Evaluation of carbohydrate and fiber consumption in patients with irritable bowel syndrome in outpatient treatment

Suzana Soares LOPES¹, Sender Jankiel MISZPUTEN¹, Anita SACHS², Maria Martha LIMA¹ and Orlando AMBROGINI JR¹

ABSTRACT – Background – Irritable bowel syndrome (IBS) is a chronic functional condition, which main symptoms of pain, discomfort and abdominal distension, constipation, diarrhea, altered fecal consistency and sensation of incomplete evacuation can be influenced by the presence of dietary fiber and fermentable carbohydrates (FODMAPs). This study aimed to assess the relationship between the quantity of fermentable carbohydrates (FODMAP) and fiber consumed by individuals diagnosed with IBS, and their classification according to the Rome III criteria. Methods – A transversal study was carried out in the Intestinal Outpatient Clinic of the Gastroenterology Discipline of UNIFESP. The nutrients of interest for the study were: fiber, general carbohydrates and FODMAPs, with intake quantity measured in grams, analyzed through portions consumed. A nutrition log was used, along with a semi-quantitative questionnaire of consumption frequency. Results – The sample included 63 adult patients; 21 with constipated IBS, 21 with diarrhea IBS, and 21 with mixed IBS. Carbohydrate intake was suboptimal in 55.6% of patients in all groups; excessive consumption was identified in 38.1% of the diarrhea group, 14.3% of the mixed group and 38.1% of the constipated group. Low consumption of carbohydrates was found in 28.6% of diarrhea patients and 47.6% of the mixed group. A mean intake of 23 g of fiber per day was identified, lower than recommended. Conclusion – The study identified a number of inadequacies in the consumption of different nutrients, excessive carbohydrate intake, especially FODMAPs, identified by the respondents as responsible for a worsening of their conditions. By contrast, other food groups such as meat, eggs and dairy were consumed by the sample population in insufficient quantities.

INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic functional condition, characterized by abdominal pain and/or discomfort and altered evacuatory function (diarrhea, constipation). Patients can also present abdominal distension, altered fecal consistency and sensation of incomplete evacuation. One of the main diagnostic criteria is the absence of evident organic structural substrate, such as inflammation, infection, enzyme deficiency, among others, that could explain the presence and persistence of symptoms(1-6).

IBS can occur in any age group, but is especially prevalent in young adults, between 20 and 40 years of age. It usually occurs in women, has no relevant geographical variations, despite appearing to have a higher prevalence in the western world, and affects 10% to 20% of the general population, according to epidemiological studies obtained through public surveys(7,8,9,10).

IBS does not have clear physiopathological mechanisms involving intestinal motor dysfunction, neuroendocrine alterations, immunological mechanisms, the composition of microbiota, psychological stress or visceral hypersensitivity. These factors can however contribute to the development of symptoms, in isolation or in conjunction, as well as cognitive impairment capable of changing the behavior of the patient(11,12).

Published in 1999, the Rome Criteria incorporate new aspects of the syndrome, something updated in its third edition (Rome III) from 2006, which establishes the diagnostic criteria for a number of functional digestive disturbances. For IBS these are; I) the presence of pain or abdominal discomfort that occurs with frequency for three or more days per month over the previous three months; II) pain present for at least the previous six months, in conjunction with at least two of the following symptoms; III) relief at defection, associated change to the frequency of defection and/or associated with a change fecal consistency and appearance(3,11).

Despite no rigorous dietary plan being recommended for patients with this condition, individual specific intolerances should be taken into consideration. It is first necessary to monitor feeding habits, before making recommendations on the basis of the findings. The patient could benefit from a low-fat diet and a restriction of foods that produce gases. Dietary fiber intake is recommended, especially for patients suffering predominantly from constipation, who can substantially benefit in terms of evacuatory frequency, when combined with increased fluid intake and physical exercise.
Some, however, do not achieve pain relief despite an improvement in evacuatory frequency. It can also help patients with diarrhea by better controlling intestinal peristalsis and compacted feces. This is, an attempt to transform multiple evacuations of low volume into fewer evacuations with increased volume\(^{27,13-19}\).

According to the literature, patients related the exacerbation of symptoms with dietary aspects\(^ {8,10,16,18,20-23}\). Monsbakken et al.\(^ {23}\) assessed 84 patients with IBS and observed that 62% reduced or excluded something from their diets, with the intention of minimizing symptoms, these included: fats (64%), milk and dairy products (54%), carbohydrates (43%), caffeine (41%), alcohol (27%) and excessive protein intake (21%).

Patients with diarrhea report symptoms as a result of intake of certain foods and opt for a restricted diet. From a physiopathological point of view, there is no clear indication for this option, since it does not pertain to any proven lactose or gluten intolerance. However, fatty foods have a notable laxative effect through the stimulation of cholecystokinin (CCK). It would be appropriate to adopt a balanced and varied diet, without many restrictions, accompanied by pharmacological treatment\(^ {8,24,25}\).

Currently, there are no official guidelines recommending specific dietary treatment for functional gastrointestinal disturbances, but a number of researchers have focused on this topic, with growing evidence suggesting the benefits that this diet could offer to some patients\(^ {8,9,26,23}\). In a 2006 study\(^ {27}\), a diet of reduced FODMAPs lead to an improvement of all intestinal symptoms in 72% of 62 patients with IBS.

At the beginning of the 2000s, Peter Gibson and Sue Shepherd created a restricted diet for FODMAPs that aimed to ameliorate the symptoms of functional gastrointestinal disturbances. They are osmotically active nutrients that ferment quickly, causing digestive effects in some individuals\(^ {28}\) (FIGURE 1).

In both diarrhea and constipation patients, the recommended treatment is a reduction in fermentable carbohydrates, especially those with short chains, such as fructose, oligosaccharides, disaccharides, monosaccharides, fermentable polysaccharides, lactose and sorbitol, described as FODMAPs. It is likely that deficiencies of specific enzymes related to these intolerances are the cause of intestinal hypersensitivity present in this population, which responds to abdominal distension and increased water secretion in an exaggerated manner due to the incomplete absorption of these carbohydrates, rather than of IBS itself\(^ {8,9,15,16,19,31}\).

Ong et al.\(^ {33}\) assessed two groups of 15 patients, an identical number of healthy volunteers and patients with IBS, and submitted them to hydrogen and methane measurement in expired air. The IBS group produced higher levels of both gases over the course of a day with a FODMAP-rich diet, demonstrating that gastrointestinal symptoms are significantly induced by the consumption of these sugars; the healthy volunteers reported only an increase in the amount of gas produced.

This study aimed to measure the quantity of fermentable carbohydrates (FODMAPs) and fiber intake in patients with IBS and correlate it with the classification model defined by the Rome III criteria.

### METHODS

This descriptive transversal study included 65 patients with irritable bowel syndrome, selected at random, with either constipation, diarrhea or mixed (alternating between constipation and diarrhea). They were treated in the Outpatient Clinic of Intestinal Disease of the Gastroenterology Department of the School of Medicine – Federal University of São Paulo, Brazil. All included patients were previously advised about the research, and were only included after signing the Terms of Consent. The study was approved by the Ethics Board of the Federal University of São Paulo (UNIFESP).

### Definition of sample

Patients between 18 and 65 years of age were analyzed with clinical diagnosis of irritable bowel syndrome. The following were exclusion criteria: pregnancy, breastfeeding, previous history of surgery on the digestive system, organic diseases that could interfere with the research objectives.

- **Nutrients chosen for the study:** the nutrients chosen for study in this research were carbohydrates and fiber, the quantity of which was calculated in grams.
- **Patients received a tailored dietary orientation, taking the type of attack and the foods that triggered the symptoms into account and provided an individualized dietary menu with FODMAP food restrictions.** In this sense, the foods that were most often identified by patients as exacerbating symptoms were bread, cake, pasta, milk and dairy products, cabbage, beans, raw vegetables, soft drinks, alcoholic drinks, fructose, sorbitol and caffeine.
- **Research materials:** the research materials used were intake diary and a semi-quantitative questionnaire on dietary eating frequency.
- **Patient data:** identification, sex, level of education, age, profession, symptom duration and associated diseases.

The semi-quantitative questionnaire of eating or dietary habits was based on the one used by Cardoso\(^ {34}\) and Slater\(^ {35}\), categorizing foods into 10 groups; cereals, wholegrain cereals, sugars, vegetables, leaves, fruits, fats, dairy, meats and beverages.

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**FIGURE 1.** Relation between FODMAPs and functional intestinal symptoms\(^ {8,22}\).

FODMAPs include foodstuffs that contain fructose (apples, pears, watermelon and honey), vegetables rich in fructan (onions, asparagus, artichoke and leek), wheat-based products (bread, doughs in general), sorbitol (artificial sweeteners) and raffinose (cabbage, lentils and beans). Although highly restrictive, a FODMAP free diet generally brings notable benefits to IBS patients\(^ {8,9,16,19,21,29,30}\).
All foods in the questionnaire were reported as domestic measures converted into grams, using the table set out by the Department of Nutrition at the Federal University of Rio de Janeiro (38), consumption patterns were recorded for one month and was applied by the interviewer.

Food consumption was analyzed by the online program Nutri-Quanti (28), which initially transforms consumed frequency into daily consumption by estimating the quantity of each foodstuff and then calculating calories, fiber and carbohydrate intake.

With the aim of increasing the accuracy of the data collected, they were categorized into daily, weekly, fortnightly, monthly and never. How frequently each group is consumed was recorded according to these categories.

Analysis and statistics

According to protocol, we considered the minimum “n” as 20 patients for each group (constipated, diarrhea and mixed); this inclusion was random up to the attainment of the target number of individuals. The data were organized according to the original values and represented visually.

Data are presented first as mean, median, minimum and maximum values, standard deviation, absolute and relative frequency (percentage), and boxplot and bar charts (39).

Inferential analyses used to confirm or refute the findings were: analysis of variation from a fixed value (38) in group comparison [constipation (C), diarrhea (D) and mixed (M)], according to carbohydrate consumption (g) and fiber (g). Pearson’s chi-squared test (40) was used for analysis of the groups (C, D and M), according to consumption (adequate, inadequate) of carbohydrates, fiber and fats.

In all conclusions obtained through inferential analysis, statistical significance was considered at 95%.

All data were analyzed using R Package v 2.15.2 and Statistica version 12.

For proper use of ANOVA to compare groups for consumption of fiber and carbohydrates, the data was processed logarithmically (log).

RESULTS

Two individuals were excluded from the analysis, because the initial screening interview revealed diabetes mellitus, meaning that from an initial 65 patients interviewed, 63 were included in the study. 53 (84.1%) were female, and the median age was 57 years.

Descriptive analysis sought to characterize patients included in the sample according to their food consumption. Therefore, quantity of fiber, general carbohydrates and FODMAPs consumed was assessed.

The patients were referred to a gastroenterologist following diagnosis of IBS by Rome III criteria according to the characteristics of bowel movements.

Patients were grouped according to predominant symptom of IBS (constipated, diarrhea or mixed) and evacuatory frequency in TABLE 1, as established during the preliminary interview.

<table>
<thead>
<tr>
<th>Bowel movement</th>
<th>(n)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constipated (&lt; 3 evacuations/week)</td>
<td>21</td>
<td>33.33</td>
</tr>
<tr>
<td>Diarrhea (&gt; 5 evacuations/day)</td>
<td>21</td>
<td>33.33</td>
</tr>
<tr>
<td>Mixed (alternating constipation/diarrhea)</td>
<td>21</td>
<td>33.33</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>100</td>
</tr>
</tbody>
</table>

Foods and symptoms

When patients were asked whether any foods worsened their symptoms, all responded affirmatively. The most commonly cited foods with low tolerance were: raw vegetables, FODMAPs such as bread, cake, potato and legumes (beans, lentils, peas, chick-peas) (TABLE 2). However, a substantial percentage of patients (79.3%) preferred not to modify or exclude them from their diets.

TABLE 2. Numerical and percentual distribution of FODMAPs cited as causes of worsening of symptoms of IBS.

<table>
<thead>
<tr>
<th>Foods (groups)</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread, cake, pasta, potato</td>
<td>47 (75.0)</td>
</tr>
<tr>
<td>Dairy products</td>
<td>40 (63.0)</td>
</tr>
<tr>
<td>Beans, peas, lentils, chickpeas</td>
<td>39 (62.0)</td>
</tr>
<tr>
<td>Raw vegetables</td>
<td>51 (81.0)</td>
</tr>
<tr>
<td>Soft drinks</td>
<td>28 (44.0)</td>
</tr>
<tr>
<td>Dried fruits</td>
<td>25 (39.6)</td>
</tr>
<tr>
<td>Alcoholic</td>
<td>10 (15.8)</td>
</tr>
<tr>
<td>Sweeteners</td>
<td>9 (14.2)</td>
</tr>
</tbody>
</table>

Carbohydrates and fiber

In the assessment of food intake, involving the sum of all the groups (TABLE 3), the semi-quantitative questionnaire identified a mean value of 256 g/day of carbohydrates and 23 g/day of fiber. The mean percentage distribution, calculated for the whole sample, was 52% for carbohydrates, as recommended (45% to 65%), which was considered adequate, and fiber, which was below the recommended levels (25-38 g/day) (33).

TABLE 3. Descriptive measures of the mean consumption of carbohydrates (CHO, g/day) and dietary fiber (g/day) for all groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Median</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO (g/day)</td>
<td>256.08</td>
<td>82.09</td>
<td>108.58</td>
<td>250.86</td>
<td>565.6</td>
</tr>
<tr>
<td>Fiber (g/day)</td>
<td>23.06</td>
<td>10.18</td>
<td>7.76</td>
<td>21.45</td>
<td>58.77</td>
</tr>
</tbody>
</table>

• Carbohydrates

The results in TABLE 4 show consumption of carbohydrates in grams in FIGURE 2.

TABLE 4. Adequate and inadequate consumption of carbohydrates (CHO, g/day) in the groups of constipation (C), diarrhea (D) and mixed (M).

<table>
<thead>
<tr>
<th>CHO consumption</th>
<th>C (n)</th>
<th>%</th>
<th>D (n)</th>
<th>%</th>
<th>M (n)</th>
<th>%</th>
<th>Total (n)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate</td>
<td>13</td>
<td>61.9</td>
<td>7</td>
<td>33.3</td>
<td>8</td>
<td>38.1</td>
<td>28</td>
<td>44.4</td>
</tr>
<tr>
<td>Inadequate</td>
<td>8</td>
<td>38.1</td>
<td>14</td>
<td>66.7</td>
<td>13</td>
<td>61.9</td>
<td>35</td>
<td>55.6</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>100.0</td>
<td>21</td>
<td>100.0</td>
<td>21</td>
<td>100.0</td>
<td>63</td>
<td>100.0</td>
</tr>
</tbody>
</table>
The majority of patients included were female (n=53); this study aimed to understand the nutritional and dietary aspects related to symptoms in outpatient patients with IBS, and the relationship with IBS classification. The majority of patients included were female (n=53); this study aimed to understand the nutritional and dietary aspects related to symptoms in outpatient patients with IBS, and the relationship with IBS classification.

Epidemiology
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is to be expected from a convenience sample given that IBS is more prevalent among women. The reason for this is unclear, but it appears, to be related to women’s willingness to seek medical attention and cultural and social factors, according to epidemiological studies.\textsuperscript{6,16,25}

As outpatients already under medication treatment, patients were classified as constipated (<3 evacuations/week), with diarrhea (>3 evacuations/day) or mixed (alternating between constipation and diarrhea). The frequency of the three types is highly variable, but generally in the West, the prevalence of each classification is relatively similar (around 30\% for each). Some studies, however, relate that IBS with diarrhea or mixed are the most prevalent.\textsuperscript{6,18,42} In the sample in this study the groups were chosen to be of equal size to help statistical analysis, and because it is a descriptive study.

### Global evaluation of intolerances for diverse food groups

All of our patients reported that their symptoms were aggravated by some foods containing dairy products, beans, peas, dried fruits, white bread, cake, pasta, raw vegetables, alcohol, soft drinks and sweeteners. Some publications have already shown that exclusion of these foods from the diet can help up to 70\% of IBS patients, and up 80\% of them specifically without FODMAPs.\textsuperscript{6,9,10,15,16,23,43} Monsbakken, et al.\textsuperscript{43} described some symptoms of intolerance in 70\% of individuals with IBS for dairy products, onion, cabbage, chocolate, coffee, teas, peas, chili, beer, apple and wheat. Halper et al.\textsuperscript{13} assessed 1242 patients with IBS, were recruited from the internal medicine and GI clinics at the University of North Carolina, Chapel Hill (UNC) and reported spontaneous restrictions that the patients made for some foods: fats, dairy products, sugars, caffeine, alcohol and meat.

### Carbohydrates

Fermentable carbohydrates (FODMAPs) present in cabbage, broccoli and beans are considered the main triggers of symptoms.\textsuperscript{8,15,16} In fact, leguminous vegetables (beans, lentils, peas, chickpeas), bread, pasta, cake, dairy were cited by patients as the main foods for which they had intolerances, and whose intake was directly correlated with complaints of IBS symptoms (TABLE 2). Halmos et al.\textsuperscript{43} studied 30 patients with IBS, were recruited between April 2009 and June 2011 via advertisements in breath testing centers, community newspapers, and through word of mouth, study by Eastern Health and Monash University Human Research, testing a FODMAP-restricted diet for 21 days. The patients’ conditions improved, and they reported reduced gastrointestinal symptoms compared to their usual eating habits.

Several clinical trials have reported that reducing intake of high-FODMAP foods achieves adequate symptom relief in approximately 70\% of IBS patients.\textsuperscript{49} A meta-analysis reported the efficacy of a low-FODMAP diet on the functional gastrointestinal symptoms associated with IBS, and found a significant improvement in symptom severity and quality of life scores compared to patients receiving a regular Western diet.\textsuperscript{49,10}

Fernandez et al.\textsuperscript{45} assessed gastrointestinal manifestations in 25 patients in the outpatient clinic with IBS and 20 healthy volunteers after consuming lactose, sorbitol, fructose and sucrose. Followed by a period these foods were excluded for the IBS patients. The results showed that malabsorption of some of the sugars tested occurred in up to 90\% of patients assessed, with most frequent complaints from individuals with IBS. In this group, 40\% improved after excluding these substances from their diets. Therefore, the authors conclude that malabsorption of these sugars is common in healthy people as well as those with IBS, who show greater sensitivity to unabsorbed carbohydrates.

In this study, carbohydrate consumption was not at the recommended level in 35 (55.6\%) cases. Excessive intake was found in 8 (38.1\%) patients with diarrhea, 3 (14.3\%) in the mixed group and 8 (38.1\%) in the constipated group and low in 6 (28.6\%) cases of diarrhea a and 10 (47.6\%) of the mixed group; 44.4\% of individuals in all groups had an adequate intake of these products (n=28) (TABLE 4).

The mean percentage distribution of carbohydrates in a normal diet is between 45\% and 65\%, values considered balanced according to recommended daily intakes (RDI).\textsuperscript{40} The mean value found among the participants in this study was 52\%; a mean intake of 256 g/day, which is considered adequate (TABLE 3).

For patients in groups D and M, where consumption of FODMAPs was excessive, patients were advised to reduce intake at the first nutritional consultation, on the basis that a diet that restricts these items generates benefits for patients with IBS.\textsuperscript{6,9,10,15,16,41,42} Equally, those classified as either C or M had this alteration suggested. Despite the exclusion/inclusion technique seeming simple, a nutritionist’s guidance is required for the patient to be able to adhere to the diet without compromising their nutritional states.\textsuperscript{10,33}

### Dietary fiber

Increasing dietary fiber content in a diet can substantially improve the fecal consistency and frequency of bowel movements in patients with IBS. However, for some with constipation excessive soluble fiber can contribute to worsening pain and luminal distension caused by excessive gas production.\textsuperscript{19,46} McFarland and Dimidi\textsuperscript{19,47} refer to the fact that fermentation of fibrous carbohydrates are poorly digested, and are not absorbed by the intestine, through the intestinal microbiota that promote the conversion of dietary fiber into short-chain fatty acids (SCFA) and gases. This seems to explain inadequate fiber consumption in part of our assessed population in this study, with a relative predominance in the constipated group.

In both sexes, 69.8\% (n=44) of patients consumed an inadequate amount of fiber, below recommended intake; 15 in the diarrhea group (71.4\%), 16 (76.2\%) in the constipated group and 13 (61.9\%) in the mixed group, while just 19 (30.2\%) from the general population had sufficient fiber consumption (TABLE 5).

Traditionally, the diet of choice for the treatment of IBS involved a large quantity of fiber, because the syndrome could be exacerbated by low intake, but according to Boasaeus’s publication differences between fiber consumption between healthy subjects and IBS patients is not confirmed.\textsuperscript{49,10}

In a simplified form, fiber is classified as either soluble or insoluble. Soluble fiber dissolves in water, forming viscous gels. They are not digested in the small intestine and are easily fermented by the microflora in the large intestine. These are pectin, gums, inulin and some hemicelluloses. Insoluble fiber does not dissolve in water, and therefore does not form a gel, meaning that its fermentation is limited. The insolubles are lignin, cellulose and some hemicelluloses. The majority of fiber containing foods are constituted by a ratio of one third of soluble fiber to two thirds of insoluble fiber.\textsuperscript{49,10}

Hammonds et al.\textsuperscript{49} observed in a systematic review of 13 studies with fiber supplementation in IBS subjects that just one identified an improvement in symptoms with this strategy. Ma- han et al.\textsuperscript{51} conclude that fiber increase up to the recommended...
levels helps to normalize gastrointestinal function in individuals with all types of IBS. However, higher quantities of whole wheat are no longer recommended, because they can exacerbate some symptoms in these patients. According to Birtwhistle\textsuperscript{42}, soluble fiber becomes effective especially in cases of constipation IBS, but not for abdominal pain.

According to the literature, a fiber-rich diet can exert a positive effect on the group of patients with constipation, particularly in those with intake below recommended\textsuperscript{3,15,19,30}. However, there is no general recommendation for soluble and insoluble fiber consumption for patients with IBS. In predominantly constipated individuals there is a significant improvement with a consumption of 25 to 38 g/day, values adequate according to RDIs\textsuperscript{34}. Mean of 23 g/day was observed in patients included in this study, below recommended (TABLE 3).

**Intake questionnaire**

Similar to other publications, this study also has a limiting factor related to the population that composes our study; memory bias with regards to foods that are not tolerated.

When presented with a list for this identification, some had difficulty in remembering foods that had worsened their symptoms with precision. This can also occur during the application of the semi-quantitative feeding frequency questionnaire. In studies of dietary evaluation it is common for researchers to depend on the memory of individuals to recognize foods that reduce or accentuate attacks\textsuperscript{10,34,54}.

**Final considerations**

Patients with IBS need to be treated and supported by a specialized multiprofessional team. Dietary intervention has become important as it is associated with an improved quality of life and, when carried out effectively, can maintain the patient nutritionally healthy as well as reducing intestinal symptoms\textsuperscript{18,19,20}.

It is recommended that follow up utilizes a wide and integrated approach that is tailored to the specific alleviating and aggravating factors of the symptomatology. A good working relationship between the professionals is essential, explaining to the patient that the symptoms are the result of functional disturbances and not serious illness.

There are no tools or recommendations specific to the diets indicated for patients with IBS, because they should be tailored according to the individual intolerances\textsuperscript{54}. A careful review of eating habits is necessary in order to suggest an appropriate diet, with special attention to those that increase symptoms, like lactose, sorbitol, fructose and fat.

During the appointment, other disturbances in patients with IBS were observed, relating to quality of life and psychological alterations, social relationship issues, worries about diet and fear of cancer.

Not every patient accepts changing eating habits, often for lack of economic resources or difficulty in finding the recommended foods.

**CONCLUSION**

This study, involving outpatients of a health public service in São Paulo, allowed the researchers to recognize various inadequacies in the consumption of different food groups, particularly excessive carbohydrates, including FODMAPs, identified by patients as responsible for their symptoms worsening. On the other hand, vital nutrients found such as dairy were consumed in quantities below the recommended levels, as well as dietary fiber.

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**Authors’ contribution**

All the authors participated sufficiently in the intellectual content, analysis of data and in the review of the writing this article.

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