **Actinobacteria**, there are contrasting results because some studies showed a reduced level [73], especially of *Bifidobacteria* than healthy control [80], while, Parkes et al. showed that they were higher in IBS – C subjects [82].

In general, populations involved in the lactate metabolism (*Bifidobacteria and Lactobacilli*) are significantly lower in IBS-C patients than healthy controls. On the contrary, sulphate reducing bacteria are 100 times increased in IBS – C patients [80]. Another study, showed an increase in Firmicutes (especially *Clostridia*) and a decrease in Actinobacteria and Bacteroidetes in IBS-C patients than healthy controls [77].

Regarding mucosal microbiota, Bacteroidetes and *Bifidobacteria* were high in IBS-C subjects than healthy controls [79].

IBS-C patients had significantly higher levels of *Enterobacteriaceae* (**Proteobacteria**) both in faecal [80,81] and in mucosal samples [79]. Chassard et al., in fact, evaluated the effects of a western diet with dietary fibers comprised between 15 and 30 g/day in both IBS-C group and healthy control group. Microbiota results showed that *Enterobacteriaceae* increased 10 times more in IBS- C patients than healthy control [80].

Maukonen et al., showed that 30% of all bacterial species in IBS-C patients were *Clostridium coccoides* and *Eubacterium rectale* (which produces butyrate), which was significantly lower than healthy control subjects (43%) and IBS-D patients (50%) [76]. Observing the ratio, Parkes et al, showed that the *C. coccoides/E. rectale* was higher in IBS-C than healthy subjects [82]. Also Okhusa et al. observed an increase in *C.Coccoides* and *E.Rectale* in the mucosal microbiota in IBS-C patients [79].

Moreover, in IBS-C subjects, methanogenesis seems to be higher than healthy controls and IBS-D patients. In fact, breath methane (CH_4) production is higher in IBS – C than IBS-D and it is correlated with higher abundance of methanogens and *Ruminococcaceae* [83]. This gas production determines also bloating and constipation [30].

In the end, even if a clear consensus does not exist, many studies suggest that IBS-C patients, compared to healthy subjects, have a lower presence of Actinobacteria in their fecal samples while the level of Bacteroidetes is higher, especially at mucosa level [79]. Another study comparing the microbiota of IBS-C patients and healthy controls found that there were not significant differences in fermentative pattern methane- and nonmethane-producing in fecal samples in both groups. On the other hand, starch fermentation by IBS-C fecal microbiota showed major differences compared to healthy subjects, especially in gaseous metabolite production. It has also been shown that the amount

of acetate and propionate produced by IBS-C microbiota were rather like the one formed by healthy subjects [80].

2.3.2. Gut microbiota in IBS-D patients

In the literature it is clear that in IBS – D subjects all the four principal microbiota phyla are altered [44]. It has been observed an overall reduction in microbial diversity and an increase in potentially detrimental species [38]. There are conflicting results on **Actinobacteria**, especially on *Bifidobacterium*. In fact, Actinobacteria seem to be less in IBS-D patients than in IBS – C ones [42,77] in particular, *Bifidobacterium* [74,75,78,84-86], especially *Bifidobacterium faecis* [87], seem to be reduced in IBS – D subjects in both fecal [73,77,82] and mucosal samples [82] but two studies described a greater abundance of the phyla Actinobacteria in IBS-D fecal microbiota [41,74].

Regarding the **Firmicutes** phylum, two studies showed greater abundance in IBS-D patients compared to healthy controls [74,78], particularly in *Lactobacillaceae* (familia) both in fecal and in mucosal microbiota [88]. *Ruminococci* [42], *Lactobacilli* [42,74,78,84,89] and *Streptococci* [90] (Firmicutes) are increased in IBS – D patients while *Lactococci* are reduced in fecal samples [91]. Regarding *Lactobacilli* there are different findings in the literature. In fact, some studies observed an increase [42,74,78,84,89] while in other study they seem to be reduced [77]. Among Firmicutes there is also the class of *Clostridia* that seems to be increased in IBS-D patients [51], but within this class, *Faecalibacterium* [75,90] - especially *Faecalibacterium prausnitzii* [51, 85] is reduced compared to healthy controls. Conflicting data are reported about the *Lachnospiraceae* family (*Clostridiales*). Indeed, a study in the literature reported that they are increased in IBS-D [78] while another study observed a reduction of this family in both mucosal and fecal microbiota [86]. Whitin this family, other studies have also reported an increased abundance of *Dorea spp.* [51,89], *Coprococcus* [51] and a reduction of *Anaerostipes* [91]. *Dorea spp.* is involved in the carbohydrates fermentation, thus its abundance determines an increased gas production [89].

Both the fecal and the mucosal microbiota in IBS-D patients seem to be poor in **Bacteroidetes** compared to healthy controls [51]. Ringel et al. showed a significant reduction of *Bacteroides* [90] meanwhile the meta – analysis of Pittayanon et al. [75] showed that 3 studies in the literature observed an increase of this class, especially *Bacteroides vulgatus* and *Bacteroides fragilis*, both in mucosal and in fecal samples, and *Bacteroides thetaiotaomicron* only in the fecal one [75,84,90]. The fecal [51,78,85,90] and mucosal samples [51,74,87] of IBS-D subjects also showed a significant reduction of *Prevotella* compared to healthy controls.

Regarding **Proteobacteria,** an increase of *Enterobacteriaceae*, especially *Escherichia coli*, both in the fecal [42,88] and in the mucosal sample [74,86] in IBS-D patients compared to healthy controls have been seen [51,74]. All these findings confirm the complexity in identifying a specific pathological profile of the intestinal microbiota in order to better define the therapeutic approach [44].

In IBS-D patients a higher breath hydrogen (H_2) has been seen and it is associated to a reduced microbial diversity and an abundance of H_2S producing bacteria, including Fusobacterium, Desulfovibrio spp. and Enterobacteriaceae [83].

CHAPTER 3 Nutritional approaches for IBS

Diet plays a pivotal role in IBS patients and more than 70% of them reported that their symptoms worsen after ingestion of foods [20,21,92], particularly fermentable carbohydrates and fats [21]. In fact, some patients exclude on their own some categories of foods that cause symptoms, leading to nutritional and energetic deficiencies [92, 93].

Foods can determine symptoms because they could exert osmotic, chemical, mechanical, neuroendocrine effects but also fermentation or alterations in the bowel distention, intraluminal pH and microbiota [94].

In a survey conducted by Halpert et al., the importance of education on IBS is extremely important. In fact, IBS patients were interested in learning what food prefer or avoid and how to manage symptoms. Moreover, more than 50% of IBS patients would like to know the effects of IBS on their life and how to prevent IBS disturbances [95]. For this reason, a nutritional education in the choice of the most appropriate foods is a valid support in the patient care [96].

3.1. Traditional dietary advices

General advice on healthy eating and lifestyle is recommended as the first-line approach in the dietary management of IBS [25]. Dietary modifications are based on nutritional advices provided by the National Institute for Health and Care Excellence (NICE) and the British Dietetic Association (BDA) [28,29].

Currently, a diet for IBS subjects has not been identified yet, thus clinical practitioners usually suggest general advices such as [25,28,29,93,96-98]:

- **To have regular meals avoiding skipping meals and to consume moderate portion sizes:** IBS patients reported to have more irregular meal habits than healthy controls and this irregularity may affect colonic motility and IBS symptoms. Moreover, also the amount of food ingested is associated to the worsening of symptoms. In fact, data obtained from studies on binge – eaters demonstrated that the risk of developing IBS-like symptoms was 2-4 times higher among who ate large amount of foods.
- **To drink between 1,5 and 3 L of liquids a day:** even if there are not specific studies on the amount of liquid suitable in IBS, it could be preferable to have an adequate amount of water, especially in case of IBS-C. It is suggested to prefer still water or caffeine-free drinks; avoiding sparkling water or other fizzy drinks that may impair symptoms like bloating.

- **To reduce caffeine intake (e.g. coffee, tea, energy drinks, chocolate):** coffee for its caffein content, increases gastric acid secretion and colonic motility also in healthy individuals. Coffee in fact, may have laxatives properties. If related to symptoms, coffee intake should be restricted to 3 cups (400 mg caffeine) per day.
- **To reduce alcoholic drinks:** in the literature there is a lack of studies that correlate alcohol and IBS but it is clear that alcohol affects the gastrointestinal motility, absorption and permeability. In women with IBS has been seen that a higher alcohol consumption (more than 4 drinks per day) and not moderate consumption (2-3 drinks per day) or light consumption (1 drink per day) is associated to symptoms worsening. In general, the alcohol intake should be reduced in relation to symptoms. Safe alcohol limits are no more than 1 drink per day for woman and no more than 2 drinks per day for men.
- **To moderate fiber intake:** Fibers intake may exacerbate IBS symptoms like bloating, abdominal pain and distention. It is important to distinguish among fibers. In fact, soluble fibers (e.g. oat, psyllium) should be preferred to insoluble ones (e.g. bran or bran based cereals, whole flour or derivatives). But, a more specific classification for fibers is necessary because fiber – foods contain both kind of fibers. Thus, fibers should be classified also according to their viscosity, fermentability and gel-forming capacity. Rapidly fermented fibers lead to higher gas formation than poorly fermented ones. The correct amount of dietary fibers is still unknown, but a target dose of 20-30 g per day is usually suggested. IBS-C patients could consume linseeds up to 2 tablespoons per day to improve their evacuation frequency and stool consistency. More pieces of information about the role of fibers in IBS are reported in the paragraph 3.3.5.
- **To reduce the consumption of fatty – foods:** lipids reduce bowel motility and induce gas retention and bloating in healthy subjects and it is increased in IBS patients. It is important to distinguish the type of fats. Polyunsaturated fats, in fact, may have a positive role on gut inflammation. However, the amount of daily fats should not exceed 30-35% of total daily energy.
- **To reduce the consumption of spicy foods:** the consumption of spicy foods triggers symptoms like abdominal pain. Capsaicin is the active component of red chili that is considered as the responsible of symptoms. It increases the gastrointestinal transit through its vanilloid receptor (TRPV1) causing pain and a burning sensation also in healthy individuals. An increased numbers of receptors has been seen in IBS subjects that suffer also for visceral hypersensitivity. Studies in the literature have shown a symptoms

exacerbation in IBS patients after high intake of spicy foods, but a moderate intake has not been evaluated. However, capsaicin has also analgesic properties if consumed moderately and often.

- **To pay attention to milk and dairy products consumption:** many IBS patients attribute symptoms to consumption of milk or dairy products even without being lactose intolerant. Typical GI complaints of lactose intolerance are similar to those of IBS such as discomfort, bloating and loose stools. Lactose exclusion may improve symptoms. It could be plausible that other milk components, such as A1 β casein, may determine symptoms onset.
- **To reduce the consumption of "resistant starch"** that is usually in pre – cooked or pre – packaged foods;
- **To avoid sorbitol**, an artificial sweetener found in chewing – gums, **and fructose** especially in IBS -D patients
- IBS-D patients should avoid also **Aloe vera** (for its laxative properties), **acupuncture** and **riflessology**.

According to these nutritional suggestions, the IBS food pyramid (*Figure 6*) has been developed. It is a 9 level pyramid in which foods are presented according to their consumption frequency in an ascending order, form the most to the least frequent [25].

At the base of the pyramid there are indications on lifestyle modifications such as physical activity and good hydration. On the second level it is recommended to have regular meals and healthy eating habits, avoiding large amount of foods and take time to eat and chew food properly. At the third step cereals and derivatives are represented. They are suggested in lower amount than general guidelines for healthy subjects. In fact they are recommended 6 times per day but in the amount of 40-60 g of bread and 60-70 g of pasta or rice. At the fourth level there are vegetables that are an important source of vitamin and bioactive compounds. The suggested portion size is 100-150 g and it is recommended to consume from 3 to 5 portions per day. Here also fruit is considered. The suggested portion size of fruit is 80 g (a small fruit) 2-3 times per day. At the sixth level there are protein – rich foods (eggs, fish, meat, legumes, soy, nuts and seeds). It is recommended to consume 2-3 portions of these foods per day. A portion of meat is 100-125 g, of fish is 125-150 g or 60-80 g of eggs or legumes and 20-30 g of nuts and seeds. At the seventh level there are milk and dairy products that should be consumed 2-3 times per day. A portion of milk or yogurt is considered to be 200-250 ml or 80-100 g of fresh cheese or 30-50 g of seasoned cheese. The next level considers fats, sweets, spicy foods, caffeine and alcohol that have to be consumed moderately. For fats, it is suggested to prefer healthy ones, like

olive oil (preferably extra-virgin), for sweets, 30 g of dark chocolate is allowed while other foods should be reduced if they are linked to symptoms. At the top of the pyramid there are fried and processed foods that should be completely exclude [25].

On the left of the pyramid there are also some indications related to fibers and probiotics intake and also to see a specialized nutritional professional if there is the intention to start a low-FODMAP diet [25].

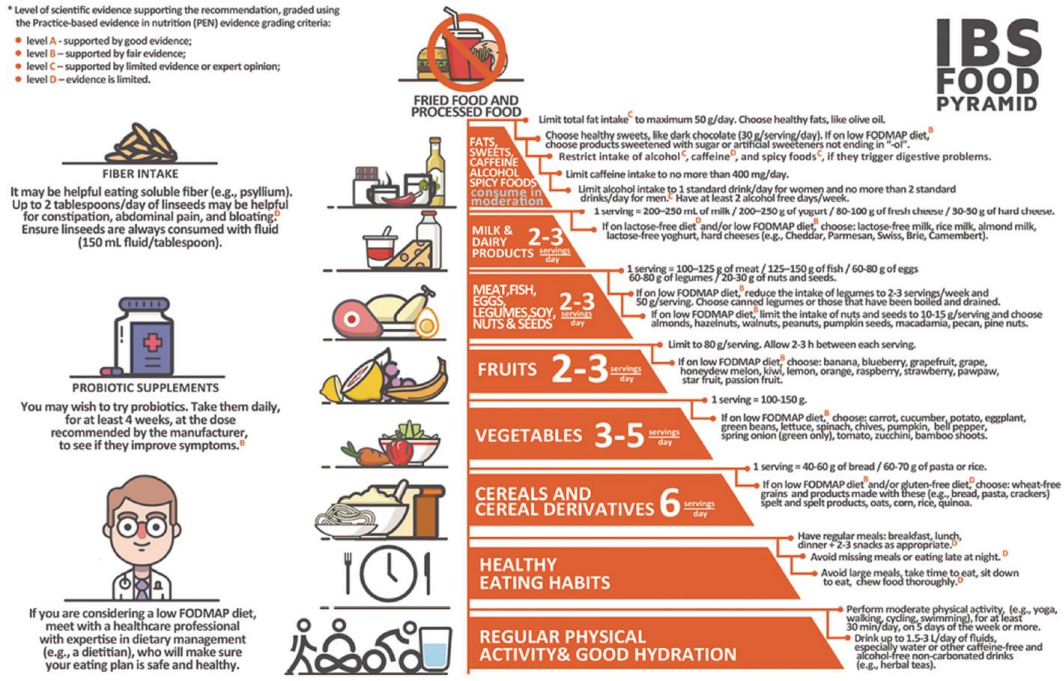

Figure 6 IBS Food Pyramid [25]

In some studies, also the mNICE diet (a modified NICE diet) is suggested. This diet, in addition to the suggestions presented above, recommends to substitute wheat products with spelt ones and to use psyllium husk as a source of fibers. Moreover, in this nutritional protocol, also beans, onions, cabbage, fat-meals, soft drinks and artificial sweeteners ending in -ol should be avoided [12]. To avoid gas production it could be necessary also to reduce some gas-producing foods such as celery, carrots, raisins, some kind of fruits (bananas, apricots, prunes), Brussel sprouts and wheat germs [3].

3.2. The low – FODMAP diet

The low – FODMAP (Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols) diet represents the second – line dietary intervention to follow if symptoms persist nevertheless the general nutritional advices mentioned above [25], although the recent published

American Gastroenterological Association clinical practice update on the role of the diet in IBS states that it is currently the most evidence – based diet intervention for IBS [99]. FODMAPs include fructans and fructo – oligosaccharides, galacto – oligosaccharides, lactose, fructose and polyols [25] and they are not absorbed in the small intestine, thus determine gas production (especially carbon dioxide and hydrogen) through fermentation by gut bacteria and the influx of water in the small bowel [25,97,98,100]. The low FODMAP diet consists in the exclusion of these short chain carbohydrates that are present in milk and dairy products, in some kinds of fruits and vegetables, in onion and garlic, in legumes, in wheat, in artificial sweeteners and in processed foods [100]. Although it is an elimination diet and it is also very restrictive, it provides a high symptoms reduction in IBS patients [100]. Both IBS patients and healthy subjects may have a reaction consuming FODMAPs, but IBS patients feel more pain due to the visceral hypersensitivity [98].

This diet is a 3-step programme. The first one, is a restricted elimination diet and it generally lasts from 6 to 8 weeks (some author reported from 2 to 6 weeks [101] or from 4 to 8 weeks [3]), under specialist dietician control [98]. This first step has not to be intended as a long – term diet [102], in fact, if individuals have an improvement in symptoms, they can start the next phase for 6-10 weeks in which FODMAPs are gradually reintroduced to have time to understand which FODMAP triggers the symptoms. Finally, patients can start the third phase that is a long-term personalization in which only FODMAPs that have induced symptoms are excluded [3,98,101]. During the second and the third phases, although the increased FODMAPs consumption, symptoms control seems to be good even if the adherence is poor without the dietician control [101]. Foods are classified as “high-FODMAP” and “low FODMAP” (*Table 1*) and patients are suggested to prefer foods belonging to the second category.

Table 1 High - FODMAP foods and alternative low FODMAP foods (25)

FODMAP	High FODMAP foods	Low FODMAP foods
Oligosaccharides: fructans and/or galacto-oligosaccharides	Vegetables: artichokes, asparagus, beetroot, Brussels sprouts, broccoli, cabbage, fennel, garlic, leeks, shallots, okra, onions, peas	Vegetables: carrot, cucumber, potato, bell pepper, eggplant, green beans, lettuce, spinach, chives, parsnip, pumpkin, silverbeet, spring onion (green only), tomato, zucchini, bamboo shoots, bok choy, choko, choy sum
	Cereals: wheat & rye when eaten in large amounts (*e.g.*, bread, pasta, crackers)	Cereals: wheat-free grains or wheat-free flours and products made with these (*e.g.*, bread, pasta, crackers), spelt and spelt products, oats, corn, rice, quinoa
	Legumes: chickpeas, lentils, red kidney beans, baked beans	Legumes: canned chickpeas
	Fruits: watermelon, custard apple, white peaches, rambutan, kaki	
Disaccharides: Lactose	Milk (cow, goat, sheep), yoghurt, soft & fresh cheeses (*e.g.*, Ricotta, Cottage), ice cream	Lactose-free milk, rice milk, almond milk, lactose-free yoghurt, hard cheeses (*e.g.*, Cheddar, Parmesan, Swiss, Brie, Camembert), butter, ice-cream substitutes (*e.g.*, dairy-free gelato, sorbet)
Monosaccharides: Fructose	Fruits: apples, pears, nashi pears, clingstone peaches, mango, sugar snap peas, watermelon, tinned fruit in natural juice, dried fruits	Fruits: banana, blueberry, grapefruit, grape, honeydew melon, kiwifruit, lemon, lime, mandarin, orange, tangelo, raspberry, strawberry, pawpaw, star fruit, passion fruit, rockmelon, carambola, durian
	Honey	Honey substitutes: maple syrup
	Sweeteners: fructose, high fructose corn syrup	Sweeteners: sugar, glucose, artificial sweeteners not ending in "-ol"
Polyols	Fruits: apples, apricots, cherries, longon, lychee, nashi pears, nectarines, pears, peaches, plums, prunes, watermelon	Fruits: banana, blueberry, grapefruit, grape, honeydew melon, kiwifruit, lemon, lime, mandarin, orange, raspberry, pawpaw, star fruit, passion fruit, rockmelon, carambola, durian
	Vegetables: avocado, cauliflower, mushrooms, snow peas	
	Sweeteners: sorbitol, mannitol, xylitol & others ending in "-ol", isomalt	Sweeteners: sugar, glucose, artificial sweeteners not ending in "-ol" (*e.g.*, sucralose, aspartame)

In many studies the low – FODMAP diet seems to have more beneficial effects compared to other nutritional protocols [103-109] and many patients see the first improvements within 1-2 weeks from the beginning even if other can take also 3-4 weeks to respond [102].

Several studies in the literature focused more on the effects of low – FODMAP diet in IBS-D [31,103-106,110]. The first RCT that evaluate the effects of a low – FODMAP diet vs a habitual diet for 4 weeks was the study of Staudacher et al. in 2012. They found an overall IBS symptoms improvement after the low – FODMAP diet compared to the habitual diet [31]. Also Eswaran et al. evaluated the effects of a low FODMAP diet and a modified NICE (mNICE) diet after 4 weeks in 84 IBS-D patients [103]. The mNICE diet provided eating small quantities of food divided in frequent meals, no trigger foods and to avoid alcohol and caffeine consumption without excluding FODMAPs that were completely absent in the low-FODMAP diet. Both diets significantly improved symptoms in 40-50% of patients but the low FODMAP diet reduced more abdominal pain, bloating and stool consistency compared to mNICE diet. Moreover, low FODMAP diet determined also a higher improvement in quality of life, anxiety, depression, activity impairment, sleep quality and fatigue compared to a mNICE diet [104]. Similar results on symptoms, stool consistency and frequency improvement were found after a 6 -week period of low- FODMAP diet vs a diet based on traditional dietary advices but the low – FODMAP diet showed better results [105]. Recently, also a prospective trial on 101 IBS-D subjects for a total of 16 weeks demonstrated that the low FODMAP diet improved symptoms and quality of life more than traditional nutritional advices, providing also the reduction

of medication use [106]. The study of Orlando et al. conducted on 20 IBS-D subjects showed that a long–term (90 days) - low FODMAP diet reduced symptoms severity and improved bowel movements. This diet showed also an anti-inflammatory effect through the reduction of prostaglandin E2 and n-6 PUFA/n-3 PUFA ratio, main fatty acids in the red-blood cell membranes [110].

The meta – analysis of Varjù et al. confirmed that low – FODMAP diet is as effective as traditional IBS diet but results on symptoms reduction remain lower in the low – FODMAP diet compared to the traditional one also during the follow – up period [111].

Nevertheless, some studies results are in contrast with those presented above. In fact, the study of Bohn et al. showed similar results on symptoms reduction after a low – FODMAP diet and a diet based on traditional dietary advices [112]. Similar results were found be Eswaran et al. on IBS symtpoms after a low – FODMAP and a mNICE diet [103].

As this diet is very restrictive, sometimes the adherence results low. A recent retrospective cross – sectional study observed up to 2 years the effects of a low – FODMAP diet in IBS patients. They found out that the long-term adherence and satisfaction was high even if the majority of patients referred that the low – FODMAP diet is complicated to follow in the daily life [113]. Clevers et al., instead, evaluated that the low – FODMAP diet adherence was still high thanks to low – FODMAP substitutions but a 25% fell of the total energy intake was observed. Also with the traditional dietary advice there was a reduction in the daily energy (11%). It is important, thus, that patients are more adherent to the diet because adherence and symptoms resulted to be negatively correlated [108].

A recent review of Rej et al. evaluated all studies in the literature who tested this diet [98]. Numerous studies focused on the first phase and they underlined the good results on symptoms [103,107,112,114,115] but some micronutrients deficiencies [116] and a lower intake of carbohydrates and fibers [112]. On the contrary, in some studies in the long-term no more deficiencies were seen, and the adequacy of the diet could be compared with the patients' usual diet [117,118]. Moreover, it is important to be followed by a specialized nutrition professional in order to avoid nutritional deficiencies [25].

This diet may negatively affect microbiota because it is lacking of fibers and short chain carbohydrates that could act as prebiotics on gut bacteria [25].

RCTs are needed to prove the low – FODMAP diet superiority to other dietary interventions [19].

3.3. Other nutritional advices for IBS

3.3.1. The Mediterranean diet

Similarly, to the traditional dietary advices for IBS, there is the Mediterranean Diet (MD). It contains foods and heating habits typical of people residing along the Mediterranean Sea [14]. It provides high consumption of fruit, vegetables, whole grains, legumes, nuts and seeds, fish, eggs (no more than 2 per week), white meats and dairy products and a low consumption of red and processed meats. Saturated fats are suggested in lower amount than mono (olive oil) and polyunsaturated (fish) fats [119]. According to this nutritional pattern it is important to have 5 meals per day (3 principals and 2 snacks) and to take time to eat. Also in this case physical activity is important to maintain a healthy status and to reduce the daily stress. It is associated with a low incidence of mortality [14] and a reduced risk of cardio-vascular diseases and high levels of plasma cholesterol [119]. Moreover, it has anti – inflammatory properties and it is suggested in prevention of non-communicable diseases (e.g. obesity, metabolic syndrome, type 2 diabetes etc.) [119]. Paduano et al. observed in 42 IBS patients the effects of three different diets: a gluten free-diet, a Mediterranean diet and a low-FODMAP diet. At the end of the study all three nutritional protocols showed positive results on bloating, abdominal pain and quality of life but the Mediterranean diet was preferred by participants [120]. In IBS population this diet has been evaluated and a low MD adherence and a higher symptoms score were observed both in 17-24 and in 25-34 year age group, especially among women [14]. This diet is less restrictive than low – FODMAP diet and could be a good option even if a proper education is necessary [14].

3.3.2. Gluten free diet (GFD)

It has been seen in few studies that IBS symptoms (especially in IBS-D) can be reduced after a gluten free diet [29,121]. Actually, celiac disease/ gluten sensitivity and IBS – D are two different entities with an overlap of symptoms [122], although the prevalence of celiac disease may be increased in IBS-D patients [121]. Recent studies have showed that IBS patients symptoms worsen with gluten/wheat ingestion, thus their consumption and gluten sensitivity are considered to be involved in IBS etiology [123,124]; may be one of the implicated mechanism is the alteration of the gut barrier permeability [125]. However, it is important to evaluate if IBS – D patients are also affected by celiac disease with serological tests before suggesting them a gluten – free diet [1].

In a prospective double blinded placebo RCT, 60 non – celiac IBS patients followed a GFD for 4 weeks and they had to report their symptoms during the whole study period. The participants who

showed a relief in symptoms were assigned to one of two arms: placebo (gluten free breads) or gluten (whole cereal breads) for the following next weeks. Patients who reintroduced gluten in their diet had a worsening of symptoms within 1 week. These results underline that IBS patients may benefit from a gluten free diet [124]. Also other studies [120,126,127] in the literature observed a relief in symptoms after a GFD in non – celiac IBS subjects.

Barmeyer et al. evaluated a long- term efficacy of a GFD in 35 non – celiac IBS-D and IBS-M patients observing also the HLA-DQ2 and DQ8 expression: 34% of patients resulted wheat sensitive and they continued to follow the diet until 1 year follow-up with a maintaining symptoms relief [126].

The study of Aziz et al. showed that the improvement in symptoms was independent from the HLA – DQ group. As in Barmeyer's study [126], some patients continued the GFD even after the end of the study (18 months) reporting an ongoing symptoms remission [127]. In the study of Algera et al., 20 IBS patients and 18 healthy controls followed a gluten free diet for 14 days in which they sprinkled gluten (14 g/day) or rice flour powder over their meals. The gluten-free diet showed better results on symptoms and stool consistency in IBS patients [128].

In another RCT, 45 non – celiac – subjects were randomized to GFD or a gluten containing diet (GCD) creating subgroups according to HLA-DQ2/8 positive or negative genotype. GFD significantly decreased stool frequency especially in HLA – DQ2/8 positive but no significant differences were seen for stool form, gastric empty and colonic transit in both groups. GCD, instead, determined an increased permeability in small bowel and rectosigmoid mucosa (reducing tight junction proteins). This effect was seen more in HLA – DQ2/8 positive than HLA – DQ2/8 negative subjects [129].

Patients who want to follow the gluten free diet to ameliorate their IBS symptoms have to be informed that there are not strong evidences in following such a diet, and, moreover, they have to be aware to nutritional deficiencies [25]. To date, it is not clear if gluten or other wheat components, such as amylase – trypsin inhibitors or fructans, trigger symptoms [123,126]. In any case, long-term studies are needed to better understand the effectiveness of this diet in IBS.

3.3.3. The lactose– free diet

The digestion of lactose takes place thanks to the lactase enzyme that splits this disaccharide in two monosaccharides: glucose and galactose. Throughout the life, the lactase activity is physiologically partially lost [130]. In lactose intolerant (LI) subjects, lactase activity is not enough

to digest lactose, thus lactose comes intact to the colon where it is fermented by the intestinal microbiota producing SCFAs and other gases leading to abdominal distension and symptoms like diarrhea, bloating and abdominal pain [130]. Nybacka et al. showed that in their study 17.5% of IBS – D women tended to consume a low amount of lactose (<2 g/day) for its role in symptoms worsening [131]. In fact, IBS patients, especially the IBS-D subgroup, usually exclude, on their own, food containing lactose (e.g. dairy products) from their diet [132] or they prefer to consume lactose – free products [132] not knowing that self – reported milk intolerance is not a good diagnostic predictor of lactose intolerance [133]. In general, it has been seen that the prevalence of lactose intolerance seems to be more present in IBS – D patients than in healthy subjects even if these two conditions are not always associated [133,134].

It has been seen that IBS-D patients with LI present an increase of mast cells on the intestinal mucosa and of serum TNFa and rectal sensitivity after lactose ingestion, compared to patients with only lactose malabsorption or healthy controls [135].

Actually Yang et al. found that the risk of developing lactose intolerance was related to ingested lactose dose that determines a worsening of symptoms as intestinal gas production [136]. Generally the safe dose of lactose for LI people is 20 g but this amount should be personalized depending on symptoms [137]. Finally, to date, it is clear that there is no link between IBS and lactose malabsorption, but these two conditions present only similar symptoms [138]. Thus, a lactose – free diet is not recommended for IBS patients who are not lactose – intolerant.

3.3.4. The fructose – free diet

In some cases a fructose free diet may be suggested to reduce IBS symptoms. As it has already been explained, fructose is a monosaccharides that belongs to FODMAPs. This kind of diet should be considered only in subjects with fructose malabsorption [139] or with positive fructose breath test [140]. A study on 80 IBS patients of which 26 with fructose breath test positive showed that diarrhea/loose stools were present only in fructose intolerant subjects. 14 out of these 26 improved their symptoms (abdominal pain, bloating, fullness, indigestion and diarrhea) with a fructose-restricted diet for approximatively 1 year [140].

3.3.5. The role of dietary fibers

Dietary fibers are defined as "the edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine" [141]. Thus, they come intact to the colon and they may or not be fermented by gut microbiota [142].

Fibers classification is very complex. Years ago, fibers were divided into soluble and insoluble but, to date, this differentiation has not been considered sufficient. In fact, they can also be subdivided according to their solubility, viscosity and gel – forming capacity.

For ages it has been suggested for IBS patients to consume fibers to improve symptoms but in the last years, it has been seen that symptoms may worsen after fibers ingestion. It is extremely important, so, to identify the right type and amount of fibers to suggest in case of IBS depending also from the IBS subtype [12]. The study of Bijkerk evaluated the administration of 10 g of psyllium, 10 g of bran and 10 g of placebo (rice flour) to 275 IBS patients divided in 3 arms for 12 weeks. After a month and also at the end of the study period (in a significant way), psyllium showed better results on symptoms compared to bran and placebo. In the bran group more patients dropped out due to worsening of symptoms. No significant changes were observed on quality of life during the three treatments [24].

A systematic review and meta – analysis of 12 trials compared dietary fibers supplementation to a control diet e did not find any significant differences in symptoms improving after any kind of fibers [143].

3.3.6. Functional foods with a focus on high-amylose flours and resistant starch

Starch is composed by amylose (20-30%) and amylopectin (70-80%) that is the branched part. In the last years some authors have been studying the effects of high- amylose (38%) flours on health [144]. It has been seen that high – amylose products induces both lower postprandial glycemic [144-146] and insulinemic [145,146] response and effects on colonic health increasing fecal mass and generating a higher production of SCFAs preventing some pathological conditions such as colon cancer [144,146,147]. Studies in vitro showed a correlation between the amount of amylose and the resistant starch (RS) formed during processing. The RS is a fraction of starch that is not digested in the small intestine [144].

The RS is classified in five different forms:

- **RS1:** it is a type of starch that is not accessible by digestive enzymes. It is entrapped in the cell wall of grains and seeds. It could be digested in the small intestine only if it is milled [142]. Examples of RS1 – containing foods are: breads made with whole grains and pasta made with durum wheat by extrusion [148].
- **RS2:** it comprises native starch granules that are protected by the digestion thanks to their crystalline structure, thus they are highly resistant to enzymatic hydrolysis. Examples of

RS2-containing foods are: uncooked potato starch, green banana starch, gingko starch and high – amylose maize starch. After cooking most of this kind of starch becomes digestible thanks to gelatinization but if the temperature is lower than the boiling water temperature, RS2 does not gelatinize and its structure remains inaccessible by enzymes [148, 142].

- **RS3:** it comprises the retrograded starch, mainly retrograded/recrystallized amylose formed during cooling of gelatinized starch and in cooked foods kept at low or room temperature [148, 142]. Amylose linear structure tends to form double helices, especially near the refrigeration temperature (4-5°C). RS3 has its gelatinization temperature above 170°C, thus it is not affected by cooking [148]. It is used as an ingredient in a huge variety of conventional foods. Cooked and cooled potatoes and cornflakes are good examples of RS3 [142].
- **RS4:** it is a chemically modified starch, formed either by cross – linking or by adding chemical derivatives. Due to the presence of cross links it cannot be hydrolysed by amylase or fermented by microbes [148, 142].
- **RS5:** it is formed through the interaction between starch and lipids. This complex amylose – lipids does not permit the enzymatic action and is thermally stable [148, 142]. These complexes are usually formed from high – amylose starches [142].

Studies in the literature are focused on the effects on postprandial glycemia and insulinemia in healthy adults to find an optimal composition to prevent the onset of type 2 diabetes and metabolic diseases [145, 146]. Foods rich in RS are characterized by slow digestibility and its inaccessibility by enzymes determines the lower postprandial glucose and insulin response. In 1981, the concept of glycemic index (GI) of foods was introduced to classify both foods rich in simple sugars and starchy foods [149]. GI is calculated as the incremental area under the curve (IAUC) expressed as a percentage after the consumption of 50 g of available carbohydrates and it is used to classify carbohydrate-containing foods according to their influence on postprandial glycemia [150]. According to GI classification, it may be considered high, medium and low when it is higher than 70, comprised between 55 and 69 or lower than 55, respectively [151]. The Food and Agriculture Organization (FAO) recommends the consumption of low – GI foods, in order to manage glycemic peaks, especially in case of diabetes or impaired glucose tolerance [142], counteracting the onset of cardiovascular diseases [152,153]. Unfortunately, GI does not take in consideration the portion size of foods, for that reason the glycemic load (GL) has been introduced. It is calculated as the product between the GI and the amount (in grams) of available carbohydrates in a food portion size/100 [154]. The GL is classified as high (above 20), medium (11-19) and low (under 10). This additional index

is related to the food portion size, admitting also smaller portion sizes of high GI foods without a dramatic impact on glycemia [155].

Belobrajdic et al. evaluated 4 different types of bread: the refined type high and low in amylose and the wholegrain type high and low in amylose. They found out that high amylose (HA) breads had a 39% lower glycemic response than low amylose (LA) counterparts. Also insulin was 24-30% lower after high amylose products than low amylose breads [145].

In some cases also the effects of high – amylose products on gastrointestinal functions and microbiota, have been evaluated, even if there are no studies specifically focused on IBS. In general, RS plays a prebiotic role. Prebiotics are defined as "non digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon and thus attempt to improve host health" [156].

3.3.7. Very Low Carbohydrate Diet (VLCD)

In the literature there is a lack of studies that evaluate the effects of a very low carbohydrate diet (ketogenic diet) in IBS. However, in 2009, Austin et al. conducted a study on 13 IBS subjects with overweight or obesity in which they had to follow a VLCD diet with no more than 20 g of carbohydrate/day. The study protocol provided 2 weeks of a standard diet and subsequently 4 weeks of a VLC diet. All participants showed a significant improvement in IBS symptoms (bowel movements, abdominal pain and stool consistency) in almost 2 weeks of VLCD and 77% in all 4 weeks, especially in the last week. Also the quality of life and sickness profile improved after VLCD [157].

3.4. The effects of different nutritional patterns on gut microbiota

A growing body of evidence supports that dietary macronutrients may induce a shift in the gut microbiota composition [44,70]. The mechanism that has been recognized to explain the link between gut microbiota and IBS symptoms is the luminal distention [70].

According to the traditional dietary advices, caffeine has been seen to increase *Bacteroides*, *Prevotella* and *Porphyromonas* concentration in high – consumers (45-500 ml/day), sweeteners, (especially polyols) increase *Bifidobacteria* in healthy subjects and they can express also a prebiotic effect. On the other hand, lactitol determines a decrease in *Bacteroides*, *Clostridium coliforms* and *Eubacterium*. A high-dose capsaicin enriched diet (10 mg/day for two weeks) determines an increase of *Faecalibacterium* in healthy humans [44].

The low – FODMAP diet presents a lower amount of carbohydrates (that could be used as a substrate available for colonic fermentation) than habitual diet [31]. It determines a detrimental effect on microbiota diversity [70] reducing the total abundance of gut bacteria, especially *Bifidobacteria* [19,26]. According to some authors, some *Clostridium* clusters increase after a low – FODMAP diet compared to the habitual diet, it may be due to different substrate availability [31] but, on the contrary, in other studies *Clostridia* decreased [44,70,157]. Primary fiber-fermenters include *Ruminococcus bromii, Roseburia* and *Eubacterium rectale* which generate also some products that could be utilized by other species, contributing to bacterial cross – feeding. The lack of FODMAPs and dietary fibers in this exclusion diet determines a reduction of all these beneficial species. Valeur et al. evaluated the effects of a 4-week low – FODMAP diet in 61 IBS patients. The 32 responders showed higher levels of *Bacteroides fragilis, Acinetobacter, Streptococcus* and *Eubacterium* and lower levels of *Clostridia, Shigella and Escherichia* [159].

Unfortunately, not all studies that evaluate the effects of different nutritional approaches were conducted on IBS patients. Regarding the Mediterranean diet, in fact, it was mostly observed in case of overweight or obesity. It has been seen that it determines microbiota changes differently from the Western diet reducing the risk of disease. In fact, in a study on 20 subjects affected by obesity, it has been seen that a Mediterranean diet followed for 1 year determines the reduction of *Prevotella* and the increase of *Roseburia* and *Oscillospora*. *Roseburia* is a butyrate producer microorganism that has anti – inflammatory properties and *Oscillospora* is linked to health [160]. Following the diet for 2 years, an increase in *Prevotella, Bacteroides, Ruminococcus, Faecalibacterium* and *Roseburia* has been seen [161]. In a cohort of healthy subjects, high adherence to a Mediterranean diet was associated to an increase of Bacteroidetes (especially *Prevotella*) and a reduction in Firmicutes (especially *Ruminococcus*). Some components of the Mediterranean Diet seem to be associated to microbiota changes such as: cereals with the genera *Bifidobacterium* and *Faecalibacterium*, olive oil with *Tenericutes* and *Dorea*, red wine with *Faecalibacterium*, vegetables with *Dorea, Alistipes* and *Ruminococcus* and legumes with *Coprocuccus* [162]. On the contrary, a Western diet, characterized by high intake of read and processed meat, refined sugars, saturated fats and refined grains, determines the development of inflammatory species of the microbiota [19] such as *Alistipes* and *Bacteroides spp*. and it is associated to a reduction of *Faecalibacterium*, *Lactobacilli* and butyrate producer bacteria. [44,163].

A carbohydrates-rich diet determines an increase in *Prevotella* and in SCFAs producer bacteria [57,87,164] (*Figure 7*). Dietary fibers act as prebiotics, promoting the growth of *Lactobacillus* and *Bifidobacterium* and an increasing in SCFAs production. Moreover, fibers can also stimulate the

mucus production and secretion by colonic epithelium and determine symptoms like bloating and abdominal pain [70]. A diet that presents the consumption of fibers from fruits and legumes is associated to an increase of *Prevotella* over *Bacteroides.*

Among carbohydrates there is also resistant starch. Although studies to understand how RS affect microbiota are limited, it is clear that it determines gut microbiota modification [148]. RS2 leds to an increase of *Ruminococchi* (in particular *R. bromii*) and *E.rectale* which are known for their saccharolytic properties [164,148] increasing also the butyrate production [164]. Similar results were found by Hughes et al. In fact, although the α diversity decreased, RS2 consumption determined the abundance of *Ruminococchi*, *Roseburia* and *Faecalibacterium* and their presence was correlated to butyrate and total SCFAs production [146]. RS promotes the growth of some beneficial bacteria and interact with other prebiotics such as β-glucan. The same characteristic may be extended also to high – amylose starch that is a source of RS2. Among all prebiotic fibers, only RS determines higher production of butyrate that acts as nutrient for colonocytes. Butyrate is implicated in improving colonic health and reducing the risk of colorectal cancer [142]. The study of Hughes et al. showed that after a RS2 – enriched wheat rolls in healthy subjects for one week determined an increase in hydrogen and methane production.

Diet rich in saturated fats is associated to a gut low – grade inflammation and it has effects on two pathobiont families, the *Erysipelotrichaceae* and the *Coriobacteriaceae* (*Figure 7*). It is not clear if these two families have a role in the onset of inflammation or if they are only a consequence of a high fat – diet [70]. Moreover, an animal based diet high in fats and proteins determines an increase of *Bilophila, Bacteroides* [57,87,164] and *Alistipes*. This last is associated to higher abdominal pain in pediatric IBS but also to depression and type 2 diabetes. Also other pathogenic species increased such as. *E. Coli, Salmonella, Yersinia, Vibrio* and *Pseudomonas*. Also a decrease in the production of antioxidants and SCFAs has been observed [164].

A high intake of proteins, especially from animals, determines the production of toxic end – products such as ammonia, phenols, branched – chain fatty acids and hydrogen sulphide (*Figure 7*). *Clostridium spp*. are involved in protein metabolism and ammonia production that can impair barrier function and stimulate the release of pro – inflammatory cytokines. Among products derived from the protein fermentation (especially cysteine) there is also hydrogen – sulphide [70]. Also *Bacteroides* increased while *F. Prausnitzii, Ruminococcus* and *Akkermansia* decreased in the high – protein diet, and consequently, also butyrate production decreased [164].

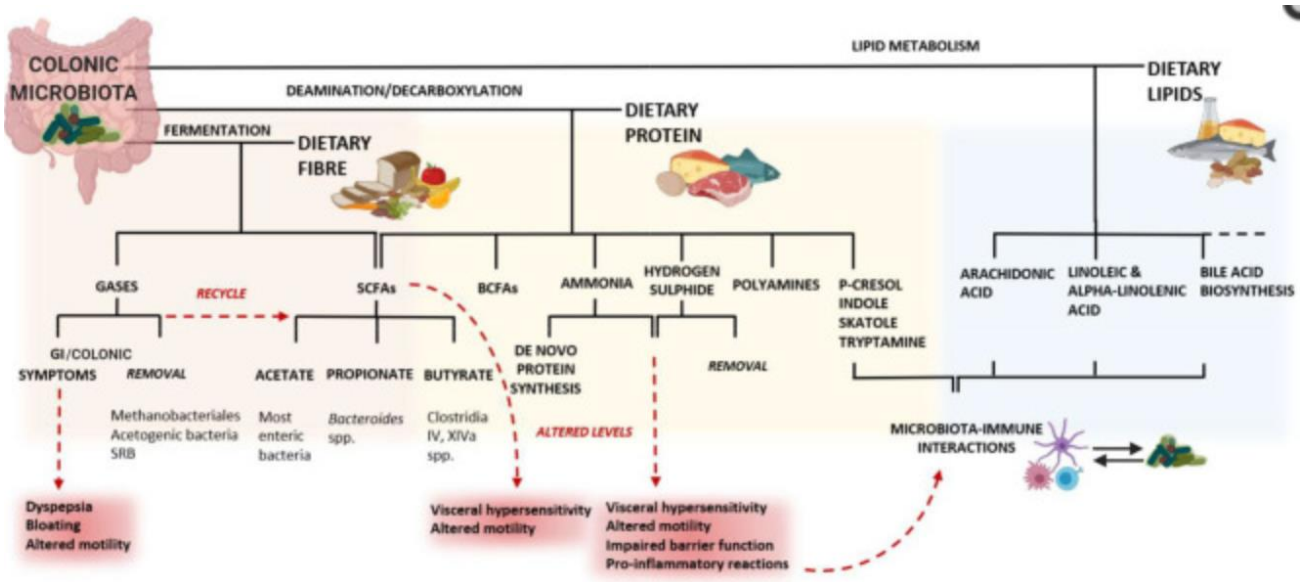

Figure 7 The consequences of diet on gut microbiota (70)

A one-month gluten free-diet, instead, determines the decrease of *Bifidobacterium, Lactobacillus, Clostridium lituseburense*, *Faecalibacterium prausnitzii* and an increase of *E.Coli* and *Enterobacteriaceae* [165]. In another study also a reduction of *R. bromii* and *Roseburia faecis* and an increase in *Victivallaceae* and *Clostridiaceae* has been observed [166]. Further investigations have also shown an increase of pathogenic species such ad *Enterococcus, Staphylococcus, Salmonella, Shigella* and *Klebsiella* that have an important effect on intestinal mucosa in healthy subjects [95]. In a Danish RCT, 60 adults were allocated to a 8 – week low - gluten diet or a high - gluten diet. The low - gluten diet determined a reduction of *Bifidobacterium* and *Dorea*, two species of *Lachnospiraceae* family and *Anaerostypes* and *Eubacterium halii* [167].

The majority of studies that evaluate the effects of a ketogenic diet on microbiota are conducted in children affected by epilepsy or in mouse models. In studies conducted in adults, the reduced carbohydrates intake during a low – carbohydrate (or ketogenic) diet has been associated to a decreased microbial diversity. *Akkermansia muciniphila* and *Lactobacillus* increased during a ketogenic diet while pro – inflammatory taxa such as *Desulfovibrio* and *Turicibacter* decreased [44, 157]. This result is linked to weight loss that usually has been obtained during a ketogenic diet [157].

In any case, the interindividual variation to different dietary approaches depends on the initial microbiota composition and it could be an area of interest for further researches [19, 148].

CHAPTER 4 The FRUMILOSIO project

The name FRUMILOSIO is an italian acronym for *FRUMento ad alto contenuto di amILOSIO*, in english *High – amylose wheat*.

This project was conducted in collaboration with University of Tuscia and University of Molise. This study was funded by Regione Lazio LazioInnova (grant number 85–2017- 14 994) and it was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

High – amylose products were obtained from high – amylose common wheat. High-amylose wheat grain cv Cadenza was grown at the Experimental Farm of University of Tuscia and milled to flour (High-Amylose Flour) by Grandi Molini Italiani SpA (Venezia, Italy).

This study was divided in two parts:

1) This ongoing study aimed to evaluate the effects of products (biscuits, taralli and pasta) obtained from high amylose flours on both symptoms and the gut microbiota composition in IBS-C subjects.
2) The aim of this study was to measure the glycemic index of three high – amylose products (biscuits, taralli and bread) and to evaluate their effects on postprandial glycemia on healthy volunteers.

4.1. PART 1: Evaluation of high-amylose products on symptoms and fecal microbiota in subjects affected by IBS-C

4.1.1. Materials and Methods

Study participants

IBS – C adults were enrolled 2 weeks before T0 (T0 -14) at Gastroenterology Unit of Campus Bio – Medico University Hospital of Rome according to the inclusion and exclusion criteria reported in *Table 2*.

Table 2 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Both sexes and Age comprised between 18 and 65 years old	Who was already involved in other experimental studies

Normal-weight or overweight subjects (BMI comprised between 18.5 and 29.9)	Patients affected by inflammatory, oncological, metabolic diseases with effects on gastrointestinal motility
IBS – C according to Rome IV criteria	Patients who used antibiotics and/or probiotics in the previous month
Negative colonoscopy for organic diseases in the previous 5 years for subjects older than 50 years old. For <45 this criterium was not considered	Patients who suffered for allergies (included asthma)
Who signed the informed consent	Patients who suffered for psychiatric or mental diseases

Participants were allocated to High Amylose (MD-HA+) (n=10; 90% women) or control group (MD – HA-) (n=22; 91% women). The study was approved by Ethical Committee of Campus Bio – Medico of Rome (Prot.: 69/20 PAR ComEt CBM) and all patients signed an informed consent before their participation. The study was conducted in accordance to the European Union Standards of Good Clinical Practice and to the last revision of Helsinki Declaration.

Study design

The study lasted 6 weeks for each patient and it was composed by the following visits (*Figure 8*):

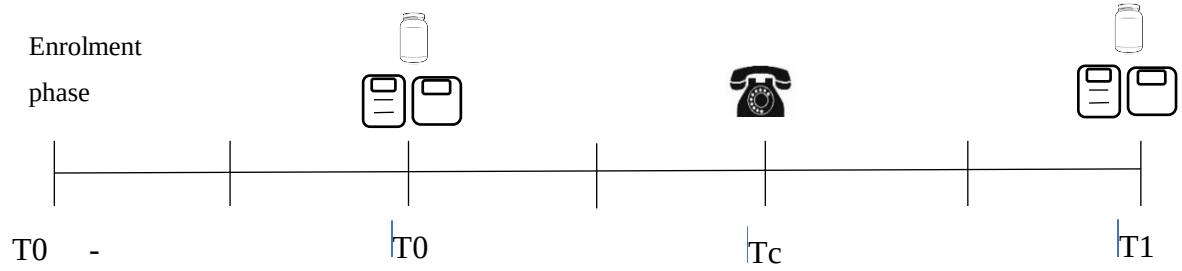

Figure 8 Study protocol

Legend:

Stool sample

Anthropometric parameters

Food diary and FAST questionnaire

Phone call

- **T0-14 – enrolment phase:** The first gastroenterological examination was carried out with an evaluation of the familiar, physiological and pathological anamnesis. The anthropometric parameters (weight, height and BMI) and the inclusion and exclusion

criteria were evaluated. The patient eligible to participate, read and signed the informed consent to adhere to the protocol. Patients were recommended to suspend the drug therapy for IBS for the entire duration of the study. During this visit, participants received a food diary to assess nutritional intake, the Food and Symptoms time (FAST) questionnaire to evaluate IBS symptoms (abdominal pain, bloating, meteorism, frequency and urgency of evacuation) and the kit to collect the fecal sample 14 days after. Patients were invited to follow their habitual diet for the following 14 days.

- **T0 - Gastroenterological and nutritional visit**: At T0, patients handed over the filled in food diary, symptom questionnaire from the previous 14 days, and the first fecal sample. They received new food diaries and FAST questionnaire to fill in, another kit to collect the fecal sample at T1 and a nutritional protocol to follow for 4 weeks (T1).
- **Tc (14 days 2) - Telephone contact for feedback:** A telephone interview was conducted to investigate the adherence to the nutritional protocol and symptomatology of the patients.
- **T1 (28 days ± 2) - Final visit**: A gastroenterological and nutritional visits were carried out. Patients received feedback on the results of the first 14-day food diary. The patients delivered the symptom questionnaire, the completed 28-day food diary and the second fecal sample of T1.

Food diaries

The dietary habits of participants were evaluated through a daily food diary. It is a simple and practical tool specifically designed to evaluate the caloric intake, and the assumption of carbohydrate, protein, lipid and dietary fiber. Patients received the same food diary twice: from T0-14 to t0 to assess the nutritional intake during their habitual diet, and from T0 to T1 to evaluate the modification of their nutritional habits during the nutritional protocol that was suggested. Participants had to complete the following sections in the food diary: 1) time and place of meals; 2) type and amount of food consumed; 3) cooking methods.

Nutritional protocol

The nutritional protocol was based on the Mediterranean diet and it was characterized by 1800 Kcal for women and 2000 Kcal for men. The portions and weekly frequencies of consumption have been established according to the *Levels of Intake and Reference of Nutrients and Energy for the Italian population* (LARN: IV Revision, 2014) [168] of the Italian Society of Human Nutrition (SINU) and to the established guidelines for the nutritional treatment of patients with IBS-C [28,29]. Based on these evidences, the energy requirement was distributed as follows:

- 50-55% of total energy from carbohydrates of which < 15% from simple sugars
- <15% from proteins
- 30-35% from lipids

The diet provided 25 – 30 g of dietary fibers daily.

In addition, a series of practical nutritional suggestions were given, essential for a greater adherence to the diet plan.

In the MD-HA+ diet, the 50% of carbohydrates–rich foods were substituted by HA foods (biscuits, taralli and pasta). The HA products were provided in a box with the correct amount of foods for 28 days to respect the portion sizes: 40 g of biscuits, 30 g of taralli and 80 g of pasta (*Figure 9*).

Figure 9 Box of high- amylose products

Evaluation of Mediterranean Diet adherence

To assess the adherence to the Mediterranean diet of each patient, the Mediterranean Diet Score Scale (MDSS) was used. This score was calculated for both periods: 14-day and 28-day food diaries. For each food class, a score interval of 0 to 3 was identified. Each score was assigned on the basis of the portion and daily or weekly frequency of each food. The score ranges varied from a minimum of 0 to a maximum of 24 points.

Food and Symptoms Time (FAST) questionnaire

To assess gastrointestinal symptoms, participants filled in the FAST questionnaire. The questionnaire was completed in the first phase of 14 days, before the beginning of the nutritional protocol during patients' habitual diet, and during the second phase of 28 days, in which the patient followed the nutritional plan. Patients were asked to report the presence or the absence or the timing (morning, afternoon, evening) of the following symptoms: 1) abdominal pain; 2) bloating; 3) meteorism; 4) evacuation associated to effort, abdominal pain and urgency. Moreover, they were asked to report the number of daily evacuations and the stool form according to the Bristol Stool Scale.

Fecal samples

Fecal samples were collected from patients with IBS-C at T0 and T1. Each fecal sample was stored in appropriate vials at -80°C and then were sent to the Human Microbiome Unit of the Pediatric Hospital Bambino Gesu in Rome in dry ice and immediately stored at -80°C until the analysis process, in order to avoid exposure to oxygen of anaerobic bacteria and to avoid bacterial proliferation before DNA extraction.

4.1.2 Statistical analysis

Food questionnaire and symptoms

Data were analysed using the statistical program GraphPad version 9.4.1. A paired t test was conducted between T1 and the baseline to assess the nutritional intake from food diaries and for symptoms (abdominal pain, bloating and meteorism and for the average number of evacuation). Variable evacuative effort, evacuative urgency, pre-evacuative abdominal pain were evaluated with a Likert scale, it was not possible to assume the distributive normality of the data, therefore it was applied the non-parametric test of Wilcoxon for paired data. The significance level was assessed for a p value <0.05.

Fecal microbiota

The fecal DNA extraction was conducted by Bambino Gesu Pediatric Hospital. DNA was extracted manually, using the Qiaamp DNA Stool Mini kit (Qiagen, Germany), in accordance with the manufacturer's instructions. V3-V4 variable region amplification from the bacterial gene 16S rRna (460 bp) was performed using primers 16S_F 5'-(TCG TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG)-3 and 16S_R 5 -(GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C)-3, according to the Miseq Rna Amplicon Sequencing protocol (Illumina, San Diego, California, USA). The polymerase chain reaction (PCR) was performed using the KAPA Hifi Hotstart ready Mix kit 2 (KAPA Bio-systems Inc., Wilmington, Massachusetts, USA). DNA amplicons were purified using Cleanngs beads kits (Cleanna, Coenecoop 75, PH Waddinxveen, the Netherlands). A second amplification step was then carried out in order to obtain a unique combination of the Illumina Nextera XT forward and reverse barcodati primers for each sample. The final libraries were purified using Cleanngs beads kit (Cleanna, Coenecoop 75, PH Waddinxveen, Netherlands), quantized with the fluorimetric method Quant-iT™ Picogreen® dsdna Assay Kit (Thermo Fisher Scientific, Waltham, MA) and normalized at 4 nm. To generate paired-end readings of 250x2 bp length, the

normalized libraries were grouped into a single pool and analysed on the Miseq Illumina platform, according to the manufacturer's specifications. The obtained reads were analysed using the software Quantitative Insights in Microbial Ecology (QIIME, v.1.9.0) and grouped into operational taxonomic units (OTUs). In order to ensure a higher level of accuracy of Otus detection, reads readings with an average score of less than 25, with a length of less than 300 bps and with an ambiguous basis have been excluded from the analysis. The denigrated sequences were chemically controlled by identify_chimeric_seqs.py, using the Blast_fragments and Chimeraslayer approaches (http://qiime.org/scripts/identify_chimeric_seqs.html). Otus were selected with a 97% sequence similarity level through Pynast, a device for sequence alignment, and UCLUST, a device for sequence grouping and taxonomic assignment (greengenes.secondgenome.com/downloads/database/13_8). The α and β diversity and the Kruskal Wallis test were calculated using the QIIME software, with the script "alpha_rarefaction.py, beta_diversity_throughugh_py, group_significance.py", respectively. Taxonomic levels of phylum and genus/species were studied and statistically significant values of p-value <0,05 and false discovery rate (pFDR) <0,05 were considered.

4.1.3 Results

Study population

Thirty-two participants were enrolled at T0, 10 in the MD – HA+ group (90% females,) and 22 in the MD – HA- group (90% females,). The two groups were homogeneous for age, height, body weight and BMI. *Table 3* shows the anthropometric parameters of participants at T0.

Table 3 Anthropometric parameters of participants at T0

	MD – HA+ group (n=10)	**MD – HA- (n=22)**
Age (years ± SD)	41.4 ± 14.81	40.68 ± 19.21
Height (m ± SD)	1.62 ± 0.07	1.63 ± 0.07
Body Weight (kg ± SD)	64.6 ± 13.79	62.17 ± 9.65
BMI (kg ± SD)	24.38 ± 4.16	23.57 ± 3.80

Of these 32 patients, only 25 completed the study: 8 in the MD – HA+ group (100% females, mean age 38.25 ± 14.39 and mean height 1.61 ± 0.04) and 17 in the MD – HA- group (88% females, mean age 45.65 ± 18.93 and mean height 1.62 ± 0.08). *Table 4* shows the anthropometric parameters of participants who completed the study at T0 and T1.

Table 4 Body weight and BMI of participants at T0 and T1

	MD – HA+ group (n=8)		**MD – HA- (n=17)**	
	T0	T1	T0	T1
Body Weight (kg ± SD)	63.75 ± 11.23	63.91 ± 9.84	61.81 ± 9.55	60.84 ± 9.37
BMI (kg ± SD)	24.57 ± 4.15	24.64 ± 3.72	23.58 ± 4.08	23.18 ± 3.75

No significant differences were observed for body weight and BMI in both groups from T0 to T1.

Symptoms

The evaluation of FAST questionnaires showed that abdominal pain significantly decreased in both groups. In the MD – HA+ group, in fact it decreased from 1.07 ± 0.85 to 0.81 ± 0.79 (p=0.0285), while in the MD – HA- group it decreased from 1.03 ± 0.78 to 0.63 ± 0.5 (p=0.0012). no significant changes were observed for bloating in both groups. Meteorism, instead, significantly decreased only in the MD – HA+ group, from 1.6 ± 0.8 to 0.92 ± 0.7 (p=0.0003) (*Table 5*). No significant differences were observed comparing the two groups for bloating, meteorism and abdominal pain.

Table 5 Bloating, meteorism and abdominal pain in both groups

		T0 (mean + SD)	**T1 (mean + SD)**	**P - value**
Bloating	MD – HA+ group	2.08 ± 1.0	1.42 ± 0.8	0.1576
	MD – HA- group	1.42 ± 0.76	1.18 ± 0.64	0.1240
Meteorism	MD – HA+ group	1.6 ± 0.8	0.92 ± 0.7	**0.0003**
	MD – HA- group	1.13 ± 0.76	0.93 ± 0.61	0.1790
Abdominal pain	MD – HA+ group	1.07 ± 0.85	0.81 ± 0.79	**0.0285**
	MD – HA- group	1.03 ± 0.78	0.63 ± 0.5	**0.0012**

Regarding evacuation effort and urgency, we did not observe any significant changes in both groups. Only pre - evacuation abdominal pain significantly changed in the MD – HA- group from 2.21 to 1.75 (p=0.0016) (*Table 6*). Comparing the two groups, significant changes were observed for urgency (p=0.0219), evacuation effort (p=0.0193) and pre – evacuation abdominal pain (p=0.0021) but we noticed that all these three parameters increased in MD-HA+ group and decreased in MD – HA- group.

Table 6 Evacuation effort, pre-evacuation abdominal pain and urgency in both groups

		T0 (median)	**T1 (median)**	**P - value**
Evacuation effort	MD – HA+ group	1.54	2.48	0.3125
	MD – HA- group	2.22	1.9	0.2633

Pre-evacuation Abdominal pain	MD – HA+ group	1.46	2.53	0.0625
	MD – HA- group	2.21	1.75	**0.0016**
Urgency	MD – HA+ group	1.29	1.55	0.0625
	MD – HA- group	2	1.7	0.0814

Both arms registered an upward trend in the weekly evacuation frequency: from 4.8 ± 2.35 to 5.37 ± 2.28 (MD-HA- group) and from 5.91 ± 2.63 to 6.17 ± 3.41 (MD-HA+ group) but without significant changes (*Table 7*).

Table 7 Weekly evacuation frequency in both groups

		T0 (mean + SD)	**T1 (mean + SD)**	**P - value**
Weekly evacuation frequency	MD – HA+ group	5.91 ± 2.63	6.17 ± 3.41	0.6257
	MD – HA- group	4.82 ± 2.35	5.37 ± 2.28	0.0925

Regarding Bristol Stool Scale, in the MD – HA+ group we observed that there was a change between T0 and T1 in the percentage of participants that had stool type of 3 and 4. In fact, at T0 33% of patients had stool type of 3 and nobody had the stool type of 4, while at the end of the study, 17% of participants had stool type of 4 and the type 3 was reduced to 16%. No changes were observed for type 1 and type 5. In the MD – HA- group, instead, type 3 and type 5 remained stable but type 1 was halved from 12% to 6% and type 2 increased. Curiously also type 4 decreased from 23% to 18%. (*Figure 10*).

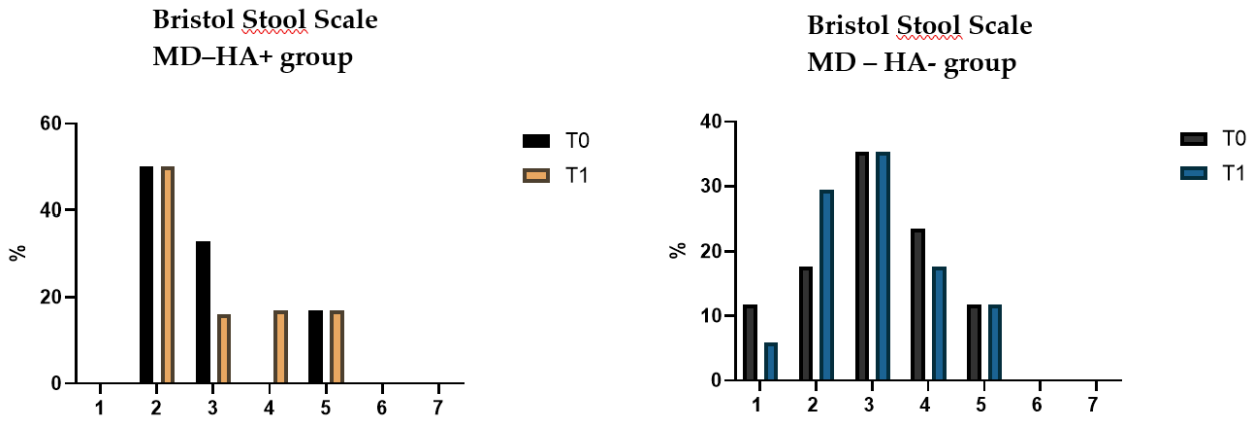

Figure 10 Bristol Stool Scale in both groups

Food diaries

Regarding the food diaries collected at T0 and T1, we observed that during their habitual diet, participants tended to consume less carbohydrates and fibers and more proteins and lipids compared to nutritional guidelines. We observed that both groups did not respect perfectly the prescribed diet. In fact, regarding kcal intake we noticed that it increased but it did not reach the suggested amount. In both groups, calorie intake significantly increased (p=0.0056 and 0.0070 for MD – HA+ and MD – HA-, respectively). Regarding carbohydrates consumption, it significantly increased in both groups. In fact, in the MD – HA+ group it increased from 42 ± 3.64 to 49 ± 4.07 (p=0.0318), while in the MD – HA- group it increased from 46.18 ± 5.10 to 50 ± 2.63 (p=0.0126) (*Figure 11*), on the contrary, simple sugars did not significantly change in both groups.

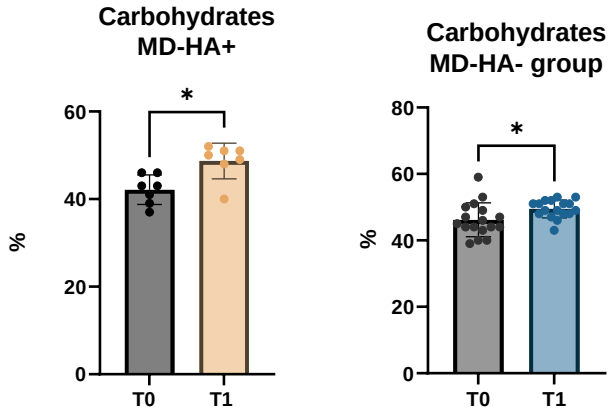

Figure 11 Carbohydrates intake in both groups

Protein intake (*Figure 12*), significantly decreased in the MD-HA- group (p=0.0310) but not in the MD-HA+ group, while lipids decreased more in the MD-HA+ group compared to MD-HA- group. In fact, in the MD-HA+ group, lipids decreased from 40 ± 2.26 to 35 ± 3.46 (p=0.0251) (*Figure 13*).

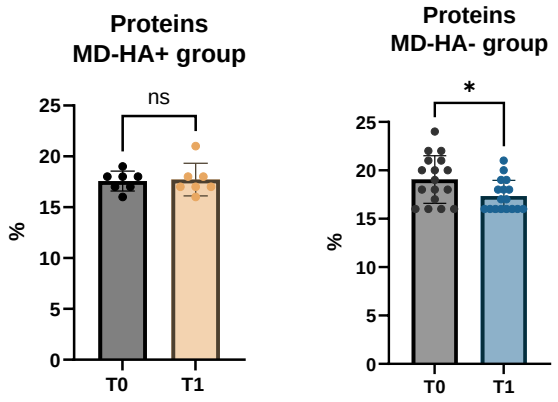

Figure 12 Proteins intake in both groups

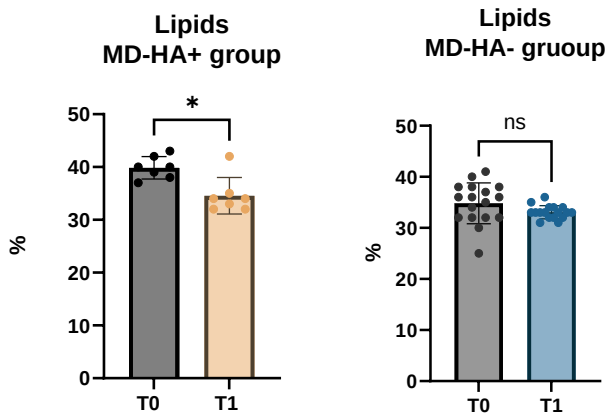

Figure 13 Lipids intake in both groups

Also dietary fibers intake significantly changed in both groups. In fact, it doubled from 11 ± 1.26 to 22 ± 3.82 (p=0.0010) in MD-HA+ group and from 11.61 ± 2.69 to 21.69 ± 5.25 (p=0.0001) in MD-HA- group (*Figure 14*).

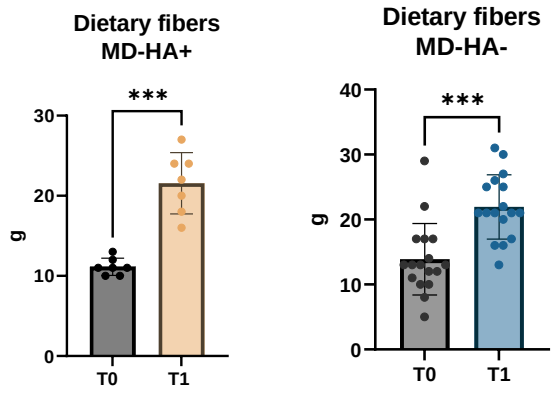

Figure 14 Dietary fibers intake in both groups

All mean macronutrients values are reported in *Table 8.*

No significant differences were observed comparing kcal, macronutrients, simple sugars and dietary fibers intake between the two groups.

Table 8 Macronutrients in both groups (Mean ± SD)

		T0	T1	p value	LARN 2014 [168]
Kcal	MD – HA+ group	1341 ± 121.02	1606 ± 206.56	**0.0056**	**1800 kcal for women and 2000 kcal for men**
	MD – HA- group	1364 ± 271.2	1528 ± 205.47	**0.0070**	
	MD – HA+ group	42 ± 3.64	49.0 ± 4.07	**0.0318**	**45-60%**

Carbohydrates (%)	MD – HA- group	46.18 ± 5.10	50.0 ± 2.63	**0.0126**	
Proteins (%)	MD – HA+ group	18 ± 1.02	18 ± 1.74	0.8049	**<15%**
	MD – HA- group	19.06 ± 2.46	17.00 ± 1.66	**0.0310**	
Lipids (%)	MD – HA+ group	40 ± 2.26	35 ± 3.46	**0.0251**	**20-35%**
	MD – HA- group	34.82 ± 4.00	33.0 ± 1.37	0.0973	
Simple sugars (%)	MD – HA+ group	9 ± 2.18	11 ± 0.89	0.0558	**<15%**
	MD – HA- group	11.29 ± 3.42	12.00 ± 1.89	0.1996	
Dietary Fibers (g)	MD – HA+ group	11 ± 1.26	22 ± 3.82	**0.0010**	**25-30 g**
	MD – HA- group	11.61 ± 2.96	21.69 ± 5.25	**0.0001**	

MDSS score significantly increased only in the MD-HA+ groups. In fact, it increased from 8.4 ± 2.4 to 13 ± 2.9 (p=0.0013), while in the MD-HA- group it remained similar between T1 and the baseline (from 13.65 ± 2.58 to 13.93 ± 2.04) (*Figure 15*). A significant difference was observed comparing the MDSS index variation between the two groups (p<0.0001).

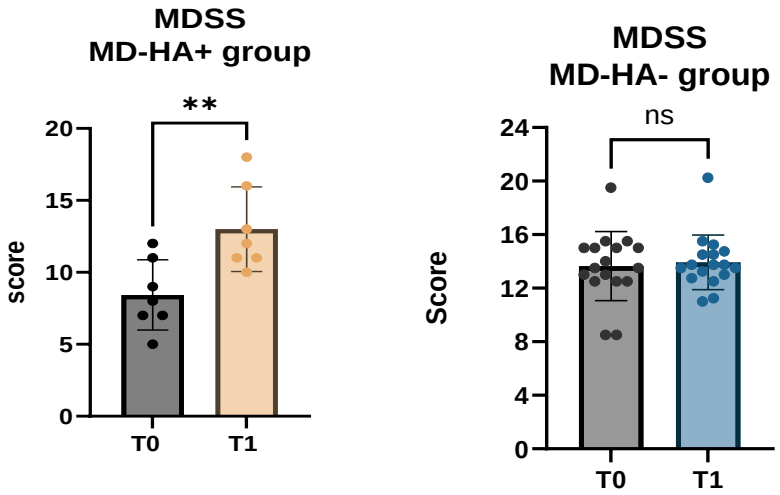

Figure 15 MDSS in both groups

Fecal microbiota

All fecal microbiota results obtained are preliminary results due to the small sample size of the two groups. In fact, no changes observed were significant.

Regarding phyla, in the MD HA+ group, we observed a decreasing trend of Bacteroidetes and Proteobacteria and an increasing trend of Actinobacteria and Firmicutes while in the MD-HA- group

Bacteroidetes showed an increasing trend while, on the contrary, Firmicutes showed a decreased trend. All other phyla in both groups were stable (*Figure 16*). No significant differences among gut microbiota phyla were observed comparing the two groups.

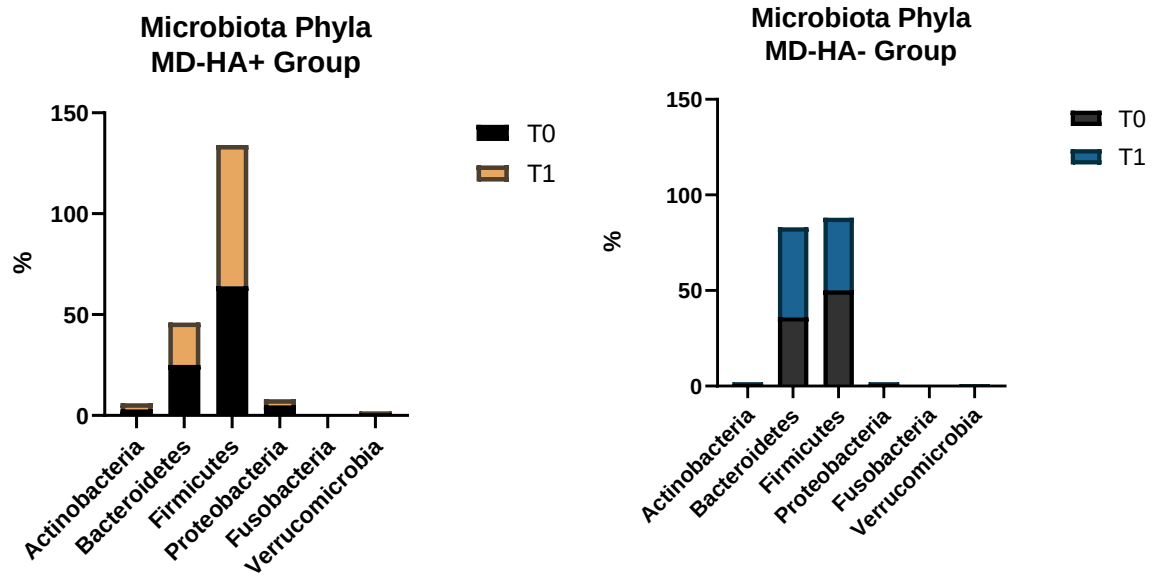

Figure 16 Microbiota phyla variations in both groups from T0 to T1

Observing the Firmicutes phyla we divided it in *Bacilli* and *Clostridia*, and we observed that in the MD-HA+ group there was a little increasing trend in *Clostridia* while in the MD-HA- group both *Bacilli* and *Clostridia* showed a downward trend.

At family level (*Figure 17*), in the MD-HA+ group an upward trend in *Bifidobacteriaceae*, *Bacteroidaceae* and *Ruminococcaceae* was observed. While in the MD-HA- group a reduction trend in *Ruminococcaceae* and *Lachnospiraceae* and an increase in *Bacteroidaceae* was observed. All other families were stable. No significant changes were observed comparing gut microbiota families variations between the two groups.

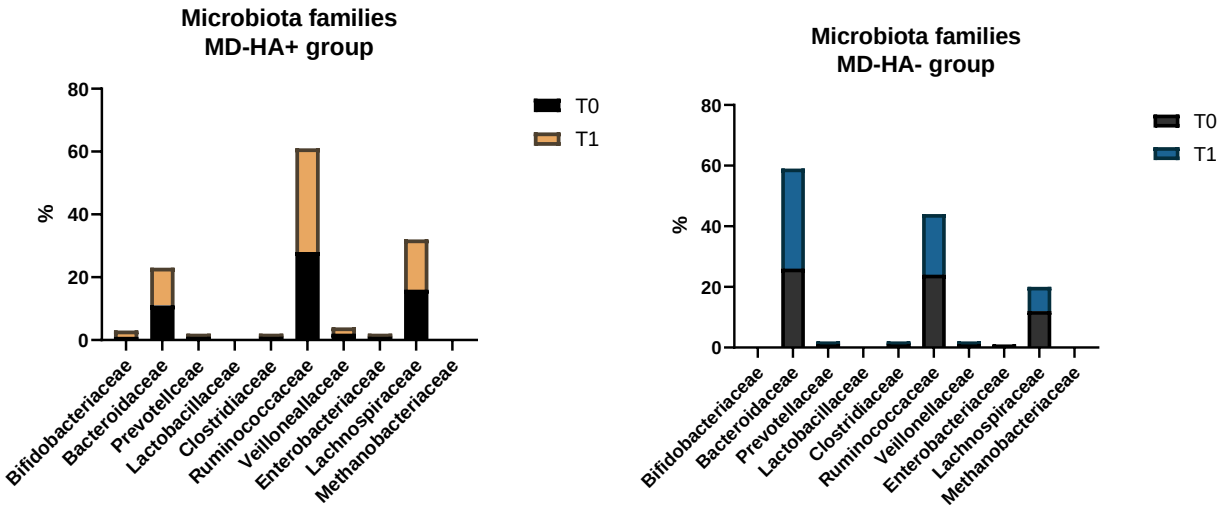

Figure 17 Microbiota families variations in both groups from T0 to T1

Regarding genus (*Figure 18*), in the MD-HA+ group we observed an increasing trend of *Bifidobacterium, Ruminococcus, Faecalibacterium and Roseburia* while in the MD-HA- group an increasing trend only of *Fecalibacterium*. All other genera remained stable in both groups. In both groups lactobacilli were almost absent, they increased in both groups but this variation was nearly undetectable. No significant changes were observed comparing gut microbiota genera variations between the two groups.

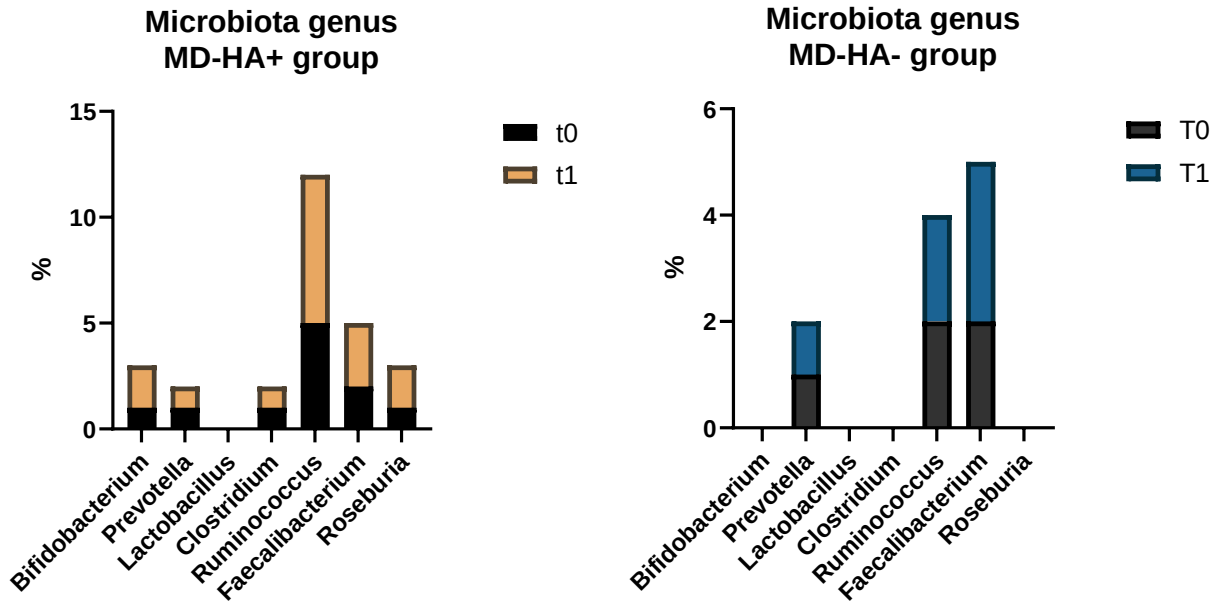

Figure 18 Microbiota genus variations in both groups from T0 to T1

4.1.4. Discussion

Our study shows that the consumption of high-amylose products included in a balanced Mediterranean diet results to be successful in improving symptoms and constipation in IBS-C patients. In fact, we observed a significant reduction of abdominal pain, that is the principal IBS symptom, in both groups ($p=0.0285$ and $p=0.0012$ in the MD-HA+ group and in the MD-HA- group, respectively) but a significant reduction in meteorism only in the MD-HA+ group ($p=0.0003$). From food diaries we noticed an improvement in both groups for carbohydrates ($p=0.0318$ and $p=0.0126$ in the MD-HA+ group and in the MD-HA- group, respectively) and fibers consumption ($p=0.0010$ and $p=0.0001$ in the MD-HA+ group and in the MD-HA- group, respectively). Only in the MD-HA+ group lipids intake significantly decreased ($p=0.0251$) while only in the MD-HA- group proteins intake significantly decreased ($p=0.0310$). Considering that most of IBS patients tend to reduce carbohydrates, lipids and fibers from their habitual diet worrying for symptoms, in this case we reached a good result because patients followed a more balanced diet (it was demonstrated also by MDSS that was significantly higher in the MD-HA+ group) and their symptoms, especially abdominal pain, decreased. In the literature, only the study of So et al. evaluated the effects of RS2 on IBS symptoms [169]. They administered per 3 days each: RS2 (10,15 and 20 g/day), sugarcane bagasse (5,10 and 15 g/day) and their combination (20,25 and 30 g/day). They observed that all RS2 doses increased bloating, unlike our results. Moreover, an amount <20 g/day of RS was well tolerated even in combination with the minimally fermented fiber (sugarcane bagasse).

IBS subjects usually have lower abundance of anti – inflammatory bacteria and a higher abundance of pro – inflammatory ones. In this case we observed an increasing trend of Actinobacteria and a decreasing trend in Proteobacteria in the MD – HA+ group. The high amylose diet had also an impact on the gut microbiota composition. After the diet period, we noted an increasing trend of *Bifidobacteria, Ruminococchi, Faecalibacterium and Roseburia* in MD-HA+ group confirming other results observed in literature on healthy subjects [146, 170]. All these specific bacterial populations, in fact, are involved in the carbohydrate – rich foods fermentation, especially with high RS2 content, and butyrate production [146]. *Ruminococchi* are considered as part of the "core gut microbiota" and they are usually involved in RS degradation [171]. An increasing trend in Firmicutes was observed but may be because also some species belonging to *Clostridia* are involved in RS digestion. In fact, the same increase in Firmicutes and reduction in Bacteroidetes was observed also in other studies in the literature [170,172].

In the MD-HA- group only *Fecalibacterium* showed an increasing trend while all other populations remained stables. Among phyla, we observed a decrease in Firmicutes and an increase in Bacteroidetes and it could be positive for Firmicutes/Bacteroidetes ratio.

As Hughes et al. we did not observe a variation of *Prevotella*, nevertheless it is usually involved in RS degradation [146]. On the contrary, we observed an increasing trend of *Roseburia*, that is a butyrate producer, confirming the findings of other studies in the literature [170]. Further observations and a broadening of the sample are needed to confirm these promising preliminary results of the effects of both high amylose and RS on IBS symptoms and microbiota.

4.2. PART 2: Evaluation of glycemic index of high-amylose products and their impact on post-prandial glycemia in healthy volunteers

4.2.1. Materials and Methods

Study population

Participants were recruited at Campus Bio – Medico University of Rome according to the criteria showed in *Table 9.*

Table 9 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age comprised between 18 and 30 years old	Subjects who were involved in other interventional studies
Normal-weight subjects (BMI comprised between 18.5 and 24.9)	Subjects who used medications
Subjects who signed the informed consent	Subjects with type 1 or 2 diabetes mellitus or used medications that could impair glucose metabolism
	Subjects who had allergies (included asthma), organic (inflammatory, neoplastic, metabolic diseases) or psychiatric diseases (anorexia, bulimia, depression),
	Subjects who were coeliac or gluten – sensitive,
	Subjects who had kidney failure (creatinine > 1.5 mg/dl) or liver failure (ALT and AST twice more than normal values), anaemia (Hb < 12 g/dl) and other chronic conditions.

All investigations were conducted according to the Declaration of Helsinki. The study was approved by University Campus Bio – Medico di Roma Ethical Committee (approval code of the study 70/20) and all the subjects signed an informed consent statement allowing their anonymized information to be used for data analysis. Participants records were anonymized and de-identified before the analysis.

Study protocol

During this study participants followed their habitual diet and they were called to measure their glycemic values with a glycemic curve after the ingestion of foods. Participants were blinded, thus they did not know if the food they ingested was a HA product or a control one. At T0 participants were submitted to: 1) anthropometrical measurements, 2) medical visit with anamnesis paying attention to diseases or intake of medications, 3) nutritional anamnesis. The first and the last day of

the study participants consumed 50 g of glucose in 250 ml of water while other days they ingested on an empty stomach control and HA foods in a randomized order every 3 days (with two days of wash out). The test day fingerstick blood glucose was measured on fasting and every 15 minutes for 2 hours after the food consumption. Subjects were not allowed to drink alcohol and to practise physical activity in the 72 h before the test and they were invited to standardize the dinner before the test consuming a light meal avoiding the consumption of fiber rich – foods (as legumes and whole cereals).

Anthropometric parameters

Anthropometric parameters (weight and height) were measured using a SECA 700 scale with integrated altimeter. These parameters were recorded only at T0 during the enrolment visit.

Glycemia measurement

A glucometer with pen and reactive stripes was used to measure the capillary glycemia after food or glucose ingestion every 15 minutes for 2h. As standard, glucose was used for the first and the last measurement.

High amylose and control products

High-amylose foods and the respective control foods contained the same amount of energy and carbohydrates (50 g) net of the fiber, but not resistant starch, in order to evaluate the metabolic response. Resistant starch was about 17% in HA flours and only 1% of the total starch in control flour. The percentage of amylose was 54.4 ± 0.50 in HA flour and 25.3 ± 0.64 in the control one, thus it was nearly double. All HA and control products were provided in transparent plastic bags and they were already portioned according to LARN 2014 [168]. Chemical analysis of high amylose and control products were conducted by University of Molise. The nutritional composition of all products is reported in *Table 10.*

Table 10 Available carbohydrates and amount of resistant starch in all products

	Available Carbohydrates (g/100 g)	**Portion size (g) with 50 g of available carbohydrates**	**Resistant starch (% total starch)**
HA biscuits	58.3	86	11.6
Control biscuits	61.9	81	0.4
HA taralli	55.0	91	12.5
Control taralli	55.8	90	1.3
HA bread	45.8	109	12.5
Control bread	51.0	99	2.8

Biscuits

The ingredients of biscuits were: high amylose flour, sugar, high – oleic sunflower seeds oil, eggs, water, yeast. *Figure 19* shows HA and control biscuits that were provided during the study in a 30 g portion size.

Figure 19 High amylose biscuits (on the left) and control biscuits (on the right)

Taralli

Taralli were made up by high amylose flour, water, extra-virgin olive oil and salt. *Figure 20* shows HA and control taralli that were provided during the study in a 30 g portion size.

Figure 20 High amylose taralli (on the left) and control taralli (on the right)

Bread

Bread was made up by high amylose flour, water, yeast and salt. *Figure 21* shows HA and control bread that were provided during the study in a 50 g portion size.

Figure 21 High amylose bread (on the left) and control bread (on the right)

Consumer test questionnaire

After the test, participants joined a blinded tasting section. They were asked to answer a questionnaire to check for differences in organoleptic characteristics, general liking and willingness to pay for HA biscuits and taralli and for their control counterparts. Participants received information about the type of products and the nutritional properties of all products (control vs. HA). They evaluated their overall liking and selected the appropriate sensory attributes (colour: yellow or brown; size: small or large, thin, thick, taste and flavour - crumbly, chewy, crispy, doughy, dry, stringy, fatty, salty, sweet, fragrant, toasted, bitter) on a 9-point scale.

After the blind sensory test, participants were asked to answer two questions about willingness to pay (WTP): 1) "How much would you be willing to pay for a snack pack of the product you just tasted?"; 2) 'How much would you be willing to pay for a snack pack of products containing natural high amylose flours?'. This second question was asked after the disclosure of the message that high amylose determines a lower rise of glycemia than a normal product. Finally, in order to obtain further indications on the intention to buy these new products, two final questions were asked: "If you found this type of product on the market, how likely would you be to buy it?" and "Would you recommend the purchase of the product with high amylose flours?", using a 5-point scale.

4.2.2. Statistical analysis

The statistical analysis was conducted through the statistical program Graphpad Prism version 9.4.1. The area under the curve (AUC) was calculated for both high-amylose and control foods through integral calculation and for glucose (taking in consideration the mean values between the first and the last measurement) and then the GI was calculated as the percentage ratio between the two areas: glicemic index = (food area/glucose area) x 100. Moreover, a One – way ANOVA with Tukey's post hoc test was conducted to evaluate the differences at each time among high – amylose food, control food and glucose. A p value < 0.05 was considered to be statistically significant.

The level of liking was analysed via the XLSTAT software with an ANOVA analysis, with the aim of investigating the role of the different attributes in the overall liking perception, and to test whether the liking of the HA and control products significantly differed. The differences for the last two questions were tested with the paired-sample t-test.

4.2.3. Results

Study participants

At the end of the enrolment phase, 10 subjects (50% females) were enrolled. The mean anthropometric parameters of participants at baseline are reported in *Table 11*.

Table 11 Mean anthropometrical parameters of participants at baseline

Parameters	Values
Age (yrs ± SD)	25.2 ± 1.69
Height (m ± SD)	1.73 ± 0.098
Weight (kg ± SD)	64.85 ± 11.34
BMI (kg/m2 ± SD)	21.45 ± 2.16

High – amylose and control products

Biscuits

Significant differences were observed between HA biscuits and glucose at 15' (p=0.0036), 30' (p<0.0001), 45' (p<0.0001), 60' (p=0.0001), 75' (p=0.0102) of the glycemic curve while between HA biscuits and control biscuits there were no significant differences at each time. Significant differences were observed also between control biscuits and glucose at 30' (p=0.0001), 45' (p<0.0001), 60' (p=0.0035). The maximum glycemic peak was observed at 45' for HA biscuits, for control biscuits and for glucose and it was 110.1 mg/dl, 118.8 mg/dl and at 158.6 mg/dl, respectively (*Figure 22*). Glycemic index was calculated as 84.28 ± 5.21 and 90.29 ± 6.15 for HA biscuits and for control ones (p=0.0116), respectively, and in fact, HA biscuits showed a lower area under the curve (810.8 ± 37.37) than control biscuits (870.15 ± 71.34) (p=0.0125).

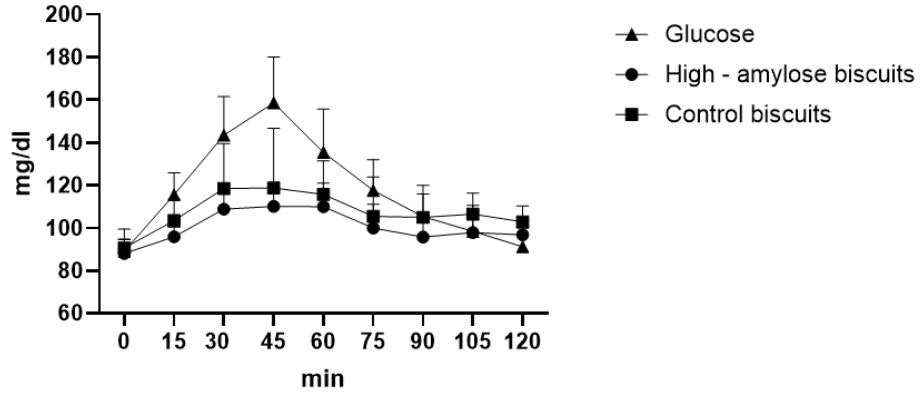

Figure 22 Biscuits glycemic curve

Also the GL of HA and control biscuits was calculated. Taking into account the portion size of 30 g and that in 100 g there were 58.3 g of available carbohydrates, we estimate that in 30 g there were 17.5 g of carbohydrates. Thus, GL was calculated as: (84.28 * 17.5) /100 = 14.75 (medium glycemic load). Instead, control biscuits had a GL of 16.77 (medium glycemic load) considering 61.9 g of available carbohydrates in 100 g of product, thus 18.57 g in 30 g.

Taralli

From the glycemic curve significant differences between HA taralli and glucose at 15' ($p=0.0217$), 30' ($p<0.0001$), 45' ($p<0.0001$), 60' ($p=0.0006$), 120' ($p=0.0228$) were observed while between HA taralli and control ones there were no significant differences at each time. We also observed significant differences between control taralli and glucose at 15' ($p=0.0004$), 30' ($p<0.0001$), 45' ($p<0.0001$). About taralli, from the glycemic curve we observed that the maximum peak for the HA product was of 116.6 mg/dl after 45 minutes while for the control one it was of 127.1 mg/dl after 60 minutes. The maximum glycemic peak of glucose was always of 158.6 mg/dl after 45 minutes (*Figure 23)*. Glycemic index was calculated as 90.32 ± 5.9 and 91.73 ± 8.32 respectively for HA taralli and for control ones. These two GIs are not statistically different, in fact the area under the curve of both products was similar (868.25 ± 27.69 and 880.9 ± 44.46 respectively for HA taralli and control ones) without significant differences.

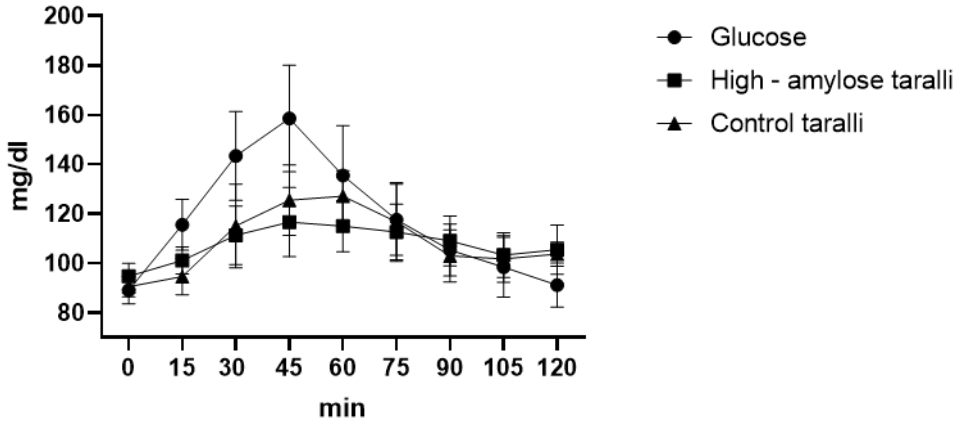

Figure 23 Taralli glycemic curve

The GL was calculated in the same way as for biscuits. Thus, considering that in 100 g of HA taralli there were 55 g of available carbohydrates we calculated that the GL of a portion of taralli (30 g) was 14.8 (medium glycemic load). For control taralli the amount of available carbohydrates in 100 g was 55.8, thus the GL was 15.2 (medium glycemic load).

Bread

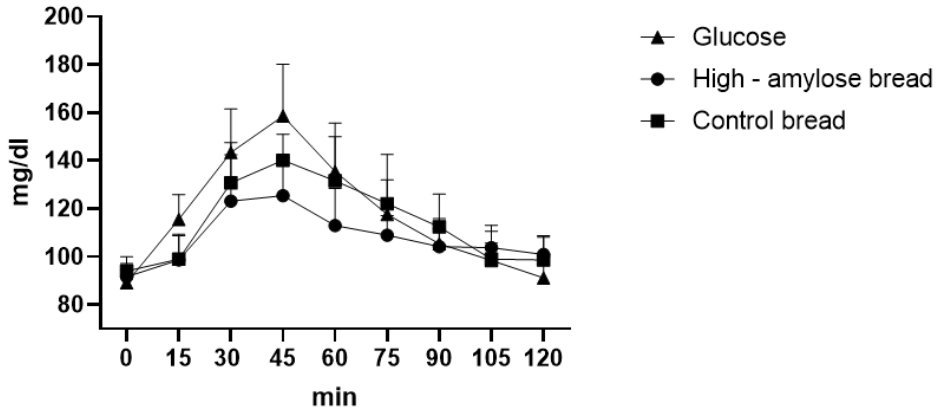

Figure 24 Bread glycemic curve

Comparing each time point of the glycemic curve we observed statistical differences between high-amylose bread and glucose at 15' (p=0.0136), 30' (p=0.0021), 45' (p<0.0001), 60' (p=0.0006) and between control bread and glucose at 15' (p=0.0158), 45' (p=0.0058). In this case we also observed a significant difference between high – amylose bread and control bread at 45' (p=0.0371) and 60' (p=0.0055) (*Figure 24*). High – amylose bread had a maximum glycemic peak at 125.4 mg/dl after 45 minutes from the ingestion and the GI was calculated as 90.6 ± 4.19 while common bread showed a maximum peak at 140 mg/dl after 45 minutes and a GI of 96.82 ± 8.23. These two GIs are significantly different (p=0.0111), indeed the AUC of HA bread was 873.35 ± 60.42 while the AUC of common bread was 931.15 ± 66.03 (p=0.0135).

We calculated also the GL of HA and control bread. In 100 g of HA bread there were 45.8 g of available carbohydrates. Considering a portion of 50 g we obtained that GL of HA bread was: 20.6 (high glycemic load) while for the control bread it was calculated as 24.5 (high glycemic load) because there were 51 g of available carbohydrates in 100 g of product.

Overall Liking and WTP of high-amylose biscuits and taralli

The liking analysis was only performed on biscuits and taralli. The results showed that, although without statistical significance due to the limited sample, the high amylose biscuits were preferred to the normal biscuits. The average liking score was 6.05 points on 1 to 9 sensory scale, compared to 5.85 points for the control. On the contrary, for the taralli, the average liking score for the control product was higher (6.00 points) than for the high-amylose product (5.20 points).

The WTP values expressed by participants after the blind test, did not show any significant differences between HA and control products. Participants declared that they would have spent an average bid of €2.24 for high amylose products that was statistically higher than the average bid for the conventional pack of €1.58 ($p < 0.001$). More than 60% of the observations showed a positive intention to buy HA bakery products, and more than 80% stated that they would have recommended HA products to other people.

4.2.4. Discussions

In the literature, the potential beneficial role for health given by a higher intake of resistant starch (RS) has been widely discussed. Indeed, the replacement of digestible starch with RS may provide higher satiety, an improvement in gut health, a potential reduction of the risk of colon cancer and the diabetes onset, thus sharing positive actions with dietary fibers [148]. The use of a high-amylose flour to make biscuits, taralli and bread determined an increase in the resistant starch content in the final product compared to control (<1%). The amount of resistant starch in the high – amylose product was around 12.5% over total starch. High-amylose flour, due to the partial replacement of total starch with resistant starch, determined a lower glycemic index in both biscuits and bread and a better glycemic control.

Regarding the effects on post-prandial glycemia, we observed that HA biscuits and bread resulted in a lower glycemic index and load than their control counterparts, even if they were not "low" according to GI and GL classification [155,173] while no significant differences were observed between HA and control taralli. Hallstrom et al. conducted a study very similar to our on 14 healthy volunteers to evaluate the postprandial glycemic and insulinemic response after the consumption of high – amylose (38%) bread. They observed that HA bread provided a lower glycemic but not insulinemic response [144]. Analysing the products, the HA had a slowly digestible starch fraction that determined a reduced absorption of glucose and, consequently, a lower glycemic response [144]. Belobrajdic et al. evaluated the effect of 4 different kind of breads (low and high-amylose -refined and low and high-amylose – wholegrain bread) on 20 healthy subjects and observed that HA products determined a lower glycemic and insulinemic response [145]. Also Corrado et al. in this study observed a lower glycemic response after the consumption of a meal providing bread rolls formulated with high-amylose flour (6.4% resistant starch over total starch) [174]. Regarding biscuits, Luhovyy et al. evaluated 3 biscuits with three different concentrations of high-amylose flour (1= control with 100% wheat flour; 2= 37% HA flour and 3= 67% HA flour) but, differently to our study, these biscuits had a different amount of available carbohydrates (53.5, 43.5 and 36.3 g, respectively). They observed

a 22% lower AUC after the ingestion of the high dose treatment ($p<0.05$), thus HA flour determined a better glycemic control [175].

Our results confirm other studies about the better glycemic control of bread and biscuits [145,176,177]. Currently, in the literature there are no studies on taralli, thus our study was the first that evaluated this traditional Italian savoury snack. Nevertheless, HA taralli showed a lower GI than control taralli, but we did not observe significant differences. This may be possible due to the effects of processing and starch modification. In fact, despite the resistant starch proportion is comparable with bread and biscuits, during the production of taralli, after shaping, the dough is shortly boiled in water before baking. This step is crucial for starch gelatinization resulting in the disruption of starch granules, with increased viscosity and enzymatic accessibility [178], possibly leading to mitigation effects on the ability of amylose in modulating glycemic response.

Other authors, moreover, evaluated the effects of high – amylose flours derived from cereals different from wheat (barley [176,179], rice [180] and corn [181,182]) on glycemic and insulinemic control. Regarding high-amylose barley authors observed in 14 women a significant reduction in the glycemic and insulinemic curve (22% and 32% respectively with a p value = 0.05 in both cases) compared to control products made of HA wheat [176]. The study on the high – amylose corn starch, instead, evaluated the insulinemic effect in 14 subjects affected by hyperinsulinemia. The consumption of the high – amylose product significantly improved the insulinemic response ($p<0.002$) after 10 weeks but without significant glycemic differences compared to the control group ($n=10$) [181]. Moreover, the consumption of high – amylose seems to be associated to the so – called "second meal effect". In fact, consuming less digestible carbohydrates may decrease glycemic response to a subsequent meal. Ten healthy subjects who consumed high-amylose starch at breakfast showed a lower glycemic response to a lunch compared to high – amylopectin breakfast [183].

No significant differences were found between HA and control products regarding liking or the specific sensory attributes used for the panel analysis of bakery products, but the WTP results were really interesting. They showed a very high premium price, representing approximately 40% of the average value recorded for the ordinary product. It could be due to the awareness of consumers to the health linked to snack consumption. In agreement with several studies in the literature [184,185], the liking analysis suggests that HA products may be well accepted by consumers and that a positive willingness to pay can be expected.

To date, managing the glycemic response after carbohydrate – rich foods consumption is one of the major health challenges, especially for subjects that suffer for DMT2 or alteration of glucose

metabolism. To the best of our knowledge, this is the first study that evaluate the effect of three high – amylose bakery products on glycemia. As a strength of this study, we conducted it both on experimental and control food produced with a soft wheat flour chemically similar to the experimental flour, but with a different amount of resistant starch. Nevertheless, some studies in literature showed that HA products did not affect satiety [145,176], it would have been interesting to evaluate the satiety and also the insulinemic response after the consumption of these products.

CHAPTER 5. Evaluation of a Mediterranean nutritional protocol on symptoms and microbiota in IBS – D patients

5.1. Aim of the study

The aims of this ongoing study were:

- To evaluate IBS – D symptoms changes after the nutritional protocol
- To determine the microbiota composition through a genetic test (LifeBrain®) before and after the nutritional protocol

5.2. Materials and methods

Study population

For this pilot study 20 IBS – D subjects had to be enrolled at gastroenterological center of Idp Medica in Colleferro. Patients should be enrolled according to the following inclusion and exclusion criteria (*Table 12*).

Table 12 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age comprised between 18 and 80 years old	Subjects who were involved in other interventional studies
Normal-weight subjects (BMI comprised between 18.5 and 24.9)	Subjects who have used antibiotics or probiotic in the last month
IBS – D diagnosed according to Rome IV criteria	Subjects who had allergies (included asthma), organic (inflammatory, neoplastic, metabolic diseases) or psychiatric diseases (anorexia, bulimia, depression)
Negative colonoscopy for organic diseases within 5 and 3 years before the enrolment in patients older than 50 years. For subjects under 45 it was not considered.	
Patients who signed the informed consent	

Study protocol

The study lasted 6 weeks. The study provided a visit 7 days before the real T0 screening visit (T0 – 7) in which participants underwent a gastroenterological consultation. During the visit, the gastroenterologist evaluated the inclusion criteria and if the patients respected those criteria, they were included in the study. During this visit patients signed the informed consent and received the

IBS symptoms questionnaire, the food diary to fill in for 7 days and the kit to collect the stool sample. Anthropometric parameters were taken.

Seven days after (T0), patients underwent a nutritional consultation. They delivered the stool sample, the symptoms questionnaire and the food diary filled in and received a new copy of symptoms questionnaire, the food diary and the nutritional protocol. They received also the second kit for the stool sample collection.

After 30 days (± 2) (T1) patients underwent the last gastroenterological and nutritional visit in which they delivered the food diary, the symptom questionnaire and the stool sample.

Anthropometric parameters

At T0, body weight (BW) and height were recorded using a Seca 700 scale with a stadiometer. Measurements were taken in the morning after an overnight fast of at least 12 h. Subjects wore underwear and were without shoes. Height was measured only at T0, while body weight was recorded every month. BMI was calculated through weight/height^2 ratio.

Nutritional protocols

The nutritional protocols were elaborated according to IBS recommendations by NICE and BDA guidelines [28,29] and they were delivered with nutritional suggestions to help patients in following the diet.

The diets consisted in 2000 kcal for men and 1800 kcal for women and they were composed by less than 15% of protein, 30-35% of lipids (especially mono and polyunsaturated fatty acids) and 50-55% of carbohydrates with less than 15% of simple sugars. The amount of dietary fiber was between 20 and 25 g /daily.

Portion sizes and frequencies of food consumption were elaborated according to IBS nutritional suggestion published in the literature [25,28,96,97,168] (*Table 13*).

Table 13 Portion sizes and food frequencies [25,96]

	PORTION SIZES		FREQUENCES
	Men	**Women**	
Grains and derivatives	Pasta/rice 80 g Bread 40 g	Pasta/rice 60 g Bread 40 g	Max. 6 portions/day
Vegetables	Raw 100 g Cooked 200 g		3-5 portions/day
Fruit	Max. 80 g		2-3 portions/day
White meat	120 g	100 g	2-3 times/week
Red meat	120 g	100 g	Once per week
Fish	150 g	120 g	2-3 times/week
Eggs	2 eggs		Once per week
Legumes	Grains 60 g + dry legumes 40 g or cooked legumes 120 g	Grains 50 g + dry legumes 30 g or cooked legumes 90 g	3 times/week
Milk	200 – 250 ml	150 – 200 ml	Once per day
Yogurt	125 ml		Once per day
Fresh Cheeses	80 – 100 g	60 – 80 g	Once per week
Extra-virgin olive oil	50 ml	40 ml	Every day
Nuts	10 – 15 g	5 – 10 g	Once per day

To patients received also suggestions on reducing sweets, caffein – rich beverages (max 3 cups/day), alcoholic beverages (max 1 alcoholic unit for women and 2 for men) with the total exclusion 2 days per week, fizzy drinks, fiber – rich foods (especially insoluble fibers e.g. whole grain cereals) were provided. Moreover, other behavioural suggestions were provided: 1) to consume 3 meals and 2 snacks avoiding to skip meals; 2) to avoid eating too late in the evening or eating during the night; 3) to avoid eating large amount of foods; 4) relaxing and eating slowly; 5) chewing accurately; 6) to avoid sweeteners and fried foods.

Symptoms questionnaire and food diary

To evaluate symptoms, we used the validated FAST questionnaire according to Rome IV criteria. Patients were asked about their daily evacuation frequency, fecal consistency according to Bristol Stool Scale (BSS), symptoms daily frequency (abdominal pain, bloating and meteorism) and intensity (on a likert scale from 1 to 5).

The food diary was filled in every day for the entire study period. Participants had to write the type and the amount of food eaten and also the time and the place of their meal. To evaluate the portion sizes, patients were asked to weight foods. If they were not able to weight foods, they could download the *Dietometer* smartphone application to have an idea about their portion sizes.

Stool sample kit

The stool sample kit (Arrow Microbiota Solution B) was provided by the Life Brain®. The stool samples were collected at T0 and after the nutritional protocol (T1). The test permits to have a precise and complete gut microbiota profile from a fecal sample.

5.3. Statistical analysis

The results were analysed through the statistical program R. A paired t-test was conducted to compare weight, BMI, food diaries results and the frequency of symptoms from T0 to T1. Symptoms intensity was evaluated by a Likert scale and it was analysed through a non parametric Wilcoxon test. To examine the correlation among symptoms frequency and the diet a Spearman test was conducted. All results were evaluated with a 95% confidence level. A p value <0.05 was considered to be statistically significant.

The genetic gut microbiota test was evaluated through the Next Generation Sequencing (NGS) by the LifeBrain®. The analysis software used was the MicrobAT. The analysis workflow provided the DNA extraction and amplification and then the barcoded sequencing of hypervariable regions (V3,V4,V6) of the bacterial ribosomal gene 16 S (rRNA).

5.4. Results

Anthropometric parameters

Actually, 6 IBS-D patients (85% females) are enrolled. Anthropometrical parameters of participants at baseline are reported in *Table 14*.

Table 14 Anthropometrical parameters of participants at baseline

Parameters	Values
Age (years)	55 ± 13
Height (m)	1.63 ± 0.09
Weight (Kg)	61.8 ± 8.4
BMI (Kg/m^2)	23.2 ± 1.4

After 4 weeks of diet, participants reduced their body weight from 61.8 ± 8.4 to 58.5 ± 7.6 but this variation was not significant, the same result was for BMI that decreased from 23.2 ± 1.4 to 21.9 ± 1.1 without any significant changes.

Symptoms

Both frequency and intensity of abdominal pain downstreamed but they did not change significantly compared to baseline. At T0, participants median intensity was 2.5 (a bit), while after 4 weeks their median values was 1 (not at all). Also for bloating and meteorism we did not found significant changes neither for frequency nor intensity (*Table 15*).

Table 15 Symptoms variation between T0 and T1

	T0	**T1**	**p value**
Abdominal pain frequency (mean ± SD)	1.64 ± 0.55	1.56 ± 1.81	0.4931
Abdominal pain intensity (median ± SD)	2.5	1	**0.0048**
Abdominal bloating frequency (mean ± SD)	0.93 ± 0.84	2.03 ± 1.82	0.1475
Abdominal bloating intensity (median ± SD)	1.0	0.9	0.5775
Meteorism frequency (mean ± SD)	0.93 ± 0.63	1.34 ± 0.60	0.4960
Meteorism intensity (median ± SD)	1.17	0.5	0.4795
Weekly evacuation frequency	23.17 ± 4.54	12.5 ± 5.37	**0.0005**

Abdominal pain frequency and intensity, and also intensity of bloating and meteorism showed a decreasing trend, while bloating and meteorism seemed to have an increasing trend.

Bristol Stool Scale

At T0, the mean Bristol Stool Scale score of all participants (100%) was 6 that corresponds to fecal fragments with jagged edges. After 4 weeks, a downstream trend was observed. In fact, 34% (n=2) of patients showed a score of 4, 50% (n=3) of patients a score of 5 and only 17% (n=1) of patients remained at a score of 6 (*Figure 25*).

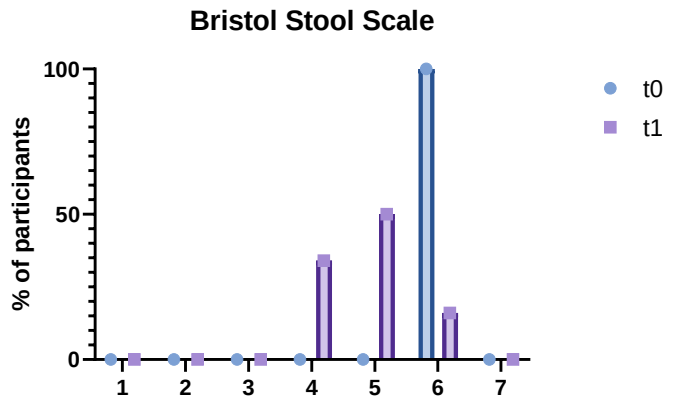

Figure 25 Bristol Stool Scale

Food diaries

Dietary fibers significantly increased from 12.95 ± 3.99 to 18.5 ± 4.37 (p=0.0088) (Figure 26). The reached amount of fibers does not reflect the quantity of dietary fibers suggested by LARN 2014 and nutritional advices for IBS. In fact, it is still low compared to nutritional suggestions.

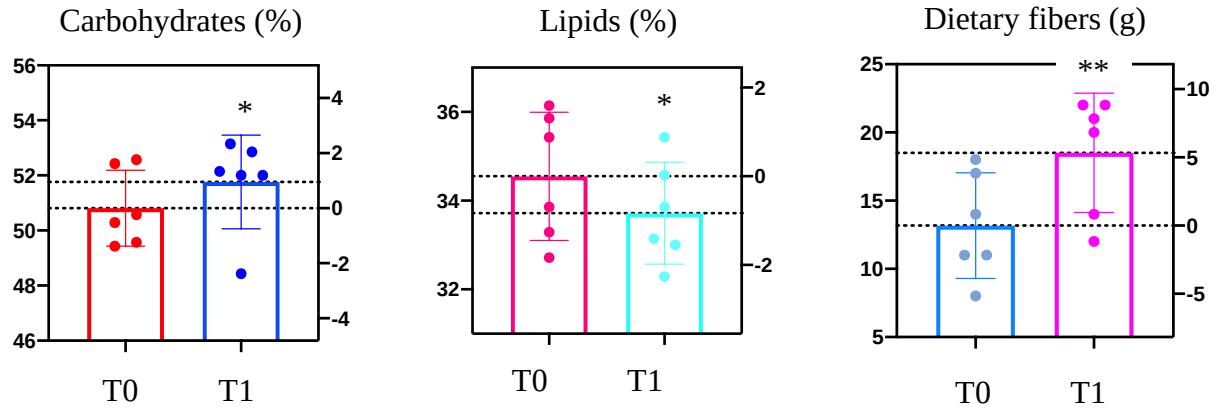

Figure 26 Intake of carbohydrates, lipids and dietary fibers

Table 16 shows how macronutrient intake changed at T1 compared to the baseline. Lipids and carbohydrates intakes significantly changed during the study. Lipids intake, in fact, decreased under the threshold of 35% (p=0.0327) while carbohydrates intake, on the contrary, increased going up to 50% (p=0.0317) as suggested by nutritional recommendations [148]. No significant differences were observed for proteins intake. However, proteins intake decreased a bit going under 15% as suggested by nutritional recommendations.

Table 16 Macronutrients intake from T0 to T1

	T0	T1	P value	**LARN 2014 [168]**
Proteins (%)	15.03 ± 1.72	14.6 ± 0.83	0.2956	**<15%**
Lipids (%)	35.69 ± 2.21	34.22 ± 2.15	**0.0327**	**20-35%**
Carbohydrates (%)	49.07 ± 3.64	51.09 ± 1.67	**0.0317**	**45-60%**
Fibers (g)	12.95 ± 3.99	18.5 ± 4.37	**0.0088**	**25 -30 g**

Gut microbiota

Among participants, there were no significant variations about dysbiosis, microbial diversity, Bacteroides/Firmicutes ratio and the microbic population compared to the baseline.

Figure 27 shows that in the whole population more than 50% showed no change in the composition of the microbiota, while in a very small percentage of patients (about 10%) a worsening was observed. An improvement was observed in about 20% of patients. Looking at microbiota of each patient, *Figure 28*, in most cases the composition of the microbiota did not show evident changes, but for example in patient 1 almost 50% of the microbiota had a positive change, patient 2 also showed a marked improvement in microbiota composition. Patients 3 and 4, on the other hand, did not show much improvement in the composition of their microbiota.

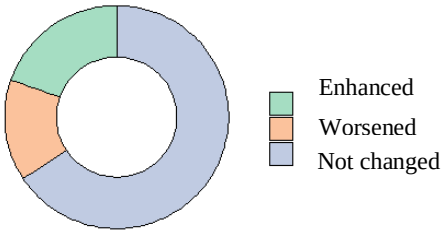

Figure 27 Interindividual Microbial variation between T0 and T1

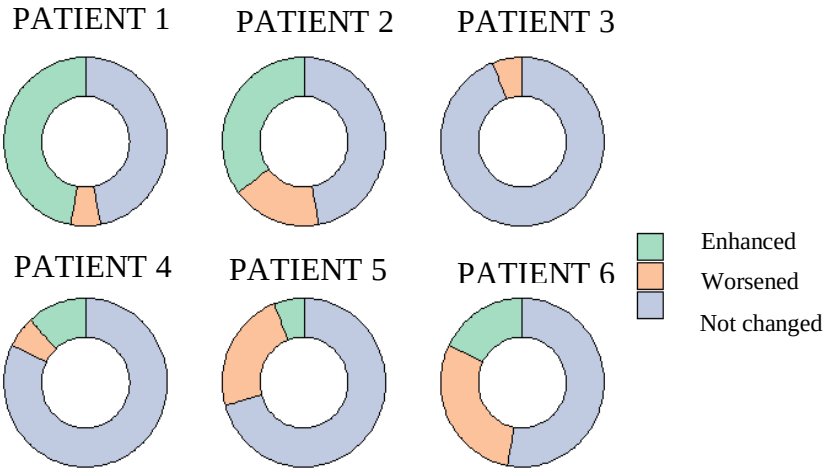

Figure 28 Intraindividual Microbial variation between T0 and T1

Considering the Bacteroidetes/Firmicutes ratio in a range from – 3 (very diminished) to + 3 (very increased) where 0 corresponds to a perfect balance between these two phyla, at T0 67% of patients had +3 and 33% had +1 while at T1 33% patients had +3, 50% had +1 and 17% had 0.

5.5. Correlation between dietary intake and IBS symptoms

Figure 29 shows the existing correlation between dietary intake and IBS symptoms. We observed, first of all that there was a strong inverse correlation between carbohydrates and lipids intake. If we match also the results from food diaries, we can deduce that at the increasing carbohydrates intake corresponds a decrease in the lipids intake. In a similar way we can describe the mild inverse relationship exhisting between carbohydrate and proteins intake.

Regarding the correlation with symptoms, a very mild inverse association was observed between dietary fibers intake and daily evacuations number. Thus, a higher fiber intake determines a reduced number of daily evacuations. About symptoms like abdominal pain, meteorism and bloating, there are no relations with food intake but there are some mild correlations between abdominal pain and meteorism but also between bloating and meteorism.

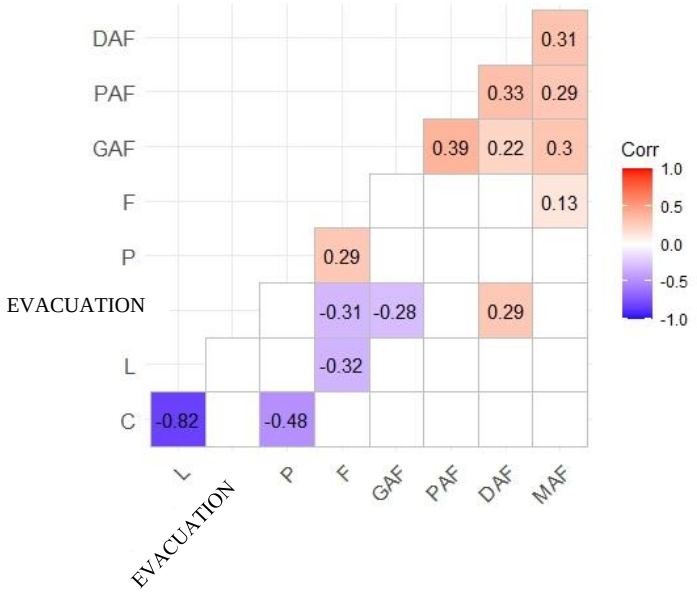

Figure 29 Correlation between dietary intake and IBS symptoms

Legend:

C: Carbohydrates

P: Protein

L: Lipids

GAF: Abdominal bloating

PAF: Abdominal fullness

DAF: Abdominal pain

MAF: Meteorism

5.6. Discussions

The present study evaluated IBS-D symptoms changes after a nutritional protocol. Due to the very small sample size, no significant changes in IBS symptoms were observed from T0 to T1.

In the prescribed nutritional protocol the amount of dietary fibers was kept between 20 and 25 g daily according to nutritional guidelines for IBS [25,96]. In our study the dietary fibers intake significantly increased from 12.95 ± 3.99 to 18.5 ± 4.37 (p=0.0088) even if it did not reach the goal suggested by guidelines [25,96,168]. Patients were recommended to avoid eating whole grains and some insoluble fibers-rich vegetables (e.g. artichoke, asparagus). Probably due to this increased consumption of (soluble) fibers, after 4 weeks most of patients reported mild abdominal pain, compared to T0 in which most had reported 'intense' and/or 'very intense' abdominal pain. Contrary to several studies that have correlated the increased consumption of fiber and carbohydrates to an increased frequency of bloating and abdominal pain [140,186,187], in our study, we found that there was a significant decrease in the intensity of abdominal pain (p=0.0048) and a reduced trend for both abdominal pain frequency and meteorism intensity.

Furthermore, looking at the Bristol Stool Scale (BSS) (Figure 22), an important change in the stool form was observed. In fact, the stool type changed towards type 4 and 5 from the type 6 that characterized the whole population at the beginning of the study. This change may probably be attributed to fact that they consumed more dietary fibers during the suggested nutritional protocol than they were used to do before. About dietary fibers consumption in IBS-D subjects there are a lot of contrasting evidences in the literature but it is known that it is not only beneficial for gut microbiota health, but it could also reduce diarrhea episodes by making stools more formed and compact [188]. The weekly evacuation frequency was significantly reduced after the nutritional protocol and contrary to Bijkerk et al. that reported that fibers accelerate the intestinal transit and increases faecal weight [24], in our study a significant and inverse correlation between the number of evacuations and the fiber consumption was observed. It is an interesting finding and would lead to the hypothesis that fibers not only regularise the intestinal transit in IBS-C [24] but also in IBS-D.

As reported by Belizário et al. [189], IBS – D patients suffer for dysbiosis, related to a low bacterial biodiversity. Even in our study at the beginning all patients had dysbiosis that improved during the study protocol even if not in a significant way maybe for the limited time span. At T1 in 83% of patients, there was an improvement in dysbiosis, maybe for the consumption of fibers, with a consequent increase in the bacterial biodiversity [190]. Only in one patient there was a worsening of dysbiosis. It is interesting to point out that this patient (patient 3) is the only one who did not have full adherence to the nutritional protocol, but it was not possible to quantify it numerically. In fact, a limitation of this study was that we did not evaluate the adherence to the nutritional protocol and the quality of life, data that could have been very useful to evaluate the effectiveness of the dietary protocol.

CHAPTER 6. DOMINO trial post – hoc analysis: evaluation of the diet effects on symptoms in IBS subtypes

6.1. Aim of the study

The recently published DOMINO trial showed that in the primary care a FODMAP - lowering diet was more effective than otilonium bromide after a 8 - week treatment [33]. This diet was provided through a smartphone application and did not completely exclude FODMAPs but only reduced them without determining nutritional deficiencies.

The present work is a post hoc analysis of the DOMINO trial [33] that aimed to evaluate the diet effects on symptoms, psychosocial aspects and quality of life (QoL) in the diet arm for each IBS subtypes (IBS – C, IBS – D, IBS – M and IBS – U).

6.2. Materials and Methods

Study design

This pragmatic study represents a subanalysis based on the DOMINO trial [33] that provides a stratification of the diet arm according to stool types. The diet arm was divided into 4 subtypes: IBS – C, IBS – D, IBS – M and IBS – U according to general practitioners evaluation. The study protocol lasted 6 months and was divided in 5 visits (*Figure 30*).

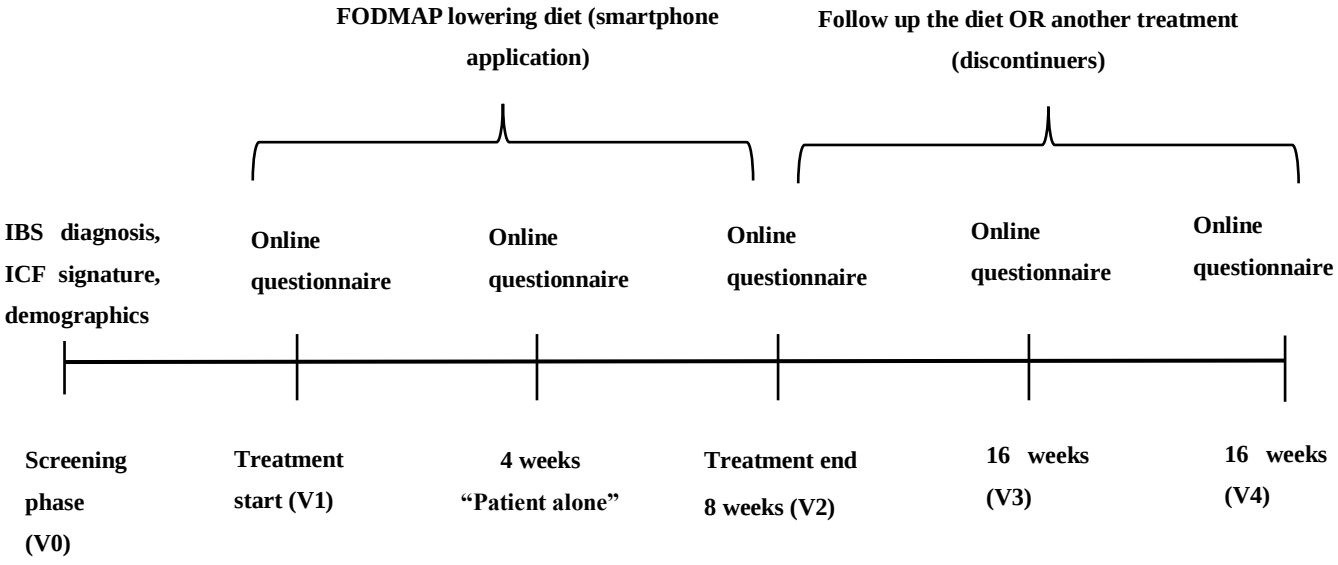

Figure 30 Study protocol

All visits were conducted by general practitioners except for the "patient alone" visit (after 4 weeks) in which patients completed online questionnaires.

Study Population

At the beginning, 227 subjects were enrolled in the diet arm according to the inclusion and exclusion criteria (*Table 17*) [33].

Table 17 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Subjects affected by every IBS subtypes	Subjects with a concurrent GI disease or with a history of abdominal surgery, diabetes mellitus or other coexisting diseases (cancer, endometriosis, thyroid disfunctions, psychiatric disorders)
Who signed the informed consent	Pregnancy women
	Subjects who had already followed a low – FODMAP diet or other elimination diets

The population was stratified according to IBS subtypes and the population was divided as reported in *Figure 31*:

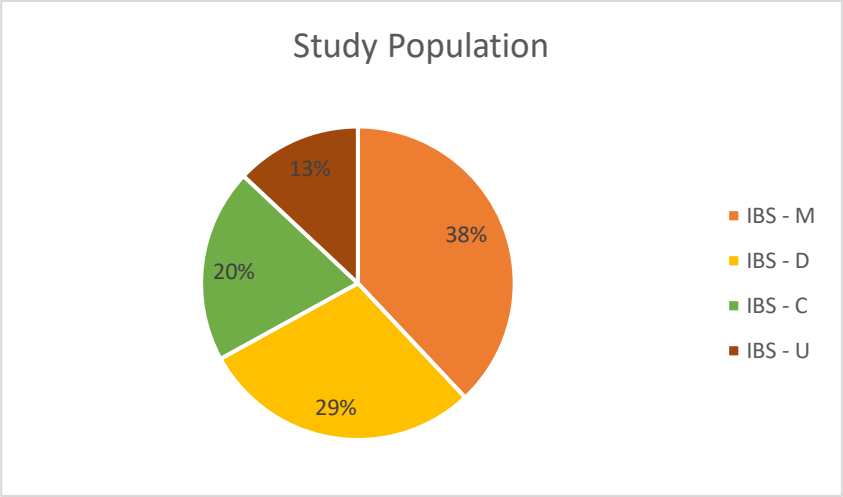

Figure 31 Study population according to subtypes

In accordance with a pragmatic trial, after 8-week period, patients could change treatment. Who changed treatment was defined as "discontinuer". Discontinuers are the patients that did not abandon the study, but they changed the treatment. Thus, after week 8, the term "whole population" includes all participants who followed the initial treatment until the end of follow up without changing.

Dietary intervention

The prescribed diet was a FODMAP - lowering diet, based on NICE and BDA dietary recommendations [28,29] enriched by suggestions on what food avoid or reduce giving also alternatives to avoid nutritional deficiencies. The diet was self – administered and the patients received pieces of information about the diet through an electronic application for smartphone and/or tablet (developed EverywhereIM, Amsterdam, the Netherlands). If participants were not able to download or to use the application they received a booklet with all information and instructions (2%) [33]. In total, 105 recipes were provided for principal meals and snacks. Patients were also able to create their own menu or a shopping list on the app.

Questionnaires

Participants were asked to complete questionnaires during the study before each study visit. They had to complete the IBS – Symptom Severity Scale (IBS– SSS) to evaluate symptoms changes during the study. The score of IBS-SSS is between 0 and 500, and higher scores indicate more severe symptoms. Patients with IBS-SSS < 175 are defined as mild IBS, 175–300 as moderate IBS, and >300 as severe IBS [191]. We considered also the responders rate. Responders were people who had a score of >50 compared to the previous visit. The IBS-SSS was composed by 7 questions:

- QA) Have you suffered for abdominal pain in the last month?
- QB) Pain intensity – how much pain from 0 to 100?
- QC) How many times in the last 10 days have you felt abdominal pain?
- QD) Do you suffer for abdominal distension (bloating, swollen, tight tummy)?
- QE) Abdominal distention intensity – how much pain from 0 to 100?
- QF) Dissatisfaction from 0 to 100 for bowel habits (0 very happy - 100 very unhappy)
- QG) How much abdominal pain, distention and discomfort interfere with life in general from 0 to 100 (0 not at all; 100 completely)?

Moreover, they were given questionnaires evaluate anxiety (GAD-7), depression (PHQ-9) and somatization (PHQ-15) status [192].

The quality of life (QoL) was evaluated through a 34-items questionnaire and it is composed by 8 subscales: dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual activities and relationship [193]. Thus, it could be considered in its entirety or for each subscale.

Participants were asked also to complete adherence and satisfaction questionnaires. About adherence, we evaluated the question: “How often have you forgotten to follow the prescribed diet for IBS?” on a 0-7 points Likert scale (from 0, never, to 7 several/most of the time or constantly). Who forgot at least 2 day/week was considered as “non adherent”. Regarding treatment satisfaction, participants answered the question: “Thinking about the past week, how are your irritable bowel symptoms compared to what they were before receiving the treatment?”. The score was calculated on a 0-9 points Likert scale (from 0, extremely worse, to 9, extremely better).

6.3. Statistical analysis

The analysis was conducted through the statistical program Graphpad Prism 9.4.1. version. Changes between each time point and baseline were analysed with a One – way ANOVA with Dunnett’s post hoc test while a Chi square test was conducted for responders rate, adherence and treatment satisfaction and also questions QA and QD of IBS-SSS questionnaire. A p value < 0.05 was considered to be statistically significant.

6.4. Results

Study participants

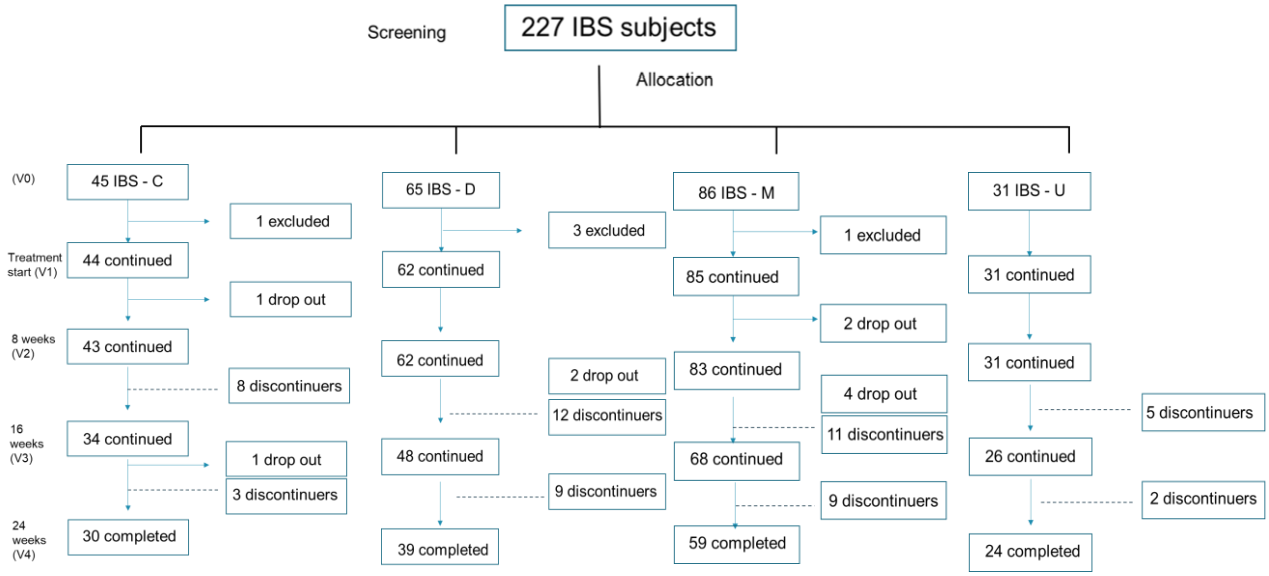

Figure 32 Study participants

Legend:

Excluded: patients who decided not to start the study

Drop out: patients who missed the visit and/or abandoned the study protocol

Discontinuers: patients who completed the trial but decided to change treatment

Completed: patients who completed the trial following the initial treatment

After the screening phase, 227 patients were allocated to different IBS subtypes according to their prevalent stool type (V0). At V1, 5 patients were excluded because they did not want to continue the trial. Thus 222 patients started the study: 85 IBS – M, 62 IBS – D, 44 IBS -C, and 31 IBS – U (*Figure 32)*.

In total, 219 patients completed the 8 weeks of treatment and 176 patients continued with the allocated treatment for an additional 16 weeks. At the end of the follow up period (24 weeks) there were 152 subjects that had followed the lowering FODMAP diet: 59 IBD – M, 39 IBS – D, 30 IBS – C and 24 IBS – U. In total, 59 patients chose to change treatment: 36 after week-8 and 23 after week-16. They could choose among "spasmolytic agents", "another diet" (different from lowering – FODMAP diet), "no new treatment" or "other" (*Figure 33*).

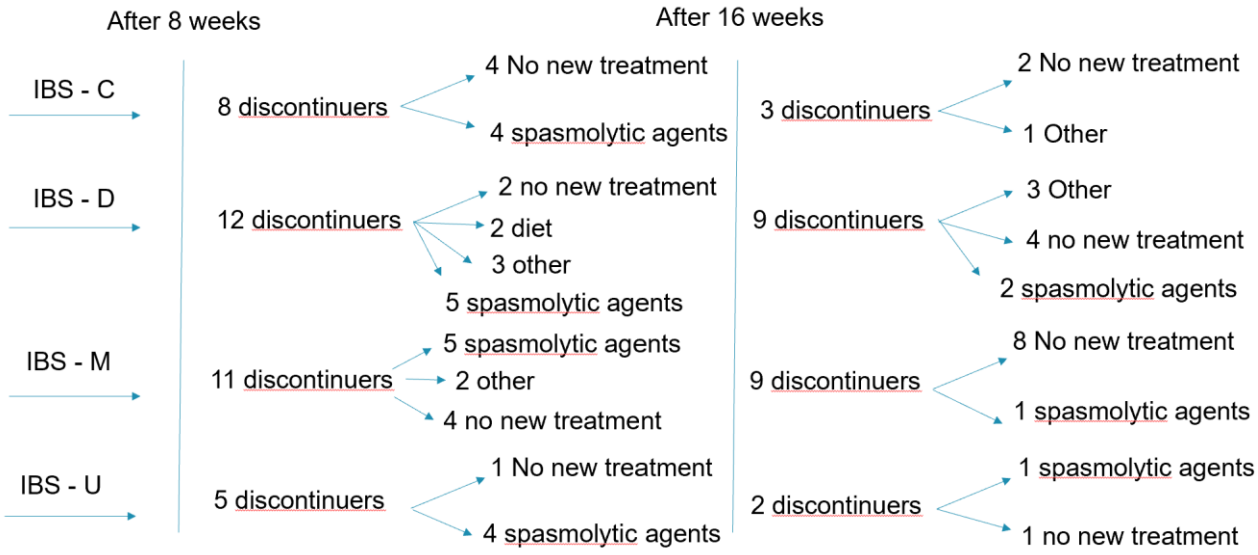

Figure 33 Discontinuers in all subtypes at each time point

Among discontinuers, we had: 11 in IBS – C, 21 in IBS – D, 20 in IBS – M and 7 in IBS - U.

In total, out of 59, 26 subjects chose not to start a new treatment, 22 chose spasmolytic agents, 9 chose other unspecified treatment and 2 chose a dietary treatment different from the FODMAP lowering diet.

Demographic results and body weight

At V1, there were no statistical differences among subtypes for age, height, weight and BMI (*Table 18*).

Table 18 Demographic parameters of the IBS subtypes at V1 (average ± SD)

Parameters	Total population (n=222)	IBS-C (n=44)	IBS – D (n=62)	IBS – M (n=85)	IBS – U (n=31)
Female (%)	168 (76)	38 (86.4)	45 (72.6)	64 (75.3)	21 (67.7)
Age (years ± SD)	40.6 ± 14.5	40.0 ± 14.2	37.5 ± 14.0	40.4 ± 14.9	43.6 ± 14.1
Height (cm ± SD)	169.4 ± 8.8	168.5 ± 8.8	170.4 ± 9.7	168.7 ± 8.0	171.3 ± 8.9
Weight (kg ± SD)	69.8 ± 14.0	65.9 ± 11.3	69.7 ± 14.5	72.2 ± 15.1	69.1 ± 12.2
BMI (kg/m2 ± SD)	24.4 ± 4.8	21.4 ± 4.7	23.9 ± 4.1	25.3 ± 4.7	23.5 ± 3.6

Legend: One way ANOVA with Dunnett's post hoc test was conducted

Regarding body weight, we observed that it did not significantly change after each visit compared to baseline in all subtypes.

IBS – SSS

The IBS – SSS score improved in each subgroup from the baseline to the end of the dietary treatment (8 weeks) ($p<0.0001$). Observing the treatment end phase (8 weeks) all subtypes had an improvement of symptoms ($p<0.0001$). Table 20 summarizes the IBS – SSS scores in the four subtypes while *Table 19* summarizes the IBS-SSS in discontinuers for each time point.

Table 19 IBS - SSS score in the whole population (average ± SD)

	Treatment start (V1)	4 weeks	8 weeks (treatment end)	16 weeks	24 weeks
IBS-C	295.9 ± 94.1 (n=44)	190.5 ± 105.1[b] (n=44)	184.0 ± 112.5[b] (n=43)	173.8 ± 99.5[b] (n=34)	184.0 ± 97.6[b] (n=30)
IBS-D	264.8 ± 89.2 (n=62)	200.5 ± 77.7[a] (n=61)	161.6 ± 93.8[b] (n=62)	158.3 ± 111.7[b] (n=48)	147.9 ± 112.7[b] (n=39)
IBS-M	269.2 ± 102.7 (n=85)	177 ± 101.4[b] (n=80)	184.5 ± 123.4[b] (n=83)	150.6 ± 116.2[b] (n=68)	170.9 ± 116.0[b] (n=59)
IBS-U	224.8 ± 79.8 (n=31)	156.5 ± 102.6[a] (n=31)	128.1 ± 82.0[b] (n=31)	127.3 ± 82.8[b] (n=26)	119.2 ± 74.9[b] (n=24)

Legend: a) $p<0.05$; b) $p<0.0001$; 4 weeks: it was a "patient alone" time point, there was no visit, thus some patients did not fill in the questionnaires; One way ANOVA with Dunnett's post hoc test was conducted

Among discontinuers, although we observed a downward trend in symptoms in all subtypes, without significant variations from the baseline except for IBS – M at 16 weeks ($p<0.05$) (*Table 20*).

Table 20 IBS - SSS score in discontinuers (average ± SD)

	Treatment start	4 weeks	8 weeks (treatment end)	16 weeks	24 weeks
IBS-C (n=11)	311.82 ± 125.68	228 ± 105.7	244.55 ± 125.25	210 ± 132.25	198.89 ± 150.20
IBS-D (n=21)	262.38 ± 97.97	227.5 ± 63.9	198.5 ± 83.43	237.78 ± 116.9	206.25 ± 87.09
IBS-M (n=20)	254± 126.67	183.16 ± 123.7	242 ± 136.02	123 ± 130.23[a]	177.65 ± 124.48
IBS-U (n=7)	241.43 ± 54.29	231.43 ± 166.58	218.57 ± 89.34	236.67 ± 122.75	176.67 ± 40.41

Legend: a) $p<0.05$; One way ANOVA with Dunnett's post hoc test was conducted

According to IBS – SSS responders rate, we could observe that an average of 65% of patients show a positive response to the diet after 4 weeks, while 72% of patients after 8 weeks and this value still remained high (70%) until the end of follow up period (24 weeks). Between subgroups, the subgroup showing the greatest benefit of the diet after 8 weeks was IBS-C with a response rate of 79%, followed by IBS-U (74%), IBS-D (70%) and IBS-M (67%). After 6 months of treatment, the IBS-C group was still above 75% (*Figure 34*).

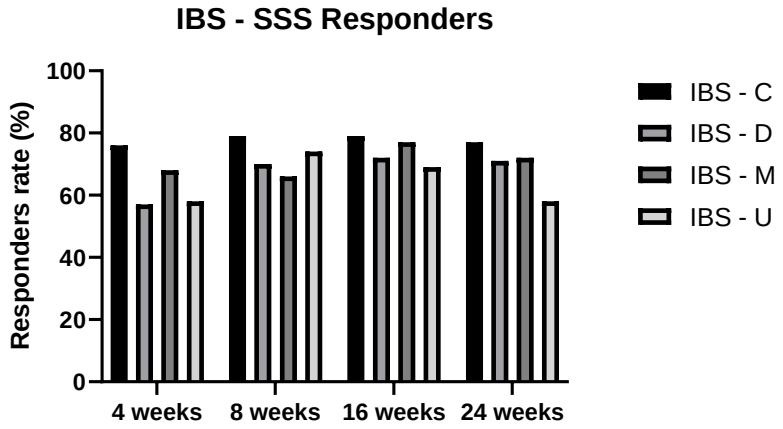

Figure 34 IBS - SSS responders rate

Table 21 shows the responders' rate percentage both in the whole population (patients without discontinuers) and in the discontinuers population (who changed treatment after 8 or 16 weeks). The mean responders' rate among discontinuers at 8 weeks was 55% and it increased after 16 weeks (60%) but decreased again after 24 weeks (57%). The IBS – U subgroup had a higher response rate (59%) compared to the other subtypes. In the whole population, without discontinuers, the mean responders rate remained similar between 8 weeks (72%), 16 weeks (74%) and 24 weeks (70%).

Table 21 IBS - SSS responders rate in the whole population and in the discontinuers group

	4 weeks	8 weeks (treatment end)	16 weeks	24 weeks	mean
Whole population (%)					
IBS-C	76 (n=41)	79 (n=43)	79 (n=34)	77 (n=30)	78
IBS-D	57 (n=61)	70 (n=61)	72 (n=47)	71 (n=38)	68
IBS-M	68 (n=80)	66 (n=83)	77 (n=66)	72 (n=58)	71
IBS-U	58 (n=31)	74 (n=31)	69 (n=26)	58 (n=24)	65
Discontinuers (%)					
IBS-C	50 (n=10)	64 (n=11)	50 (n=10)	56 (n=9)	55
IBS-D	45 (n=20)	55 (n=20)	50 (n=18)	50 (n=16)	50
IBS-M	58 (n=19)	45 (n=20)	71 (n=17)	53 (n=15)	57
IBS-U	43 (n=7)	57 (n=7)	67 (n=6)	67 (n=3)	59

Observing the single questions of the IBS – SSS for the whole population (*Table 22*), we noticed that pain intensity decreased from 53.4 ± 23.5 at V1 to 36.5 ± 25.2 at 8 weeks in IBS – C ($p<0.05$), from 51.9 ± 26.5 to 32.9 ± 24.8 for IBS – D ($p<0.05$) and from 49.8 ± 24.9 to 38.9 ± 25.4 ($p<0.05$) in IBS – M, but no significant changes were noticed in IBS - U. Also bloating intensity changed significantly from 61.8 ± 21.7 at V1 to 39.1 ± 29.3 ($p<0.05$) at 8 weeks in IBS – C, from 50.6 ± 28.4 to 29.8 ± 26.0 ($p<0.05$) in IBS – D, from 52.9 ± 27.9 to 37.1 ± 28.5 ($p<0.05$) in IBS – M and, from 46.1 ± 24.2 to 24.8 ± 25.0 in IBS-U. Except for IBS – U, at 16 weeks abdominal pain and bloating continued to decrease in all subgroups ($p<0.05$) and the reduction was higher in IBS – M group ($p<0.0001$) for abdominal pain. In IBS – U subgroup we observed only a significant reduction in bloating at 16 weeks ($p<0.05$).

The percentage of participants who observed a reduction in their abdominal pain decreased significantly from 98% to 84% in IBS – C, from 92% to 77% in IBS – D, from 94% to 84% in IBS – M while for bloating it decreased from 98% to 81% ($p<0.05$) in IBS – C and from 84% to 67% ($p<0.05$) in IBS – D at 8 weeks. No significant changes were in IBS – M and IBS – U.

The number of days in which participants felt abdominal pain in the last 10 days also decreased significantly in all subgroups ($p<0.05$ for IBS – M and IBS – U and $p<0.0001$ for IBS – D) except for IBS-C.

Finally, we observed also the dissatisfaction for their proper bowel habits and the interference of abdominal symptoms on daily life. For the first question we observed a significant reduction in IBS-

C, IBS-D, IBS-M (P<0.001 for all), and in IBS – U (P<0.05) from 8 weeks compared to the baseline. This result was maintained also at 16 and 24 weeks. Evaluating also how much the abdominal symptoms interfere with the daily life, we observed a significant reduction in IBS – C (p<0.0001), IBS – D (p<0.0001), IBS – M (p<0.05), and IBS – U (p<0.05) and this result was maintained also during the follow up period.

Table 22 IBS-SSS questions in the whole population

IBS - C					
	V1 (n=44)	**4 weeks (n=41)**	**8 weeks (n=43)**	**16 weeks (n=34)**	**24 weeks (n=30)**
QA (%)	98	85	84[a]	76[a]	87
QB (average)	53.4 ± 23.5	35.4 ± 24.2[a]	36.5 ± 25.2[a]	34.4 ± 24.3[a]	40.3 ± 26.6
QC (average)	5.1 ± 3.0	3.2 ± 2.1	3.2 ± 2.5	2.8 ± 2.2	2.9 ± 2.4
QD (%)	98	83[a]	81[a]	79[a]	83[a]
QE (average)	61.8 ± 21.7	39.3 ± 27.6[a]	39.1 ± 29.3[a]	37.6 ± 24.7[a]	43.3 ± 25.8[a]
QF (average)	72.3 ± 23.3	52.4 ± 28.4[a]	45.8 ± 26.7[b]	46.8 ± 26.9[a]	44.7 ± 25.8[b]
QG (average)	57.7 ± 26.9	31.7 ± 26.9[b]	30.9 ± 27.9[b]	26.8 ± 21.0[b]	26.3 ± 21.7[b]
IBS – D					
	V1 (n=62)	**4 weeks (n=61)**	**8 weeks (n=61)**	**16 weeks (n=47)**	**24 weeks (n=38)**
QA (%)	92	85	77[a]	72[a]	66[a]
QB (average)	51.9 ± 26.5	37.9 ± 25.2[a]	32.9 ± 24.8[a]	33.8 ± 28.6[a]	28.4 ± 26.6[b]
QC (average)	4.6 ± 2.8	4.7 ± 1.2	2.6 ± 2.4[b]	2.6 ± 2.6[b]	2.3 ± 2.7[b]
QD (%)	84	77	67[a]	70	63[a]
QE (average)	50.6 ± 28.4	33.3 ± 25.4[a]	29.8 ± 26.0[a]	34.5 ± 27.2[a]	25.5 ± 27.7[b]
QF (average)	68.1 ± 22.5	48.0 ± 24.0[b]	44.3 ± 44.1[b]	40.2 ± 26.4[b]	45.0 ± 30.3[b]
QG (average)	48.5 ± 25.5	34.6 ± 25.3[a]	28.2 ± 22.5[b]	27.7 ± 26.6[b]	25.8 ± 25.0[b]
IBS - M					
	V1 (n=85)	**4 weeks (n=80)**	**8 weeks (n=83)**	**16 weeks (n=66)**	**24 weeks (n=58)**
QA (%)	94	79[a]	84[a]	74[a]	78[a]
QB (average)	49.8 ± 24.9	34.9 ± 25.5[a]	38.9 ± 25.4[a]	30.6 ± 26.5[b]	34.0 ± 26.8[a]
QC (average)	5.1 ± 2.9	3.1 ± 2.6[b]	3.4 ± 2.8[a]	2.7 ± 2.6[b]	3.2 ± 2.8[a]
QD (%)	88	79	78	66[a]	69[a]
QE (average)	52.9 ± 27.9	36.3 ± 26.2[a]	37.1 ± 28.5[a]	33.8 ± 28.9[a]	34.5 ± 26.0[a]
QF (average)	67.4 ± 25.0	46.5 ± 27.6[b]	43.7 ± 29.2[b]	37.0 ± 26.7[b]	42.8 ± 28.1[b]
QG (average)	48.4 ± 26.0	28.6 ± 25.0[b]	31.2 ± 28.9[a]	22.6 ± 25.5[b]	27.9 ± 27.5[b]
IBS – U					

	V1 (n=31)	4 weeks (n=31)	8 weeks (n=31)	16 weeks (n=26)	24 weeks (n=24)
QA (%)	90	65[a]	74	73	63[a]
QB (average)	36.1 ± 22.6	23.5 ± 23.9	23.5 ± 19.9	23.1 ± 20.5	22.1 ± 21.9
QC (average)	4.3 ± 3.3	2.4 ± 2.6[a]	2.2 ± 1.7[a]	2.1 ± 1.8[a]	2.4 ± 2.4[a]
QD (%)	84	65	61	77	54[a]
QE (average)	46.1 ± 24.2	31.3 ± 30.4	24.8 ± 25.0[a]	26.9 ± 22.2[a]	21.7 ± 23.2[a]
QF (average)	57.7 ± 24.5	49.7 ± 28.9	37.1 ± 24.7[a]	37.3 ± 26.2[a]	35.8 ± 28.3[a]
QG (average)	41.9 ± 24.7	28.4 ± 26.5	21.0 ± 23.1[a]	19.2 ± 18.5[a]	17.9 ± 17.2[a]

Legend: p-value Baseline vs W4,8,16,24; a) $p<0.05$; b) $p<0.0001$; % = chi square test; average = One Way ANOVA

Table 23 shows the different questions of IBS – SSS in the discontinuers' groups divided according to stool types. Among discontinuers, we did not observe a lot of differences compared to baseline. Only IBS – M showed a significant reduction of days in which participants had suffered for abdominal pain ($p<0.05$) and the interference on their daily life ($p<0.05$).

Table 23 IBS-SSS questions in the discontinuers' group

IBS - C					
	V1 (n=11)	**4 weeks (n=10)**	**8 weeks (n=11)**	**16 weeks (n=10)**	**24 weeks (n=9)**
QA (%)	100	100	100	90	89
QB (average)	53.6 ± 25.0	36.0 ± 25.0	47.3 ± 26.9	35.0 ± 23.2	33.3 ± 26.5
QC (average)	6.0 ± 3.5	4.1 ± 2.6	3.9 ± 2.7	3.9 ± 2.6	3.4 ± 2.4
QD (%)	100	100	100	90	89
QE (average)	62.7 ± 26.1	53.0 ± 24.5	57.3 ± 28.7	47.0 ± 30.6	44.4 ± 38.8
QF (average)	78.2 ± 29.3	60.0 ± 33.3	57.3 ± 29.3	52.0 ± 32.9	52.2 ± 38.3
QG (average)	57.3 ± 32.2	38.0 ± 28.2	43.6 ± 34.1	37.0 ± 33.7	34.4 ± 36.1
IBS - D					
	V1 (n=21)	**4 weeks (n=20)**	**8 weeks (n=20)**	**16 weeks (n=18)**	**24 weeks (n=16)**
QA (%)	86	95	90	78	88
QB (average)	50.5 ± 26.0	45.5 ± 20.6	40.5 ± 21.9	43.9 ± 30.7	41.3 ± 23.1
QC (average)	4.7 ± 3.2	5.1 ± 0.4	3.6 ± 2.2	4.1 ± 3.0	3.1 ± 2.0
QD (%)	86	90	90	83	81
QE (average)	55.7 ± 24.6	44.5 ± 21.8	41.5 ± 22.8	49.4 ± 26.9	45.6 ± 22.8
QF (average)	64.3 ± 26.0	51.5 ± 19.8	49.5 ± 19.3	62.8 ± 20.2	50.6 ± 21.7
QG (average)	44.8 ± 26.9	40.0 ± 18.6	31.5 ± 20.3	41.1 ± 29.1	37.5 ± 24.1
IBS - M					

	V1 (n=20)	4 weeks(n=19)	8 weeks (n=20)	16 weeks (n=17)	24 weeks (n=15)
QA (%)	90	68	90	65	80
QB (average)	48.0 ± 27.5	31.6 ± 26.3	52.0 ± 25.9	30.0 ± 29.6	46.7 ± 26.4
QC (average)	5.2 ± 3.1	2.8 ± 2.8	4.3 ± 3.1	2.4 ± 2.9 [a]	3.3 ± 2.6
QD (%)	80	68	90	59	73
QE (average)	49.0 ± 30.2	40.0 ± 35.4	51.5 ± 30.7	34.7 ± 33.2	41.3 ± 28.3
QF (average)	62.0 ± 29.1	54.2 ± 31.7	54.0 ± 30.1	37.6 ± 31.1	49.3 ± 31.0
QG (average)	45.3 ± 28.5	28.9 ± 31.9	42.0 ± 30.5	18.2 ± 27.0 [a]	30.7 ± 22.8
IBS - U					
	V1 (n=7)	**4 weeks (n=7)**	**8 weeks (n=7)**	**16 weeks (n=6)**	**24 weeks (n=3)**
QA (%)	100	57	100	100	67
QB (average)	34.3 ± 16.2	31.4 ± 34.8	37.1 ± 12.5	36.7 ± 29.4	23.3 ± 5.8
QC (average)	4.7 ± 3.9	3.7 ± 4.4	4.0 ± 1.9	3.8 ± 2.8	2.7 ± 1.5
QD (%)	100	71	86	100	67
QE (average)	60.0 ± 14.1	45.7 ± 41.2	42.9 ± 34.5	56.7 ± 30.8	56.7 ± 32.1
QF (average)	61.4 ± 21.1	71.4 ± 29.1	52.9 ± 18.0	61.7 ± 31.3	70.0 ± 10.0
QG (average)	38.6 ± 25.4	45.7 ± 39.1	45.7 ± 28.2	43.3 ± 30.1	13.3 ± 15.8

Legend: p-value Baseline vs W4,8,16,24; a) $p<0.05$; % = chi square test; average = One Way ANOVA

Quality of life (QoL)

At 8 weeks quality of life improved only in the IBS – D ($p<0.05$) and in the IBS – M ($p<0.05$) subtypes from the beginning of the study. Regarding QoL subscales we noticed significant variations in the IBS – D and IBS – M subtypes for dysphoria ($p<0.05$ and $p<0.0001$, respectively); interference with activity ($p<0.05$, in both cases); body image ($p<0.05$, in both cases), social reaction ($p<0.05$, in both cases). Only dysphoria changed significantly also in the IBS – C subgroup ($p<0.05$) at 8 weeks.

At V1 participants in each subgroup were: 85 IBS – M, 62 IBS – D, 44 IBS – C, 31 IBS – U while after 4 weeks they were 85 IBS-M, 60 IBS-D, 41 IBS – C, 31 IBS – U. At 4 weeks not all patients completed questionnaires but they still continued the study. At treatment end, 8 weeks, we had: 83 IBS – M, 62 IBS – D, 43 IBS – C and 31 IBS – U. During the follow up period, the number of patients who continued to follow the initial treatment decreased due to drop outs and discontinuers. Thus, at 16 weeks we had 68 IBS – M, 48 IBS – D, 34 IBS – C and 26 IBS – U and after 24 weeks the number of participants decreased again, in fact we had 9 participants less in both IBS – M and IBS – D, 4 less in IBS – C and 2 less in IBS – U than the previous visit.

No significant changes were seen in every subtype for health worrying, food avoidance, sexual activities and relationship.

Results of QoL questionnaire and its subscales are reported in *Table 24.*

Table 24 Subscales of QoL questionnaires in the IBS subtypes in the whole population (average ± SD)

	Treatment start (V1)	**4 weeks**	**8 weeks (treatment end)**	**16 weeks**	**24 weeks**
QUALITY OF LIFE					
IBS-C	79.6 ± 27.4	71.7 ± 28.8	70.4 ± 27.1	64.4 ± 20.8	63.4 ± 19.0[a]
IBS-D	75.6 ± 20.6	68.7 ± 18.7	65.2 ± 19.6[a]	65.1 ± 21.6[a]	58.6 ± 15.1[a]
IBS-M	78.9 ± 22.5	72.1 ± 24.6	67.3 ± 24.3[a]	61.8 ± 22.9[a]	62.1 ± 19.0[b]
IBS-U	68.1 ± 18.0	62.2 ± 18.9	59.0 ± 18.4	57±17.1	54.4 ± 19.6[a]
DYSPHORIA					
IBS-C	19.1 ± 7.8	16 ± 8.0	15.2 ± 7.1[a]	13.4 ± 5.9[a]	13.1 ± 4.5[a]
IBS-D	17.6 ± 6.5	14.9 ± 5.4[a]	65.2 ± 19.6[a]	14 ± 5.7[a]	12.3 ± 4.1[b]
IBS-M	18.1 ± 6.2	15.1 ± 6.5[a]	14.0 ± 6.3[b]	12.5 ± 5.2[b]	12.8 ± 4.8[b]
IBS-U	16.0 ± 5.5	13.7 ± 5.7	13.1 ± 5.9	12.6 ± 5.2	11.9 ± 4.5[a]
INTERFERENCE WITH ACTIVITY					
IBS-C	14.2 ± 5.6	13.2 ± 5.3	13.6 ± 5.3	12.2 ± 3.6	12.0 ± 3.6
IBS-D	17.6 ± 5.5	15.2 ± 5.7	13.9 ± 5.3[a]	14.4 ± 6.1[a]	13.3 ± 5.1[a]
IBS-M	16.1 ± 5.7	14.6 ± 5.5	14.0 ± 5.4[a]	13 ± 5.5[a]	13.2 ± 4.9[a]
IBS-U	13.7 ± 4.2	12.6 ± 4.1	11.8 ± 3.8	11.3 ± 3.7	11.8 ± 3.9
BODY IMAGE					
IBS-C	10.7 ± 3.9	9.2 ± 3.8	9.0 ± 3.6	8.3 ± 2.9[a]	8.2 ± 2.9[a]
IBS-D	9.2 ± 2.9	7.7 ± 2.7[a]	7.6 ± 2.9[a]	7.5 ± 2.8[a]	6.5 ± 2.1[b]
IBS-M	10.0 ± 3.4	9.3 ± 3.7	8.4 ± 3.5[a]	7.7 ± 3.6 [a]	8.0 ± 3.1 [a]
IBS-U	8.3 ± 2.9	7.2 ± 2.7	7.1 ± 2.6	6.8 ± 2.2	6.6 ± 2.3[a]
HEALTH WORRYING					
IBS-C	8.3 ± 3.1	7.2 ± 3.3	7.1 ± 3.0	6.7 ± 2.5	6.5 ± 2.5[a]
IBS-D	5.9 ± 1.9	5.7 ± 2.0	5.4 ± 1.8	5.2 ± 1.9	4.7 ± 1.6[a]
IBS-M	7.3 ± 2.5	6.6 ± 2.6	6.4 ± 2.6	5.8 ± 2.7[a]	5.6 ± 2.3[a]
IBS-U	6.2 ± 2.3	5.9 ± 2.8	5.5 ± 2.5	5.0 ± 1.7	5.3 ± 1.9
FOOD AVOIDANCE					
IBS-C	8.4 ± 3.5	9.2 ± 3.6	9.3 ± 3.4	8.8 ± 2.8	9.1 ± 2.8
IBS-D	8.3 ± 3.1	9.1 ± 2.5	8.8 ± 2.8	8.9 ± 2.6	8.2 ± 2.8
IBS-M	8.4 ± 3.1	9.0 ± 3.1	8.4 ± 3.1	7.8 ± 2.9	8.0 ± 2.9
IBS-U	7.9 ± 3.4	8.1 ± 2.7	7.6 ± 2.6	7.1 ± 2.2	7.4 ± 2.6
SOCIAL REACTION					

IBS-C	8.9 ± 3.9	8.2 ± 4.1	7.3 ± 3.5	6.8 ± 3.1[a]	6.3 ± 2.6[a]
IBS-D	8.0 ± 3.1	7.2 ± 2.6	6.8 ± 2.8[a]	6.9 ± 2.7	6.3 ± 2.2[a]
IBS-M	9.1 ± 3.6	8.4 ± 3.4	7.5 ± 3.4[a]	7.1 ± 3.3[a]	6.7 ± 2.7[a]
IBS-U	7.5 ± 3.1	6.6 ± 2.5	6.4 ± 2.4	6.4 ± 2.4	6.3 ± 2.3
SEXUAL ACTIVITIES					
IBS-C	4.2 ± 2.5	3.8 ± 2.5	3.4 ± 2.0	3.3 ± 1.9	2.9 ± 1.4
IBS-D	3.3 ± 1.7	3.4 ± 1.7	3.2 ± 1.5	3.1 ± 1.4	2.8 ± 1.2
IBS-M	3.7 ± 1.7	3.4 ± 1.9	3.2 ± 1.9	3.1 ± 1.6	3.0 ± 1.4
IBS-U	2.9 ± 1.1	2.9 ± 1.0	2.6 ± 1.0	2.8 ± 1.3	2.6 ± 0.8
RELATIONSHIP					
IBS-C	5.7 ± 2.6	5.0 ± 2.6	5.0 ± 2.4	4.5 ± 1.8	4.7 ± 2.0
IBS-D	5.2 ± 2.1	5.0 ± 1.9	4.7 ± 1.8	4.8 ± 2.2	4.5 ± 1.4
IBS-M	5.4 ± 2.2	5.0 ± 2.2	4.8 ± 2.2	4.4 ± 2.0[a]	4.3 ± 1.6[a]
IBS-U	4.7 ± 2.1	4.5 ± 2.0	4.4 ± 1.9	4.5 ± 2.0	4.4 ± 1.9

Legend: a) $p<0.05$; b) $p<0.0001$; One way ANOVA with Dunnett's post hoc test was conducted

Somatization status (PHQ – 15), PHQ – 9 Depression Scale and GAD – 7 Anxiety Severity

The PHQ – 15 score decreased only in IBS – D and IBS – M subtypes. In the first one we observed a significant reduction after 4,8,16 and 24 weeks ($p=0.0006$; $p=0.0006$; $p=0.0004$ and $p<0.0001$, respectively), while in the second group we observed a significant reduction only after 16 weeks ($p=0.0269$). In the IBS – C and IBS – U subtypes we did not appreciate any significant changes. Depression scale (PHQ – 9) decreased only in the IBS – D subtype after 16 ($p=0.0301$) and 24 weeks ($p=0.0202$) while anxious severity scale (GAD – 7) significantly changed after 24 weeks ($p=0.0200$) (*Table 25*).

The number of participants who completed these questionnaires was the same as the QoL questionnaire.

Table 25 PHQ-15, PHQ - 9 and GAD - 7 questionnaire in the IBS subtypes in the whole population (average ± SD)

	Treatment start (V1)	**4 weeks**	**8 weeks (treatment end)**	**16 weeks**	**24 weeks**
PHQ 15					
IBS-C	13.2 ± 5.3	11.9 ± 6.0	10.7 ± 5.7	10.3 ± 4.3	10.8 ± 4.05
IBS-D	13.1 ± 4.6	10.0 ± 4.3[a]	9.9 ± 5.0[a]	9.5 ± 4.5[a]	8.6 ± 4.3[b]
IBS-M	13.0 ± 5.7	11.1 ± 5.4	10.9 ± 5.7	10.4 ± 5.8[a]	10.6 ± 5.9
IBS-U	11.1 ± 5.2	10.4 ± 5.6	9.2 ± 4.1	8.6 ± 5.2	9.4 ± 6.1
PHQ 9					
IBS-C	7.7 ± 5.9	6.7 ± 5.2	6.2 ± 5.4	5.0 ± 4.3	5.0 ± 3.9

IBS-D	6.6 ± 4.5	5.1 ± 4.0	4.9 ± 3.7	4.5 ± 3.5[a]	4.3 ± 3.3[a]
IBS-M	6.8 ± 4.7	6.1 ± 5.2	5.8 ± 5.1	5.6 ± 5.3	5.8 ± 5.4
IBS-U	7.3 ± 5.4	5.5 ± 5.5	5.0 ± 4.1	4.4 ± 4.0	4.7 ± 4.9
GAD 7					
IBS-C	7.3 ± 5.2	6.6 ± 5.7	5.7 ± 4.6	5.7 ± 4.7	4.7 ± 3.6
IBS-D	7.0 ± 5.1	5.3 ± 4.4	5.2 ± 4.3	4.8 ± 4.6	4.4 ± 3.8[a]
IBS-M	6.6 ± 4.7	5.7 ± 4.9	5.9 ± 4.9	5.2 ± 5.0	5.2 ± 4.7
IBS-U	6.6 ± 4.5	5.3 ± 5.5	5.0 ± 4.2	4.3 ± 4.7	5.7 ± 4.7

Legend: a) $p<0.05$; b) $p<0.0001$; One way ANOVA with Dunnett's post hoc test was conducted

Treatment adherence and satisfaction

Participants who forgot more than twice/week to follow the diet were considered as non-adherent. Thus, we observed that adherence values were high in all subtypes (70% in IBS - D, 67% in IBS - C and IBS - M and 55% in IBS - U). After 8 weeks IBS-D had the higher adherence rate (72%) followed by IBS – C (67%), IBS – M (65%) and IBS – U (53%). At the end of the follow up period, IBS – M subgroup was the one who remained more adherent to the protocol (77%), followed by IBS – D (66%), IBS – U (56%) and IBS – C (54%) (*Figure 35*).

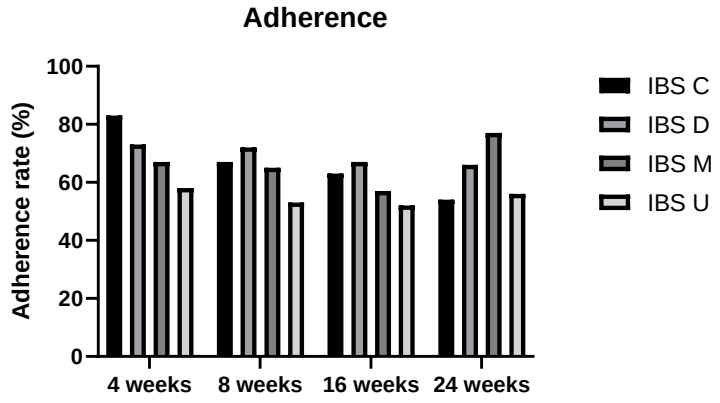

Figure 35 Adherence in the IBS subtypes in the whole population

Table 26 shows the percentage of patients (among who continued to follow the initial treatment until the end of the follow up) who were adherent. The number of patients in the week 4 was fewer than week 8 because it was a "patient alone" time point in which some patients did not fill in the questionnaire, thus we have some missing data.

Table 26 Adherence in the IBS subtypes in the whole population (%)

	4 weeks	**8 weeks (treatment end)**	**16 weeks**	**24 weeks**	**mean**

IBS-C	83 (n=36)	67 (n=43)	63 (n=30)	54 (n=28)	**67**
IBS-D	73 (n=55)	72 (n=60)	67 (n=39)	66 (n=35)	**70**
IBS-M	67 (n=73)	65 (n=79)	57 (n=46)	77 (n=46)	**67**
IBS-U	58 (n=26)	53 (n=30)	52 (n=23)	56 (n=18)	**55**

Regarding treatment satisfaction (*Figure 36*), the mean values ranged from 45% to 53%. After 16 and 24 weeks, the satisfaction scores increased more than after the first 8 weeks (>50%). This was significant for IBS-U (58%) and IBS – M (56%) after 16 weeks and for IBS – C and IBS – M (57% in both cases) after 24 weeks.

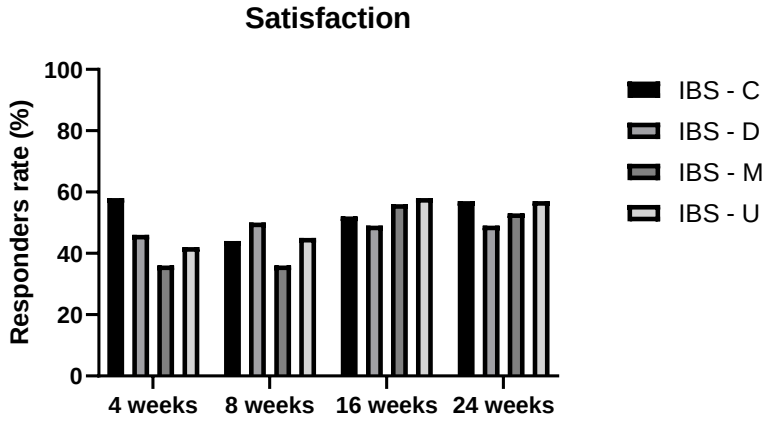

Figure 36 Satisfaction in the IBS subtypes in the whole population

Table 27 shows the percentage of patients without discontinuers who were satisfied of the treatment they followed.

Table 27 Satisfaction in the IBS subtypes in the whole population (%)

	4 weeks	**8 weeks (treatment end)**	**16 weeks**	**24 weeks**	**mean**
IBS-C	58 (n=36)	44 (n=43)	52 (n=33)	57 (n=30)	**53**
IBS-D	46 (n=56)	50 (n=60)	49 (n=45)	49 (n=35)	**49**
IBS-M	36 (n=74)	36 (n=80)	56 (n=64)	53 (n=55)	**45**
IBS-U	42 (n=26)	45 (n=31)	58 (n=26)	57 (n=23)	**51**

6.5. Discussions

To date, it is well established that a low FODMAP diet can improve symptoms in IBS patients [see paragraph 3.2 and references 105,107,170], but there is a lack of evidence in the literature of large randomised controlled trials (RCTs) on the effects of this diet both in the primary care setting and in the different IBS subtypes. The DOMINO study developed nutritional advices that merge the indication of a lowering FODMAP diet and traditional dietary advice for IBS [28,29], with the support of a smartphone application. It was not a strict FODMAP diet and, in fact, it resulted in high adherence to the dietary plan (between 55-70%). Furthermore, thanks to the fact that the mobile application was self-instructed, the patients were involved in the management of their disease being awareness of which foods were most related to the onset of symptoms.

In this specific, post - hoc analysis, we focused on the therapeutic impact of the DOMINO diet application in the different IBS subgroups. In the literature, a limited number of studies stratify the IBS population according to stool type. Due to the heterogeneity of IBS symptoms [15], it is still difficult to determine a single nutritional protocol for the treatment of IBS. The diet suggested did not determined weight loss at each time in all IBS subgroups. The diet, in fact, was not a hypocaloric diet thus it helped to maintain the participants' body weight until 8 weeks and also during the follow up period. Symptom relief was observed in all IBS subtypes with the use of the FODMAP-lowering diet application until week 24 of follow-up for all different IBS-subtypes. IBS – C, IBS – U and IBS – M showed a higher response rate, and IBS – C still maintain more than 70% of response also after 24 weeks.

Generally, IBS patients have higher levels of anxiety and depression compared to healthy controls and they are more associated to IBS – M [194,195] even if the prevalence seems to be higher in IBS – C subtype followed by IBS – M and IBS – D. Our results showed that even if IBS-D started with the lowest depression score, it was the only that had a significant improvement during the follow up period. Eswaran et al. noted that a low FODMAP diet resulted in a greater improvement in anxiety and depression than the group that followed NICE advice in 92 patients with IBS - D [104]. Regarding IBS M and IBS - C, we did not observe any significant changes in anxiety and depression even if they had a downstream trend. In IBS-D also the somatization score significantly decreased from week 4 to the end of the follow up. Moreover, psychosocial status is linked to the QoL, that is often impaired in IBS patients. In fact, IBS – D had a significant improvement in QoL from week 4 to 24, as the same as IBS – M whereas in IBS - C and IBS - U the improvement was only observed after 24 weeks. With regard to QoL there are conflicting results in the literature, with Pedersen et al. observing no

improvement after a low FODMAP diet [114] while Goyal et al. observed it in 166 patients with IBS - D [106]. Even if the study of Rafferty et al. showed that the use of the application improved QoL and symptoms [196], in the literature there are no large – scale studies that use the application as an actual therapeutic strategy as the DOMINO did. In fact, in the literature, also other studies used electronical devices, such as smartphone applications, but they were focused on symptom monitoring and diet adherence (generally low FODMAP) [197,198]. We observed higher psychosocial status scores in the IBS-C subtype compared to other subgroups at baseline, but no significant improvement after the nutritional protocol. Regarding the somatization status (PHQ 15), the IBS- D and IBS- M subtypes had an improvement from week 4 to week 24 and after 16 weeks, respectively. Only IBS – D subgroup showed an improvement on anxiety and depression scales, but it was only observed during the follow up period. The study of Clevers et al. showed that the diet adherence is of paramount importance for symptom improvement because they found a positive correlation between these two parameters [108]. In our study, we had a high rate of adherence in every subgroup (between 55-70%) and, in fact, we noticed that every subtype had a significant symptom improvement during the trial. The reduction in symptoms also resulted in a high satisfaction rate, which was almost 50% among groups.

There is lack of literature evaluating nutritional intervention in the different subgroups of IBS. The study by Nybacka et al. showed that FODMAP intake was generally similar among the various subtypes of IBS and that higher FODMAP intake correlated with greater symptom severity in all subtypes [131]. Ong et al. conducted a single-blind crossover study to evaluate FODMAP restriction on symptoms in 15 healthy volunteers and 15 subjects with IBS (7 IBD - C, 4 IBS - D, 2 IBS - M and 2 IBS - U). They followed a FODMAP-restricted diet (9 g/day) or a high FODMAP diet (50 g/day) for 2 days. Different IBS symptoms decreased significantly in the IBS group compared to healthy controls, although IBS subjects produced more gas during both nutritional protocols. Methane production was more associated with IBS - C subjects possibly due to the slower transit [199]. Halmos et al. conducted a single-blind, placebo-controlled, crossover study of 30 patients with IBS randomised to a low FODMAP diet or a traditional Australian diet. The low FODMAP group showed greater symptom improvement after 3 weeks, but they noted that after the first week, all subtypes of IBS showed symptom improvement with no statistical differences between them [107].

Moreover, primary care studies are lacking in the literature. The study by Seamark et al. evaluated long-term (11 months) symptom changes in 221 patients with IBS enrolled in primary care. These patients received nutritional advices (low FODMAP or other dietary recommendations) from experienced dietitians. The results showed that both nutritional interventions resulted in symptom

improvement without significant differences. This study, however, was innovative because it evaluated the long-term low FODMAP diet, but nutritional advices were still provided by dieticians or health professionals [4].

Thus, the novel aspect of the DOMINO study is that patients downloaded the app and were able to self-manage their disease through nutritional advice on foods to be restricted or preferred according to FODMAPs content or even by choosing a recipe. Sometimes a practical problem is that patients with IBS do not know which foods to consume and how to combine them in a meal [33]. For this reason, a practical guide is absolutely helpful to manage not only the symptoms, but also the time taken to prepare meals, which in some cases can lead to anxiety. Moreover, the low – FODMAP diet is too strict and need the supervision of a specialized dietitian, while using the app, that suggest a less strict diet, patients can play an active role in the management of their disease. Dietitians are expensive and not always available or well – prepared in the low – FODMAP diet, thus, the app provides a useful solution.

A limitation of our study is that we did not have a daily diary of symptoms and foods, so we cannot be completely sure of the actual amount of FODMAP ingested. However, this study was conducted in a large population and resulted in favourable changes, so it could be considered a viable alternative treatment option for patients who do not have many options. To date, the application device is not available worldwide, but only in Belgium. A future perspective is to make it available internationally with traditional country-related recipes.

CHAPTER 7 Conclusions and future perspectives

Conclusions

The irritable bowel syndrome affects quality of life through symptoms such as abdominal pain, bloating and meteorism and presents a high rate of dysbiosis. It could be managed with pharmacological and non – pharmacological approaches. Among this second category there are different nutritional protocols evaluated in the literature and extensively discussed in this work. In fact, specific dietary patterns are able to induce short- and long-term changes in symptoms and in the gut microbiota composition, selecting specific beneficial bacterial species.

Generally, subjects affected by IBS, tend to exclude certain foods from their diet, increasing the risk of nutritional and energy deficiencies. The studies included in my PhD thesis showed that both IBS-C and in IBS-D patients had an unbalanced nutrition before starting the study. In fact, they had an increased consumption of proteins and fats and a decreased consumption of carbohydrates and fibers compared to LARN 2014 and Mediterranean Diet Recommendations. The nutritional protocols suggested in the studies provided a controlled amount of dietary fibers (preferably soluble at the expense of the insoluble one) and a macronutrient composition according to the aforementioned nutritional recommendations and they showed improvements in symptoms, stool consistency and microbiota composition in both IBS – C and IBS-D. Moreover, the consumption of high – amylose wheat, and, consequently of resistant starch, determined a decrease in abdominal pain and also an increasing trend of beneficial bacterial genera such as *Ruminococcus*, *Faecalibacterium* and *Bifidobacteria* especially in IBS-C patients.

The DOMINO study subanalysis showed that a FODMAP lowering diet reduces symptoms in all IBS subtypes. This diet is not restricted as the low – FODMAP diet but it is a merge between the low – FODMAP diet and the traditional dietary advices for IBS. In addition, very often patients tend to reduce their food intake either because of concern about their symptoms or because they are unaware of which foods they might consume. Furthermore, the electronic device helped patients to self-manage their diet and to know which foods can actually exacerbate their symptoms providing pieces of information and also substitutions for foods that should be avoided.

In conclusion, to date, there is not a consensus of the most suitable diet to suggest in case of IBS considering also the different IBS subtypes [200]. Several studies highlighted that symptoms improvement may depend on the initial microbiota composition it would be useful to develop personalized nutritional approaches considering also the initial microbiota in IBS patients to have

more beneficial effects on symptoms. Further studies are necessary to better understand the most suitable dietary approach for IBS management.

Future perspectives

Looking towards the future, there are some projects that are ready to start.

Firstly, two of the projects presented in this thesis are still ongoing. In fact, the high amylose products and IBS-C and the Mediterranean diet on IBS-D projects are not completed yet. The sample size is still small and we are working on implementing it.

Another study that is at its beginning is the so-called "Keto and microbiota" project. This ongoing project aimed to compare the effects of a one-month very low calorie ketogenic diet (VLCKD) with meal replacements on anthropometrical parameters, body composition, IBS symptoms and microbiota composition changes. In this pilot study 40 subjects with overweight or obesity will be recruited as candidates for a ketogenic diet by the endocrinologist according to the SIE guidelines, 2019 [16]. Out of 40 participants, 20 with IBS will be allocated to the IBS group and other 20 without symptoms to the control group. At T0 and T1 (1 month after) anthropometric parameters, body composition, food diary, symptoms and stool sample will be assessed. The stool sample will be analysed by Wellmicro®.

From the collaboration with the KU Leuven other two projects are going to start. Firstly, the great result of the DOMINO trial in the Belgian population took Prof. Tack and Dr. Carbone thinking to extend the smartphone application to other countries. Thus, one of these projects is to adapt the recipes suggested in the Belgian app to Italian typical dishes. In this way, the DOMINO app could also be extended to Italy to help IBS subjects and also clinicians to manage IBS symptoms.

Secondly, during my Erasmus+ period at KU Leuven, I had the opportunity to learn how to use Ussing Chambers. Thanks to this equipment it is possible to evaluate the intestinal permeability. Thus, it would be of great interest to study the intestinal permeability in IBS patients after different nutritional protocols.

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Scientific publications, posters and oral communications

Scientific Publications

1. Soare A., Khazrai Y.M., Fontana L., Del Toro R., Lazzaro M.C., Di Rosa C., Buldo A., Fioriti E., Maddaloni E., Angeletti S., Di Mauro A., Gesuita R., Skrami E., Tuccinardi D., Fallucca S., Pianesi M., Pozzilli P. **Treatment of reactive hypoglycemia with the macrobiotic Ma-Pi 2 diet as assessed by continuous glucose monitoring: The MAHYP randomized crossover trial**; *Metabolism Journal – Clinical and Experimental (2017)*

2. Defeudis G., Khazrai Y.M., Di Rosa C., Secchi C., Montedoro A., Maurizi A.R., Palermo A., Pozzilli P., Manfrini S**. Conversation Map™, an Effective Tool for the management of males and females with Type 2 Diabetes and mildly impaired glycemic control;** *Hormones (2018); 17: 113-117*
3. Guarino M.P.L., Altomare A., Emerenziani S., **Di Rosa C.**, Ribolsi M., Balestrieri P., Iovino P., Rocchi G., Cicala M. **Mechanisms of action of Prebiotics and their effects on gastro-intestinal disorders in adults;** *Nutrients (2020); 12(4):1037.*
4. **Di Rosa C.**, Lattanzi G., Taylor S.F., Manfrini S., Khazrai Y.M. **Very low calorie ketogenic diets in overweight and obesity treatment: Effects on anthropometric parameters, body composition, satiety, lipid profile and microbiota**; *Obesity Research & Clinical Practice (2020)*; *S1871-403X(20)30577-9*
5. Altomare A., **Di Rosa C.**, Imperia E., Emerenziani S., Cicala M., Guarino M.P.L. **Diarrhea Predominant-Irritable Bowel Syndrome (IBS-D): Effects of Different Nutritional Patterns on Intestinal Dysbiosis and Symptoms;** *Nutrients (2021); 13, 1506.*
6. Altomare A., **Di Rosa C.**, Putignani L., Emerenziani S., Fabris S., Ristori M.V., Crucillà S., Calò C., Nicolò R., Ciccozzi M., Cicala M., Guarino M.P.L. **T.04.9. Role of dietary habits on symptom control and gut microbiota composition in patients with irritable bowel syndrome with constipation (ibs-c);** *Digestive and Liver Disease (2022); 54:S131-S132*
7. Defeudis G., Di Tommaso A.M., **Di Rosa C.**, Cimadomo D., Khazrai Y.M., Faggiano A., Cincione R.I., Napoli N., Mazzilli R. **The Role of Antihyperglycemic Drugs and Diet on Erectile Function: Results from a Perspective Study on a Population with Prediabetes and Diabetes;** *Journal of Clinical Medicine (2022); 11, 3382*
8. **Di Rosa C.,** Lattanzi G., Spiezia C., Imperia E., Piccirilli S., Beato I.,Gaspa G., Micheli V., De Joannon F., Vallecorsa N., Ciccozzi M., Defeudis G., Manfrini S., Khazrai Y.M. **Mediterranean diet versus Very Low-Calorie Ketogenic diet: Effects of reaching 5%**

body weight loss on body composition in subjects with overweight and with obesity. A cohort study. *International Journal of Environmental research and Public Health (2022)*

9. Gaspa G., Naciu A.M., **Di Rosa C.,** Lattanzi G., Beato I., Micheli V., Turriziani C., Khazrai Y.M., Cesareo R. **Short- and long- term efficacy of Very Low and Low Calorie Ketogenic Diets on metabolic and cardiometabolic risk factors: a narrative review.** *Minerva Endocrinologica (2022)*
10. **Di Rosa C.**, Altomare A., Imperia E., Spiezia C., Khazrai Y.M., Guarino M.P.L. **The role of dietary fibers in the management of IBD symptoms.** *Nutrients (2022)*
11. Lattanzi G., **Di Rosa C**., Spiezia C., Sacco R., Cattafi S., Romano L., Benvenuto D., De Gara L., Khazrai Y.M. **"Nutripiatto" as a tool for nutritional education: survey to assess dietary habits in preschool children.** *Plos One,* Under review.
12. **Di Rosa C.**, De Arcangelis E., Vitelli V., Crucillà S., Angelicola M., Trivisonno M.C., Lafiandra D., Masci S., Messia M.C., De Gara L., Marconi E., Khazrai Y.M. **Effect of three bakery products formulated with high-amylose bread wheat flour on post-prandial glycemia in healthy volunteers.** *Foods,* Under review.
13. Altomare A., **Di Rosa C.**, Terrigno V., Carbone F., Tack J., Guarino M.P.L. **Constipation Predominant-Irritable Bowel Syndrome (IBS-C): Effects of Different Nutritional Patterns on Intestinal Dysbiosis and Symptoms.** *Nutrients,* Under review.

Posters and Oral Communications

Posters

1. Y.M. Khazrai, **C. Di Rosa**, G. Lattanzi, C. Spiezia, I. Beato, D. Benvenuto, S. Manfrini. **Effectiveness of a very low calorie ketogenic diet with meal replacements on body weight and composition. Obesity Reviews (2020).** *Poster Number: EP -566; Meeting: ECOICO Online 2020 - European and International Congress on Obesity, 2020*
2. Altomare A., **C. Di Rosa,** L. Putignani, S. Emerenziani, S. Fabris, M.V. Ristori, S. Crucillà, C. Calò, M. Cicala, M.P.L. Guarino. **Effect of a targeted nutritional intervention on symptom control and modulation of the fecal microbiota in patients with IBS-C (2021)** *Poster Number: NGS15527-92***;** *Meeting: NeuroGASTRO Online 2021 – Biennal Meeting of the European Society of Neurogastroenterology and Motility*
3. Altomare A., C. **Di Rosa,** L. Putignani, S. Emerenziani, S. Fabris, M.V. Ristori, S. Crucillà, C. Calò, R. Nicolo', M. Ciccozzi, M. Cicala, M.P.L. Guarino. **Role of dietary habits on symptom control and gut microbiota composition in patients with irritable bowel**

syndrome with constipation (ibs-c) (2022). *Poster number:* T.04.9**;** *Meeting: Federazione Italiana Società Malattie dell'Apparato Digerente (FISMAD), 28° Convegno Nazionale delle Malattie Digestive (CNMD) 2022*

4. De Arcangelis E., **Di Rosa C.,** Khazrai Y. M., Blasi E., Angelicola M., Trivisonno M. C., Cicatiello C., Sestili F., De Gara L., Marconi E. **Valutazione di prodotti da forno alto-amilosio: indice glicemico e gradimento al consumo (2022).** Poster number: P66**;** Meeting: 12° Convegno AISTEC CEREALI E SCIENZA: resilienza, sostenibilità e innovazione, Portici (NA)

Oral communications

1. Y.M. Khazrai, G. Defeudis, **C. Di Rosa**, E. Roncella, C. Secchi, P. Pozzilli, S. Manfrini**. Diabetes, Conversation Map™, an Effective Tool for Type 2 Diabetes Treatment *(2014)*** *Abstract Number: 697 – P; Meeting: 74th Scientific Session of American Diabetes Association (2014), San Francisco, CA*
2. A.Soare, R. Del Toro, L. Fontana, Y.M. Khazrai, **C. Di Rosa**, E. Fioriti, E. Maddaloni, R. Gesuita, D. Tuccinardi, S. Fallucca, M. Pianesi, P. Pozzilli. **The macrobiotic Ma-Pi 2 diet for treating reactive hypoglycemia underling pre-diabetes (MAIP randomized crossover trial) (2015).** *Abstract Number: 0287 – PD; Meeting: The International Diabetes Federation (IDF), World Diabetes Congress 2015, Vancouver, Canada*
3. **C. Di Rosa,** A. Altomare, L. Putignani, S. Emerenziani, S. Fabris, M.V. Ristori, S. Crucillà, C. Calò, M. Cicala, M.P.L. Guarino. **Effect of a targeted nutritional intervention on symptom control and modulation of the fecal microbiota in patients with IBS-C (2021)** *Abstract number:* C10**;** *Meeting:* 2° Congresso Nazionale SINGEM – dalla fisiopatologia alla terapia: la sfida dei non responders