The connection between irritable bowel syndrome and lactose intolerance, and the role of small intestinal bacterial overgrowth: from the diagnosis to the therapy.

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ABBREVIATIONS

CI	confidence interval
CMA	Comprehensive Meta-Analysis Software
DIM	difference in means
ES	effect size
FODMAP	Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols
GI	gastrointestinal
HC	healthy control
HIV	human immunodeficiency virus
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
IBS-D/C/M/A/ U	irritable bowel syndrome diarrheal/constipation/ mixed/alternating/ unclassified form
IBS-SSS	irritable bowel syndrome symptom severity score
IQR	interquartile range

LBT	lactose breath test
LI	lactose intolerance
LM	lactose maldigestion
LTT	lactose tolerance test
MINORS	Methodological Index for Non-Randomized Studies
mm, ml	millimetre, milliliter
MRI	magnetic resonance imaging
NICE	National Institute for Health and Care Excellence
NOS	Newcastle-Ottawa Scale
OR	odds ratio
PICO	Population/Problem, Intervention, Comparison, Outcome
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analysis
RCT	randomized controlled trial
SD	standard deviation
s.e.	standard error
SIBO	small intestinal bacterial overgrowth

S/Suppl.	supplementary material
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
VAS	visual analog scale

INTRODUCTION

Irritable bowel syndrome

Irritable bowel syndrome (IBS) is a common chronic functional gastrointestinal disorder, which can be defined by the Rome IV criteria [1-3]. It is characterized by abdominal pain related to defecation, and associated with a change in stool frequency or consistency (diarrhea, constipation or a combination of these), without any organic disease and routine histologic examination reveals no mucosal abnormality of the gut-wall [4]. Four subtypes of IBS can be separated: diarrheal (IBS-D), constipation (IBS-C), mixed or alternating (IBS-M/A), and unclassified (IBS-U) form [5, 6]. It can lead to significant impairment of quality of life (e.g. social isolation or stigmatization [7, 8]), decreased work productivity, and an increase of health care and societal costs [9-12]. The incidence of the disease is high in Western countries, affecting 10–20% of the adult population, and it is twice more common among women [13-15]. The exact pathomechanism remains unclear, but visceral hypersensitivity, altered gastrointestinal motility, changes in gut microbiota, altered brain-gut axis, low-grade digestive tract inflammation, and psychological factors may play a role [16-18]. Because of the uncertain etiology and pathophysiology, only a few effective, non-specific, multimodal treatment options exist (laxatives, antidiarrheal agents, antispasmodics, antidepressants, dietary, and psychiatric interventions), improving only some key symptoms but not leading to the healing of IBS [11, 19, 20]. Several studies have proven that certain foods worsen the symptoms in most IBS patients because they play an important role in the development of those symptoms [21-27]. The most commonly reported foods are those containing lactose (milk, ice cream and yogurt) or fructose (honey, dates, oranges, cherries, apples and pears), gas-producing foods (beans, peas, broccoli, cabbage, and bran), wheat and wheat-containing products, and sweeteners (sorbitol, mannitol and xylitol) [22]. These findings suggest that dietary intervention that excludes symptom-triggering food components could

be a promising treatment option for IBS. Standard dietary interventions are detailed in some guidelines, e.g. the British Dietary Association and National Institute for Health and Care Excellence (NICE) guidelines [28, 29]. They recommend that patients regularly eat three meals and three snacks a day, never too much or too little, eat in peace, chew thoroughly, avoid certain foods (fatty or spicy foods, alcohol, coffee, onions, cabbage, beans, carbonated beverages, etc.), and eat fiber but distribute its intake over the day. A suggested main dietary approach is increased daily fiber intake; however, while improving general IBS symptoms in some subgroups, it can worsen them in others [30-33]. Reduction of dietary fat intake improved symptoms in patients because fatty acids can trigger symptoms in IBS [24, 27]. The effect of a gluten-exclusion diet is also controversial [34, 35]. A novel treatment option is a diet low in FODMAPs (Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols). Many popular, healthy foods have a high-FODMAP content, such as fruits (apples, pears, peaches, and watermelons), vegetables (onions, garlic, squash, and mushrooms), dairy products, grains (wheat and rye), sweeteners (sorbitol and mannitol), etc. [36]. FODMAPs can trigger symptoms in IBS patients, based on two major mechanisms [11, 37-41]. The 'small bowel hypothesis' states that FODMAPs are unabsorbed, osmotically active molecules (carbohydrates), so they increase the intraluminal water content in the small intestine. This leads to distension, which causes symptoms such as bloating and discomfort. The increased distension also leads to faster oro-cecal transit, which impairs absorption in the small bowel [40]. The second mechanism ('large bowel hypothesis') describes FODMAPs reaching the colon unabsorbed, where they are rapidly fermented by colonic bacteria. This causes flatulence, bloating, and discomfort through increased gas production and distension of the colonic wall [40]. Because of visceral hypersensitivity, the same magnitude of distension will produce different degrees of symptoms, depending on individual susceptibility [42]. These findings suggested that the exclusion of FODMAPs from the diet could improve IBS symptoms. A growing number of studies have shown a

positive effect of FODMAPs on IBS symptoms. The need has thus arisen for a meta-analysis with a focus on effectiveness in comparison with standard IBS diet to provide evidence and underpin recommendations for wider therapeutic use.

Lactose intolerance

Lactose intolerance (LI) is a clinical syndrome characterized by abdominal symptoms after the ingestion of lactose-containing products caused by lactose maldigestion (LM) [43-45]. The most common cause of primary LM is adult-type hypolactasia [3, 46]. Acquired organic disorders (e.g. small intestinal bacterial overgrowth [SIBO], celiac disease, inflammatory bowel disease [IBD], and infectious enteritis /e.g. giardiasis/), can lead to both downregulation of lactase expression and reduction of absorptive capacity and therefore to secondary lactose malabsorption [3, 46]. Approximately 47% of the Eastern European population is affected; however, LI is more common in Asia, Africa, and South America. It affects males and females equally [44, 47]. The prevalence of LM increases with age, however, the LI symptoms decrease in the elderly [48, 49]. Because of insufficient lactase activity, lactose can reach the large intestine, where it is fermented by colonic bacteria; gases (H_2, CO_2, and CH_4), short-chain fatty acids, and other products that are formed there. Excessive gas production causes luminal distension and leads to different gastrointestinal symptoms. The most common complaints are abdominal pain and discomfort, bloating, flatulence, and diarrhea as with IBS or SIBO [43, 45, 50-52]. The diagnostic methods available for LM or LI are based on the lactose breath test (LBT), lactose tolerance test (LTT), genetic test, and assessment of lactase activity in jejunal biopsy specimens, the LBT and LTT being the most popular methods [3]. However, in most studies and at most centers, only one of the last two methods (LBT or LTT) is used, resulting in higher rates of incorrect diagnosis caused by SIBO, for example, which can lead to carbohydrate malabsorption and therefore to false-positive results during the LBT and LTT. Moreover, in some patients

with methanogenic microbiota (e.g. Methanobrevibacter smithii), the bacteria convert hydrogen to methane, leading to false negative LBT results [44]. Restricting lactose intake or replacing the lactase enzyme can alleviate unpleasant lactose-induced symptoms [3, 44-46].

Due to the potential pathogenetic factors of IBS (altered gastrointestinal motility, changes of the gut microbiome, visceral hypersensitivity, anxiety, etc.), food intolerances, such as LI, are more frequent in this disease, however, the prevalence of LM does not differ compared with the healthy population. More IBS patients have symptoms at lower lactose doses and their symptoms are more severe. Moreover, many IBS patients think that their abdominal symptoms are related to lactose intake, even though no objective tests of LM were carried out [53-57]. Numerous clinical trials are investigating the connection between IBS, LM, and LI, but to our best knowledge, no meta-analyses have been performed up to this day.

Small intestinal bacterial overgrowth

SIBO is a condition in which the small intestine is excessively colonized by aerobic and anaerobic bacteria. Normally, there are fewer than 10^5 bacteria per milliliter (ml) in the duodenal and jejunal part of the small intestine, with ileal counts reaching 10^8 per ml [58]. The prevalence of SIBO is unclear, depending on the population and the diagnostic test used. It is more frequent among the elderly due to reduced gastric acid secretion and medications causing hypomotility [59]. Disorders disturbing mucosal defense mechanisms can predispose one to SIBO, intestinal motility disorders, and chronic pancreatitis being the most common causes [60-62]. Other etiological factors are motility disorders (diabetes mellitus, IBS, use of narcotics, intestinal pseudo-obstruction, etc.), anatomic disorders (adhesions, strictures, diverticulosis, etc.), immunological disorders (e.g. human immunodeficiency virus [HIV]), metabolic and systemic diseases (e.g. cirrhosis) [58, 63-65]. SIBO causes mucosal damage and altered motility and therefore leads to complex

malabsorption (of carbohydrate, fatty acids, proteins, and vitamins), diarrhea, bloating, flatulence, and abdominal discomfort [59, 66-69]. A diagnosis of this disease can be based on carbohydrate breath tests or an assessment of bacterial concentration from the jejunal aspirate. Although jejunal aspirate culture is the gold standard method, it is not widely used due to its invasiveness, poor reproducibility, possible contamination, and patchy disease localization. Carbohydrate breath tests are simple, non-invasive, inexpensive, and therefore widely used [70-72]. The treatment comprises the correction of the underlying cause, antibiotic therapy, and nutritional support (e.g. lactose-free diet, vitamin replacement, and correction of nutrient deficiencies). Rifaximine antibiotic therapy is effective in 80% of patients [73, 74]. Higher doses (1200 or 1600 mg/day) are more effective compared to standard ones (600 or 800 mg/day) [75]. The length of antibiotic therapy is not clearly defined. A single 7–10-day course can alleviate symptoms in most patients [76]. Repeated or continuous antibiotic therapy should be useful if symptoms recur [59]. The effectiveness of probiotics is inconclusive, and generally, they are not recommended in SIBO [64, 77].

AIMS

1. Given the uncertain connection between IBS and lactose consumption-related disorders, we performed a systematic literature search and meta-analysis in this important topic intending to assess the prevalence of:
   a) LM
   b) objective LI, and
   c) subjective LI

   in IBS patients compared to healthy controls (HC) [78].
2. We aimed to:
   a) assess the prevalence of LM and LI in South-West Hungary (Baranya County, except for the Mohács district, with a population of 317,000 people),

b) show that parallel testing for SIBO could reduce false positive cases determined by LBT and/or LTT, and
c) investigate the effect of a combined diagnostic method (parallel use of LBT and LTT) compared to standard LBT method in improving diagnostic accuracy.

A retrospective observational study was performed to answer these questions [79].

3. Our third goal was to carry out a meta-analysis to prove whether a low-FODMAP diet improves the symptoms of adult IBS patients more effectively than other (standard) dietary interventions (i.e. without the restriction of FODMAP content) recommended by the latest guidelines [80].

METHODS

Methods for AIM 1

Our work was planned and conducted according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2009 statement [81].

Searching strategy

Our systematic literature search was based on the PICO format: Participants: subjects who underwent any form of LM or LI assessment; Intervention: IBS patients; Comparison: HCs; Outcomes: prevalence of LM, subjective/objective LI. It was conducted by two independent reviewers (JC and PV) to find all relevant articles on the prevalence of LM, subjective and objective LI in IBS compared to HCs, up to 24 April 2018 (first search: 20 June 2017). The search covered three major databases (PubMed, Embase, and the Cochrane Library) with the terms '('irritable bowel syndrome' OR 'IBS') AND ('lactose intolerance' OR 'lactose maldigestion' OR 'lactose malabsorption')'. The reference lists of the relevant articles were hand-searched and all appropriate

records identified were included in the screening process. After this search process, language (only English) and species (only humans) filters were used. Duplicates were removed with EndNote X4 and manually, and then the title and abstract screening was performed by the two reviewers to identify potentially eligible articles. Disagreements were resolved by consensus.

Eligibility criteria

In our meta-analysis, we included all studies investigating the connection between IBS, lactose consumption-related symptoms, and maldigestion in comparison with the HC group. Retrospective studies were also included. The length of follow-up was not a reason for either inclusion or exclusion. Only articles written in English and those examining the effect of lactose ingestion in human IBS patients were included in this study. Short conference abstracts or papers not available in full-text format were excluded. By definition, adult IBS patients (17 years or above) had to be diagnosed according to the Rome or, in articles that were not recently published, according to any other well-defined criteria system. Articles without clear definitions of IBS, or in which SIBO or any other organic diseases (IBD, celiac disease, etc.) were reported or suspected in the background, were excluded from the analysis. We enrolled controlled studies which included healthy adult participants (without organic disease) who did not fulfill IBS criteria, as a control group. Only articles reporting data about the prevalence of LM and/or subjective/objective LI in IBS and HC group were analyzed statistically.

Quality assessment of the individual studies

The quality and the biases of the included studies were analyzed with the Newcastle-Ottawa Scale (NOS) for case-control studies [82]. Two authors (IMC, PV) independently assessed the risk of bias in each paper included in the statistical analysis. Disagreements were resolved by consensus. If the discussion did not result in consensus, a third author was consulted (PH). The NOS for case-control studies contains eight items covering three main domains (selection,

comparability and exposure). A study can be awarded a maximum of one star for each numbered item; on the other hand, a maximum of two stars can be given for comparability. Each item was rated as 'high risk' (zero stars), 'low risk' (one star), or 'unclear risk' (zero stars) corresponding to the definitions.

Data extraction

At the end of the screening process, relevant data were independently extracted from studies by two independent reviewers (JC and PV). These included: prevalence of LM and LI (subjective or objective) as the outcome parameters, first author, year of publication and country of origin, study design, basic characteristics of the study population (age, percentage of females and IBS subtypes, size of the study groups), diagnostic criteria for IBS, diagnostic methods, thresholds and lactose dose used to diagnose maldigestion. Data for the risk of bias (NOS) assessment were collected as well. Extracted data were validated by five co-authors (ZsSz; DP; MB; ÁV; JT).

Outcome measure

The prevalence of LM, subjective and objective LI were the main outcome parameters in our analysis. LM can be diagnosed in different ways [83], the non-invasive and inexpensive LBT, and LTT being the most common methods. The sensitivity and specificity of these tests depends on the lactose dose, but they are relatively high (78% and 93%) [84]. Before (baseline) and after the ingestion of a given amount of lactose, breath and blood samples are collected at different time points for a period and end-alveolar H_2 and blood glucose concentrations are measured. A certain rise of H_2 (or additionally methane) and/or no rise of blood glucose (or additionally galactose) above the baseline levels are considered diagnostic for LM. The amount of ingested lactose and the diagnostic thresholds were different in the studies. Testing of lactase activity in mucosal biopsy samples from duodenum or jejunum is the gold standard method in the diagnosis of LM, but due to the invasiveness, high costs, and patchy expression of the enzyme it is performed less frequently, compared to the tests mentioned

above. The availability of genetic testing of the genes associated with lactase non-persistence (C/T_13910 with CC genotype; G/A_22018 with GG genotype) is variable, its costs are relatively high, and the sensitivity depends on the patients'genetical origin (the different regional mutations are not examined) [83].

Participants with LM who had abdominal symptoms during or shortly after lactose test were defined as objectively lactose intolerant. Participants reporting before any tests, that their symptoms can be in connection with the ingestion of lactose-containing products, were defined as subjectively lactose intolerant.

Statistical analysis

Pooled odds ratios (OR) were calculated with 95% confidence intervals (CI). Random effects and fixed models were applied at all of the analyses with DerSimonian-Laird [85] estimation. Statistical heterogeneity was analyzed using the I^2 and the chi-square test to gain probability-values; $p < 0.1$ was defined to indicate significant heterogeneity [86]. Subgroups of test type (LBT, LTT, lactase activity, and genetic test) and lactose dosages (10-18 g, 20-25 g, and 40-50 g) were created in the analysis on the outcomes. Statistical analyses were performed using the Comprehensive Meta-Analysis Software (CMA). Forest plots were used to present the results of the meta-analyses. To check for publication bias, the visual inspection of funnel plots and Eggers' tests were performed.

Methods for AIM 2

The key points of the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guideline [87] were followed in planning and reporting this study. We retrospectively analyzed data from adult symptomatic patients who underwent the LBT and LTT in parallel at our center (Division of Gastroenterology, First Department of Medicine, University of Pécs) between

15 February 2016 and 14 February 2017. The LBT and LTT were carried out with 50 g lactose (equal to the content of 1 liter of milk), H_2 levels were measured with Micro H_2 instrument (Micro Medical Limited, P.O. Box 6. Rochester, Kent ME1 2AZ ENGLAND). Before lactose ingestion, baseline end-alveolar H_2 and blood glucose levels were measured (0 min). Then patients drank the set amount of lactose dissolved in 250 ml water. After this process, end-alveolar H_2 and blood glucose levels were measured every 30 minutes over three hours (in the case of glucose over two hours). Depending on the clinical situation and patients' compliance, in clinically uncertain (but not in all) cases, a lactulose breath test with 10 g lactulose was carried out to prove or reject the diagnosis of SIBO or slow oro-cecal transit [72]. A significant, ≥20 ppm elevation of H_2 level during the LBT and/or less than 1.1 mmol/l rise of blood glucose during the LTT was diagnostic for LM. Patients with negative LBT and LTT are lactose digesters. Patients with LM who had symptoms during the test were defined as lactose intolerant. Patients with an early (≤90 min) significant (≥20 ppm) rise of H_2 during the LBT and/or lactulose breath test were determined to have SIBO [72]. The diagnostic criteria of the different conditions are summarized in **Table 1**. For optimal preparation, patients stopped taking laxatives, antibiotics, and prokinetics, avoided high fiber-containing foods and fasted for 12 hours, avoided smoking, and exercise for at least two hours before the test. Antiseptic mouthwash was not given routinely, only for those with high initial H_2 value (>20 ppm). We excluded patients with inappropriate preparation for the test (baseline H_2 level >20 ppm) and those with suspected rapid or slow oro-cecal transit (clinical symptoms of gastroparesis and a negative LBT with a positive LTT or no significant rise of H_2 during a 180-min lactulose breath test compared to the baseline value). We collected data on the baseline characteristics of the analyzed population (mean age, gender differences, and their correlation with the outcome measures), the diagnostic tests (baseline and maximum H_2 and glucose levels, time of glucose and H_2 peak, and the presence of LM), the presence and type of symptoms occurring during the test (abdominal pain,

cramps, discomfort, bloating, diarrhea, nausea/vomiting, borborygmi, and other gastrointestinal symptoms, such as increased bowel motility, flatulence, belching, a sensation of fullness in the stomach, a burning sensation in the stomach, increased sensation for defecation or headache [45, 88]), and the presence of LI and SIBO. The data collection and research were approved by the director of the Clinical Center and the director of the First Department of Medicine of the University of Pécs (Institutional Review Board), and the study process was carried out following current laws and regulations (Case Number: PTE/98494/2018). All patient data were fully anonymized after the specific parameters necessary for our research were collected. However, our analysis was made retrospectively; therefore, we have not included patients' data who had refused scientific purpose data handling.

Table 1. The summary of the different diagnostic criteria used in our study.

Lactose maldigester	**LBT**: ≥20 ppm elevation of H_2 compared to baseline level and/or **LTT**: <1.1 mmol/l rise of blood glucose level compared to baseline value
Normal lactose digestion	Negative LBT (<20 ppm elevation of H_2 level) and Negative LTT (≥1.1 mmol/l elevation of blood glucose)
Lactose intolerance	Lactose maldigesters, who had symptoms during the test period
Small intestinal bacterial overgrowth	Significant (≥20 ppm) rise of H_2 during lactose and/or lactulose breath test, within 90 minutes

Slow oro-cecal transit (excluded)	Clinical symptoms of gastroparesis and a negative LBT with a positive LTT or no significant rise of H_2 during a 180-min lactulose breath test compared to the baseline value

LBT: lactose breath test; LTT: lactose tolerance test.

Statistical analysis

Data were analyzed using SPSS 25.0 software. Means, standard deviation, minimum and maximum values, and relative frequency were calculated for descriptive statistics. The Pearson correlation, the Mann–Whitney test, and ORs with 95% CI were used for other analyses. A p-value of less than 0.05 was accepted as statistically significant.

Methods for AIM 3

Search for articles

Our work was planned according to the PRISMA 2009 statement [81]. Following the PRISMA 2009 guidelines, we used the PICO format to formulate our question (P: patients with IBS; I: low-FODMAP diet; C: high-FODMAP/standard IBS diet; O: IBS Symptom Severity Score [IBS-SSS]). A systematic literature search was conducted by two independent reviewers (JC and PV) to find relevant articles on the effect of low-FODMAP dietary intervention in IBS up to 19 September 2016. The search covered three databases (PubMed, EMBASE, and the Cochrane Library) with the terms 'FODMAP AND irritable bowel syndrome'. For better targeting of synonymous phrases, we used the search terms: 'FODMAP' OR 'FODMAPS' OR 'Fermentable poorly absorbed short-chain carbohydrates' OR 'Fermentable oligosaccharides disaccharides monosaccharides and polyols' as was done in a recent meta-analysis by Marsh et al. [89]. After this search process, language (only English) and species (only humans) filters were used and a title and abstract screening

was performed by the reviewers to identify potentially eligible articles. Disagreements were resolved by discussion. Duplicates were removed.

Study selection

We included randomized controlled trials (RCT), non-randomized controlled trials, and non-controlled prospective trials in our meta-analysis. Retrospective studies were excluded. The length of follow-up was not a reason for either inclusion or exclusion. Only articles written in English and those examining the effect of a low-FODMAP diet in human IBS patients were included in the meta-analysis. By definition, adult IBS patients (18 years or above) had to be diagnosed according to the Rome II, Rome III, Rome IV, NICE criteria. We enrolled controlled studies which included adult IBS patients as a control group. In the control groups, IBS patients had to follow a standard IBS diet (according to the guidelines) with significantly higher FODMAP content than in the intervention (low-FODMAP) group. As a standard, validated output measure, we searched for studies reporting the IBS-SSS. The measurement of the severity of individual symptoms among the studies showed great heterogeneity (e.g. Visual Analogue Scale [VAS], different types of Likert scale, etc.), so we used only the complex IBS-SSS in our analysis as an outcome measure. Articles examining the results of patients with an organic disease (for example, IBD) with functional gastrointestinal symptoms, which are similar to IBS symptoms, were excluded from the analysis.

Quality assessment of the individual studies

The quality of RCTs was assessed with the frequently used Jadad score [90], while non-randomized and non-controlled prospective studies were evaluated according to the Methodological Index for Non-Randomized Studies (MINORS) [91]. Both scores were evaluated by JC and PV. Any disagreements were resolved by consensus.

Data extraction

At the end of the screening process, relevant data were independently extracted from studies by the two reviewers (JC and PV). These included: IBS-SSS as the main outcome parameter, study design RCTs, non-randomized controlled trials, etc.), basic characteristics of the study population (age, percentage of females, and IBS subtypes), length of follow-up, diagnostic criteria for IBS, and the size of the low-FODMAP and control (high-FODMAP) groups. Extracted data were validated by five co-authors (AG, IS, GP, ÁV, and ÁS).

Outcome measure

Irritable bowel syndrome symptom severity score

This score provides a measure of overall IBS severity. It was validated by Francis et al. [92] in 1997 and consists of five questions that measure abdominal pain severity, abdominal pain frequency, abdominal bloating, bowel habit dissatisfaction, and interference with quality of life on a 100 mm VAS. Patients should rate every symptom with a score from 0–100, so the theoretical range is 0–500 mm, with higher scores indicating a more severe disease. A final score of less than 175 indicates mild disease, 175–300 shows moderate severity, and >300 points refer to severe IBS [89].

Statistical Analysis

Data analysis was conducted with the CMA (Version 3.0, Biostat Inc.). In the forest plot analysis, mean differences with 95% confidence intervals were calculated from studies that contained means, standard deviation (SD) or mean differences, and SD of differences and p-values. In one study (Pedersen et al. [93]), where the results were expressed as median, minimum, and maximum values, we converted the data using the Hozo method [94].

The studies we included in the meta-analysis indicated that there is a considerable heterogeneity (different clinical methods, diverse participants, etc.), so the random-effects model was used according to the DerSimonian and

Laird method [95]. Statistically, heterogeneity was tested by Q test ($\chi 2$) and I^2 indicator [96]. I^2 indicator and Q test were performed to assess whether the heterogeneity observed among effect sizes could be attributed to random chance or if other factors may play a role. The similar effect of non-investigated variables such as food intolerances and functional digestive tract disorders other than IBS could also cause IBS-like symptoms. I^2 statistics represent the percentage of effect size heterogeneity that cannot be explained by random chance, but by other factors noted above. If the Q test is significant, it implies that the heterogeneity among effect sizes reported in the studies selected is more diverse than could be explained by random error only. The Q test was considered significant when $p < 0.1$.

We used subgroup analysis, with a p-value of less than 0.05 indicating a significant difference to compare the differences in the IBS-SSS between the control and low-FODMAP diet groups. Results from the meta-analysis were displayed graphically using forest plots. The potential for "small study effects", including publication bias, was examined by visual inspection of funnel plots, in which the standard error was plotted against the net change for each study.

RESULTS

Results for AIM 1

Search results

Using the terms mentioned above, we found 647 articles in the three databases for evaluation, 213 in PubMed, 413 in Embase, and 21 in Cochrane Library. We also examined 14 further articles from the reference lists of relevant articles, so 661 articles were found in total. After using the language (only English) and species (only humans) filters in Embase, PubMed, and the Cochrane Library, 520 of 647 studies were further assessed and none of the articles from the reference lists were excluded. After removing duplicates, title and abstract screening, 89 articles reporting on lactose consumption-related disorders in IBS

and eligible for further evaluation were found. The detailed screening of the full-text papers identified 16 articles for further assessment, of which two were not suitable for statistical analysis. Altogether 14 case-control studies met the inclusion criteria and remained for quantitative analysis [53, 54, 97-108]. The flow chart of the systematic literature search was based on the PRISMA 2009 guideline [81] and is detailed in **Figure 1**.

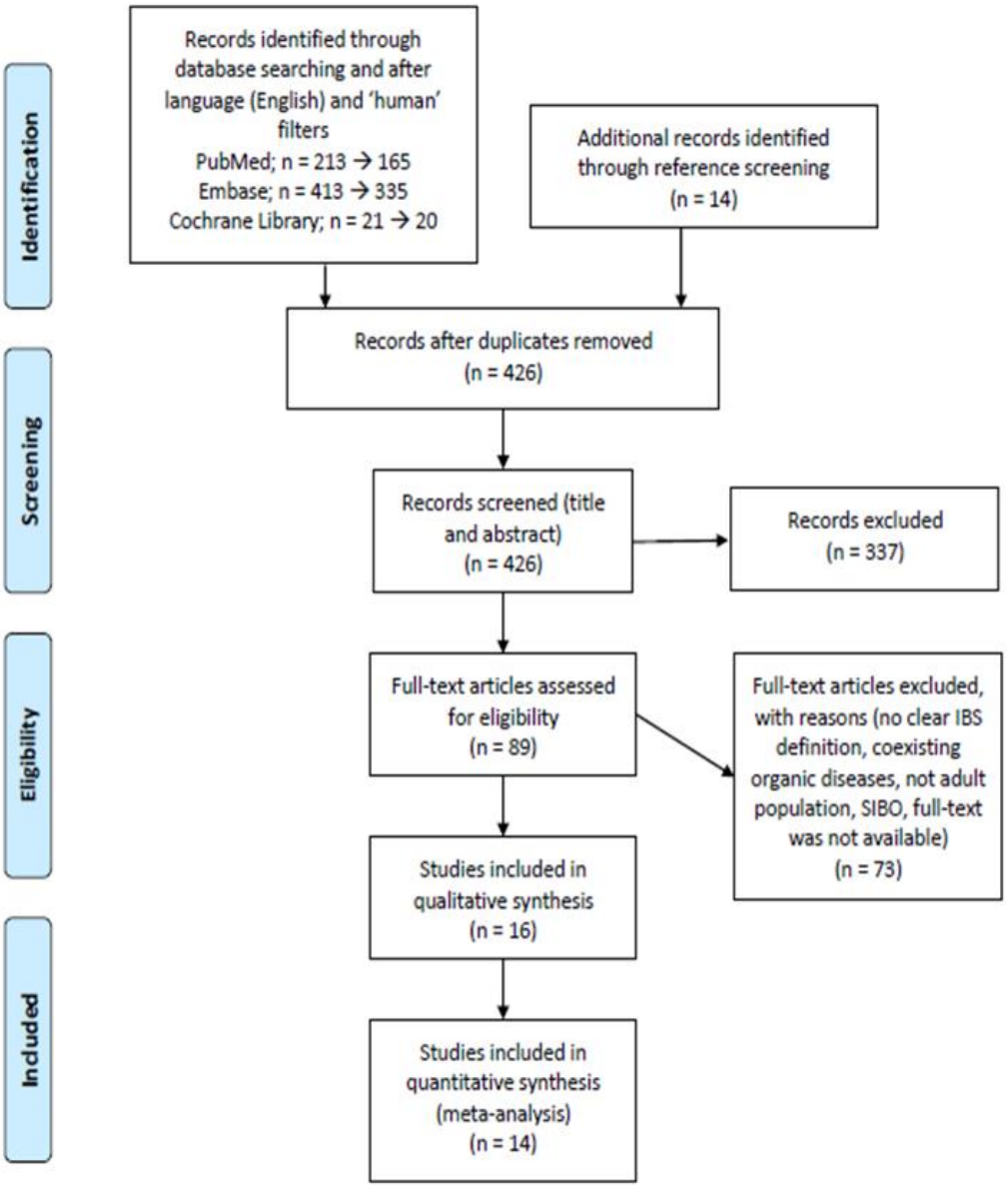


Figure 1. PRISMA-flowchart of the systematic literature search. IBS: irritable bowel syndrome; SIBO: small intestinal bacterial overgrowth.

At the time of the literature search, we found no eligible paper that used the most recent diagnostic criteria (Rome IV) for IBS. The basic characteristics of the articles are summarized in **Table 2**. The proportion of each IBS subtype and the used lactose doses, diagnostic methods for LM, and thresholds in the studies included in the meta-analysis are detailed in **Table 3**. A quality assessment (NOS) of the articles is summarized in **Table 4**.

Table 2. Characteristics of the studies included in the statistical analyses.

First author, year, reference number	Country	Study design	IBS diagnostic criteria	Number of participants (IBS / HC)	Age (IBS / HC), years	Percentage of females (IBS / HC)
Bianchi Porro et al., 1983 [97]	Italy	case-control	Unspecific abdominal complaint (primarily colicky abdominal pain and / or intermittent diarrhea or dyspepsia) for at least 1 year without any organic disease	77 / 40	group 1: mean 42.2 (range: 19-53); group 2: mean 41.2 (range: 18-54) / mean 35.05 (range: 18-55)	51.9 / 52.5
Gwee et al, 1996 [98]	UK	case-control	Rome criteria	22 / 53	median 34 (IQR: 23.5-51) / median 36 (IQR: 27-52)	77 / 36
Vesa et al., 1998 [99]	Finland	case-control	Rome criteria	63 / 364	mean 51 ± 9 SD / mean 50 ± 9 SD	68 / 50

First author, year, reference number	Country	Study design	IBS diagnostic criteria	Number of participants (IBS / HC)	Age (IBS / HC), years	Percentage of females (IBS / HC)
Goldstein et al., 2000 [100]	Israel	case-control	Rome criteria	94 / 145	women: mean 44.4 ± 17.5 SD; men: mean 42.7 ± 18.6 SD / women: mean 49.6 ± 18.9 SD men: mean 35.7 ± 15.7 SD	no data
Vernia et al., 2001 [54]	Italy	case-control	Rome criteria	503 / 336	women: mean 37.8 ± 13.9 SD men: mean 36.2 ± 13.9 SD / women: mean 36.1 ± 14.8 SD men: mean 32.1 ± 13.5 SD	66.7 / 65.1
Parry et al., 2002 [101]	UK	case-control	Rome II	16 / 18	mean 44.4 (range 25–76)	no data / 55.5

First author, year, reference number	Country	Study design	IBS diagnostic criteria	Number of participants (IBS / HC)	Age (IBS / HC), years	Percentage of females (IBS / HC)
Lanng et al., 2003 [102]	Denmark	case-control	Kay and Jørgensen criteria: More than weekly experience of abdominal pain and distension and in addition either borborygmi or altering stool consistency	32 / 26	mean 51.8 / mean 52.8	75 / 53.8
Farup et al., 2004 [103]	Norway	case-control	Rome II	82 / 105	mean 48.8 / mean 46.3	68 / 78
Saberi-Firoozi et al., 2007 [104]	Iran	case-control	Rome II	215 / 1763	mean 49.9 ± 11.14 SD	64.2
Corlew-Roath et al., 2009 [105]	USA	case-control	Rome III	66 / 55	all participants over 18 years	86 / 62
Yakoob et al., 2011 [106]	Pakistan	case-control	Rome III	119 /115	mean 35 ± 13 SD (range: 18-74) / mean 36 ± 15 SD (range: 18-80)	26.05 / 33.04

First author, year, reference number	Country	Study design	IBS diagnostic criteria	Number of participants (IBS / HC)	Age (IBS / HC), years	Percentage of females (IBS / HC)
Kumar et al., 2012 [107]	India	case-control	Rome III	150 / 252	mean 36.7 ± 11.8 SD / mean 37.2 ± 11.5 SD	24 / 22
Yang et al., 2013 [53]	China	case-control	Rome III	60 / 60	mean 40.8 ± 11.7 SD / mean 40.8 ± 15.2 SD	51.6 / 48.3
Xiong et al., 2017 [108]	China	case-control	Rome III	109 / 50	mean 36.0 ± 12.2 SD / mean 34.8 ± 13.3 SD	47.7 / 48

HC: healthy control; IBS: irritable bowel syndrome; IQR: interquartile range; SD: standard deviation.

Table 3. The percentage of IBS subtypes and the diagnostic methods and thresholds used in the analyzed studies.

First author, year, reference number	IBS subtypes (%)	Diagnostic method for LM	Amount of lactose (g)	Diagnostic threshold for LM
Bianchi Porro et al., 1983 [97]	no data	LBT, LTT, lactase activity (jejunal biopsy)	LBT: 50 LTT: 100	LBT: > 20 ppm H_2 rise LTT: < 20 mg / 100 ml rise of blood glucose lactase activity: ≤ 39 IU / g protein
Gwee et al., 1996 [98]	IBS-D: 86 IBS-C: 9 IBS-M / A: 5	LBT	50	no data
Vesa et al., 1998 [99]	no data	LTT	50	Blood glucose elevation < 1.1 mmol / l (20 mg / 100 ml) and maximal rise in blood galactose concentration ≤ 0.3 mmol / l (5 mg / 100 ml)

First author, year, reference number	IBS subtypes (%)	Diagnostic method for LM	Amount of lactose (g)	Diagnostic threshold for LM
Goldstein et al., 2000 [100]	no data	LBT	18	≥ 20 ppm rise of H_2 or ≥ 5 ppm rise of CH_4 over baseline value
Vernia et al., 2001 [54]	IBS-D: 24.8 IBS-C: 13.3 IBS-M / A: 17.1	LBT	0.5 g / kg body weight up to a maximum of 25 g	H_2 peak exceeding 20 ppm over the baseline values
Parry et al., 2002 [101]	no data	LBT, LTT	50	A failure of plasma glucose to rise by more than 1.1 mmol / l from baseline. A rise in the breath hydrogen value above 20 ppm from baseline
Lanng et al., 2003 [102]	no data	LTT	50	Glucose level rise ≤ 1.3 mmol / l
Farup et al., 2004 [103]	no data	LBT	25	Peak values of H_2 breath excretion > 20 ppm above the lowest preceding value, peak CH_4 excretion > 12 ppm above baseline, and / or combined H_2 and CH_4 increase > 15 ppm were considered diagnostic
Saberi-Firoozi et al., 2007 [104]	no data	-	-	-
Corlew-Roath et al., 2009 [105]	no data	LBT	50	H_2, CH_4, and CO_2 were tested (threshold: no data)
Yakoob et al., 2011 [106]	IBS-D: 100	LBT	50	H_2 rise above baseline of 20 ppm

First author, year, reference number	IBS subtypes (%)	Diagnostic method for LM	Amount of lactose (g)	Diagnostic threshold for LM
Kumar et al., 2012 [107]	IBS-D: 52 IBS-C: 35 IBS-M / A: 13	genetic test	-	C/T_13910 (CC genotype) / G/A_22018 genetic variant (GG genotype)
Yang et al., 2013 [53]	IBS-D: 100	LBT, genetic test	10, 20, 40	≥ 20 ppm H_2 rise above the baseline, C/T_13910 (CC genotype)
Xiong et al., 2014 [108]	IBS-D: 100	LBT	25	Peak hydrogen breath excretion of 20 ppm above the baseline level

IBS-D / C / M / A: irritable bowel syndrome-diarrheal / constipation / mixed / alternating subtype; LBT: lactose breath test; LM: lactose maldigestion; LTT: lactose tolerance test.

Table 4. The quality and risk of bias assessment of the included studies according to the Newcastle-Ottawa Scale for case-control studies [82].

First author, year	SELECTION				COMPARABILITY	EXPOSURE			NOS score
	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	
Bianchi Porro et al., 1983 [97]	0	*	*	*	**	*	*	0	7 / 9
Gwee et al., 1996 [98]	0	*	0	0	*	*	*	0	4 / 9
Vesa et al., 1998 [99]	*	0	*	*	*	*	*	0	6 / 9
Goldstein et al., 2000 [100]	*	*	0	0	**	*	*	0	6 / 9

First author, year	SELECTION				COMPARABILITY	EXPOSURE			NOS score
	Item 1	Item 1	Item 1	Item 1	Item 5	Item 6	Item 7	Item 8	
Vernia et al., 2001 [54]	*	*	*	*	0	*	*	0	6 / 9
Parry et al., 2002 [101]	*	*	0	*	*	*	*	0	6 / 9
Lanng et al., 2003 [102]	*	*	*	*	**	*	*	0	8 / 9
Farup et al., 2004 [103]	*	*	*	*	**	*	*	0	8 / 9
Saberi-Firoozi et al., 2007 [104]	*	*	*	0	0	*	0	0	4 / 9
Corlew-Roath et al., 2009 [105]	*	*	0	0	0	*	*	0	4 / 9
Yakoob et al., 2011 [106]	*	*	0	*	**	*	*	*	8 / 9
Kumar et al., 2012 [107]	*	*	0	0	**	*	*	0	6 / 9
Yang et al., 2013 [53]	*	*	0	*	**	*	*	0	7 / 9
Xiong et al., 2017 [108]	*	*	*	*	**	*	*	*	9 / 9

The NOS consists of eight numbered items, divided into three main sections (selection, comparability, and exposure). Each numbered item can be rewarded with a maximum one star; comparability can be awarded with two stars. The studies with a maximum of nine stars representing the highest-quality trials with the lowest risk of bias. NOS: Newcastle-Ottawa Scale.

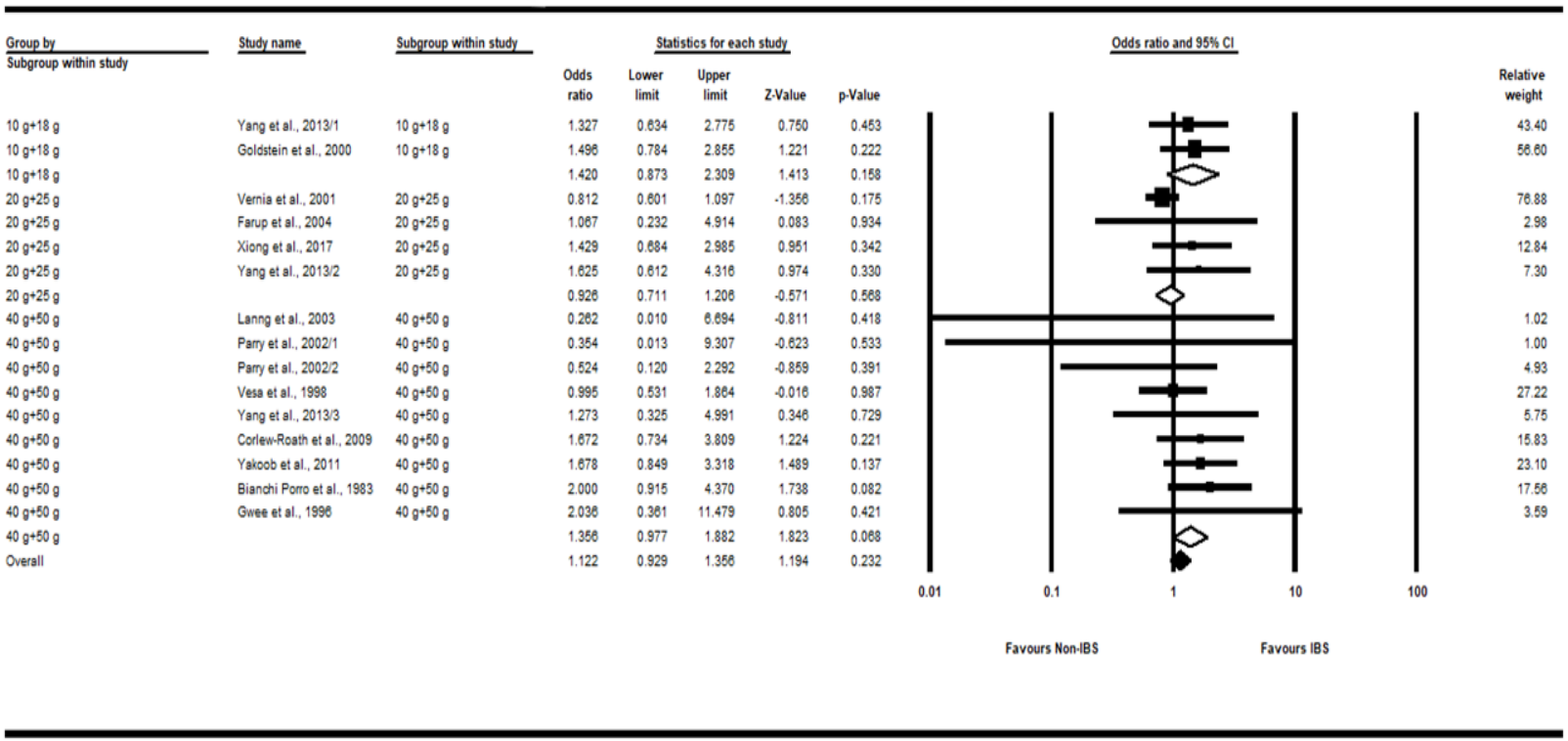
Lactose maldigestion and irritable bowel syndrome

In 13 of the 14 articles, LM was objectively tested with LBT, LTT, or genetic testing. There were not enough controlled studies with lactase activity

measurement to carry out a correct statistical analysis. In one of the included case-control studies, only subjective LI was assessed [104].

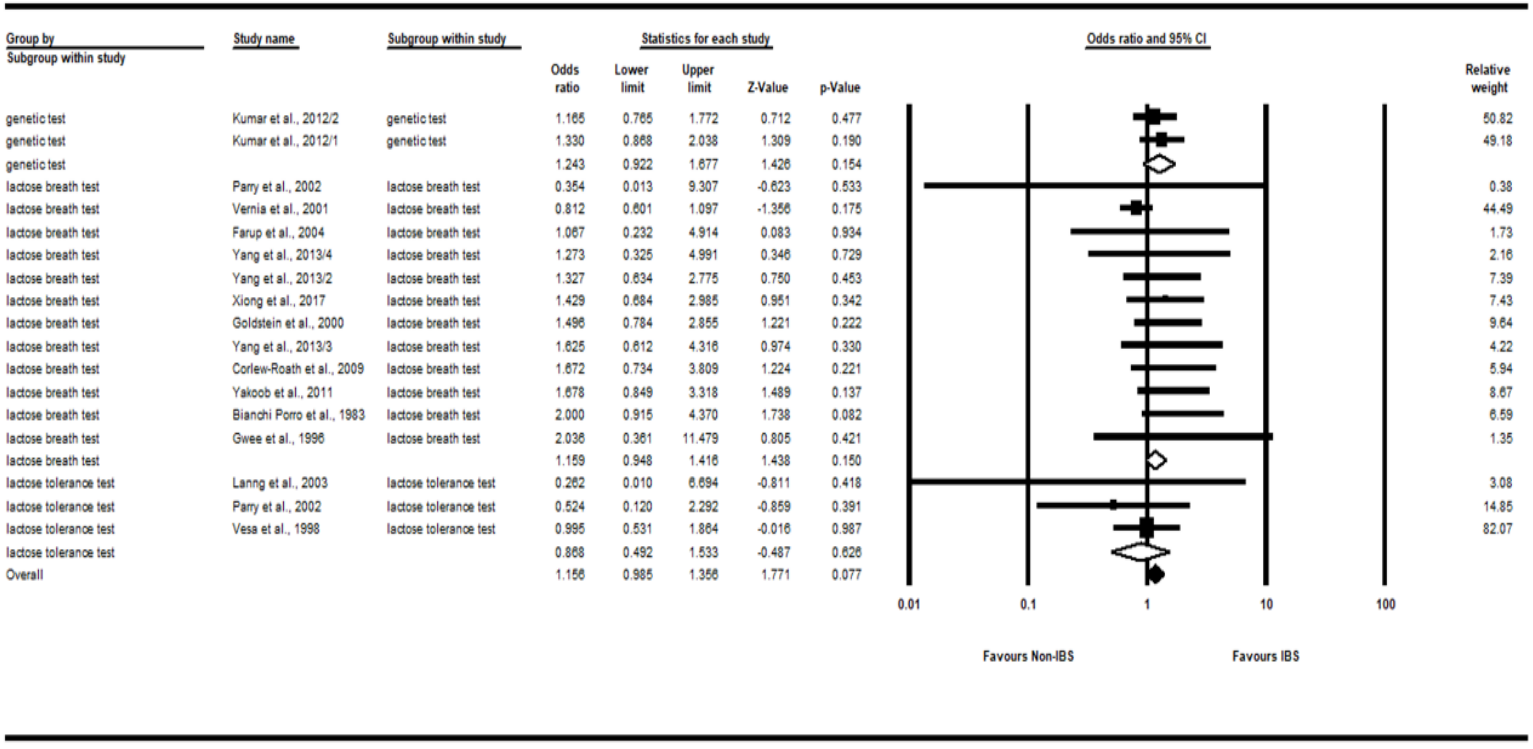
Based on the ingested lactose dose used in the different studies three subgroups were made: (1) 10 g-18 g; (2) 20-25 g; (3) 40-50 g (**Figure 2**). Overall there was no significant difference in the prevalence of LM between IBS and HC groups (OR = 1.122; 95% CI: 0.929 – 1.356; p = 0.232). The I^2 test showed no significant heterogeneity (I^2 = 0.000%; p = 0.479). We did not find significant difference either between (p = 0.121), or within the subgroups: (1) OR = 1.420, 95% CI: 0.873 – 2.309, p = 0.158 (I^2 = 0.000%; p = 0.810); (2) OR = 0.926, 95% CI: 0.711 – 1.206, p = 0.568 (I^2 = 11.037%; p = 0.338); (3) OR = 1.356, 95% CI: 0.977 – 1.882, p = 0.068 (I^2 = 0.000%; p = 0.651). There was no significant heterogeneity within the subgroups.

Figure 2. The difference of LM between IBS and HCs, based on the ingested lactose dose (10-18 g, 20-25 g, 40-50 g). There was no significant difference either overall, or in the subgroups. HC: healthy control; IBS: irritable bowel syndrome; LM: lactose maldigestion.



According to the test methods, three subgroups were made: (1) genetic test; (2) LBT, and (3) LTT (**Figure 3**). Overall, there was no significant difference in the prevalence of LM between IBS patients and HCs (OR = 1.156; 95% CI: 0.985 – 1.356; p = 0.077) and the analyzed studies were homogeneous (I^2 = 0.548%; p = 0.590). We did not find significant difference either between (p = 0.548) or within the subgroups: (1) OR = 1.243, 95% CI: 0.922 – 1.677, p = 0.154 (I^2 = 0.000%; p = 0.664); (2) OR = 1.159, 95% CI: 0.948 – 1.416, p = 0.150 (I^2 = 4.977%; p = 0.396); (3) OR = 0.868, 95% CI: 0.492 – 1.533, p = 0.626 (I^2 = 0.000%; p = 0.561). There was no significant heterogeneity within the subgroups.

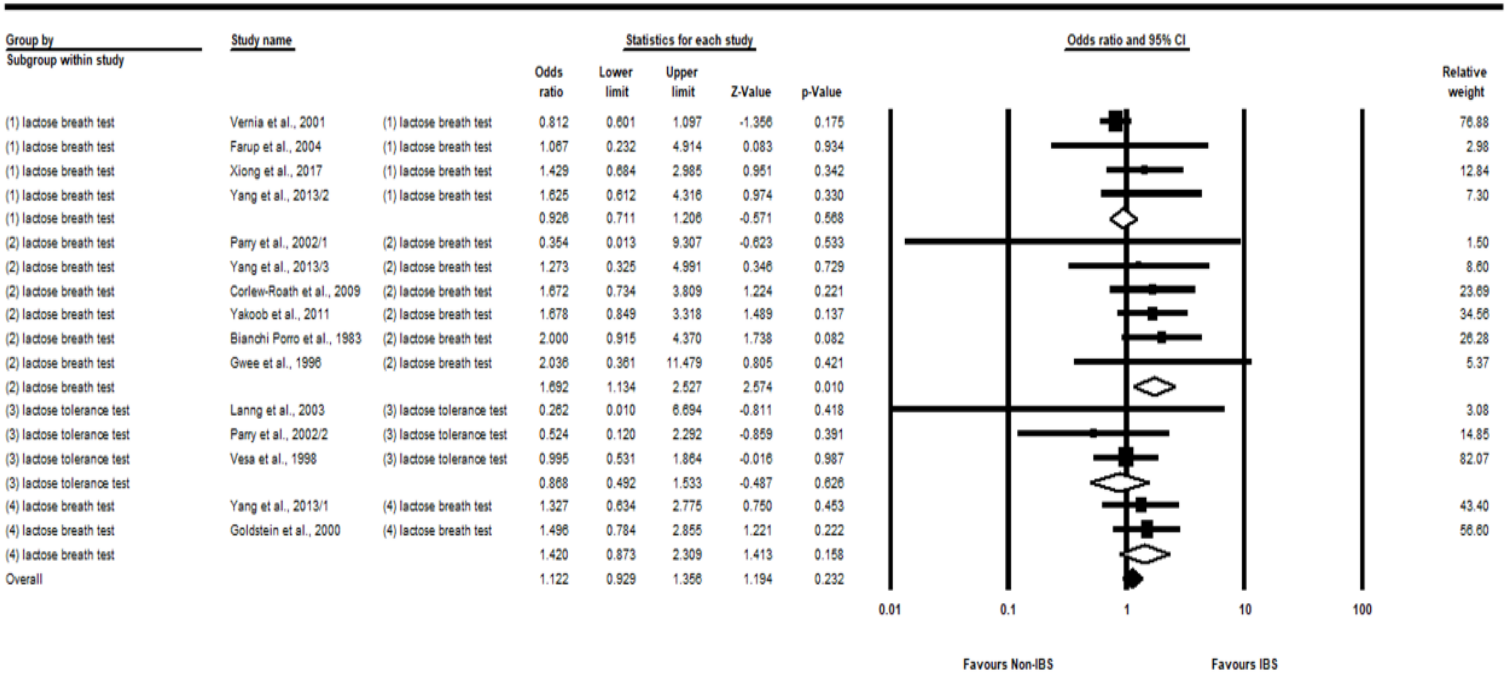
Figure 3. The difference of LM between IBS and HCs, based on the diagnostic method (LBT, LTT, genetic test). There was no significant difference either overall, or in the subgroups. HC: healthy control; IBS: irritable bowel syndrome; LBT: lactose breath test; LM: lactose maldigestion; LTT: lactose tolerance test.



Meta Analysis

Based on the test type and ingested amount of lactose, four subgroups were made: (1) 20-25 g LBT; (2) 40-50 g LBT; (3) 40-50 g LTT and (4) 10 g-18 g LBT (**Figure 4**). Overall there was no significant difference between IBS and control groups in the prevalence of LM (OR = 1.122; 95% CI: 0.929 – 1.356; p = 0.232) and the analyzed studies were homogeneous (I^2 = 0.000%; p = 0.479). LM was more frequent among IBS patients who underwent LBT with 40-50 g lactose (2) compared to HCs (OR = 1.692; 95% CI: 1.134 – 2.527; p = 0.010; I^2= 0.000%; p = 0.938). Between (p = 0.051) and within the other subgroups there was no significant difference: (1) OR = 0.926, 95% CI: 0.711 – 1.206, p = 0.568 (I^2 = 11.037%; p = 0.338); (3) OR = 0.868, 95% CI: 0.492 – 1.533, p = 0.626 (I^2 = 0.000%; p = 0.561); (4) OR = 1.420, 95% CI: 0.873 – 2.309, p = 0.158 (I^2 = 0.000%; p = 0.479). There was no significant heterogeneity within the subgroups.

Figure 4. The difference of LM between IBS and HCs, based on the lactose dose and diagnostic method. LM was significantly more frequent in IBS only at the LBT with the highest lactose dose (40-50 g). HC: healthy control; IBS: irritable bowel syndrome; LBT: lactose breath test; LM: lactose maldigestion.



Lactose intolerance

Only four case-control studies published data about self-reported (subjective) LI [53, 99, 103, 104]. Our results (**Figure 5**) showed that subjective LI was more common in IBS compared to HCs, patients reported more often that their abdominal symptoms can be related to lactose-containing products (OR = 3.499; 95% CI: 1.622 – 7.551; p = 0.001). The examined population was significantly heterogeneous (I^2 = 86.774%; p = 0.000).

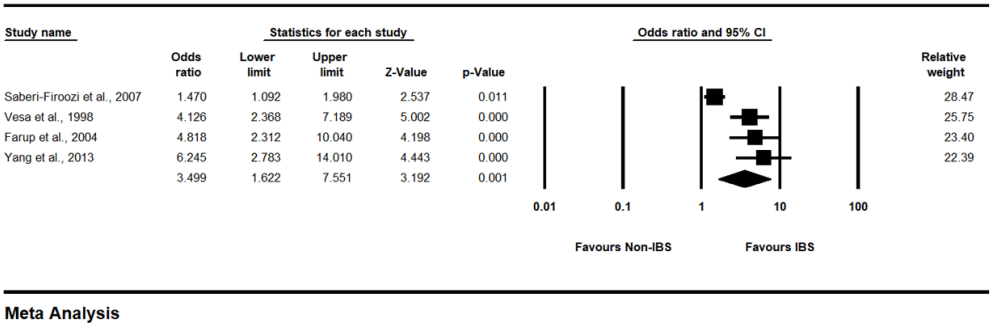


Figure 5. The difference of subjective (self-reported) LI between IBS and HCs. Subjective LI was significantly (p = 0.001) more frequent in IBS compared to the control group. HC: healthy control; IBS: irritable bowel syndrome; LI: lactose intolerance.

There were three articles available reporting on objective LI (**Figure 6**) [53, 54, 108]. Significantly more maldigester IBS patients reported abdominal symptoms during or shortly after the diagnostic test compared to controls (OR = 2.521; 95% CI: 1.280 – 4.965; p = 0.008), but our result is limited by the heterogeneity of the analyzed population (I^2 = 74.866%; p = 0.003).

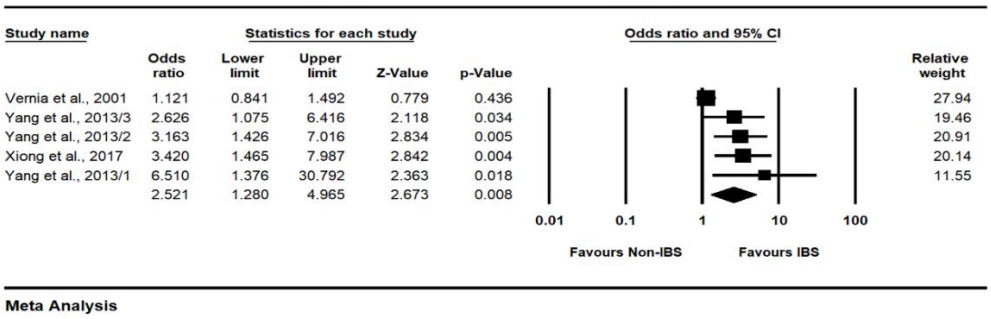


Figure 6. The difference of objective LI between IBS and HCs. Objective LI was significantly (p = 0.008) more frequent in IBS compared to the control group. HC: healthy control; IBS: irritable bowel syndrome; LI: lactose intolerance.

Results for AIM 2

A total of 310 patients were assessed in the period noted above. Twenty-four of them were excluded because of inappropriate preparation and 22 (7.6% of the well-prepared patients) were ruled out because of slow oro-cecal transit, leaving 264 patients, 185 females (F: 70.1%), and 79 males (M: 29.9%), for statistical analysis. No patient had rapid transit in our study group. The mean age of the analyzed study group was 40.3 years (F: 40.6 years; M: 39.5 years).

Based on the LBT and/or LTT results, 49.6% (131/264) of the study population had LM (LBT and/or LTT positivity), as represented in **Figure 7**. Seventy-eight (78/131, 59.5%) of them had symptoms after lactose ingestion and were therefore defined as lactose intolerant (78/264, 29.5%, **Figure 7**). Combined positivity (LBT+LTT) was found in 30.7% (81/264) of the patients (see **Figure 7**). There was no significant difference between females and males in the prevalence of normal lactose digestion, LM, and LI (p > 0.05). There was no significant correlation between age and digester (p = 0.352), maldigester (p =

0.352), and LI (p = 0.098) status. The basic results of the analyzed population are summarized in **Figure 7**. The gender-related results are represented in **Figure 8**.

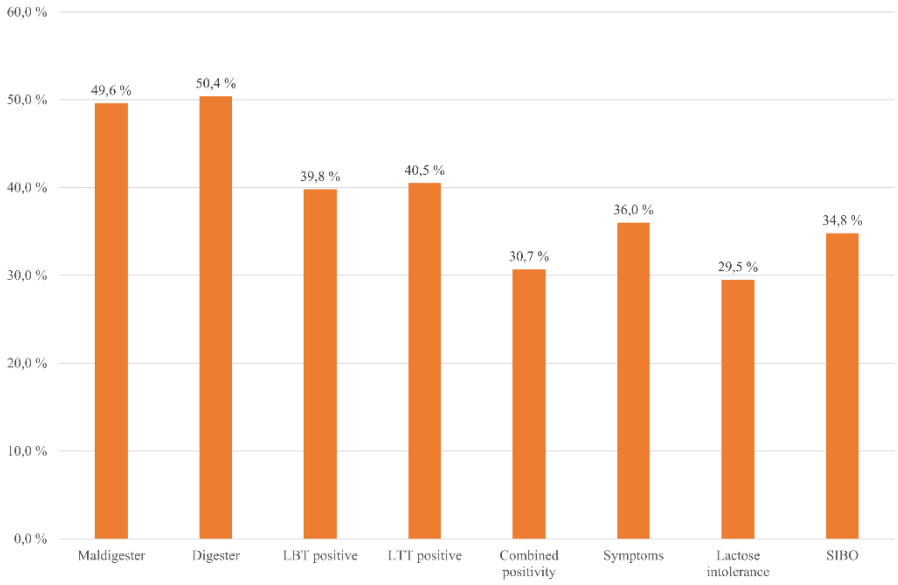
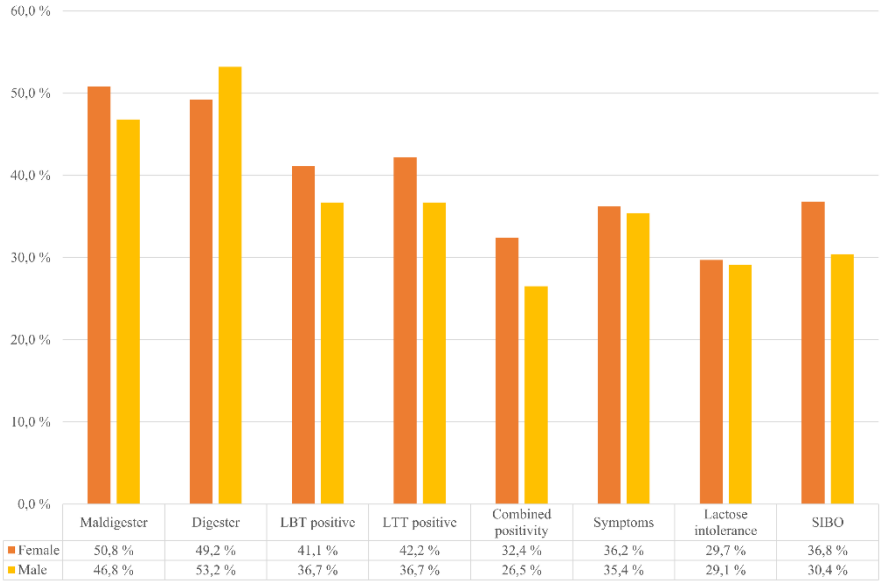


Figure 7. Summary of the basic results in the analyzed study population. A significant, ≥20 ppm elevation of H_2 level during LBT and/or less than 1.1 mmol/l rise of blood glucose during LTT were diagnostic for LM. Patients with negative LBT and LTT are lactose digesters. Patients with LM who had symptoms during the test were defined as lactose intolerant. Patients with an early (≤90 min) significant (≥20 ppm) rise of H_2 during LBT and/or lactulose breath tests were defined to have SIBO. LBT: lactose breath test; LTT: lactose tolerance test; SIBO: small intestinal bacterial overgrowth.



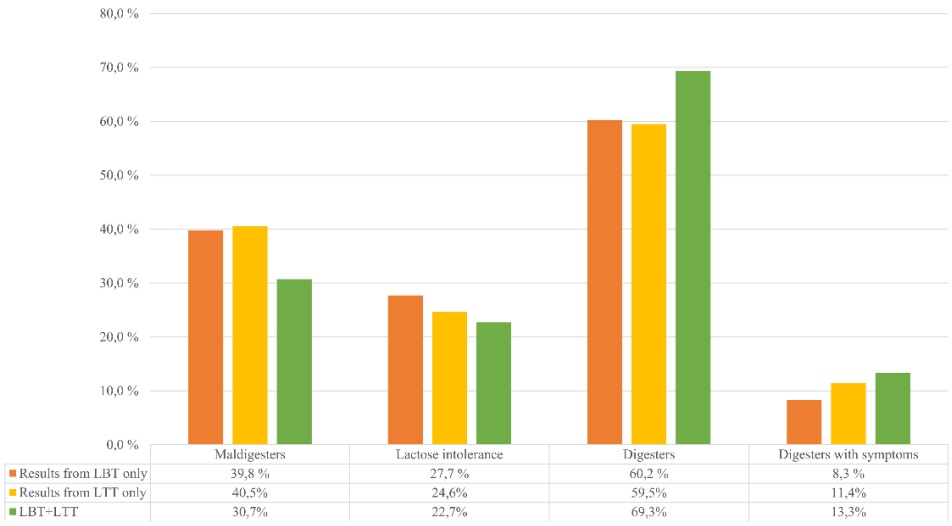
	Maldigester	Digester	LBT positive	LTT positive	Combined positivity	Symptoms	Lactose intolerance	SIBO
Female	50,8 %	49,2 %	41,1 %	42,2 %	32,4 %	36,2 %	29,7 %	36,8 %
Male	46,8 %	53,2 %	36,7 %	36,7 %	26,5 %	35,4 %	29,1 %	30,4 %

Figure 8. **The summary of the basic results among females/males.** A significant, ≥20 ppm elevation of H_2 level during LBT and/or less than 1.1 mmol/l rise of blood glucose during the LTT were diagnostic for LM. Patients with negative LBT and LTT are lactose digesters. Patients with LM who had symptoms during the test were defined as lactose intolerant. Patients with an early (≤90 min) significant (≥20 ppm) rise of H_2 during LBT and/or lactulose breath tests were defined to have SIBO. LBT: lactose breath test; LTT: lactose tolerance test; SIBO: small intestinal bacterial overgrowth.

Lactose maldigestion and intolerance based on the lactose breath test

Based on the LBT only, 39.8% of the tested study population (105/264) were LM, and 73 of them (69.5%) had symptoms during the test; therefore, 27.7% (73/264) of the population was defined as lactose intolerant (see **Figure 9**). The majority (159/264, 60.2%) of the patients had a negative LBT, however; 13.8% (22/159) of them had symptoms after lactose ingestion, meaning that 8.3% (22/264) of the analyzed patients had symptoms without a positive test result, as represented in **Figure 9**. There was a weak negative correlation between age and baseline H_2 ($p = 0.009$; $r = -0.161$). There was no significant connection between

gender, age, and LBT positivity (gender: $p > 0.05$; age: $p = 0.792$). The results are summarized in **Figure 9**.



	Maldigesters	Lactose intolerance	Digesters	Digesters with symptoms
Results from LBT only	39,8 %	27,7 %	60,2 %	8,3 %
Results from LTT only	40,5%	24,6%	59,5%	11,4%
LBT+LTT	30,7%	22,7%	69,3%	13,3%

Figure 9. Summary of the results based separately on the LBT, LTT, and the combination of them. LBT: lactose breath test; LTT: lactose tolerance test.

Lactose maldigestion and intolerance based on the lactose tolerance test

Based on an analysis of the LTT alone measured in parallel, 40.5% of the same study population (107/264) were maldigesters and 65 of them (60.7%) had symptoms during the test. Therefore, 24.6% (65/264) of the population was defined as lactose intolerant (see **Figure 9**). The majority (157/264, 59.5%) of the patients had a negative LTT; however, 19.1% (30/157) of them had symptoms after lactose ingestion, meaning that 11.4% (30/264) of the analyzed patients had symptoms without a positive test result (**Figure 9**). Men had a significantly higher baseline ($p < 0.001$) and maximum ($p = 0.015$) glucose level. There was a moderate positive correlation between age and glucose levels (baseline: $p < 0.001$; $r = 0.338$; maximum: $p < 0.001$; $r = 0.222$). There was no

significant connection between gender, age, and LTT positivity (gender: $p > 0.05$; age: $p = 0.378$). The results are summarized in **Figure 9**.

Combined lactose breath test and lactose tolerance test positivity

Combined positivity (LBT+LTT) was found in 30.7% (81/264) of the patients, 74% of them (60/81) had symptoms. Therefore, 22.7% (60/264) of the study population was lactose intolerant based on the combined results (see **Figure 9**). In the majority (183/264, 69.3%) of the population, one or both tests were negative; however, 19.1% (35/183) of them had symptoms meaning that 13.3% (35/264) of the analyzed patients had symptoms without combined test positivity (**Figure 9**). The results are summarized in **Figure 9**.

Clinical symptoms

Thirty-six percent (95/264) of the patients had symptoms after lactose ingestion (see **Figure 7**), bloating being the most frequent (60/264; 22.7%), as seen in **Figure 10**. There was no statistically significant difference between females and males in the presence of symptoms ($p > 0.05$). Those who had nausea/vomiting were significantly older ($p = 0.014$). Otherwise, there was no statistically significant correlation between age and symptoms ($p = 0.204$). 12.8% (17/133) of the lactose digester patients (the LBT and LTT are negative) and 59.5% (78/131) of the maldigester patients (at least one of the tests is positive) had clinical symptoms (see **Figure 11**). Based on the latest meta-analysis conducted by our workgroup [78], we hypothesize that IBS may be a contributing factor in LI among lactose maldigesters. **Figures 10 and 11** show the frequency of the different symptoms in the study population, and among lactose maldigesters/digesters and lactose intolerant/tolerant patients. Female/male data regarding symptoms are represented in **Figure 12**.

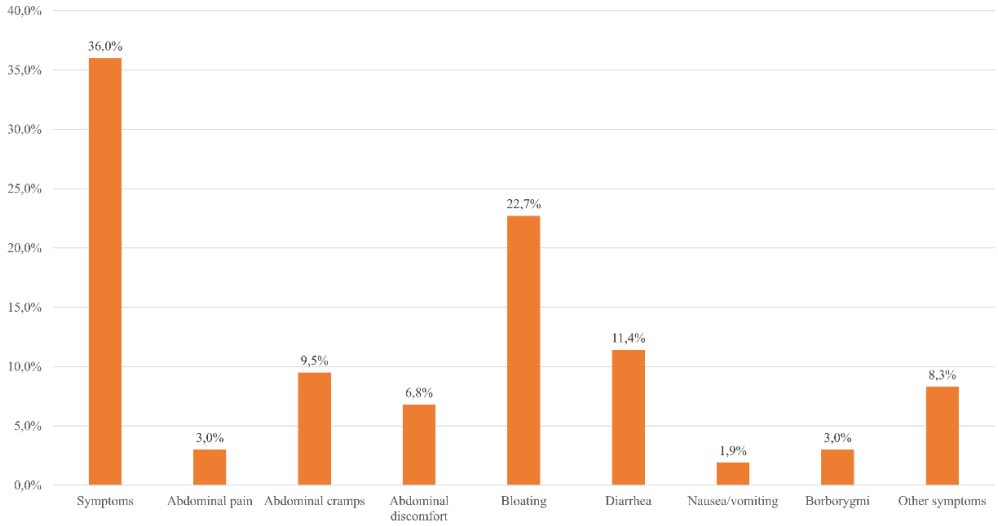
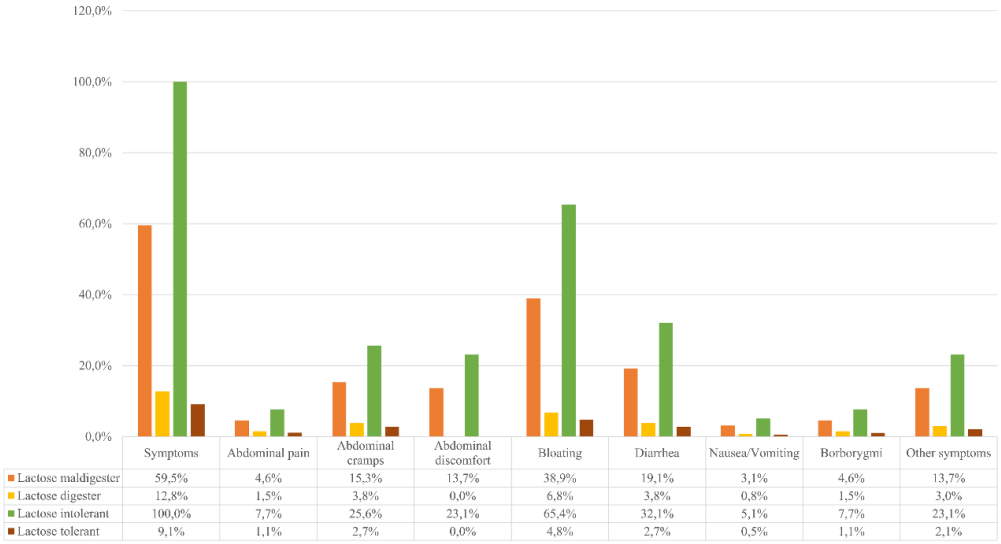
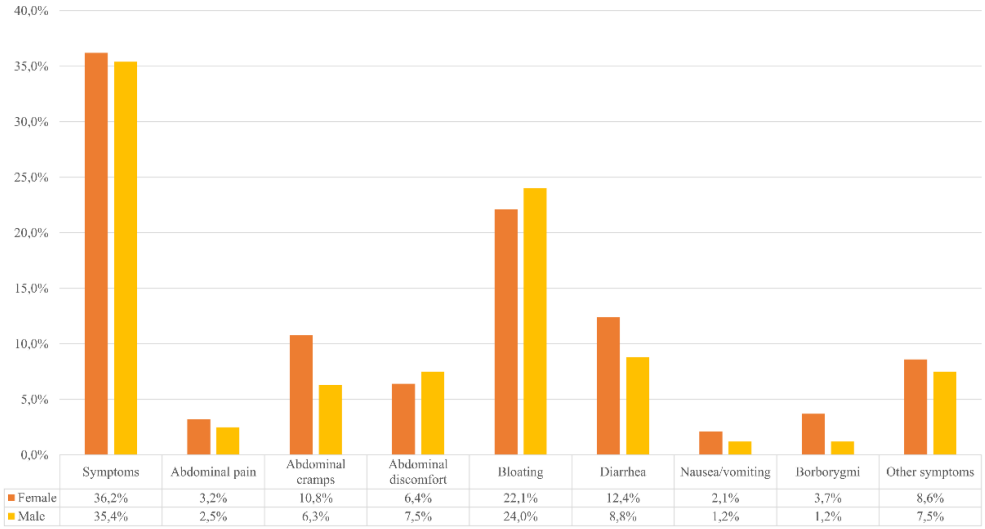


Figure 10. The frequency and distribution of different symptoms in the entire study population. Other symptoms comprise increased bowel motility, flatulence, belching, sensation of fullness in the stomach, headache, burning sensation in the stomach, or increased sensation for defecation.



	Symptoms	Abdominal pain	Abdominal cramps	Abdominal discomfort	Bloating	Diarrhea	Nausea/Vomiting	Borborygmi	Other symptoms
Lactose maldigester	59,5%	4,6%	15,3%	13,7%	38,9%	19,1%	3,1%	4,6%	13,7%
Lactose digester	12,8%	1,5%	3,8%	0,0%	6,8%	3,8%	0,8%	1,5%	3,0%
Lactose intolerant	100,0%	7,7%	25,6%	23,1%	65,4%	32,1%	5,1%	7,7%	23,1%
Lactose tolerant	9,1%	1,1%	2,7%	0,0%	4,8%	2,7%	0,5%	1,1%	2,1%

Figure 11. The frequency and distribution of different symptoms among lactose digesters/maldigesters, and among lactose tolerant/intolerant patients. A significant, ≥20 ppm elevation of H_2 level during LBT and/or less than 1.1 mmol/l rise of blood glucose during LTT were diagnostic for LM. Patients with negative LBT and LTT are lactose digesters. Patients with LM who had symptoms during the test were defined as lactose intolerant. Other symptoms comprise increased bowel motility, flatulence, belching, sensation of fullness in the stomach, headache, burning sensation in the stomach, or increased sensation for defecation.



	Symptoms	Abdominal pain	Abdominal cramps	Abdominal discomfort	Bloating	Diarrhea	Nausea/vomiting	Borborygmi	Other symptoms
Female	36,2%	3,2%	10,8%	6,4%	22,1%	12,4%	2,1%	3,7%	8,6%
Male	35,4%	2,5%	6,3%	7,5%	24,0%	8,8%	1,2%	1,2%	7,5%

Figure 12. **The frequency and distribution of different symptoms among females/males.** Other symptoms comprise increased bowel motility, flatulence, belching, sensation of fullness in the stomach, headache, burning sensation in the stomach, or increased sensation for defecation.

The role of small intestinal bacterial overgrowth

Approximately one-third (92/264; 34.8%) of the study population (see **Figure 7**) and 60% (57/95) of the symptomatic patients had SIBO based on the definition (see **Table 1**). There was no significant difference in the presence of SIBO between females and males (F: 68/185, 36.8%; M: 24/79, 30.4%, $p > 0.05$); furthermore, there was no significant correlation between age and SIBO ($p = 0.848$). SIBO patients had significantly higher maximum H_2 levels ($p < 0.001$), and they reached the H_2 peak later ($p < 0.001$). Moreover, they had lower maximum glucose levels ($p < 0.001$), and LTT positivity was significantly more frequent in this patient group (OR = 5.833; 95% CI: 3.356–10.138). Symptoms were more common in SIBO patients compared to non-SIBO patients (OR = 5.743; 95% CI: 3.300–9.994), especially abdominal discomfort (OR = 3.201; 95% CI: 1.196–8.565), bloating (OR = 4.798; 95% CI: 2.606–8.833), diarrhea

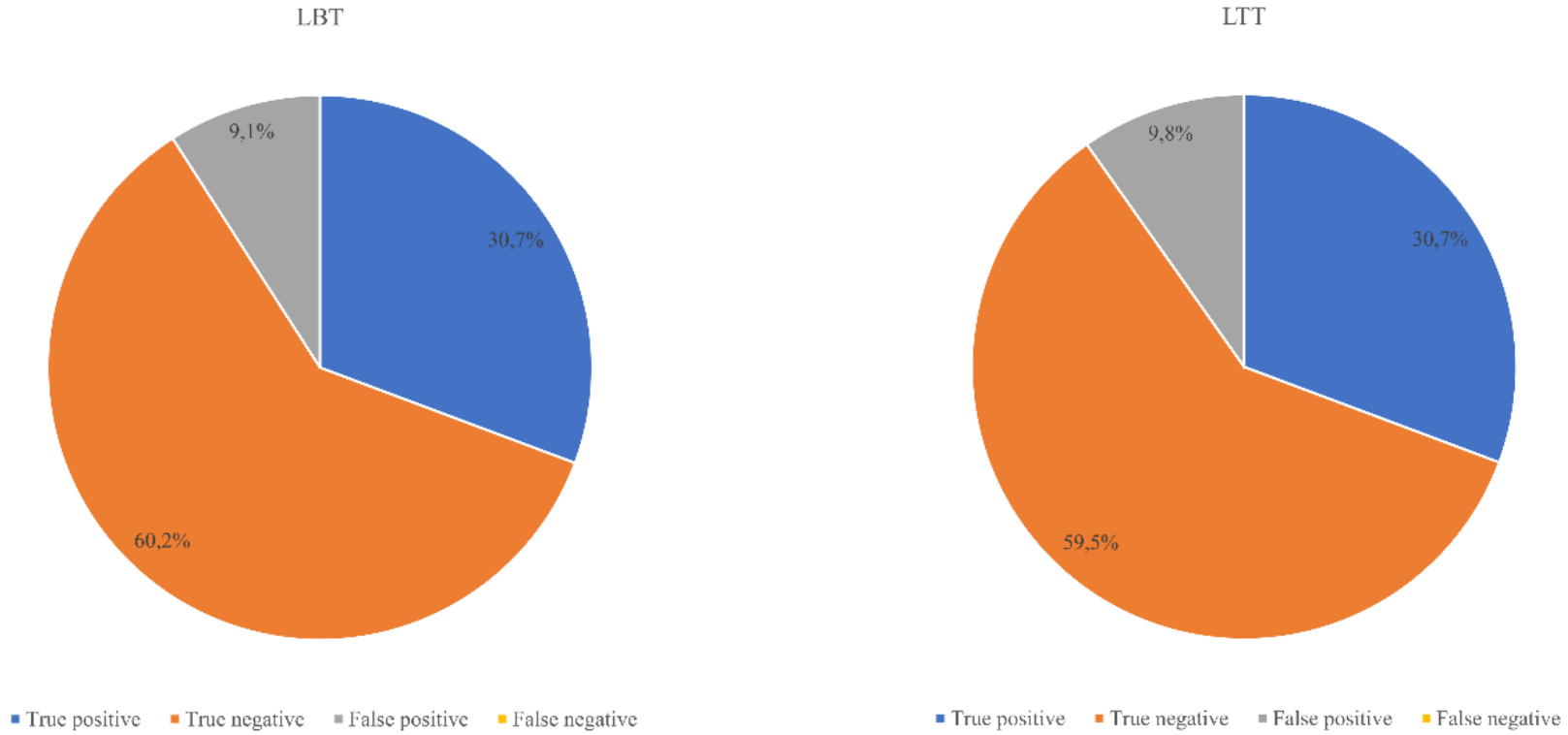
(OR = 6.443; 95% CI: 2.737–15.168), and other symptoms (OR = 5.825; 95% CI: 2.193–15.469).

In 90.9% (240/264) of the patients, the LBT gave correct diagnosis (30.7% true positive: 81/264, 60.2% true negative: 159/264) of LM (or the lack of it) using combined LBT and LTT as reference. False-positive results were found in 9.1% (24/264) of the cases; however, there are no false negatives in this setting (see **Figure 13**). LBT in this setting has 100% sensitivity, 86.9% specificity, 77.1% positive predictive value, and 100% negative predictive value. SIBO was found in 76.5% (62/81) of the true positive and 75% (18/24) of the false-positive patients.

In 90.2% (238/264) of the patients, the LTT gave correct diagnosis (30.7% true positive: 81/264, 59.5% true negative: 157/264) of LM (or the lack of it) using combined LBT and LTT as reference. False-positive results were found in 9.8% (26/264) of the cases; however, there are no false negatives in this setting (see **Figure 13**). Therefore, LTT has 100% sensitivity, 85.8% specificity, 75.7% positive predictive value, and 100% negative predictive value. SIBO was found in 76.5% (62/81) of the true positive, but surprisingly in 0% (0/26) of the false-positive patients.

Based on these findings the combination of the LBT and LTT and the careful monitoring of results (e.g. early H_2 rise, parallel performed lactulose breath test) can decrease false results caused by e.g. SIBO.

Figure 13. The diagnostic accuracy of the LBT and LTT verified by the combined results of the tests. LBT: lactose breath test; LTT: lactose tolerance test.



Results for AIM 3

Searching results

Using the terms above, we found 880 articles in the three databases for evaluation, 261 in PubMed, 87 in the Cochrane Library, and 532 in EMBASE. We also examined 22 further articles from the recent meta-analysis noted above [89], so 902 articles were found in total. After using the language (only English) and species (only humans) filters in EMBASE, PubMed and the Cochrane Library, 673 of 880 studies remained, and one of 22 was excluded from the meta-analysis by Marsh et al. [89] because it failed to meet the English-language inclusion criterion. After title and abstract screening and removing duplicates, ten articles reporting on IBS-SSS eligible for further evaluation were found (**Figure 14**). Of these studies, six were available in full-text format, and four were short abstracts or supplements. The number of controlled trials was seven, and there were three non-controlled prospective studies. Of the controlled trials, five were RCTs, and two were non-randomized studies. At the time of the literature search, we found no eligible paper that used the most recent diagnostic criteria (Rome IV) for IBS. The basic characteristics of the articles are summarized in **Table 5**. The proportion of each IBS subtype in the studies included in the meta-analysis is detailed in **Table 6**. A quality assessment of the articles is summarized in **Table 7**.

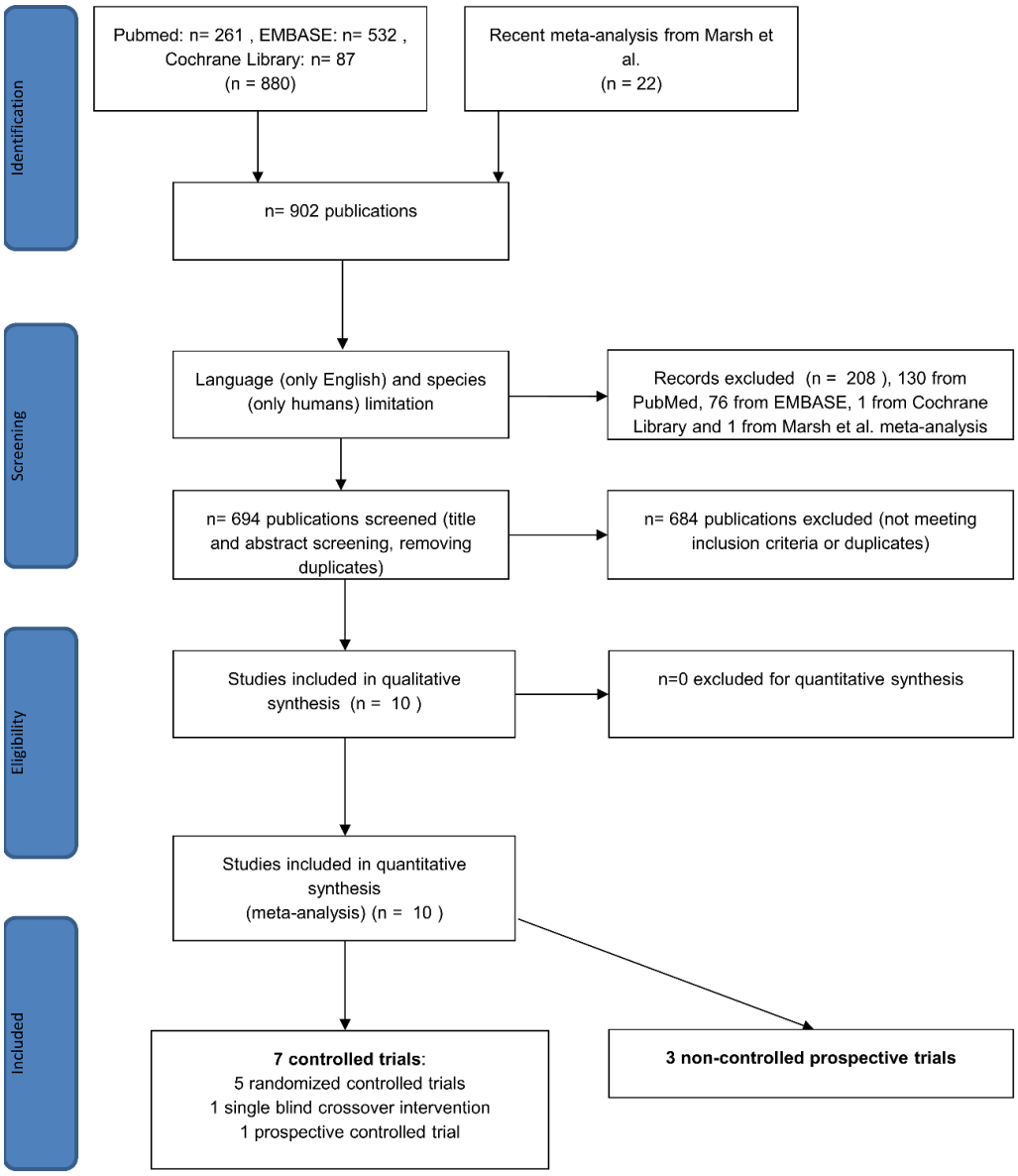


Figure 14. Flow chart for the systematic literature search.

Table 5. Baseline characteristics of the studies involved in the meta-analysis.

Reference	Country	Study design	Study duration	total or low-FODMAP/ control cohort size	IBS diagnostic criteria	Age (years)	Females (%)
Böhn et al. [11]	Sweden	multi-centre, parallel, randomized, controlled, single-blind, comparative trial	4 weeks	33 / 34	Rome III	low-FODMAP: mean: 44 (18–69); control: mean: 41 (18–68)	total: 81; low-FODMAP: 79; control: 84
McIntosh et al. [109]	Canada	prospective, randomized, single-blind, parallel study	3 weeks	18 / 19	Rome III	low-FODMAP: mean: 50.28 (26–77); control: mean: 51.47 (24–83)	total: 86; low-FODMAP: 83; control: 89
Pedersen et al. (B) [20]	Denmark	randomized, non-blind, controlled trial	6 weeks	42 / 40 started, 34 / 37 finished	Rome III	low-FODMAP: median: 37 (18–71); control: median: 32 (18–73)	total: 77; low-FODMAP: 81; control: 72.5
Laatikainen et al. [110]	Finland	randomized, double-blind, 2x2 cross-over study	13 weeks (1- week run-in, 2x4-week intervention, 4 -week wash-out period)	80 started, 73 finished	Rome III	mean: 42.9 (21–64)	91
Schultz et al. (Suppl.) [111]	New - Zealand	randomized controlled trial	12 weeks	23 / 27	Rome III	no data	no data
Pedersen et al. (A) [93]	Denmark	single-blind, cross-over intervention	12 weeks (0–6 and 7–12 weeks)	19	Rome III	median 35 (18–74)	74
Piacentino et al. (Suppl.) [112]	Italy	prospective controlled trial	4 weeks	28 / 28	no data (Rome III)	21–68	68
Ones et al. (Suppl.) [113]	Norway	non-controlled prospective study	6 weeks	23	Rome III	mean: 35 ± 11	87
Rossi et al. (Suppl.) [114]	Italy	non-controlled prospective study	8 weeks	12	Rome III	mean: 44.2 ± 15.5	92

Reference	Country	Study design	Study duration	total or low-FODMAP/ control cohort size	IBS diagnostic criteria	Age (years)	Females (%)
Valeur et al. [115]	Norway	non-controlled prospective study	4 weeks	63	Rome III	mean: 38.4 (19–67)	89

FODMAP = Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols; IBS = irritable bowel syndrome; Suppl. = supplementary material.

Table 6. **The proportion of each IBS subtype in the studies included in the meta-analysis.**

References	IBS subtype	
	Low-FODMAP group (%)	**Control diet group (%)**
Böhn et al.	IBS-D: 26; IBS-C: 24; IBS-M/U: 50	IBS-D: 22; IBS-C: 35; IBS-M/U: 43
McIntosh et al.	IBS-D: 22; IBS-C: 6; IBS-M: 67; IBS-U: 5	IBS-D: 32; IBS-C: 5; IBS-M: 58; IBS-U: 5
Pedersen et al. (B)	IBS-D: 45; IBS-C: 12; IBS-M: 33; IBS-U: 10	IBS-D: 45; IBS-C: 17.5; IBS-M: 35; IBS-U: 2.5
Laatikainen et al.	IBS-D: 32.5; IBS-M: 62.5; IBS-U: 5	
Schultz et al. (Suppl.)	no data	no data
Pedersen et al. (A)	IBS-D: 42; IBS-C: 21; IBS-A: 37	
Piacentino et al. (Suppl.)	no data	no data
Ones et al. (Suppl.)	no data	-
Rossi et al. (Suppl.)	IBS-D: 25; IBS-C: 17; IBS-M: 58	-
Valeur et al.	IBS-D: 54; IBS-C: 16; IBS-M: 30	-

FODMAP = Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols; IBS = irritable bowel syndrome; IBS-D = IBS-diarrhoeal subtype; IBS-C = IBS-constipation subtype; IBS-M/A = IBS-mixed/alternation subtype; IBS-U = IBS-unsubtyped; Suppl. = supplementary material.

Table 7. **Quality assessment of the studies included in the meta-analysis.**

References	Study design	Jadad score	MINORS
Böhn et al.	multi-center, parallel, randomized, controlled, single-blind, comparative trial	**3 / 5**	**-**
McIntosh et al.	prospective, randomized, single-blind, parallel study	**3 / 5**	**-**
Pedersen et al. (B)	randomized, non-blind, controlled trial	**3 / 5**	**-**
Laatikainen et al.	randomized, double-blind, 2x2 cross-over study	**5 / 5**	**-**
Schultz et al. (Suppl.)	randomized controlled trial	**0 / 5**	**-**
Pedersen et al. (A)	single-blind, cross-over intervention	**-**	**16 / 24**
Piacentino et al. (Suppl.)	prospective controlled trial	**-**	**15 / 24**
Ones et al. (Suppl.)	non-controlled prospective study	**-**	**8 / 16**
Rossi et al. (Suppl.)	non-controlled prospective study	**-**	**9 / 16**
Valeur et al.	non-controlled prospective study	**-**	**12 / 16**

RCTs were evaluated with the Jadad score (0=very poor, 5=rigorous) [90]. Non-randomized studies were evaluated with the MINORS [91], in which 12 items are scored (0: not reported; 1: reported, but inadequate; 2: reported and adequate). The global ideal score is 16 for non-comparative studies and 24 for comparative studies. Suppl. = supplementary material.

Low Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols and control diets

Patients received dietary advice from a dietitian on a low- or high-FODMAP diet in seven [11, 20, 93, 109, 111, 113, 115] of the ten studies analyzed. Two [112, 114] abstracts included in the meta-analysis failed to detail any information about dietitian involvement in the introduction of a low- or high-FODMAP diet. In a study by Laatikainen et al. [110], a special low-FODMAP diet containing rye bread was prepared for the patients, which had been developed and supplied by a bakery. Its FODMAP (fructan and mannitol) content was lower than that of regular rye bread. The control group was not homogeneous among the studies, but it always had a significantly higher FODMAP content. The precise content of the foods used was only detailed in two trials [11, 110]; the others probably followed dietary guidelines. This uncertainty could have influenced our results.

Irritable bowel syndrome symptom severity score

First, we wanted to see if a low-FODMAP diet is an effective treatment for IBS. We compared the pre- vs. post-intervention IBS-SSS in control groups (four publications) and low-FODMAP groups (eight publications) (**Figure 15**). There was a significant reduction in IBS-SSS in both control (difference in means [DIM], post- minus pre-values: -59.816 (95% CI: -108.922 – -10.710); p = 0.017) and low-FODMAP groups (DIM: -105.339 (95% CI: -140.773 – -69.905); p = 0.000). This means that both standard (high-FODMAP) and low-FODMAP diets are effective in improving symptoms and quality of life among IBS patients. The forest plot suggests that a low-FODMAP diet is more effective, but we cannot prove this statistically because of the overlapping CIs. Significant

heterogeneity was found between the studies: control group IBS-SSS values: Q = 9.837; df = 3; p = 0.02; I^2 = 69.504%; low-FODMAP group IBS-SSS values: Q = 26.321; df = 7; p < 0.001; I^2 = 73.405%.

We compared (**Figure 16**) the pre- and post-intervention scores between the control and low-FODMAP groups in the controlled trials (six publications for each group). This shows that there is no statistically significant difference in pre-values between the groups (DIM: control minus low-FODMAP values: -8.675 (95% CI: -40.043 – +22.693); p = 0.588), but a significant difference between post-values (DIM: +51.537 (95% CI: +18.891 – +84.183); p = 0.002) could be observed. These results confirm that the therapeutic effect of a low-FODMAP diet is better than standard dietary advice in patients with IBS. The meta-analysis also showed a significant heterogeneity: pre-IBS-SSS values: Q = 21.242; df = 5; p = 0.001; I^2 = 76.462; post-IBS-SSS values: Q = 20.675; df = 5; p = 0.001; I^2 = 75.816.

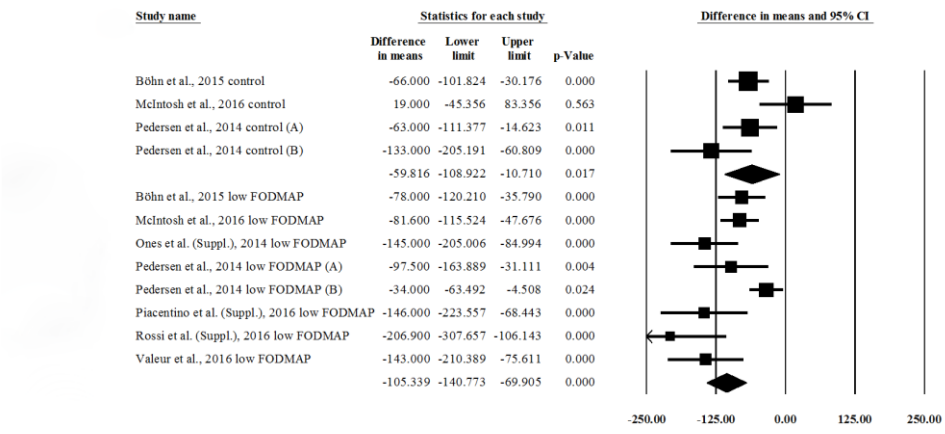


Figure 15. Forest plot of IBS-SSS DIMs, comparing pre- vs. post-intervention values within groups (low-FODMAP and control). IBS-SSS = irritable bowel syndrome symptom severity score (0–500); DIM = difference in means; FODMAP = Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols.

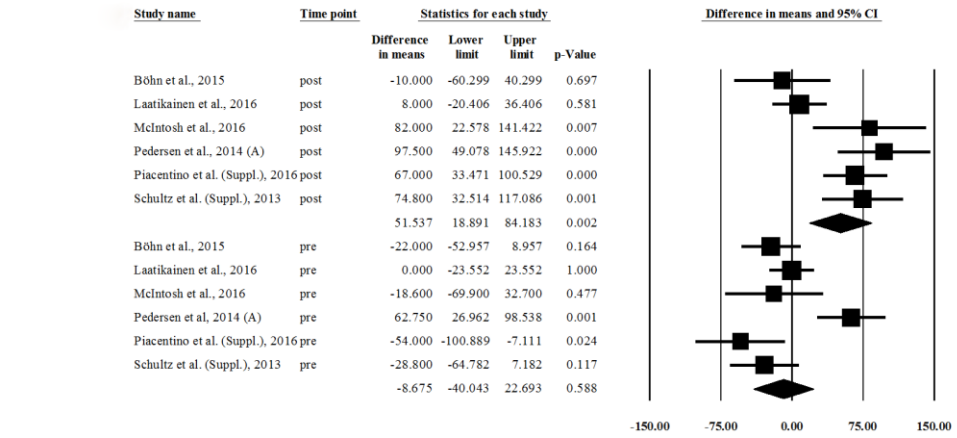


Figure 16. Forest plot of IBS-SSS DIMs, comparing pre- and post-intervention values between groups (low-FODMAP vs. control). IBS-SSS = irritable bowel syndrome symptom severity score (0–500); DIM = difference in means; FODMAP = Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols.

DISCUSSION

The connection between irritable bowel syndrome and lactose consumption-related disorders (meta-analysis)

A growing number of studies have shown that intolerance to lactose-containing products and other food types is more frequent among patients with IBS than among healthy subjects, but to our best knowledge, no meta-analysis investigated the association between these two conditions so far. Only two recent reviews by Borghini and Bayless et al. [3, 116] discuss the correlation between IBS and LI.

We carried out a systematic literature search and quantitative data (meta-) analysis on the topic. A pooled analysis of 14 case-control trials confirmed a significantly higher prevalence of subjective and objective LI, whereas nearly the same prevalence of LM in IBS patients compared to healthy participants. The

underlying mechanism remains unknown, but common etiological factors like psychological (e.g., anxiety) and gastrointestinal dysfunctions (e.g., visceral hypersensitivity and altered gut transit) might play a role [55-57]. The visceral hypersensitivity can also be in connection with altered gut microbiome. Gut microbiota of IBS patients is generally reduced and has lower diversity, compared to HCs [117]. It has been shown that potentially pathogenic bacteria (e.g. Clostridium spp., Ruminococcus spp., Streptococcus spp., Enterobacteriaceae members) are more concentrated in IBS patients than in controls [118-121]. A recent MRI (magnetic resonance imaging) study concluded that visceral hypersensitivity, rather than excessive gas production is responsible for carbohydrate associated symptoms in patients with IBS [40]. The hypersensitivity to colonic distension can be transferred to mice by fecal transplantation which highlights the role of the microbiome [122]. Moreover, gut microbiota produces many neuroactive or neuromodulatory metabolites (histamine, serotonin, gamma-aminobutyric acid, brain-derived neurotrophic factor, etc.), which can potentially lead to peripheral or central neural sensitization [123, 124].

Most studies have shown a beneficial effect of lactose-free or restricted diet in IBS [52, 125, 126]. One reason might be that lactose belongs to FODMAPs, which are poorly absorbed carbohydrates leading to increased water content in the bowel based on the compounds' osmotic effect and increased gas production by colonic bacterial flora, inducing symptoms in patients with IBS and numerous patients with functional gastrointestinal disorders. Based on these findings a low-FODMAP diet could be beneficial in these patients [36, 80, 127].

In the present study, the pooled sample size was large concerning the key question and the random effects and fixed model were used with the DerSimonian and Laird method [85] for analysis. Study data reflected no publication bias according to the analyses of LM status, but showed significant

bias (small study effect) based on heterogeneity in forest plots of subjective and objective LI. Eggers's test was performed to assess publication bias.

We evaluated the quality of the studies included in the meta-analysis with the NOS for case-control studies, which showed satisfactory scores of the trials with low or medium risk of bias (**Table 4**).

The strength of our study is that standardized, well-defined, rigorous outcome measures were used to assess the role of lactose consumption-related disorders in IBS patients, and a sufficient number of articles were found to carry out a detailed statistical analysis. Only full-text papers were enrolled, where IBS patients with appropriate control groups were present. According to our results, more IBS patients reported themselves lactose intolerant before any objective tests compared to HCs, which can be highlighted with objective measures: significantly more maldigester IBS patients reported abdominal symptoms during or shortly after the diagnostic test (objective LI). However, except for the LBT with the highest lactose doses (40-50 g), the prevalence of LM was similar in the study groups. Our meta-analysis is the first to provide evidence for the connection between IBS and LI and our former [80] data suggests that a lactose-free or lactose-restricted diet (low-FODMAP) in the treatment of IBS could improve the therapeutic effect on IBS symptoms and might decrease healthcare-related and societal costs.

There are some limitations to our study. Firstly, we focused on the prevalence of LM and subjective/objective LI, and due to the lack of detailed, uniform, controlled, published data, we could not perform a statistical analysis of individual symptoms. Uniform, consensus-based, well-comparable measurement of symptom severity, for example, VAS is suggested for use in future studies. Because of the same reasons, we could not analyze the role of lactose-restricted diet or lactase replacement in this patient group; therefore, a network meta-analysis could be a useful future perspective to establish which

treatment is better in IBS. Secondly, because of the lack of data in the different IBS subtypes, it is not clear which subgroup is mostly affected by LI. Moreover, the diagnostic criteria for IBS and the diagnostic thresholds of LBT and LTT were different in some studies which could influence the results. The sensitivity and specificity of these noninvasive tests are relatively high, however, false positive or negative results could affect our findings. It should be taken into account that similar activity of lactase in two persons might result in different LBT results due to the different activity and composition of the intestinal microbiota and the lactase non-persistence allele is not always associated with LM [83]. Another difficulty is that it is hard to identify the food, responsible for the symptoms. The correlation between self-reported and objective LI increases with the ingested lactose dose [53]. Finally, we found significant heterogeneity in the analysis of the subjective and objective LI. We could not perform subgroup analysis with different amounts of lactose in LI, however, it can influence the frequency and severity of the abdominal symptoms and therefore the prevalence of objective LI, as presented by Yang et al [53].

More trials with standardized parameters are necessary in the future to provide the best quality of evidence regarding the correlation between IBS and LI. Only patients fulfilling the most recent diagnostic criteria for IBS (Rome IV) should be included in such studies. Outcomes should be reported for each IBS subtypes. Uniform outcome measures (e.g., VAS) regarding abdominal symptoms should be used to make the different studies scientifically comparable. More randomized controlled trials are needed to provide evidence about the role of lactose-free or restricted diet in IBS compared to placebo or lactase replacement. In these studies, a more accurate IBS-SSS should be used in each IBS subtype, which measures not only the severity of the main symptoms, but also the quality of life. Clinical trials with different lactose doses are also suggested to test the role of IBS in LI among lactose maldigesters. Yao et al. [128] discuss the crucial

points and difficulties of designing clinical trials in dietary interventions in patients with functional gastrointestinal disorders.

The role of small intestinal bacterial overgrowth and false-positive diagnosis of lactose intolerance (retrospective observational study)

In this retrospective, single-center study, we analyzed the epidemiological characteristics of LI in South-West Hungary and assessed the role of combined diagnostic method and SIBO in the accuracy of the diagnosis.

LI is a relatively common problem in the white population, affecting approximately 47% of Eastern European adults [44, 47]. There are widely-used, inexpensive, non-invasive, diagnostic methods based on the measurement of end-alveolar H_2 concentration (LBT) or blood glucose (LTT) [3, 44-46]. The sensitivity and specificity of these tests are relatively high, but they depend on the ingested lactose dose (25 g LBT: 82% and 95%; 25 g LTT: 78% and 93%; 50 g LBT: 92% and 83%; 50 g LTT: 94% and 90%) [84, 129]. Other circumstances, such as SIBO, antibiotic usage, lung diseases, inappropriate preparation, and abnormal gastric emptying can influence their diagnostic accuracy. A combination of these tests and careful evaluation of the results can reduce the false positive or negative cases; however, due to the lack of evidences, in most studies, they are used separately [130].

The gold standard diagnostic method is the testing of lactase activity in duodenal and jejunal biopsy samples taken from the mucosa. However, due to the invasiveness, high costs, and patchy enzyme expression, it is less frequently performed compared to the tests noted above. Moreover, it should be considered that similar lactase activity in two patients might result in different LBT results due to the different activity and composition of the intestinal microbiota. There are several genes associated with lactase non-persistence (C/T_13910 with CC genotype; G/A_22018 with GG genotype), but the availability of genetic testing is variable, and its costs are relatively high. Moreover, the lactase non-

persistence allele is not always associated with LM [44-46, 78]. A Hungarian study, published by Nagy et al., determined the applicability of the LBT in comparison with genetic screening (C/T_13910). They found that 37% of the analyzed population had lactase non-persistence, which correlated well with positive LBT results in symptomatic children [131]. We found similar LBT positivity among symptomatic adults. Another retrospective study from Hungary, conducted by Buzás et al., also underlined that both genetic and breath tests are sufficiently accurate [132].

In this study, we presented epidemiological data on the prevalence of LM and LI in South-West Hungary, we analyzed the frequency of the most common symptoms, we demonstrated that combined analysis of LBT and LTT can improve diagnostic accuracy and the parallel testing for SIBO could reduce false cases caused for example by SIBO. It should also be mentioned that the study population had a very large female representation (185 vs 79); however, there were no statistically significant gender-related differences regarding LM, LI, LBT/LTT positivity, symptoms frequency, and prevalence of SIBO, which underlines the literature data in case of LI [44]. Moreover, despite the literature data [48, 49, 59], we did not find any age-related correlations in the outcomes mentioned above.

The limitations of our results should be considered for a correct interpretation, thus possibly influencing outcomes. Firstly, our results are based on a single-center retrospective medical database analysis. Secondly, we only analyzed the results in one year; therefore, the number of enrolled patients is relatively low. Thirdly, the amount of ingested lactose can influence the prevalence of LM and LI, and the frequency of symptoms. We used a relatively high dose of lactose and we did not perform blinded testing with placebo. Based on the retrospective character, follow-up after antibiotic treatment or low-lactose diet could not be performed to confirm the diagnosis of SIBO and LI based on symptom relief. Moreover, the lactulose breath test was performed only in clinically uncertain

cases, not on all patients. Therefore, the true diagnosis and prevalence of LI and SIBO could not be assessed correctly. Only patients with high initial end-alveolar H_2 concentration got antiseptic mouthwash. Another significant limitation is that our study group comprises symptomatic patients referred to our clinic, thus potentially leading to sampling bias. It also should be considered that we did not measure methane levels in the end-alveolar gas samples to determine false-negative LBT caused by methane-producing bacteria. Based on the recent results [133] false-negative LBT (5-15%) is mainly caused by methane production. Finally, the symptoms of the patients are subjective, thus possibly prompting inaccurate conclusions. Interpretation of patient-reported symptoms will differ between clinicians; therefore, standardized symptom definitions should have been used to minimize errors. According to the Oxford Centre for Evidence-Based Medicine 2011, the evidence level of our findings is level 3 [134].

The role of low Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols diet (meta-analysis)

The standard dietary approach for IBS dietary therapy (high-fiber, low-fat, etc., as detailed above) recommended by guidelines only improves IBS symptoms to a limited extent. A growing number of recent studies have shown a beneficial effect of a low-FODMAP diet on IBS symptoms. Several of them have compared its efficacy to a standard IBS diet and challenged us to review the latest literature on the issue. A recent meta-analysis by Marsh et al. [89] analyzed the beneficial effect of a low-FODMAP diet on symptoms and quality of life in adult and pediatric patients with IBS and IBD in the literature up to 24 March 2015. They only investigated the complex IBS-SSS only in four articles, and it was not stated whether the low-FODMAP diet is significantly better than a control diet or not. We carried out our analysis on IBS-SSS, using more (ten) articles, and we only focused on adult patients with IBS. A previous meta-analysis by Khan et al. and systematic review by Rao et al. [36, 135] also proved the efficacy of this diet on

symptom improvement and suggested its introduction as a baseline treatment, but they could not state clearly whether it is better than standard dietary advice or not. Rao et al. [135] also investigated the high-fiber diet on chronic constipation and IBS. They performed a literature search up to September 2014 and did not conduct a statistical analysis due to heterogeneity and methodological quality. To our knowledge, this is the first meta-analysis to compare the effectiveness of low-FODMAP foods to a regular IBS diet recommended by the guidelines.

A pooled analysis of seven controlled trials (five randomized and two non-randomized) and three non-controlled trials confirmed that a low-FODMAP diet significantly improves general symptoms (IBS-SSS) in patients with IBS compared to standard dietary recommendations and a high-FODMAP diet. FODMAPs are poorly absorbed carbohydrates that cause an increase of water content in the bowel based on the osmotic effect and increased gas production by colonic bacterial flora. These effects of FODMAPs induce several symptoms in patients with IBS and numerous patients with functional gastrointestinal disorders mainly by distension and the osmotic laxative effect [11, 40, 41].

The pooled sample size was large, and the expression of the data from the studies enrolled was acceptably homogeneous concerning the key question of the meta-analysis.

Because of the considerable heterogeneity of the expressed data, the random-effects model was used with the DerSimonian and Laird [95] method for analysis. This is possible because of the similar effect of non-investigated variables such as food intolerances and functional digestive tract disorders other than IBS that could cause IBS-like symptoms as well. It would be important to study the effect of a low-FODMAP diet in these groups to better understand the role of a food challenge in provoking uncompliant symptoms of functional digestive tract disorders, as in IBS. Nevertheless, the beneficial effects of a low-

FODMAP diet on IBS-SSS were statistically significant even in the heterogeneous population analyzed, thus supporting the high impact of this diet on IBS symptoms. Study data reflected some publication bias based on heterogeneity.

We evaluated the quality of the studies included in the meta-analysis (**Table 7**) using the Jadad score for RCTs and MINORS for non-randomized studies. Among RCTs, the Schultz et al. trial [111] was an outlier (Jadad score=0). This could be because it is only available in abstract form; there is therefore a lack of information on the study design. The scores given to the other trials were satisfactory.

The strength of our study is that a standardized complex outcome score (IBS-SSS) was used to measure the therapeutic effect. This score measures abdominal pain frequency and severity, bloating, dissatisfaction with bowel habit, and quality of life together on a 0–500 mm VAS. This scoring system provides information not only about symptoms, but also about quality of life. A sufficient number of articles were found to carry out an accurate statistical analysis, using this important outcome score. With this work, we proved not only the positive effect of a low-FODMAP diet on IBS-SSS, but also its superiority to a high-FODMAP standard IBS diet. Our meta-analysis is the first to provide unambiguous, high-level evidence for the superiority of a low-FODMAP diet to a standard dietary approach in the improvement of general symptoms and well-being among patients with IBS. These data suggest that the first-line introduction of a low-FODMAP diet in the treatment of IBS could improve the therapeutic effect on IBS symptoms and might decrease healthcare-related and societal costs [12].

There are some limitations to our study. First, we focused on the complex IBS-SSS, and due to the lack of detailed published data, we did not perform a statistical analysis of the individual symptoms in the symptom score. Therefore,

it is not clear which of the five elements play a key role in the improvement of IBS symptom severity toward better personalization of this dietary approach. The main reason was the lack of data and control groups, as well as the heterogeneity in the literature in measuring symptom severity (e.g. VAS and different types of Likert scale). A uniform, consensus-based, well-comparable measurement of symptom severity (e.g. IBS-SSS) is suggested for use in future studies. Second, we included not only full-text articles, but also four short supplements [111-114] in our analysis, thus increasing the quantity of data on control groups. Third, because of the lack of data in the different IBS subtypes, it is not clear which subgroup experienced the greatest symptom improvement. Finally, the standard IBS diet group was not homogeneous. The control diet always contained a significant number of FODMAPs; however, only two out of ten studies detailed exact food contents [11, 110]. Others probably used IBS dietary guidelines; thereafter, some differences were realized between contents, thus potentially influencing our results.

To prove the effectiveness of a low-FODMAP diet on bowel movement frequency in IBS patients and to demonstrate which IBS subgroup could profit significantly from this diet, more double-blind, randomized controlled trials should be conducted with the following standardized parameters. Only patients fulfilling the most recent diagnostic criteria for IBS (Rome IV) should be included in studies. A precise description of the contents of the diets studied is crucial for an accurate analysis. It is highly recommended dietitians be involved in guiding patients on diets to avoid significant differences within study groups and inadequate nutrient intake. Patients should also be adequately monitored during trials to ensure their adherence to a particular diet. Uniform outcome measures should be used to make studies scientifically comparable. Except for measuring symptom severity on the VAS only, it is suggested that a more detailed IBS-SSS be used in each subtype of the IBS patient group, which measures not only the severity of the main symptoms, but also the quality of life.

As mentioned above, Yao et al. [128] discuss the crucial points and difficulties of designing clinical trials in dietary interventions in patients with functional gastrointestinal disorders.

CONCLUSIONS

I. Our meta-analysis is the first to confirm that:

a) LM has the same prevalence, but

b) objective LI and

c) subjective LI

are more common in IBS patients compared to the healthy population.

Based on these findings and literature data, **IBS can be a contributing factor of LI among people with LM.** Further studies are needed to determine whether a confirmed diagnosis of IBS is an etiological factor in determining whether LM patients present with LI.

II. Based on our results, we can conclude that:

a) **the prevalence of LI is lower in South-West Hungary compared to the Eastern European values** (29.5% vs 47%) and that is worth to perform a population-based prospective analysis in this area. During the provocation tests, **59.5% of lactose maldigesters had IBS-like symptoms (LI)**, but the role of IBS in the background is unknown.

b) SIBO was relatively common among symptomatic patients (60%), which may significantly influence the H_2 breath test based diagnostic accuracy of LM.

c) **To use a combination of LBT and LTT testing can be a reasonable alternative of H_2 lactose- and H_2 lactulose breath test combination to exclude false positivity (caused by e.g. SIBO)**, that approach needs further validation.

III. Our meta-analysis confirms that **a diet low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) significantly improves general symptoms and quality of life in patients with IBS.** Our analysis of the appropriate literature data also confirms that **a low-FODMAP diet is more effective than standard IBS dietary therapy** in patients diagnosed with IBS. However, a low-FODMAP diet raises certain issues, such as the alteration of gut microbiota and inadequate nutrient intake without dietitian assistance. The possible health advantages of a low-FODMAP diet – when it is effective – compared to medical treatment require further evaluation. In consideration of its possible limitations and based on findings from this meta-analysis, **a low-FODMAP diet could be a potential first-line and supplementary dietary therapeutic approach with the aid of a dietitian for patients with IBS to improve abdominal discomfort, abdominal pain, bloating and quality of life.** Because of the lack of published data, it is not possible to prove the effectiveness of a low-FODMAP diet on bowel movement frequency in IBS patients. It also remains unclear which IBS subgroup could profit most from this diet. More RCTs are needed to analyze these effects of dietary approaches.

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First author

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Publications serving as the basis of the thesis

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RESEARCH ARTICLE

Low fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) diet improves symptoms in adults suffering from irritable bowel syndrome (IBS) compared to standard IBS diet: A meta-analysis of clinical studies

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Abstract

Background

Irritable bowel syndrome (IBS) and functional digestive tract disorders, e.g. functional bloating, carbohydrate maldigestion and intolerances, are very common disorders frequently causing significant symptoms that challenge health care systems. A low Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols (FODMAP) diet is one of the possible therapeutic approaches for decreasing abdominal symptoms and improving quality of life.

Objectives

We aimed to meta-analyze data on the therapeutic effect of a low-FODMAP diet on symptoms of IBS and quality of life and compare its effectiveness to a regular, standard IBS diet with high FODMAP content, using a common scoring system, the IBS Symptom Severity Score (IBS-SSS).

Methods

A systematic literature search was conducted in PubMed, EMBASE and the Cochrane Library as well as in the references in a recent meta-analysis. Adult patients diagnosed with IBS according to the Rome II, Rome III, Rome IV or NICE criteria were included in the analysis.

Abbreviations: CI, Confidence interval; DIM, Difference in means; ES, Effect Size; Fig, Figure; FODMAP, Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols; GI, Gastrointestinal; IBD, Inflammatory bowel disease; IBS, Irritable bowel syndrome; IBS-SSS, Irritable Bowel Syndrome Symptom Severity Score; MINORS, Methodological Index for Non-Randomized Studies; mm, millimetre; NICE, National Institute for Health and Care Excellence; PICO, Population/Problem, Intervention, Comparison, Outcome; PRISMA-P, Preferred Reporting Items for Systematic Review and Meta-Analysis Protocol; RCT, Randomized controlled trial; SD, Standard deviation; s.e., Standard error; S/Suppl., Supplementary material; VAS, Visual Analogue Scale.

Statistical methods

Mean differences with 95% confidence intervals were calculated from studies that contained means, standard deviation (SD) or mean differences and SD of differences and p-values. A random effect model was used because of the heterogeneity (Q test ($\chi 2$) and I^2 indicator). A p-value of less than 0.05 was chosen to indicate a significant difference.

Results

The literature search yielded 902 publications, but only 10 were eligible for our meta-analysis. Both regular and low-FODMAP diets proved to be effective in IBS, but post-diet IBS-SSS values were significantly lower (p = 0.002) in the low-FODMAP group. The low-FODMAP diet showed a correlation with the improvement of general symptoms (by IBS-SSS) in patients with IBS.

Conclusions

This meta-analysis provides high-grade evidence of an improved general symptom score among patients with irritable bowel syndrome who have maintained a low-FODMAP diet compared to those on a traditional IBS diet, therefore showing its superiority to regular IBS dietary therapy. These data suggest that a low-FODMAP diet with dietitian control can be a candidate for first-line therapeutic modality in IBS. Because of a lack of data, well-planned randomized controlled studies are needed to ascertain the correlation between improvement of separate key IBS symptoms and the effect of a low-FODMAP diet.

Introduction

Irritable bowel syndrome (IBS) is a common chronic functional gastrointestinal disorder, which can be defined by the Rome IV criteria [1, 2]. IBS causes abdominal pain or discomfort, bloating and altered bowel habits (diarrhoea, constipation or a combination of these), without any pathological abnormality of the intestinal wall [3]. It can lead to significant impairment of quality of life (e.g. social isolation or stigmatization [4, 5]), decreased work productivity and an increase of health care and societal costs [6–9]. The incidence of the disease is high in Western countries, affecting 10–20% of the adult population, and it is twice more common among women [10, 11]. The exact pathomechanism remains unclear, but visceral hypersensitivity, altered gastrointestinal motility, changes in gut microbiota, altered brain–gut axis, low-grade digestive tract inflammation and psychological factors may play a role [12–14]. Because of the uncertain aetiology and pathophysiology, only a few effective, non-specific treatment options exist, improving only some key symptoms but not leading to the healing of IBS (laxatives, anti-diarrhoeal agents, antispasmodics, antidepressants, and dietary and psychiatric interventions) [8, 15, 16]. Treatment is often multimodal, comprising dietary, psychological and pharmacological methods [15]. Several studies have proven that certain foods worsen the symptoms in most IBS patients because they play an important role in the development of those symptoms [17–23]. The most commonly reported foods are those containing lactose (milk, ice cream and yogurt) or fructose (honey, dates, oranges, cherries, apples and pears), gas-producing foods (beans, peas, broccoli, cabbage and bran), wheat and wheat-containing products, and sweeteners (sorbitol, mannitol and xylitol) [18]. These findings suggest that dietary intervention that

excludes symptom-triggering food components could be a promising treatment option for IBS. Standard dietary interventions are detailed in some guidelines, e.g. the British Dietary Association and NICE guidelines [24, 25]. They recommend that patients regularly eat three meals and three snacks a day, never too much or too little, eat in peace and quiet, chew thoroughly, avoid certain foods (e.g. fatty or spicy foods, alcohol, coffee, onions, cabbage, beans, carbonated beverages, etc.) and eat fibre but distribute its intake over the day. A suggested main dietary approach is increased daily fibre intake; however, while improving general IBS symptoms in some subgroups, it can worsen them in others [26–29]. Reduction of dietary fat intake improved symptoms in patients because fatty acids can trigger symptoms in IBS [20, 23]. The effect of a gluten-exclusion diet is also controversial [30, 31]. A novel treatment option is a diet low in FODMAPs (Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols). Many popular, healthy foods have a high-FODMAP content, such as fruits (apples, pears, peaches and watermelons), vegetables (onions, garlic, squash and mushrooms), dairy products, grains (wheat and rye), and sweeteners (sorbitol and mannitol), etc. [32]. FODMAPs can trigger symptoms in IBS patients, based on two major mechanisms [8, 33–37]. The 'small bowel hypothesis' states that FODMAPs are unabsorbed, osmotically active molecules (carbohydrates), so they increase the intraluminal water content in the small intestine. This leads to distension, which causes symptoms such as bloating and discomfort. The increased distension also leads to faster oro-cecal transit, which impairs absorption in the small bowel [36]. The second mechanism ('large bowel hypothesis') describes FODMAPs reaching the colon unabsorbed, where they are rapidly fermented by colonic bacteria. This causes flatulence, bloating and discomfort through increased gas production and distension of the colonic wall [36]. Because of visceral hypersensitivity, the same magnitude of distension will produce different degrees of symptoms, depending on individual susceptibility [38]. These findings suggested that the exclusion of FODMAPs from the diet could improve IBS symptoms. A growing number of studies have shown a positive effect of FODMAPs on IBS symptoms. The need has thus arisen for a meta-analysis with a focus on effectiveness to provide evidence and underpin recommendations for wider therapeutic use. Our aim was to carry out a meta-analysis to prove whether a low-FODMAP diet improves the symptoms of adult IBS patients more effectively than other (standard) dietary interventions (i.e. without restriction of FODMAP content) recommended by the latest guidelines. Following the PRISMA 2009 guidelines, we used the PICO format to formulate our question (P: patients with IBS; I: low-FODMAP diet; C: high-FODMAP/standard IBS diet; O: IBS Symptom Severity Score (IBS-SSS)).

Methods

Search for articles

Our work was planned according to the PRISMA 2009 statement (S1 File). A systematic literature search was conducted by two independent reviewers (JC and PV) to find relevant articles on the effect of low-FODMAP dietary intervention in IBS up to 19 September 2016. The search covered three databases (PubMed, EMBASE and the Cochrane Library) with the terms 'FODMAP AND irritable bowel syndrome'. For better targeting of synonymous phrases, we used the search terms: 'FODMAP' OR 'FODMAPS' OR 'Fermentable poorly absorbed short chain carbohydrates' OR 'Fermentable oligosaccharides disaccharides monosaccharides and polyols' as was done in a recent meta-analysis by Marsh et al. [39]. After this search process, language (only English) and species (only humans) filters were used and a title, and abstract screening was performed by the reviewers to identify potentially eligible articles. Disagreements were resolved by discussion. Duplicates were removed.

Study selection

We included randomized controlled trials (RCT), non-randomized controlled trials and non-controlled prospective trials in our meta-analysis. Retrospective studies were excluded. The length of follow-up was not a reason for either inclusion or exclusion. Only articles written in English and those examining the effect of a low-FODMAP diet in human IBS patients were included in the meta-analysis. By definition, adult IBS patients (18 years or above) had to be diagnosed according to the Rome II, Rome III, Rome IV or NICE criteria. We enrolled controlled studies which included adult IBS patients as a control group. In the control groups, IBS patients had to follow a standard IBS diet (according to the guidelines) with significantly higher FODMAP content than in the intervention (low-FODMAP) group. As a standard, validated output measure, we searched for studies reporting the IBS Symptom Severity Score (IBS-SSS). The measurement of the severity of individual symptoms among the studies showed great heterogeneity (e.g. Visual Analogue Scale, different types of Likert scale, etc.), so we used only the complex IBS-SSS in our analysis as an outcome measure. Articles examining the results of patients with an organic disease (for example, inflammatory bowel disease and IBD) with functional gastrointestinal symptoms, which are similar to IBS symptoms, were excluded from the analysis.

Quality assessment of the individual studies

The quality of RCTs was assessed with the frequently used Jadad score [40], while non-randomized and non-controlled prospective studies were evaluated according to the Methodological Index for Non-Randomized Studies (MINORS) [41]. Both scores were evaluated by JC and PV. Any disagreements were resolved by consensus.

Data extraction

At the end of the screening process, relevant data were independently extracted from studies by the two reviewers (JC and PV). These included: IBS-SSS as the main outcome parameter, study design (e.g. randomized controlled trials, non-randomized controlled trials, etc.), basic characteristics of the study population (age, percentage of females and IBS subtypes), length of follow-up, diagnostic criteria for IBS and the size of the low-FODMAP and control (high-FODMAP) groups. Extracted data were validated by five co-authors (AG, IS, GP, ÁV and ÁS).

Outcome measure

IBS-Symptom Severity Score (IBS-SSS). This score provides a measure of overall IBS severity. It was validated by Francis et al. [42] in 1997 and consists of five questions that measure abdominal pain severity, abdominal pain frequency, abdominal bloating, bowel habit dissatisfaction and interference with quality of life on a 100 mm visual analogue scale (VAS). Patients should rate every symptom with a score from 0–100, so the theoretical range is 0–500 mm, with higher scores indicating a more severe disease. A final score of less than 175 indicates mild IBS, 175–300 shows moderate IBS, and >300 points to severe IBS [39].

Statistical analysis

Data analysis was conducted with the Comprehensive Meta-Analysis software (Version 3.0, Biostat Inc.). In the forest plot analysis, mean differences with 95% confidence intervals were calculated from studies that contained means, standard deviation (SD) or mean differences and SD of differences and p-values. In one study (Pedersen et al. [43]), where the results were

expressed as median, minimum and maximum values, we converted the data using the Hozo method [44].

The studies we included in the meta-analysis indicated that there is a considerable heterogeneity (different clinical methods, diverse participants, etc.), so the random effects model was used according to the DerSimonian and Laird method [45]. Statistically, heterogeneity was tested by Q test ($\chi 2$) and I^2 indicator [46]. I^2 indicator and Q tests were performed to assess whether the heterogeneity observed among effect sizes could be attributed to random chance or if other factors may play a role. The similar effect of non-investigated variables such as food intolerances and functional digestive tract disorders other than IBS could also cause IBS-like symptoms. I^2 statistics represent the percentage of effect size heterogeneity that cannot be explained by random chance, but by other factors noted above. If the Q test is significant, it implies that the heterogeneity among effect sizes reported in the studies selected is more diverse than could be explained by random error only. The Q test was considered significant when $p < 0.1$.

We used subgroup analysis, with a p-value of less than 0.05 indicating a significant difference to compare the differences in the IBS-SSS between the control and low-FODMAP diet groups. Results from the meta-analysis were displayed graphically using forest plots. The potential for "small study effects", including publication bias, was examined by visual inspection of funnel plots, in which the standard error was plotted against the net change for each study. In both funnel plots, an asymmetry could be observed, which could be caused by the subgroups. In S1 Fig the subgroup analysis indicates some publication bias, but in S2 Fig the same analysis suggests no such bias.

Results

Searching results

Using the terms above, we found 880 articles in the three databases for evaluation, 261 in PubMed, 87 in the Cochrane Library and 532 in EMBASE. We also examined 22 further articles from the recent meta-analysis noted above [39], so 902 articles were found in total. After using the language (only English) and species (only humans) filters in EMBASE, PubMed and the Cochrane Library, 673 of 880 studies remained, and one of 22 was excluded from the meta-analysis by Marsh et al. [39] because it failed to meet the English-language inclusion criterion. After title and abstract screening and removing duplicates, 10 articles reporting on IBS-SSS eligible for further evaluation were found (Fig 1). Of these studies, 6 were available in full-text format, and 4 were short abstracts or supplements. The number of controlled trials was 7, and there were 3 non-controlled prospective studies. Of the controlled trials, 5 were randomized controlled trials (RCT), and 2 were non-randomized studies. At the time of the literature search, we found no eligible paper that used the most recent diagnostic criteria (Rome IV) for IBS. The basic characteristics of the articles are summarized in Table 1. The proportion of each IBS subtype in the studies included in the meta-analysis is detailed in Table 2. A quality assessment of the articles is summarized in Table 3.

Low-FODMAP and control diets

Patients received dietary advice from a dietitian on a low- or high-FODMAP diet in 7 [8, 16, 43, 47, 49, 51, 53] of the 10 studies analyzed. 2 [50, 52] abstracts included in the meta-analysis failed to detail any information about dietitian involvement in the introduction of a low- or high-FODMAP diet. In a study by Laatikainen et al. [48], a special low-FODMAP diet containing rye bread was prepared for the patients, which had been developed and supplied by a bakery. Its FODMAP (fructan and mannitol) content was clearly lower than that of regular rye

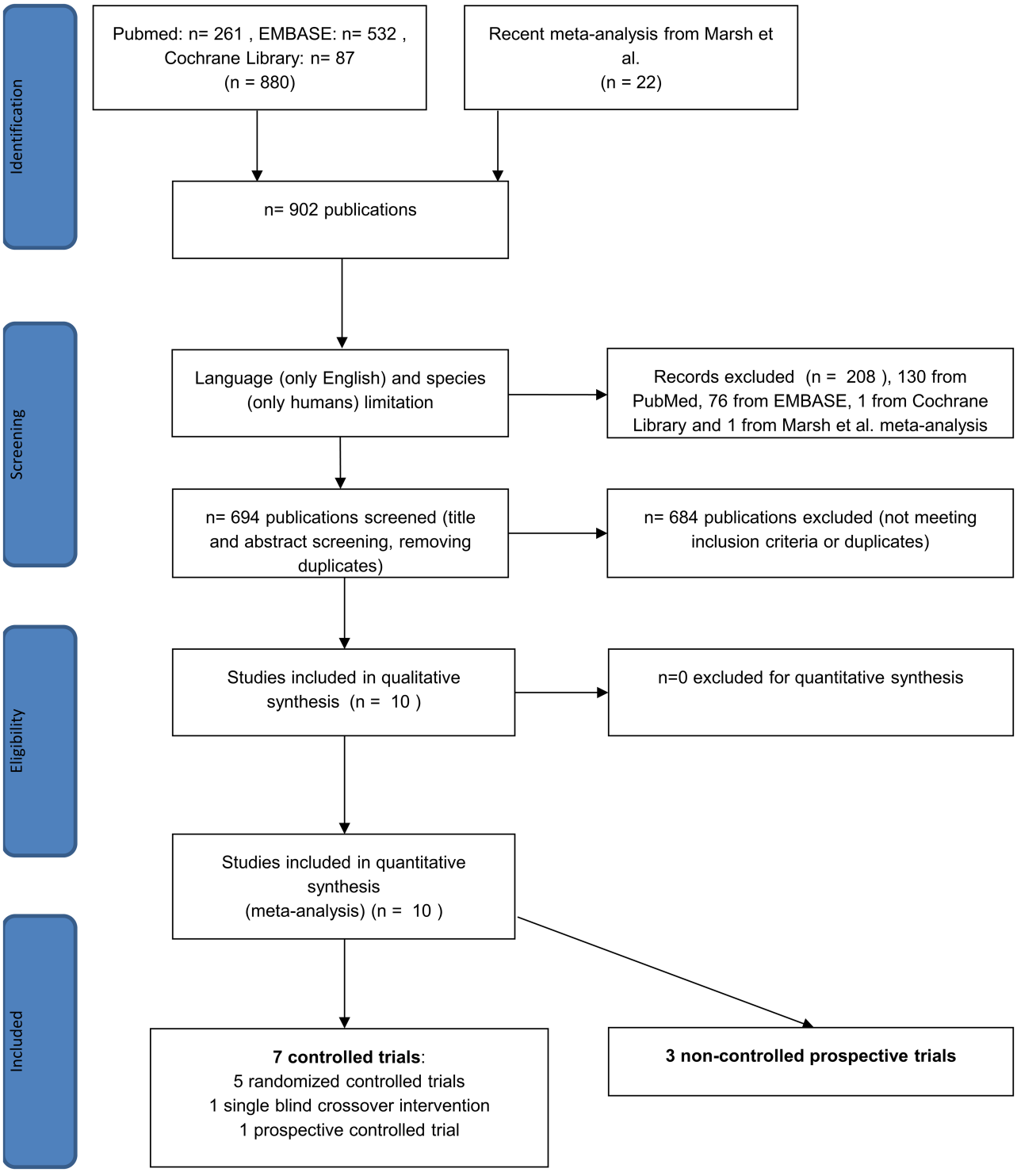

Fig 1. Flow chart for the systematic literature search.

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Table 1. Baseline characteristics of the studies involved in the meta-analysis.

References	Country	Study design	Study duration	total or low-FODMAP/control cohort size	IBS diagnostic criteria	Age (years)	Percentage of females (%)
Böhn et al. [8]	Sweden	multi-centre, parallel, randomized, controlled, single-blind, comparative trial	4 weeks	33/34	Rome III	low-FODMAP: mean: 44 (18–69); control: mean: 41 (18–68)	total: 81; low-FODMAP: 79; control: 84
McIntosh et al. [47]	Canada	prospective, randomized, single-blind, parallel study	3 weeks	18/19	Rome III	low- FODMAP: mean: 50.28 (26–77); control: mean: 5147 (24–83)	total: 86; low-FODMAP: 83; control: 89
Pedersen et al. (B) [16]	Denmark	randomized, non-blind, controlled trial	6 weeks	42/40 started, 34/37 finished	Rome III	low-FODMAP: median: 37 (18–71); control: median: 32 (18–73)	total: 77; low-FODMAP: 81; control: 72.5
Laatikainen et al. [48]	Finland	randomized, double-blind, 2x2 cross-over study	13 weeks (1- week run-in, 2x4-week intervention, 4 -week wash-out period)	80 started, 73 finished	Rome III	mean: 42.9 (21–64)	91
Schultz et al. (Suppl.) [49]	New -Zealand	randomized controlled trial	12 weeks	23/27	Rome III	no data	no data
Pedersen et al. (A) [43]	Denmark	single-blind, cross-over intervention	12 weeks (0–6 and 7–12 weeks)	19	Rome III	median 35 (18–74)	74
Piacentino et al. (Suppl.) [50]	Italy	prospective controlled trial	4 weeks	28/28	no data	21–68	68
Ones et al. (Suppl.) [51]	Norway	non-controlled prospective study	6 weeks	23	Rome III	mean: 35±11	87
Rossi et al. (Suppl.) [52]	Italy	non-controlled prospective study	8 weeks	12	Rome III	mean: 44.2±15.5	92
Valeur et al. [53]	Norway	non-controlled prospective study	4 weeks	63	Rome III	mean: 38.4 (19–67)	89

FODMAP = Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols; IBS = Irritable Bowel Syndrome; Suppl. = Supplementary material

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Table 2. The proportion of each IBS subtype in the studies included in the meta-analysis.

References	IBS subtype	
	Low-FODMAP group (%)	Control diet group (%)
Böhn et al.	IBS-D: 26; IBS-C: 24; IBS-M/U: 50	IBS-D: 22; IBS-C: 35; IBS-M/U: 43
McIntosh et al.	IBS-D: 22; IBS-C: 6; IBS-M: 67; IBS-U: 5	IBS-D: 32; IBS-C: 5; IBS-M: 58; IBS-U: 5
Pedersen et al. (B)	IBS-D: 45; IBS-C: 12; IBS-M: 33; IBS-U: 10	IBS-D: 45; IBS-C: 17.5; IBS-M: 35; IBS-U: 2.5
Laatikainen et al.	IBS-D: 32.5; IBS-M: 62.5; IBS-U: 5	
Schultz et al. (Suppl.)	no data	no data
Pedersen et al. (A)	IBS-D: 42; IBS-C: 21; IBS-A: 37	
Piacentino et al. (Suppl.)	no data	no data
Ones et al. (Suppl.)	no data	-
Rossi et al. (Suppl.)	IBS-D: 25; IBS-C: 17; IBS-M: 58	-
Valeur et al.	IBS-D: 54; IBS-C: 16; IBS-M: 30	-

FODMAP = Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols; IBS = Irritable Bowel Syndrome; IBS-D = IBS-Diarrhoeal subtype; IBS-C = IBS-Constipation subtype; IBS-M/A = IBS-Mixed/Alternation subtype; IBS-U = IBS-Unsubtyped; Suppl. = Supplementary material. The number of patients in each IBS subtype groups is expressed in S1 Table.

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Table 3. Quality assessment of the studies included in the meta-analysis.

	Study design	Jadad score	MINORS
Böhn et al.	multi-centre, parallel, randomized, controlled, single-blind, comparative trial	**3/5**	**-**
McIntosh et al.	prospective, randomized, single-blind, parallel study	**3/5**	**-**
Pedersen et al. (B)	Randomized, non-blind, controlled trial	**3/5**	**-**
Laatikainen et al.	randomized, double-blind, 2x2 cross-over study	**5/5**	**-**
Schultz et al. (Suppl.)	randomized controlled trial	**0/5**	**-**
Pedersen et al. (A)	single-blind cross-over intervention	**-**	**16/24**
Piacentino et al. (Suppl.)	prospective controlled trial	**-**	**15/24**
Ones et al. (Suppl.)	non-controlled prospective study	**-**	**8/16**
Rossi et al. (Suppl.)	non-controlled prospective study	**-**	**9/16**
Valeur et al.	non-controlled prospective study	**-**	**12/16**

Randomized controlled trials were evaluated with the Jadad score (0 = very poor, 5 = rigorous) [40]. Non-randomized studies were evaluated with the MINORS (Methodological Index for Non-Randomized Studies) [41], in which 12 items are scored (0: not reported; 1: reported, but inadequate; 2: reported and adequate). The global ideal score is 16 for non-comparative studies and 24 for comparative studies. Suppl. = Supplementary material.

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bread. The control group was not homogeneous among the studies, but it always had a significantly higher FODMAP content. The precise content of the foods used was only detailed in 2 trials [8, 48]; the others probably followed dietary guidelines. This uncertainty could have influenced our results.

IBS-SSS

First, we wanted to see if a low-FODMAP diet is an effective treatment for irritable bowel syndrome. We compared the pre- vs. post-intervention IBS-SSS in control groups (4 publications) and low-FODMAP groups (8 publications) (Fig 2). There was a significant reduction in IBS-SSS in both control (difference in means (DIM), post- minus pre-values: –59.816 (95% CI: –108.922 ––10.710); p = 0.017) and low-FODMAP groups (DIM: –105.339 (95% CI: –140.773 ––69.905); p = 0.000). This means that both standard (high-FODMAP) and low-FODMAP diets are effective in improving symptoms and quality of life among IBS patients. The forest plot suggests that a low-FODMAP diet is more effective, but we cannot prove this statistically because of the overlapping confidence intervals. Significant heterogeneity was found between the studies: control group IBS-SSS values: Q = 9.837; df = 3; p = 0.02; I^2 = 69.504%; low-FODMAP group IBS-SSS values: Q = 26.321; df = 7; p< 0.001; I^2 = 73.405%.

We compared (Fig 3) the pre- and post-intervention scores between the control and low-FODMAP groups in the controlled trials (6 publications for each group). This shows that there is no statistically significant difference in pre-values between the groups (DIM: control minus low-FODMAP values: –8.675 (95% CI: –40.043 –+22.693); p = 0.588), but a significant difference between post-values (DIM: +51.537 (95% CI: +18.891 –+84.183); p = 0.002) could be observed. These results confirm that the therapeutic effect of a low-FODMAP diet is better than standard dietary advice in patients with IBS. The meta-analysis also showed a significant heterogeneity: pre-IBS-SSS values: Q = 21.242; df = 5; p = 0.001; I^2 = 76.462; post-IBS-SSS values: Q = 20.675; df = 5; p = 0.001; I^2 = 75.816.

Discussion

The standard dietary approach for IBS dietary therapy (e.g. high-fibre, low-fat, etc., as detailed above) recommended by guidelines only improves IBS symptoms to a limited extent. A growing number of recent studies have shown a beneficial effect of a low-FODMAP diet on IBS

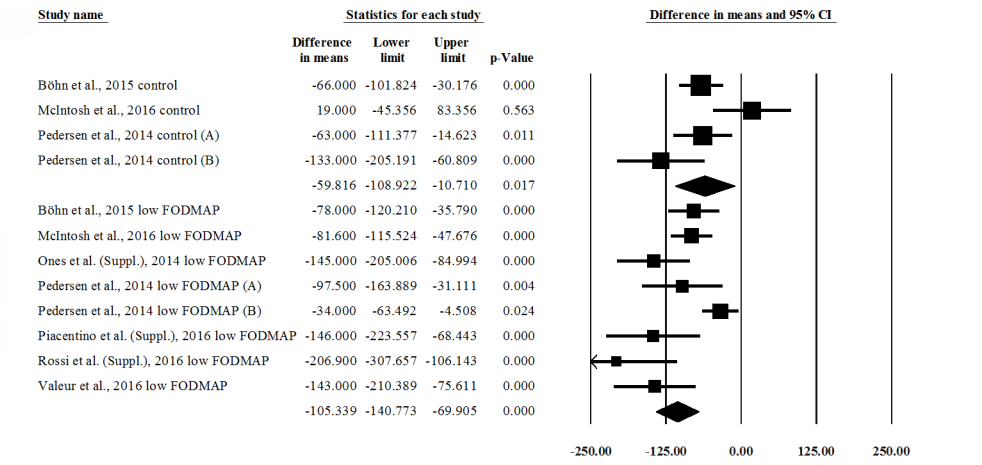

Fig 2. Forest plot of IBS-SSS DIMs, comparing pre- vs. post-intervention values within groups (low-FODMAP and control). IBS-SSS = Irritable Bowel Syndrome Symptom Severity Score (0–500); DIM = Difference in Means; FODMAP = Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols.

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symptoms. Several of them have compared its efficacy to a standard IBS diet and challenged us to review the latest literature on the issue. A recent meta-analysis by Marsh et al. [39] analyzed the beneficial effect of a low-FODMAP diet on symptoms and quality of life in adult and pediatric patients with IBS and inflammatory bowel disease in the literature up to 24 March 2015. They only investigated the complex IBS-SSS only in 4 articles, and it was not stated whether low-FODMAP diet is significantly better than a control diet or not. We carried out our analysis on IBS-SSS, using more (10) articles, and we only focused on adult patients with IBS. A

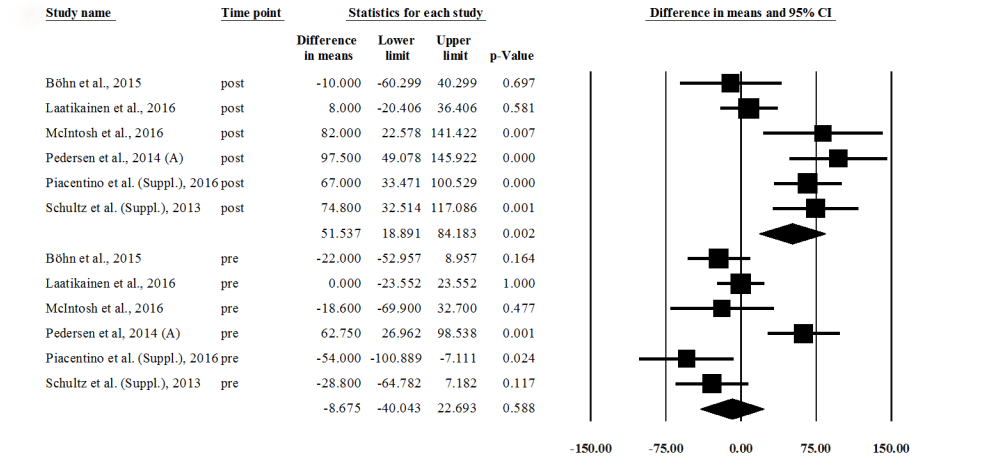

Fig 3. Forest plot of IBS-SSS DIMs, comparing pre- and post-intervention values between groups (low-FODMAP vs. control). IBS-SSS = Irritable Bowel Syndrome Symptom Severity Score (0–500); DIM = Difference in Means; FODMAP = Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols.

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previous meta-analysis by Khan et al. and systematic review by Rao et al. [32, 54] also proved the efficacy of this diet on symptom improvement and suggested its introduction as a baseline treatment, but they could not state clearly whether it is better than standard dietary advice or not. Rao et al. [54] also investigated the high-fibre diet on chronic constipation and IBS. They performed a literature search up to September 2014 and did not conduct a statistical analysis due to heterogeneity and methodological quality. To our knowledge, this is the first meta-analysis to compare the effectiveness of low-FODMAP foods to a regular IBS diet recommended by the guidelines.

A pooled analysis of 7 controlled trials (5 randomized and 2 non-randomized) and 3 non-controlled trials confirmed that a low-FODMAP diet significantly improves general symptoms (IBS-SSS) in patients with IBS compared to standard dietary recommendations and a high-FODMAP diet. FODMAPs are poorly absorbed carbohydrates that cause an increase of water content in the bowel based on the osmotic effect and increased gas production by colonic bacterial flora. These effects of FODMAPs induce several symptoms in patients with irritable bowel syndrome and numerous patients with functional gastrointestinal disorders mainly by distension and the osmotic laxative effect [8, 36, 37].

The pooled sample size was large, and the expression of the data from the studies enrolled was acceptably homogeneous with regard to the key question of the meta-analysis.

Because of the considerable heterogeneity of the expressed data, the random effects model was used with the DerSimonian and Laird [45] method for analysis. This is possible because of the similar effect of non-investigated variables such as food intolerances and functional digestive tract disorders other than IBS that could cause IBS-like symptoms as well. It would be important to study the effect of a low-FODMAP diet in these groups to better understand the role of a food challenge in provoking uncompliant symptoms of functional digestive tract disorders, as in IBS. Nevertheless, the beneficial effects of a low-FODMAP diet on IBS-SSS were statistically significant even in the heterogeneous population analyzed, thus supporting the high impact of this diet on IBS symptoms.

Study data reflected some publication bias based on heterogeneity (S1 Fig) and no significant bias based on time-based comparisons of the low-FODMAP diet (S2 Fig).

We evaluated the quality of the studies included in the meta-analysis (Table 2, S2 Table) using the Jadad score for RCTs and MINORS for non-randomized studies. Among RCTs, the Schultz et al. trial [49] was an outlier (Jadad score = 0). This could be due to the fact that it is only available in abstract form; there is therefore a lack of information on the study design. The scores given to the other trials were satisfactory.

The strength of our study is that a standardized complex outcome score (IBS-SSS) was used to measure the therapeutic effect. This score measures abdominal pain frequency and severity, bloating, dissatisfaction with bowel habit and quality of life together on a 0–500 mm Visual Analogue Scale (VAS). This scoring system provides information not only about symptoms, but also about quality of life. A sufficient number of articles were found to carry out an accurate statistical analysis, using this important outcome score. With this work, we proved not only the positive effect of a low-FODMAP diet on IBS-SSS, but also its superiority to a high-FODMAP standard IBS diet. Our meta-analysis is the first to provide unambiguous, high-level evidence for the superiority of a low-FODMAP diet to a standard dietary approach in the improvement of general symptoms and well-being among patients with IBS. These data suggest that the first-line introduction of a low-FODMAP diet in the treatment of IBS could improve the therapeutic effect on IBS symptoms and might decrease health care-related and societal costs [9].

There are some limitations to our study. First, we focused on the complex IBS-SSS, and due to the lack of detailed published data, we did not perform a statistical analysis of the individual symptoms in the symptom score. Therefore, it is not clear which of the five elements play a key

role in the improvement of IBS symptom severity toward better personalization of this dietary approach. The main reason was the lack of data and control groups, as well as the heterogeneity in the literature in measuring symptom severity (e.g. VAS and different types of Likert scale). A uniform, consensus-based, well-comparable measurement of symptom severity (e.g. IBS-SSS) is suggested for use in future studies. Second, we included not only full-text articles, but also 4 short supplements [49–52] in our analysis, thus increasing the quantity of data on control groups. Third, because of the lack of data in the different IBS subtypes, it is not clear which subgroup experienced the greatest symptom improvement. Finally, the standard IBS diet group was not homogeneous. The control diet always contained a significant number of FODMAPs; however, only 2 out of 10 studies detailed exact food contents [8, 48]. Others probably used IBS dietary guidelines; thereafter, some differences were realized between contents, thus potentially influencing our results.

To prove the effect of a low-FODMAP diet on bowel movement frequency in IBS patients and to demonstrate which IBS subgroup could profit significantly from this diet, more double-blind, randomized controlled trials should be conducted with the following standardized parameters. Only patients fulfilling the most recent diagnostic criteria for IBS (Rome IV) should be included in studies. A precise description of the contents of the diets studied is crucial for an accurate analysis. It is highly recommended dietitians be involved in guiding patients on diets to avoid significant differences within study groups and inadequate nutrient intake. Patients should also be adequately monitored during trials to ensure their adherence to a particular diet. Uniform outcome measures should be used to make studies scientifically comparable. Except for measuring symptom severity on the VAS scale only, it is suggested that a more detailed IBS-SSS be used in each subtype of the IBS patient group, which measures not only the severity of the main symptoms, but also the quality of life. Yao et al. [55] discuss the crucial points and difficulties of designing clinical trials in dietary interventions in patients with functional gastrointestinal disorders.

Conclusion

This meta-analysis confirms that a diet low in fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) significantly improves general symptoms and quality of life in patients with irritable bowel syndrome. Our analysis of the appropriate literature data also confirms that a low-FODMAP diet is more effective than standard IBS dietary therapy in patients diagnosed with IBS. However, a low-FODMAP diet raises certain issues, such as the alteration of gut microbiota and inadequate nutrient intake without dietitian assistance. The possible health advantages of a low-FODMAP diet—when it is effective—compared to medical treatment require further evaluation. In consideration of its possible limitations and based on findings from this meta-analysis, a low-FODMAP diet could be a potential first-line and supplementary dietary therapeutic approach with the aid of a dietitian for patients with irritable bowel syndrome to improve abdominal discomfort, abdominal pain, bloating and quality of life. Because of the lack of published data, it is not possible to prove the effect of a low-FODMAP diet on bowel movement frequency in IBS patients. It also remains unclear which IBS subgroup could profit most from this diet. More randomized controlled trials are called for to analyze these effects of dietary approaches.

Supporting information

S1 Table. Raw data material.
(XLSX)

S2 Table. Jadad and MINOR scores of the articles included in the meta-analysis.
(XLSX)

S1 Fig. Funnel plot of publication biases among the studies in Fig 2. ES = effect size; s.e. of ES = standard error of effect size.
(TIF)

S2 Fig. Funnel plot of publication biases among the studies in Fig 3. ES = effect size; s.e. of ES = standard error of effect size.
(TIF)

S1 File. PRISMA 2009 checklist.
(DOC)

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RESEARCH ARTICLE

The role of small intestinal bacterial overgrowth and false positive diagnosis of lactose intolerance in southwest Hungary—A retrospective observational study

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Abstract

Background

Lactose intolerance is a frequent gastrointestinal disease affecting 47% of the Eastern European population. Small intestinal bacterial overgrowth (SIBO) leads to carbohydrate malabsorption and therefore to false results during lactose breath and tolerance tests.

Objectives

We aimed to assess the prevalence of lactose maldigestion and intolerance in Hungary and to investigate the role of combined diagnostic method and testing for SIBO in reducing false results.

Methods

We retrospectively analyzed data from 264 adult symptomatic patients who underwent 50g lactose breath and tolerance tests in parallel over a one-year period at our center. A $\geq$20 ppm elevation of H_2 or less than 1.1 mmol/l rise of blood glucose was diagnostic for lactose maldigestion. Patients with maldigestion who had symptoms during the test were defined as lactose intolerant. Patients with an early ($\leq$90 min) significant ($\geq$20 ppm) rise of H_2 during lactose and/or lactulose breath tests were determined to have SIBO. Patients with slow/rapid oro-cecal transit and inappropriate preparation before the test were excluded.

Results

49.6% of the 264 patients had lactose maldigestion, and 29.5% had lactose intolerance. The most frequent symptom was bloating (22.7%), while 34.8% of the study population and 60% of the symptomatic patients had SIBO. In 9.1% and 9.8% of the patients, the lactose

decision to publish, or preparation of the manuscript.

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Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; LBT, lactose breath test; LI, lactose intolerance; LM, lactose maldigestion; LTT, lactose tolerance test; SIBO, small intestinal bacterial overgrowth; OR, odds ratio.

breath and tolerance test alone gave false positive result compared with the combined method. SIBO was present in 75% of the false positives diagnosed with breath test only.

Conclusions

The prevalence of lactose intolerance is lower in Hungary compared to the Eastern European value (29.5% vs 47%), so it is worth performing a population-based prospective analysis in this area. A combination of lactose breath and tolerance tests and the careful monitoring of results (with early H2 rise, lactulose breath test, etc.) can decrease the false cases caused by e.g. SIBO.

Introduction

Lactose intolerance (LI) is a clinical syndrome characterized by abdominal symptoms after ingestion of lactose-containing products caused by lactose maldigestion (LM) [1–3]. The most common cause of primary LM is adult-type hypolactasia [4, 5]. Acquired organic disorders (e.g. small intestinal bacterial overgrowth (SIBO), celiac disease, inflammatory bowel disease (IBD), and infectious enteritis (e.g. giardiasis)), can lead to both downregulation of lactase expression and reduction of absorptive capacity and therefore to secondary lactose malabsorption [4, 5]. Approximately 47% of the Eastern European population is affected; however, LI is more common in Asia, Africa, and South America. It affects males and females equally [2, 6]. The prevalence of LM increases with age, however, the LI symptoms decrease in elderly [7, 8]. Because of insufficient lactase activity, lactose can reach the large intestine, where it is fermented by colonic bacteria, gases (H_2, CO_2, and CH_4), short-chain fatty acids, and other products that are formed there. Excessive gas production causes luminal distension and leads to different gastrointestinal symptoms. The most common complaints are abdominal pain and discomfort, bloating, flatulence, and diarrhea as with SIBO [1, 3, 9–11]. The diagnostic methods available for LM or LI are based on the lactose breath test (LBT), lactose tolerance test (LTT), genetic test, and assessment of lactase activity in jejunal biopsy specimens, the LBT and LTT being the most popular methods [4]. However, in most studies and at most centers, only one of the last two methods (LBT or LTT) is used, resulting in higher rates of incorrect diagnosis caused by SIBO, for example, which can lead to carbohydrate malabsorption and therefore to false positive results during the LBT and LTT. Moreover, in some patients with methanogenic microbiota (e.g. Methanobrevibacter smithii), the bacteria convert hydrogen to methane, leading to false negative LBT results [2]. Restricting lactose intake or replacing the lactase enzyme can alleviate unpleasant lactose-induced symptoms [2–5].

SIBO is a condition in which the small intestine is excessively colonized by aerobic and anaerobic bacteria. Normally, there are fewer than 10^5 bacteria per milliliter in the duodenal and jejunal part of the small intestine, with ileal counts reaching 10^8 per milliliter [12]. The prevalence of SIBO is unclear, depending on the population and the diagnostic test used. It is more frequent among the elderly due to reduced gastric acid secretion and medications causing hypomotility [13]. Disorders disturbing mucosal defense mechanisms can predispose one to SIBO, intestinal motility disorders and chronic pancreatitis being the most common causes [14–16]. Other etiological factors are motility disorders (diabetes mellitus, irritable bowel syndrome [IBS], use of narcotics, intestinal pseudo-obstruction, etc.), anatomic disorders (adhesions, strictures, diverticulosis, etc.), immunological disorders (e.g. HIV), and metabolic and systemic diseases (e.g. cirrhosis) [12, 17–19]. SIBO causes mucosal damage and altered motility

and therefore leads to complex malabsorption (of carbohydrate, fatty acids, proteins, and vitamins), diarrhea, bloating, flatulence, and abdominal discomfort [13, 20–23]. A diagnosis of this disease can be based on carbohydrate breath tests or on an assessment of bacterial concentration from the jejunal aspirate. Although jejunal aspirate culture is the gold standard method, it is not widely used due to its invasiveness, poor reproducibility, possible contamination, and patchy disease localization. Carbohydrate breath tests are simple, non-invasive, inexpensive, and therefore widely used [24–26]. The treatment comprises correction of the underlying cause, antibiotic therapy, and nutritional support (e.g. lactose-free diet, vitamin replacement, and correction of nutrient deficiencies). Rifaximine is effective in 80% of patients [27, 28]. Higher doses (1200 or 1600 mg/day) are more effective compared to standard ones (600 or 800 mg/day) [29]. The length of antibiotic therapy is not clearly defined. A single 7–10-day course can alleviate symptoms in most patients [30]. Repeated or continuous antibiotic therapy should be useful if symptoms recur [13]. The effectiveness of probiotics is inconclusive, and generally, they are not recommended in SIBO [18, 31].

In this single-center retrospective study, we aimed to assess the prevalence of LM and LI in southwest Hungary (Baranya County, except for the Mohács district, with a population of 317,000 people), to investigate the role of a combined diagnostic method (LBT and LTT) in improving diagnostic accuracy, and to show that parallel testing for SIBO could reduce false positive cases determined by LBT and/or LTT.

Materials and methods

The key points of the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guideline [32] were followed in planning and reporting this study (S1 File). We retrospectively analyzed data from adult symptomatic patients who underwent the LBT and LTT in parallel at our center (Division of Gastroenterology, First Department of Medicine, University of Pécs) between 15 February 2016 and 14 February 2017. The LBT and LTT were carried out with 50g lactose (equal to the content of 1 liter of milk), H_2 levels were measured with Micro H_2 instrument (Micro Medical Limited, P.O. Box 6. Rochester, Kent ME1 2AZ ENGLAND). Before lactose ingestion, baseline end-alveolar H_2 and blood glucose levels were measured (0 min). Then patients drank the set amount of lactose dissolved in 250 ml water. After this process, end-alveolar H_2 and blood glucose levels were measured every 30 minutes over a three-hour period (in the case of glucose over a two-hour period). Depending on the clinical situation and patients' compliance, in clinically uncertain (but not in all) cases, a lactulose breath test with 10g lactulose was carried out to prove or reject the diagnosis of SIBO or slow oro-cecal transit [26]. A significant, $\geq$20 ppm elevation of H_2 level during the LBT and/or less than 1.1 mmol/l rise of blood glucose during the LTT was diagnostic for LM. Patients with negative LBT and LTT are lactose digesters. Patients with LM who had symptoms during the test were defined as lactose intolerant. Patients with an early ($\leq$90 min) significant ($\geq$20 ppm) rise of H_2 during the LBT and/or lactulose breath test were determined to have SIBO [26]. The diagnostic criteria of the different conditions are summarized in Table 1. For optimal preparation, patients stopped taking laxatives, antibiotics, and prokinetics, avoided high fiber-containing foods and fasted for 12 hours, avoided smoking and exercise for at least two hours before the test. Antiseptic mouthwash was not given routinely, only for those with high initial H_2 value ($>$20 ppm). We excluded patients with inappropriate preparation for the test (baseline H_2 level $>$20 ppm) and those with suspected rapid or slow oro-cecal transit (clinical symptoms of gastroparesis and a negative LBT with a positive LTT or no significant rise of H_2 during a 180-min lactulose breath test compared to the baseline value). We collected data on the baseline characteristics of the analyzed population (mean age, gender differences, and their

Table 1. The summary of the different diagnostic criteria used in our study.

Lactose maldigester (LM)	**LBT**: ≥20 ppm elevation of H_2 compared to baseline level and/or **LTT**: <1.1 mmol/l rise of blood glucose level compared to baseline value
Normal lactose digestion	Negative LBT (<20 ppm elevation of H_2 level) and Negative LTT (≥1.1 mmol/l elevation of blood glucose)
Lactose intolerance (LI)	Lactose maldigesters, who had symptoms during the test period
Small intestinal bacterial overgrowth (SIBO)	Significant (≥20 ppm) rise of H_2 during lactose and/or lactulose breath test, within 90 minutes
Slow oro-cecal transit (excluded)	Clinical symptoms of gastroparesis and a negative LBT with a positive LTT or no significant rise of H_2 during a 180-min lactulose breath test compared to the baseline value

LBT: lactose breath test; LTT: lactose tolerance test.

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correlation with the outcome measures), the diagnostic tests (baseline and maximum H_2 and glucose levels, time of glucose and H_2 peak, and the presence of LM), the presence and type of symptoms occurring during the test (abdominal pain, cramps, discomfort, bloating, diarrhea, nausea/vomiting, borborygmi, and other gastrointestinal symptoms, such as increased bowel motility, flatulence, belching, a sensation of fullness in the stomach, a burning sensation in the stomach, an increased sensation for defecation or headache [3, 33]), and the presence of LI and SIBO. The data collection and research were approved by the director of the Clinical Center and the director of the First Department of Medicine of the University of Pécs (Institutional Review Board), and the study process was carried out in accordance current laws and regulations (Case Number: PTE/98494/2018). All patient data were fully anonymized after the specific parameters necessary for our research were collected. However, our analysis was made retrospectively; therefore, we have not included patients' data who had refused scientific purpose data handling.

Statistical analysis

Data were analyzed using SPSS 25.0 software. Means, standard deviation, minimum and maximum values, and relative frequency were calculated for descriptive statistics. The Pearson correlation, the Mann–Whitney test, and odds ratios (OR) with 95% confidence interval (CI) were used for other analyses. A p-value of less than 0.05 was accepted as statistically significant.

Results

A total of 310 patients were assessed in the period noted above. Twenty-four of them were excluded because of inappropriate preparation and 22 (7.6% of the well-prepared patients) were ruled out because of slow oro-cecal transit, leaving 264 patients, 185 females (F: 70.1%), and 79 males (M: 29.9%), for statistical analysis. No patient had rapid transit in our study group. The mean age of the analyzed study group was 40.3 years (F: 40.6 years; M: 39.5 years).

Based on the LBT and/or LTT results, 49.6% (131/264) of the study population had LM (LBT and/or LTT positivity), as represented in Fig 1. Seventy-eight (78/131, 59.5%) of them had symptoms after lactose ingestion and were therefore defined as lactose intolerant (78/264, 29.5%, Fig 1). Combined positivity (LBT+LTT) was found in 30.7% (81/264) of the patients (see Fig 1). There was no significant difference between females and males in the prevalence of normal lactose digestion, LM, and LI ($p > 0.05$). There was no significant correlation between

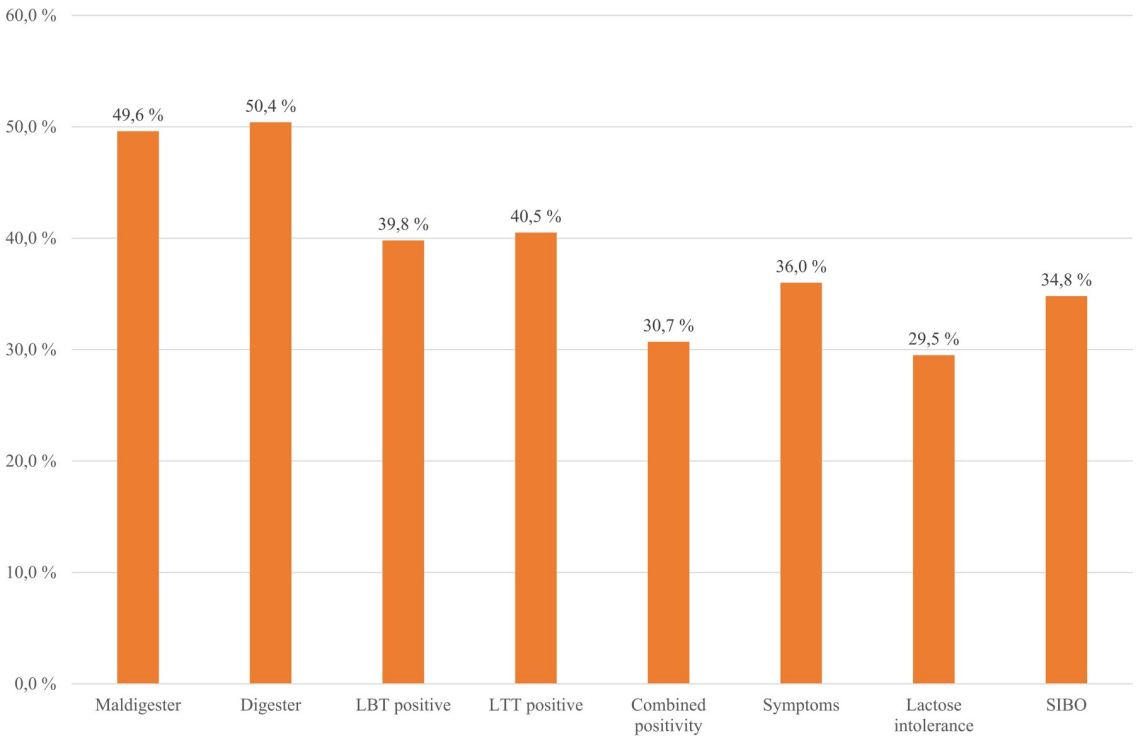

Fig 1. Summary of the basic results in the analyzed study population. A significant, ≥20 ppm elevation of H2 level during LBT and/or less than 1.1 mmol/l rise of blood glucose during LTT were diagnostic for lactose maldigestion. Patients with negative LBT and LTT are lactose digesters. Patients with LM who had symptoms during the test were defined as lactose intolerant. Patients with an early (≤90 min) significant (≥20 ppm) rise of H_2 during LBT and/or lactulose breath test were defined to have SIBO. LBT: lactose breath test; LTT: lactose tolerance test; SIBO: small intestinal bacterial overgrowth.

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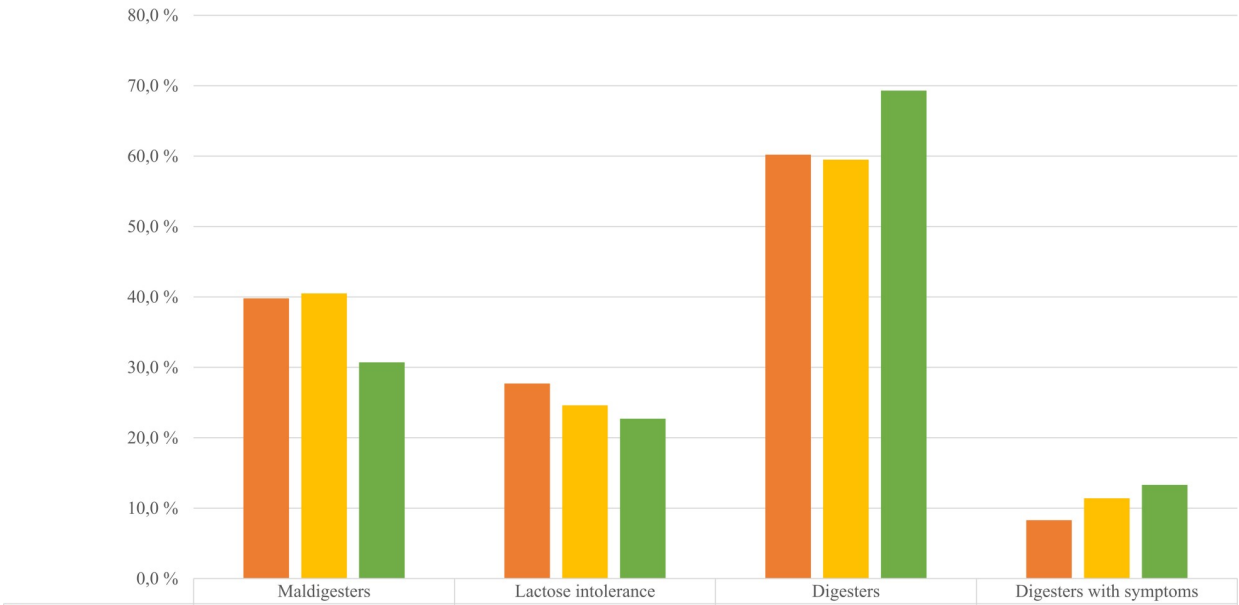
age and digester (p = 0.352), maldigester (p = 0.352), and LI (p = 0.098) status. The basic results of the analyzed population are summarized in Fig 1. The gender-related results are represented in S1 Fig.

Lactose maldigestion and intolerance based on the LBT

Based on the LBT only, 39.8% of the tested study population (105/264) were LM, and 73 of them (69.5%) had symptoms during the test; therefore, 27.7% (73/264) of the population was defined as lactose intolerant (see Fig 2). The majority (159/264, 60.2%) of the patients had a negative LBT, however; 13.8% (22/159) of them had symptoms after lactose ingestion, meaning that 8.3% (22/264) of the analyzed patients had symptoms without a positive test result, as represented in Fig 2. There was a weak negative correlation between age and baseline H_2 (p = 0.009; r = -0.161). There was no significant connection between gender, age, and LBT positivity (gender: $p > 0.05$; age: p = 0.792). The results are summarized in Fig 2.

Lactose maldigestion and intolerance based on the LTT

Based on an analysis of the LTT alone measured in parallel, 40.5% of the same study population (107/264) were maldigesters and 65 of them (60.7%) had symptoms during the test. Therefore, 24.6% (65/264) of the population was defined as lactose intolerant (see Fig 2). The majority (157/264, 59.5%) of the patients had a negative LTT; however, 19.1% (30/157) of them had symptoms after lactose ingestion, meaning that 11.4% (30/264) of the analyzed patients had symptoms without a positive test result (Fig 2). Men had a significantly higher



	Maldigesters	Lactose intolerance	Digesters	Digesters with symptoms
Results from LBT only	39,8 %	27,7 %	60,2 %	8,3 %
Results from LTT only	40,5%	24,6%	59,5%	11,4%
LBT+LTT	30,7%	22,7%	69,3%	13,3%

Fig 2. Summary of the results based separately on the LBT, LTT, and on the combination of them. LBT: lactose breath test; LTT: lactose tolerance test.

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baseline ($p < 0.001$) and maximum ($p = 0.015$) glucose level. There was a moderate positive correlation between age and glucose levels (baseline: $p < 0.001$; $r = 0.338$; maximum: $p < 0.001$; $r = 0.222$). There was no significant connection between gender, age, and LTT positivity (gender: $p > 0.05$; age: $p = 0.378$). The results are summarized in Fig 2.

Combined LHBT and LTT positivity

Combined positivity (LBT+LTT) was found in 30.7% (81/264) of the patients, 74% of them (60/81) had symptoms. Therefore, 22.7% (60/264) of the study population was lactose intolerant based on the combined results (see Fig 2). In the majority (183/264, 69.3%) of the population one or both tests were negative; however, 19.1% (35/183) of them had symptoms meaning that 13.3% (35/264) of the analyzed patients had symptoms without combined test positivity (Fig 2). The results are summarized in Fig 2.

Clinical symptoms

Thirty-six percent (95/264) of the patients had symptoms after lactose ingestion (see Fig 1), bloating being the most frequent (60/264; 22.7%), as seen in Fig 3. There was no statistically significant difference between females and males in the presence of symptoms ($p > 0.05$). Those who had nausea/vomiting were significantly older ($p = 0.014$). Otherwise, there was no statistically significant correlation between age and symptoms ($p = 0.204$). 12.8% (17/133) of the lactose digester patients (the LBT and LTT are negative) and 59.5% (78/131) of the maldigester patients (at least one of the tests is positive) had clinical symptoms (see Fig 4). Based on the latest meta-analysis conducted by our workgroup [34], we hypothesize that IBS may be a contributing factor in LI among lactose maldigesters. Figs 3 and 4 show the frequency of the different symptoms in the study population, and among lactose maldigesters/digesters and

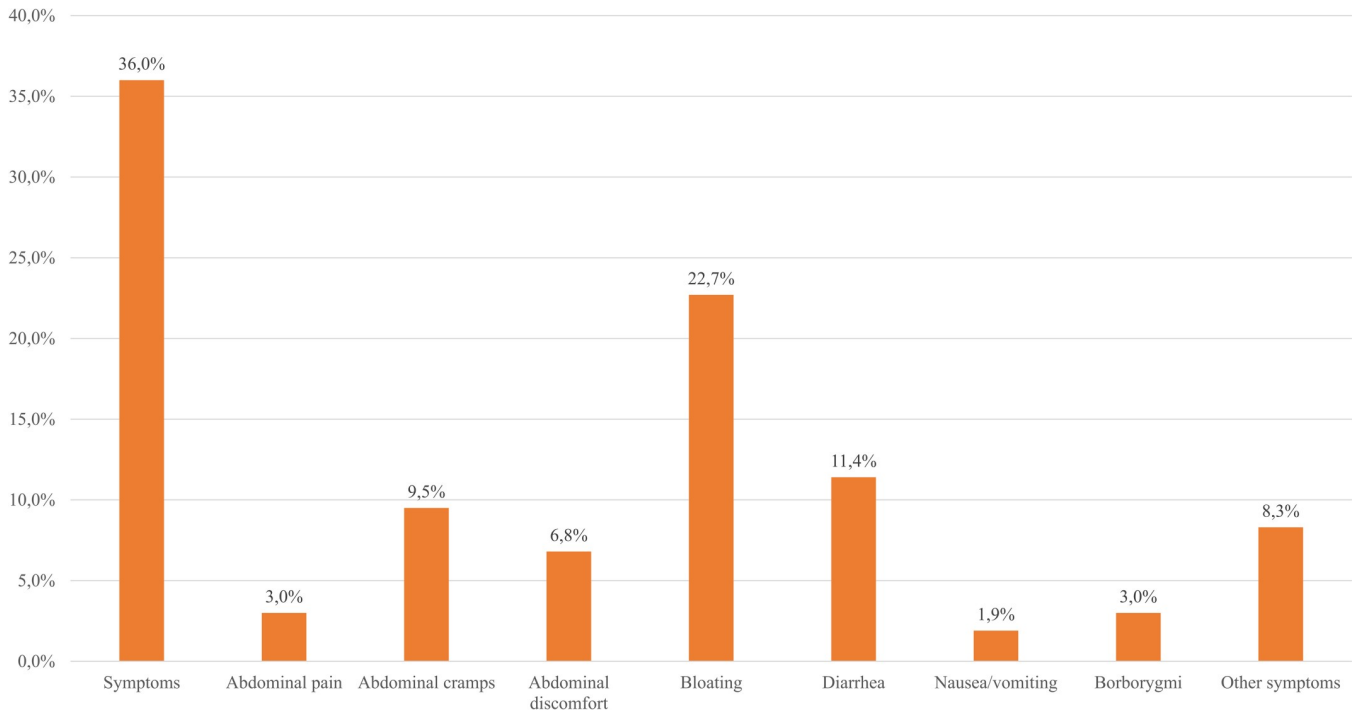

Fig 3. The frequency and distribution of different symptoms in the entire study population. Other symptoms comprise increased bowel motility, flatulence, belching, sensation of fullness in the stomach, headache, burning sensation in the stomach, or increased sensation for defecation.

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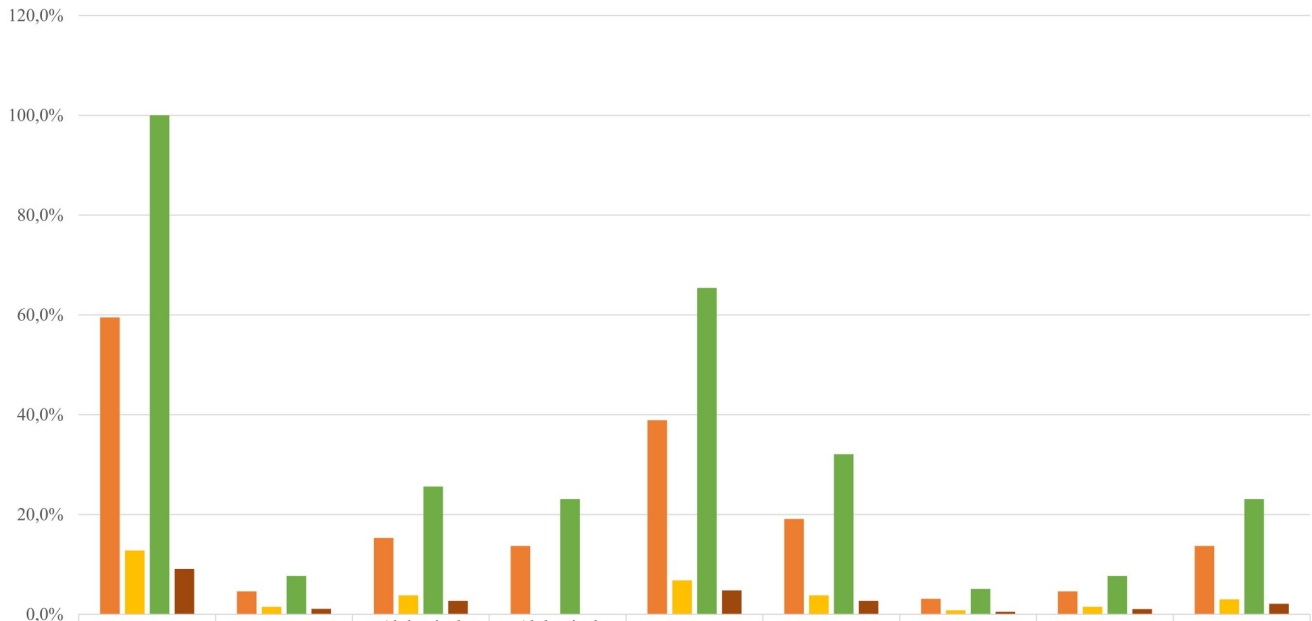
lactose intolerant/tolerant patients. Female/male data regarding symptoms are represented in S2 Fig.

The role of SIBO

Approximately one-third (92/264; 34.8%) of the study population (see Fig 1) and 60% (57/95) of the symptomatic patients had SIBO based on the definition (see Table 1). There was no significant difference in the presence of SIBO between females and males (F: 68/185, 36.8%; M: 24/79, 30.4%, $p > 0.05$); furthermore, there was no significant correlation between age and SIBO ($p = 0.848$). SIBO patients had significantly higher maximum H_2 levels ($p < 0.001$), and they reached the H_2 peak later ($p < 0.001$). Moreover, they had lower maximum glucose levels ($p < 0.001$), and LTT positivity was significantly more frequent in this patient group (OR = 5.833; 95% CI: 3.356–10.138). Symptoms were more common in SIBO patients compared to non-SIBO patients (OR = 5.743; 95% CI: 3.300–9.994), especially abdominal discomfort (OR = 3.201; 95% CI: 1.196–8.565), bloating (OR = 4.798; 95% CI: 2.606–8.833), diarrhea (OR = 6.443; 95% CI: 2.737–15.168), and other symptoms (OR = 5.825; 95% CI: 2.193–15.469).

In 90.9% (240/264) of the patients the LBT gave correct diagnosis (30.7% true positive: 81/264, 60.2% true negative: 159/264) of LM (or the lack of it) using combined LBT and LTT as reference. False positive results were found in 9.1% (24/264) of the cases; however, there are no false negatives in this setting (see Fig 5). LBT in this setting has 100% sensitivity, 86.9% specificity, 77.1% positive predictive value, and 100% negative predictive value. SIBO was found in 76.5% (62/81) of the true positive and in 75% (18/24) of the false positive patients.

In 90.2% (238/264) of the patients the LTT gave correct diagnosis (30.7% true positive: 81/264, 59.5% true negative: 157/264) of LM (or the lack of it) using combined LBT and LTT as



	Symptoms	Abdominal pain	Abdominal cramps	Abdominal discomfort	Bloating	Diarrhea	Nausea/Vomiting	Borborygmi	Other symptoms
Lactose maldigester	59,5%	4,6%	15,3%	13,7%	38,9%	19,1%	3,1%	4,6%	13,7%
Lactose digester	12,8%	1,5%	3,8%	0,0%	6,8%	3,8%	0,8%	1,5%	3,0%
Lactose intolerant	100,0%	7,7%	25,6%	23,1%	65,4%	32,1%	5,1%	7,7%	23,1%
Lactose tolerant	9,1%	1,1%	2,7%	0,0%	4,8%	2,7%	0,5%	1,1%	2,1%

Fig 4. The frequency and distribution of different symptoms among lactose digesters/maldigesters, and among lactose tolerant/intolerant patients. A significant, ≥20 ppm elevation of H2 level during LBT and/or less than 1.1 mmol/l rise of blood glucose during LTT were diagnostic for lactose maldigestion. Patients with negative LBT and LTT are lactose digesters. Patients with LM who had symptoms during the test were defined as lactose intolerant. Other symptoms comprise increased bowel motility, flatulence, belching, sensation of fullness in the stomach, headache, burning sensation in the stomach, or increased sensation for defecation.

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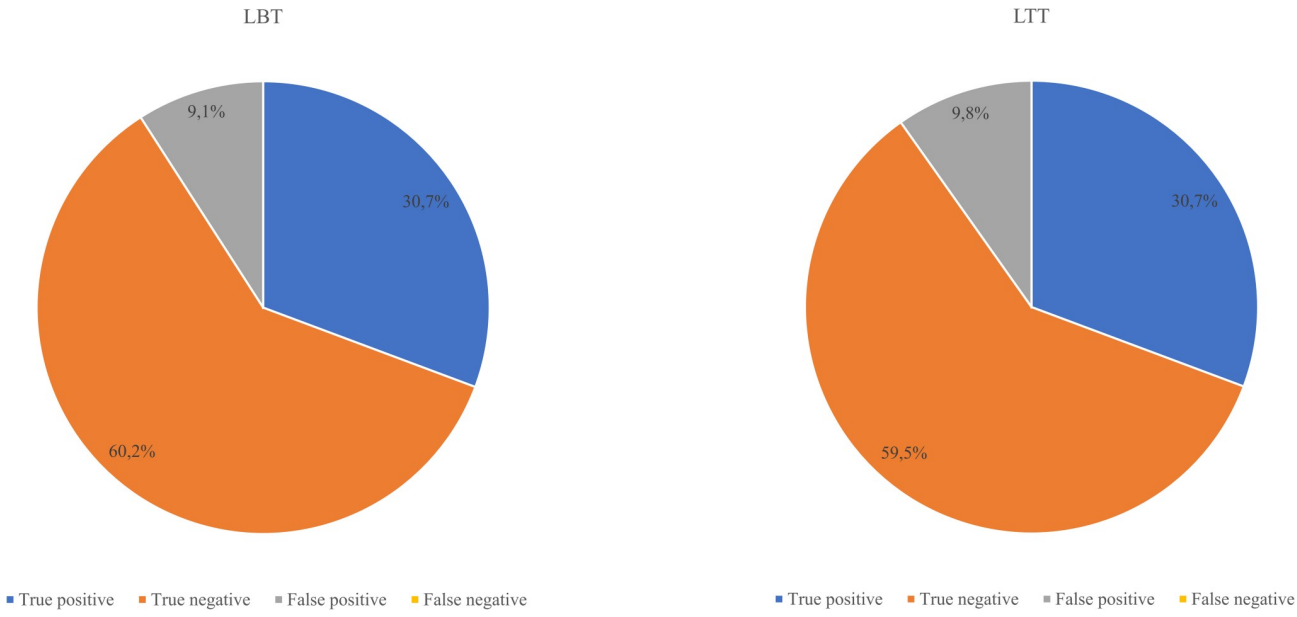

Fig 5. The diagnostic accuracy of the LBT and LTT verified by the combined results of the tests. LBT: lactose breath test; LTT: lactose tolerance test.

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reference. False positive results were found in 9.8% (26/264) of the cases; however, there are no false negatives in this setting (see Fig 5). Therefore, LTT has 100% sensitivity, 85.8% specificity, 75.7% positive predictive value, and 100% negative predictive value. SIBO was found in 76.5% (62/81) of the true positive, but surprisingly in 0% (0/26) of the false positive patients.

Based on these findings the combination of the LBT and LTT and the careful monitoring of results (with e.g. early H_2 rise, parallel performed lactulose breath test) can decrease false results caused by e.g. SIBO.

Discussion

In this retrospective, single-center study, we analyzed the epidemiological characteristics of LI in southwest Hungary and assessed the role of combined diagnostic method and small intestinal bacterial overgrowth in the accuracy of the diagnosis.

LI is a relatively common problem in the white population, affecting approximately 47% of Eastern European adults [2, 6]. There are widely-used, inexpensive, non-invasive, diagnostic methods based on measurement of end-alveolar H_2 concentration (LBT) or blood glucose (LTT) [2–5]. The sensitivity and specificity of these tests are relatively high, but they depend on the ingested lactose dose (25g LBT: 82% and 95%; 25g LTT: 78% and 93%; 50g LBT: 92% and 83%; 50g LTT: 94% and 90%) [35, 36]. Other circumstances, such as SIBO, antibiotic usage, lung diseases, inappropriate preparation, and abnormal gastric emptying can influence their diagnostic accuracy. A combination of these tests and careful evaluation of the results can reduce the false positive or negative cases; however, in most studies, they are used separately [37].

The gold standard diagnostic method is the testing of lactase activity in duodenal and jejunal biopsy samples taken from the mucosa. However, due to the invasiveness, high costs, and patchy enzyme expression, it is less frequently performed compared to the tests noted above. Moreover, it should be considered that similar lactase activity in two patients might result in different LBT results due to the different activity and composition of the intestinal microbiota. There are several genes associated with lactase non-persistence (C/T_13910 with CC genotype; G/A_22018 with GG genotype), but the availability of genetic testing is variable, and its costs are relatively high. Moreover, the lactase non-persistence allele is not always associated with LM [2, 3, 5, 34]. A Hungarian study, published by Nagy et al., determined the applicability of the LBT in comparison with genetic screening (C/T_13910). They found that 37% of the analyzed population had lactase non-persistence, which correlated well with positive LBT results is symptomatic children [38]. We found similar LBT positivity among symptomatic adults. Another retrospective study from Hungary, conducted by Buzás et al., also underlined that both genetic and breath tests are sufficiently accurate [39].

In this study, we presented epidemiological data on the prevalence of LM and LI in southwest Hungary, we analyzed the frequency of the most common symptoms, we demonstrated that combined analysis of LBT and LTT can improve diagnostic accuracy and the parallel testing for SIBO could reduce false cases caused for example by SIBO. It should also be mentioned that the study population had a very large female representation (185 vs 79); however, there were no statistically significant gender-related differences regarding LM, LI, LBT/LTT positivity, symptoms frequency, and prevalence of SIBO, which underlines the literature data in case of LI [2]. Moreover, despite the literature data [7, 8, 13], we did not find any age-related correlations in the outcomes mentioned above.

The limitations of our results should be considered for a correct interpretation, thus possibly influencing outcomes. Firstly, our results are based on a single-center retrospective medical database analysis. Secondly, we only analyzed the results in a one-year period; therefore, the number of enrolled patients is relatively low. Thirdly, the amount of ingested lactose can

influence the prevalence of LM and LI, and the frequency of symptoms. We used a relatively high dose of lactose and we did not perform blinded testing with placebo. Based on the retrospective character, follow-up after antibiotic treatment or low-lactose diet could not be performed to confirm the diagnosis of SIBO and LI based on symptom relief. Moreover, lactulose breath test was performed only in clinically uncertain cases, not on all patients. Therefore, the true diagnosis and prevalence of LI and SIBO could not be assessed correctly. Only patients with high initial end-alveolar H_2 concentration got antiseptic mouthwash. Another significant limitation is that our study group comprises symptomatic patients referred to our clinic, thus potentially leading to sampling bias. It also should be considered that we did not measure methane levels in the end-alveolar gas samples to determine false negative LBT caused by methane producing bacteria. Based on the recent results [40] false negative LBT (5–15%) are mainly caused by methane production. Finally, the symptoms of the patients are subjective, thus possibly prompting inaccurate conclusions. Interpretation of patient-reported symptoms will differ between clinicians; therefore, standardized symptom definitions should have been used to minimize errors. According to the Oxford Centre for Evidence-Based Medicine 2011, the evidence level of our findings is level 3 [41].

Conclusions

Based on our results, we can conclude that the prevalence of LI is lower in Hungary compared to the Eastern European value (29.5% vs 47%) and that it is worth performing a population-based prospective analysis in this area. During the provocation tests, 59.5% of lactose maldigesters had IBS-like symptoms (lactose intolerance), but the role of IBS in the background is unknown. SIBO was relatively common among symptomatic patients (60%), and this may influence the diagnostic accuracy of lactose maldigestion, based on the LBT and LTT as the only diagnostic test. Therefore, a combination of the LBT and LTT and careful monitoring of results may decrease the false cases caused by e.g. SIBO.

Supporting information

S1 File. STROBE checklist. STROBE: Strengthening the Reporting of Observational Studies in Epidemiology [32].
(DOCX)

S1 Fig. The summary of the basic results among females/males. A significant, $\geq$20 ppm elevation of H2 level during LBT and/or less than 1.1 mmol/l rise of blood glucose during the LTT were diagnostic for lactose maldigestion. Patients with negative LBT and LTT are lactose digesters. Patients with LM who had symptoms during the test were defined as lactose intolerant. Patients with an early ($\leq$90 min) significant ($\geq$20 ppm) rise of H_2 during LBT and/or lactulose breath test were defined to have SIBO. LBT: lactose breath test; LTT: lactose tolerance test; SIBO: small intestinal bacterial overgrowth.
(TIF)

S2 Fig. The frequency and distribution of different symptoms among females/males. Other symptoms comprise increased bowel motility, flatulence, belching, sensation of fullness in the stomach, headache, burning sensation in the stomach, or increased sensation for defecation.
(TIF)

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REVIEW ARTICLE

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Lactose intolerance but not lactose maldigestion is more frequent in patients with irritable bowel syndrome than in healthy controls: A meta-analysis

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Abstract

Background and Purpose: Irritable bowel syndrome (IBS) affects 10%-20% of the adult population and is characterized by abdominal symptoms without relevant organic disease. There are numerous clinical trials available investigating the relationship between IBS, lactose maldigestion (LM), and lactose intolerance (LI), but there have been no meta-analyses on this topic yet. We aimed to assess the prevalence of LM, objective and subjective (self-reported) LI in IBS patients compared to healthy controls (HC) without IBS.

Methods: A systematic literature search was conducted up to 24 April 2018 in PubMed, Embase, and Cochrane Library. Adult IBS patients had to be diagnosed according to the Rome criteria or other well-defined criteria system. We enrolled controlled studies including healthy adult participants without IBS, as control group. Odds ratios with 95% confidence intervals were calculated.

Key Results: Altogether 14 articles were suitable for statistical analyses. IBS patients reported themselves significantly more frequently lactose intolerant than HCs (odds ratio [OR] = 3.499; 95% confidence interval [CI] = 1.622-7.551). Generally, there was no significant difference in the prevalence of LM based on ingested lactose dose

Abbreviations: CI, confidence interval; CMA, Comprehensive Meta-Analysis Software; FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; HC, healthy control; IBS, irritable bowel syndrome; IBS-D/C/M/A/U, irritable bowel syndrome diarrheal/constipation/mixed/alternating/unclassified form; IQR, interquartile range; LBT, lactose breath test; LI, lactose intolerance; LM, lactose maldigestion; LTT, lactose tolerance test; MRI, magnetic resonance imaging; NOS, Newcastle-Ottawa Scale; OR, odds ratio; PICO, Participants, Intervention, Comparison, Outcomes; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; SD, standard deviation; VAS, Visual Analog Scale.

(OR = 1.122; 95% CI = 0.929-1.356) and test type (OR = 1.156; 95% CI = 0.985-1.356). However, significantly more IBS patients had objective LI (OR = 2.521; 95% CI = 1.280-4.965).

Conclusions and Inferences: Lactose intolerance, but not LM is more frequent among patients with IBS compared to HCs. According to our results, IBS among other functional bowel disorders is a possible contributing factor of LI in people with LM.

KEYWORDS

irritable bowel syndrome, lactose intolerance, lactose maldigestion

1 | INTRODUCTION

Irritable bowel syndrome (IBS) is one of the most frequently diagnosed disorders in gastroenterology, which can be defined by the Rome IV criteria system.[1-3] It is characterized by abdominal pain related to defecation, and associated with a change in stool frequency or consistency (diarrhea, constipation, or a combination of these), without any organic disease or pathological abnormality of the gut-wall.[4] Four subtypes of IBS can be separated: diarrheal (IBS-D), constipation (IBS-C), mixed or alternating (IBS-M/A) and unclassified (IBS-U) form.[5,6] IBS can lead to significant quality of life impairment, decreased work productivity and an increase of health care and social costs.[7-10] The prevalence of IBS is high in Western countries, affecting 10%-20% of the adult population.[11-13] Its pathogenesis remains unknown, but numerous factors may contribute to its development.[3,14-16] Treatment is often multimodal, comprising of non-pharmacological and pharmacological methods. A novel effective treatment option is a low-FODMAP diet (Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols), which suggests that certain food types, containing disaccharides like lactose, can trigger symptoms of patients with IBS.[17-19]

Lactose intolerance (LI) is a condition characterized by clinical symptoms after ingestion of lactose-containing products, caused by lactose maldigestion (LM).[20] The most common cause of LM is primary (adult-type) hypolactasia.[3] LI affects 25% of the Caucasian population. Males and females are equally affected.[21,22] Because of lactase deficiency, lactose can reach the large intestine where it is fermented by colonic bacteria. Short-chain fatty acids, gases (H_2, CO_2 and CH_4) and other products will be produced by the fermentation which can cause luminal distension and lead to different gastrointestinal symptoms. The most common complaints are abdominal pain and discomfort, bloating, flatulence, and diarrhea, similarly as in IBS.[20,23-25] Due to the potential pathogenetic factors of IBS (altered gastrointestinal motility, changes of gut microbiome, visceral hypersensitivity, anxiety, etc), food intolerances, such as LI, are more frequent in this disease, however, the prevalence of LM does not differ compared with the healthy population. More IBS patients have symptoms at lower lactose doses and their symptoms are more severe. Moreover, many IBS patients think that their abdominal symptoms are related to lactose intake, even though no objective tests of LM were carried out.[26-30] The available diagnostic methods for diagnosing LM or LI are based on several approaches, including lactose breath test (LBT), lactose tolerance test (LTT), genetic test and assessment of lactase activity in jejunal biopsy specimens.[3] The restriction of lactose intake or the replacement of the lactase enzyme can alleviate these symptoms.[3,21]

Key Points

- The connection between IBS and lactose intolerance is not clearly described yet, therefore we performed meta-analysis to explore this association.
- We proved that lactose intolerance is more common in IBS, however, the frequency of lactose maldigestion is almost the same compared to healthy people.
- This suggests that IBS is a possible contributing factor in lactose intolerance among lactose maldigesters.

There are numerous clinical trials investigating the connection between IBS, LM, and LI, but to our best knowledge no meta-analyses have been performed up to this day.

Given the uncertain connection between IBS and lactose consumption-related disorders, we performed a systematic literature search and meta-analysis in this important topic with the aim to assess the prevalence of LM, objective and subjective LI in IBS patients compared to healthy controls (HC).

2 | MATERIALS AND METHODS

Our work was planned and conducted according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2009 Statement (Table S1).

2.1 | Searching strategy

Our systematic literature search was based on the PICO format: Participants: subjects who underwent any form of LM or LI assessment; Intervention: IBS patients; Comparison: healthy controls;

Outcomes: prevalence of LM, subjective/objective LI. It was conducted by two independent reviewers (JC and PV) to find all relevant articles on the prevalence of LM, subjective and objective LI in IBS compared to HCs, up to 24 April 2018 (first search: 20 June 2017). The search covered three major databases (PubMed, Embase, and the Cochrane Library) with the terms "('irritable bowel syndrome' OR 'IBS') AND ('lactose intolerance' OR 'lactose maldigestion' OR 'lactose malabsorption')." The reference lists of the relevant articles were hand searched and all appropriate records identified were included in the screening process. After this search process, language (only English) and species (only humans) filters were used. Duplicates were removed with EndNote X4 and manually, and then title and abstract screening was performed by the two reviewers to identify potentially eligible articles. Disagreements were resolved by consensus.

2.2 | Eligibility criteria

In our meta-analysis, we included all studies investigating the connection between IBS, lactose consumption-related symptoms, and maldigestion in comparison with HC group. Retrospective studies were also included. The length of follow-up was not a reason for either inclusion or exclusion. Only articles written in English and those examining the effect of lactose ingestion in human IBS patients were included in this study. Short conference abstracts or papers not available in full-text format were excluded. By definition, adult IBS patients (17 years or above) had to be diagnosed according to the Rome or, in articles that were not recently published, according to any other well-defined criteria system. Articles without clear definitions of IBS, or in which small intestinal bacterial overgrowth or any other organic diseases (inflammatory bowel disease, celiac disease, etc) were reported or suspected in the background, were excluded from the analysis. We enrolled controlled studies which included healthy adult participants (without organic disease) who did not fulfill IBS criteria, as a control group. Only articles reporting data about the prevalence of LM and/or subjective/objective LI in IBS and HC group were analyzed statistically.

2.3 | Quality assessment of the individual studies

The quality and the biases of the included studies were analyzed with the Newcastle-Ottawa Scale (NOS) for case-control studies.[31] Two authors (IMC, PV) independently assessed the risk of bias in each paper included in the statistical analysis. Disagreements were resolved by consensus. If the discussion did not result in consensus, a third author was consulted (PH). The NOS for case-control studies contains eight items covering three main domains (selection, comparability and exposure). A study can be awarded a maximum of one star for each numbered item; on the contrary, a maximum of two stars can be given for comparability. Each item was rated as "high risk" (zero stars), "low risk" (one star) or "unclear risk" (zero stars) corresponding to the definitions.

2.4 | Data extraction

At the end of the screening process, relevant data were independently extracted from studies by two independent reviewers (JC and PV). These included: prevalence of LM and LI (subjective or objective) as the outcome parameters, first author, year of publication and country of origin, study design, basic characteristics of the study population (age, percentage of females and IBS subtypes, size of the study groups), diagnostic criteria for IBS, diagnostic methods, thresholds and lactose dose used to diagnose maldigestion. Data for the risk of bias (NOS) assessment were collected as well. Extracted data were validated by five co-authors (ZsSz; DP; MB; ÁV; JT).

2.5 | Outcome measure

The prevalence of LM, subjective and objective LI were the main outcome parameters in our analysis. LM can be diagnosed through different ways,[21] the non-invasive and inexpensive LBT and LTT being the most common methods. The sensitivity and specificity of these tests depends on the lactose dose, but they are relatively high (78% and 93%).[32] Before (baseline) and after the ingestion of a given amount of lactose, breath and blood samples are collected at different time points for a period of time and end-alveolar H_2 and blood glucose concentrations are measured. A certain rise of H_2 (or additionally methane) and/or no rise of blood glucose (or additionally galactose) above the baseline levels are considered diagnostic for lactose maldigestion. The amount of ingested lactose and the diagnostic thresholds were different in the studies. Testing of lactase activity in mucosal biopsy samples from duodenum or jejunum is the gold standard method in the diagnosis of LM, but due to the invasiveness, high costs and patchy expression of the enzyme it is performed less frequently, compared to the tests mentioned above. The availability of genetic testing of the genes associated with lactase non-persistence (C/T_13910 with CC genotype; G/A_22018 with GG genotype) is variable, and its costs are relatively high.[21]

Participants with LM who had abdominal symptoms during or shortly after lactose test were defined as objectively lactose intolerant. Participants reporting before any tests, that their symptoms can be in connection with ingestion of lactose-containing products, were defined as subjectively lactose intolerant.

2.6 | Statistical analysis

Pooled odds ratios (OR) were calculated with 95% confidence intervals (CI). Random effects and fixed model were applied at all of analyses with DerSimonian-Laird[33] estimation. Statistical heterogeneity was analyzed using the I^2 and the chi-square test to gain probability-values; $P < 0.1$ was defined to indicate significant heterogeneity.[34] Subgroups of test type (LBT, LTT, lactase activity, and genetic test) and lactose dosages (10-18 g, 20-25 g, and 40-50 g) were created in the analysis on the outcomes. Statistical analyses were performed using the Comprehensive Meta-Analysis Software (CMA, Biostat, NJ, USA). Forest plots were used to present the results of

the meta-analyses. To check for publication bias, the visual inspection of funnel plots and Eggers' tests were performed.

3 | RESULTS

3.1 | Search results

Using the terms mentioned above, we found 647 articles in the three databases for evaluation, 213 in PubMed, 413 in Embase and 21 in Cochrane Library. We also examined 14 further articles from the reference lists of relevant articles, so 661 articles were found in total. After using the language (only English) and species (only humans) filters in Embase, PubMed and the Cochrane Library, 520 of 647 studies were further assessed and none of the articles from the reference lists were excluded. After removing duplicates, title and abstract screening, 89 articles reporting on lactose consumption-related disorders in IBS and eligible for further evaluation were found. The detailed screening of the full-text papers identified 16 articles for further assessment, of which two were not suitable for statistical analysis. Altogether 14 case-control studies met the inclusion criteria and remained for quantitative analysis.[26,27,35-46] The flow chart of the systematic literature search was based on the PRISMA 2009 guideline and is detailed on Figure 1. At the time of the literature search, we found no eligible paper that used the most recent diagnostic criteria (Rome IV) for IBS. The basic characteristics of the articles and the raw data are summarized in Tables 1 and S2. The proportion of each IBS subtype and the used lactose doses, diagnostic methods for LM and thresholds in the studies included in the meta-analysis are detailed in Table 2. A quality assessment (NOS) of the articles is summarized in Tables 3 and S3.

3.2 | Lactose maldigestion and IBS

In 13 of the 14 articles, LM was objectively tested with LBT, LTT or genetic testing. There were not enough controlled studies with lactase activity measurement to carry out a correct statistical analysis. In one of the included case-control studies, only subjective LI was assessed.[42]

Based on the ingested lactose dose used in the different studies three subgroups were made: 10-18 g; 20-25 g; 40-50 g (Figure 2). Overall there was no significant difference in the prevalence of LM between IBS and HC groups (OR = 1.122; 95% CI: 0.929-1.356; $P = 0.232$). The I^2 test showed no significant heterogeneity ($I^2 = 0.000\%$; $P = 0.479$). We did not find significant difference either between ($P = 0.121$), or within the subgroups: (1) OR = 1.420, 95% CI: 0.873-2.309, $P = 0.158$ ($I^2 = 0.000\%$; $P = 0.810$); (2) OR = 0.926, 95% CI: 0.711-1.206, $P = 0.568$ ($I^2 = 11.037\%$; $P = 0.338$); (3) OR = 1.356, 95% CI: 0.977-1.882, $P = 0.068$ ($I^2 = 0.000\%$; $P = 0.651$). There was no significant heterogeneity within the subgroups.

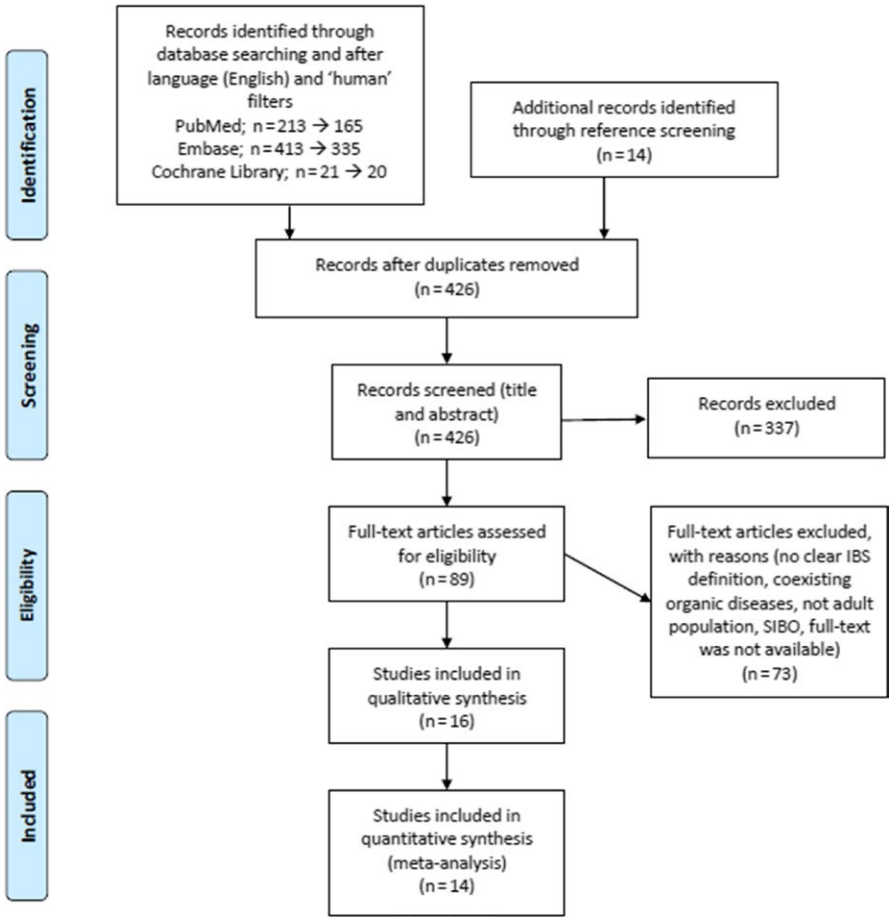


FIGURE 1 PRISMA-flowchart of the systematic literature search. IBS: irritable bowel syndrome; SIBO: small intestinal bacterial overgrowth

TABLE 1 Characteristics of the studies included in the statistical analyses

First author, year, reference number	Country	Study design	IBS diagnostic criteria	Number of participants (IBS/HC)	Age (IBS/HC), years	Percentage of females (IBS/HC)
Bianchi Porro et al. (1983)[35]	Italy	Case-control	Unspecific abdominal complaint (primarily colicky abdominal pain and/or intermittent diarrhea or dyspepsia) for at least 1 year without any organic disease	77/40	Group 1: mean 42.2 (range: 19-53); group 2: mean 41.2 (range: 18-54)/mean 35.05 (range: 18-55)	51.9/52.5
Gwee et al. (1996)[36]	UK	Case-control	Rome criteria	22/53	Median 34 (IQR: 23.5-51)/median 36 (IQR: 27-52)	77/36
Vesa et al. (1998)[37]	Finland	Case-control	Rome criteria	63/364	Mean 51 ± 9 SD/mean 50 ± 9 SD	68/50
Goldstein et al. (2000)[38]	Israel	Case-control	Rome criteria	94/145	Women: mean 44.4 ± 17.5 SD; men: mean 42.7 ± 18.6 SD/women: mean 49.6 ± 18.9 SD men: mean 35.7 ± 15.7 SD	No data
Vernia et al. (2001)[27]	Italy	Case-control	Rome criteria	503/336	Women: mean 37.8 ± 13.9 SD men: mean 36.2 ± 13.9 SD / women: mean 36.1 ± 14.8 SD men: mean 32.1 ± 13.5 SD	66.7/65.1
Parry et al. (2002)[39]	UK	Case-control	Rome II	16/18	Mean 44.4 (range 25-76)	No data/55.5
Lanng et al. (2003)[40]	Denmark	Case-control	Kay and Jørgensen criteria: More than weekly experience of abdominal pain and distension and in addition either borborygmi or altering stool consistency	32/26	Mean 51.8/mean 52.8	75/53.8
Farup et al. (2004)[41]	Norway	Case-control	Rome II	82/105	Mean 48.8/mean 46.3	68/78
Saberi-Firoozi et al. (2007)[42]	Iran	Case-control	Rome II	215/1763	Mean 49.9 ± 11.14 SD	64.2
Corlew-Roath et al. (2009)[43]	USA	Case-control	Rome III	66/55	All participants over 18 years	86/62
Yakoob et al. (2011)[44]	Pakistan	Case-control	Rome III	119 /115	Mean 35 ± 13 SD (range: 18-74)/mean 36 ± 15 SD (range: 18-80)	26.05/33.04
Kumar et al. (2012)[45]	India	Case-control	Rome III	150/252	Mean 36.7 ± 11.8 SD/mean 37.2 ± 11.5 SD	24/22
Yang et al. (2013)[26]	China	Case-control	Rome III	60/60	Mean 40.8 ± 11.7 SD/mean 40.8 ± 15.2 SD	51.6/48.3
Xiong et al. (2017)[46]	China	Case-control	Rome III	109/50	Mean 36.0 ± 12.2 SD/mean 34.8 ± 13.3 SD	47.7/48

HC, healthy control; IBS, irritable bowel syndrome; IQR, interquartile range; SD, standard deviation.

TABLE 2 The percentage of IBS subtypes and the diagnostic methods and thresholds used in the analyzed studies

First author, year, reference number	IBS subtypes (%)	Diagnostic method for LM	Amount of lactose (g)	Diagnostic threshold for LM
Bianchi Porro et al. (1983)[35]	No data	LBT, LTT, lactase activity (jejunal biopsy)	LBT: 50 LTT: 100	LBT: >20 ppm H_2 rise LTT: <20 mg/100 mL rise of blood glucose lactase activity: ≤39 IU/g protein
Gwee et al. (1996)[36]	IBS-D: 86 IBS-C: 9 IBS-M/A: 5	LBT	50	No data
Vesa et al. (1998)[37]	No data	LTT	50	Blood glucose elevation <1.1 mmol/L (20 mg/100 mL) and maximal rise in blood galactose concentration ≤0.3 mmol/L (5 mg/100 mL)
Goldstein et al. (2000)[38]	No data	LBT	18	≥20 ppm rise of H_2 or ≥5 ppm rise of CH_4 over baseline value
Vernia et al. (2001)[27]	IBS-D: 24.8 IBS-C: 13.3 IBS-M/A: 17.1	LBT	0.5 g/kg body weight up to a maximum of 25 g	H_2 peak exceeding 20 ppm over the baseline values
Parry et al. (2002)[39]	No data	LBT, LTT	50	A failure of plasma glucose to rise by more than 1.1 mmol/L from baseline. A rise in the breath hydrogen value above 20 ppm from baseline
Lanng et al. (2003)[40]	No data	LTT	50	Glucose level rise ≤1.3 mmol/L
Farup et al. (2004)[41]	No data	LBT	25	Peak values of H_2 breath excretion >20 ppm above the lowest preceding value, peak CH_4 excretion >12 ppm above baseline, and/or combined H_2 and CH_4 increase >15 ppm were considered diagnostic
Saberi-Firoozi et al. (2007)[42]	No data	-	-	-
Corlew-Roath et al. (2009)[43]	No data	LBT	50	H_2, CH_4, and CO_2 were tested (threshold: no data)
Yakoob et al. (2011)[44]	IBS-D: 100	LBT	50	H_2 rise above baseline of 20 ppm
Kumar et al. (2012)[45]	IBS-D: 52 IBS-C: 35 IBS-M/A: 13	Genetic test	-	C/T_13910 (CC genotype)/G/A_22018 genetic variant (GG genotype)
Yang et al. (2013)[26]	IBS-D: 100	LBT, genetic test	10, 20, 40	≥20 ppm H_2 rise above the baseline, C/T_13910 (CC genotype)
Xiong et al. (2014)[46]	IBS-D: 100	LBT	25	Peak hydrogen breath excretion of 20 ppm above the baseline level

IBS-D/C/M/A, irritable bowel syndrome-diarrheal/constipation/mixed/alternating subtype; LBT, lactose breath test; LM: lactose maldigestion; LTT, lactose tolerance test.

According to the test methods, three subgroups were made: (1) genetic test; (2) LBT and (3) LTT (Figure 3). Overall, there was no significant difference in the prevalence of LM between IBS patients and HCs (OR = 1.156; 95% CI: 0.985-1.356; $P = 0.077$) and the analyzed studies were homogeneous ($I^2 = 0.548\%$; $P = 0.590$). We did not find significant difference either between ($P = 0.548$) or within the subgroups: (1) OR = 1.243, 95% CI: 0.922-1.677, $P = 0.154$ ($I^2 = 0.000\%$; $P = 0.664$); (2) OR = 1.159, 95% CI: 0.948-1.416, $P = 0.150$ ($I^2 = 4.977\%$; $P = 0.396$); (3) OR = 0.868, 95% CI: 0.492-1.533, $P = 0.626$ ($I^2 = 0.000\%$; $P = 0.561$). There was no significant heterogeneity within the subgroups.

Based on the test type and ingested amount of lactose, four subgroups were made: (1) 20-25 g LBT; (2) 40-50 g LBT; (3) 40-50 g LTT and (4) 10-18 g LBT (Figure 4). Overall there was no significant difference between IBS and control groups in the prevalence of LM (OR = 1.122; 95% CI: 0.929-1.356; $P = 0.232$) and the analyzed studies were homogeneous ($I^2 = 0.000\%$; $P = 0.479$). LM was more frequent among IBS patients who underwent LBT with 40-50 g lactose (b) compared to HCs (OR = 1.692; 95% CI: 1.134-2.527; $P = 0.010$; $I^2 = 0.000\%$; $P = 0.938$). Between ($P = 0.051$) and within the other subgroups there was no significant difference: (1) OR = 0.926, 95% CI: 0.711-1.206, $P = 0.568$ ($I^2 = 11.037\%$; $P = 0.338$); (c) OR = 0.868,

TABLE 3 The quality and risk of bias assessment of the included studies according to Newcastle-Ottawa Scale for case-control studies[31]

First author, year, reference number	Selection				Comparability	Exposure			NOS summarized score
	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	
Bianchi Porro et al. (1983)[35]	0	*	*	*	**	*	*	0	7/9
Gwee et al. (1996)[36]	0	*	0	0	*	*	*	0	4/9
Vesa et al. (1998)[37]	*	0	*	*	*	*	*	0	6/9
Goldstein et al. (2000)[38]	*	*	0	0	**	*	*	0	6/9
Vernia et al. (2001)[27]	*	*	*	*	0	*	*	0	6/9
Parry et al. (2002)[39]	*	*	0	*	*	*	*	0	6/9
Lanng et al. (2003)[40]	*	*	*	*	**	*	*	0	8/9
Farup et al. (2004)[41]	*	*	*	*	**	*	*	0	8/9
Saberi-Firoozi et al. (2007)[42]	*	*	*	0	0	*	0	0	4/9
Corlew-Roath et al. (2009)[43]	*	*	0	0	0	*	*	0	4/9
Yakoob et al. (2011)[44]	*	*	0	*	**	*	*	*	8/9
Kumar et al. (2012)[45]	*	*	0	0	**	*	*	0	6/9
Yang et al. (2013)[26]	*	*	0	*	**	*	*	0	7/9
Xiong et al. (2017)[46]	*	*	*	*	**	*	*	*	9/9

The NOS consists of eight numbered items, divided into three main sections (selection, comparability, and exposure). Each numbered item can be rewarded with maximum one star; comparability can be awarded with two stars. The studies with maximum of nine stars representing the highest-quality trials with the lowest risk of bias. The detailed analysis of each study is represented in Table S3.
NOS, Newcastle-Ottawa Scale.

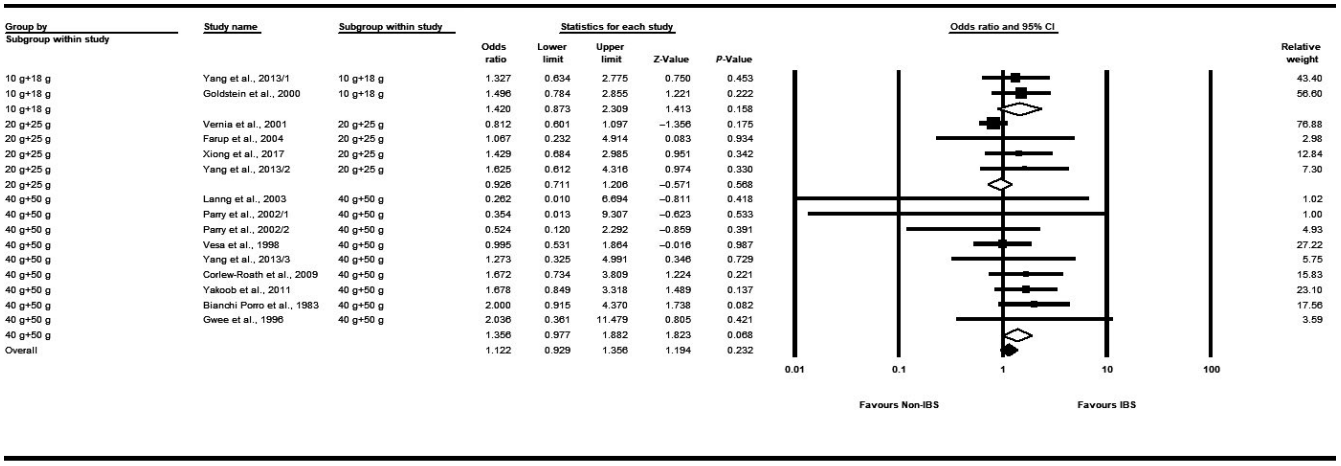


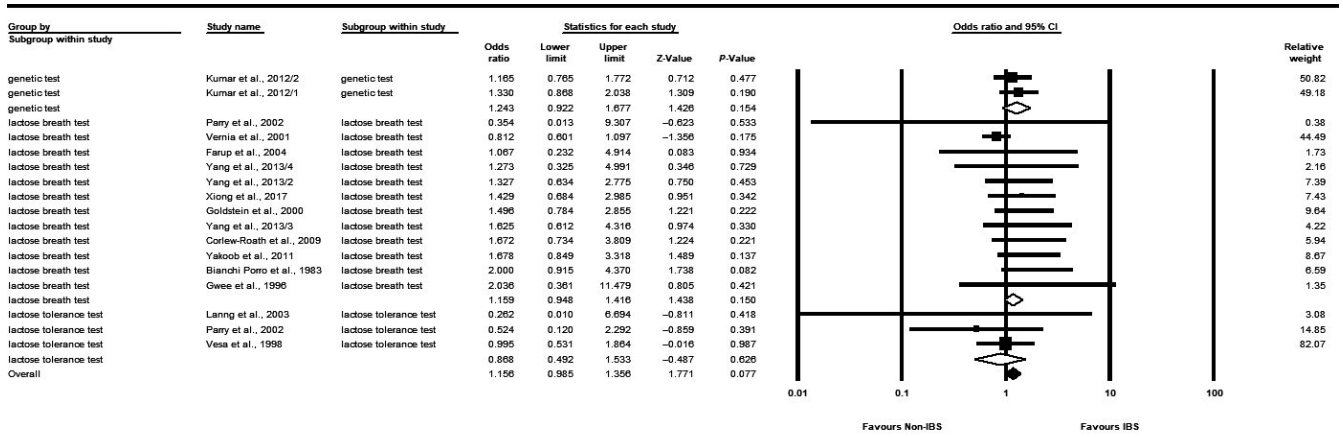
FIGURE 2 The difference of LM between IBS and HCs, based on the ingested lactose dose (10-18 g, 20-25 g, 40-50 g). There was no significant difference either overall, or in the subgroups. HC, healthy controls; IBS, irritable bowel syndrome; LM, lactose maldigestion

95% CI: 0.492-1.533, $P = 0.626$ ($I^2 = 0.000\%$; $P = 0.561$); (4) OR = 1.420, 95% CI: 0.873-2.309, $P = 0.158$ ($I^2 = 0.000\%$; $P = 0.479$). There was no significant heterogeneity within the subgroups.

3.3 | Lactose intolerance

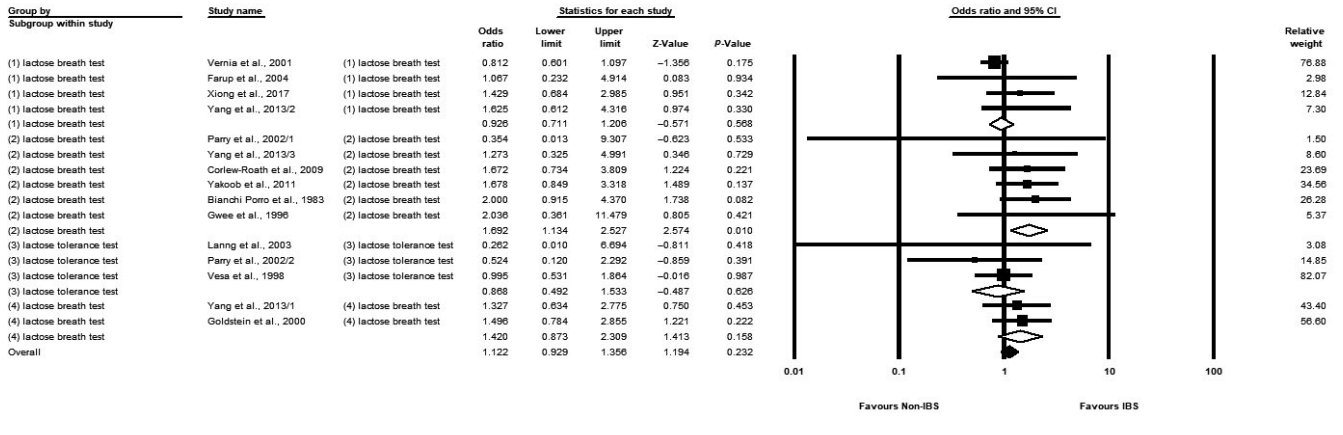
Only four case-control studies published data about self-reported (subjective) LI.[26,37,41,42] Our results (Figure 5) showed that subjective LI was more common in IBS compared to HCs, patients reported more often that their abdominal symptoms can be related to lactose-containing products (OR = 3.499; 95% CI: 1.622-7.551; $P = 0.001$). The examined population was significantly heterogeneous ($I^2 = 86.774\%$; $P = 0.000$).

There were three articles available reporting on objective LI (Figure 6).[26,27,46] Significantly more maldigester IBS patients reported abdominal symptoms during or shortly after the diagnostic test compared to controls (OR = 2.521; 95% CI: 1.280-4.965; $P = 0.008$), but our result is limited by the heterogeneity of the analyzed population ($I^2 = 74.866\%$; $P = 0.003$).



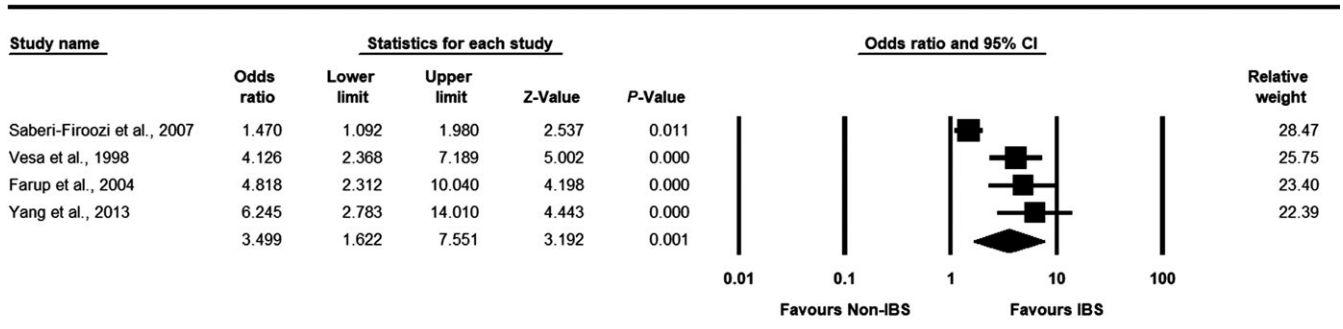
Meta Analysis

FIGURE 3 The difference of LM between IBS and HCs, based on the diagnostic method (LBT, LTT, genetic test). There was no significant difference either overall, or in the subgroups. HC, healthy controls; IBS, irritable bowel syndrome; LBT, lactose breath test; LM, lactose maldigestion; LTT, lactose tolerance test



Meta Analysis

FIGURE 4 The difference of LM between IBS and HCs, based on the lactose dose and diagnostic method. LM was significantly more frequent in IBS only at the LBT with the highest lactose dose (40-50 g). HC, healthy controls; IBS, irritable bowel syndrome; LBT, lactose breath test; LM, lactose maldigestion



Meta Analysis

FIGURE 5 The difference of subjective (self-reported) LI between IBS and HCs. Subjective LI was significantly ($P = 0.001$) more frequent in IBS compared to the control group. HC, healthy controls; IBS, irritable bowel syndrome; LI, lactose intolerance

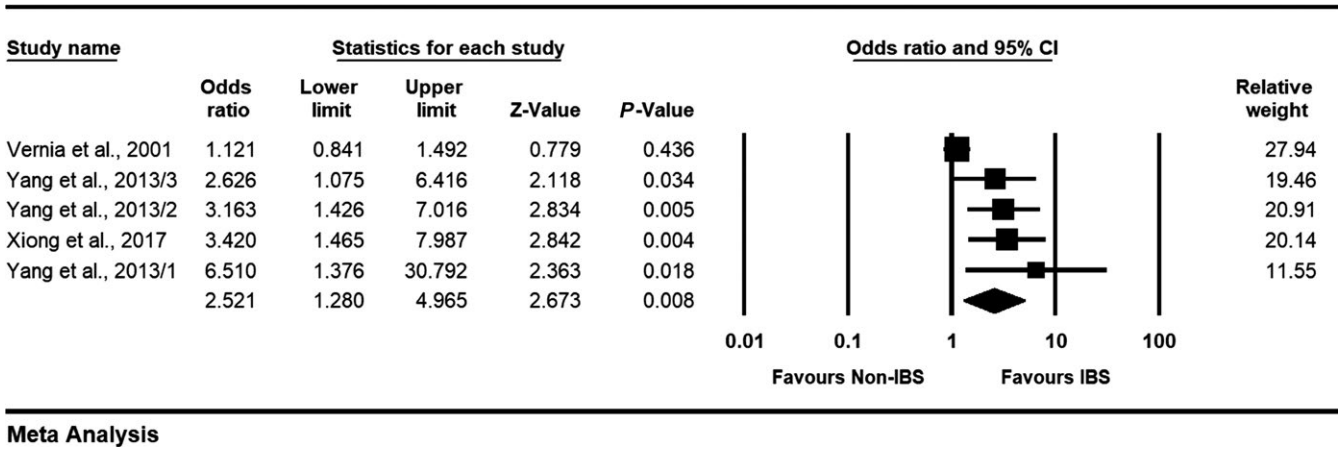


FIGURE 6 The difference of objective LI between IBS and HCs. Objective LI was significantly ($P = 0.008$) more frequent in IBS compared to the control group. HC, healthy controls; IBS, irritable bowel syndrome; LI, lactose intolerance

4 | DISCUSSION

A growing number of studies have shown that intolerance to lactose-containing products and other food types is more frequent among patients with IBS than among healthy subjects, but to our best knowledge, no meta-analysis investigated the association between these two conditions so far. Only two recent reviews by Borghini and Bayless et al.[3,47] discuss the correlation between IBS and LI.

We carried out a systematic literature search and quantitative data (meta-) analysis on the topic. A pooled analysis of 14 case-control trials confirmed a significantly higher prevalence of subjective and objective LI, whereas nearly the same prevalence of LM in IBS patients compared to healthy participants. The underlying mechanism remains unknown, but common etiological factors like psychological (eg anxiety) and gastrointestinal dysfunctions (eg visceral hypersensitivity and altered gut transit) might play a role.[28-30] The visceral hypersensitivity can also be in connection with altered gut microbiome. Gut microbiota of IBS patients is generally reduced and has lower diversity, compared to healthy controls.[48] It has been shown that potentially pathogenic bacteria (eg Clostridium spp, Ruminococcus spp, Streptococcus spp, Enterobacteriaceae members) are more concentrated in IBS patients than in controls.[49-52] A recent MRI (magnetic resonance imaging) study concluded that visceral hypersensitivity, rather than excessive gas production is responsible for carbohydrate associated symptoms in patients with IBS.[53] The hypersensitivity to colonic distension can be transferred to mice by fecal transplantation which highlights the role of microbiome.[54] Moreover, gut microbiota produces many neuroactive or neuromodulatory metabolites (histamine, serotonine, gamma-aminobutyric acid, brain derived neurotrophic factor, etc), which can potentially lead to peripheral or central neural sensitization.[55,56]

Most studies have shown a beneficial effect of lactose-free or restricted diet in IBS.[25,57,58] One reason might be that lactose belongs to FODMAPs, which are poorly absorbed carbohydrates leading to increased water content in the bowel based on the compounds' osmotic effect and increased gas production by colonic bacterial flora, inducing symptoms in patients with IBS and numerous patients with functional gastrointestinal disorders. Based on these findings, a low-FODMAP diet could be beneficial in these patients.[17-19]

In the present study, the pooled sample size was large concerning the key question and the random effects and fixed model were used with the DerSimonian and Laird method[33] for analysis. Study data reflected no publication bias according to the analyses of LM status (Figures S1, S2 and S3), but showed significant bias (small study effect) based on heterogeneity in forest plots of subjective and objective LI (Figures S4 and S5).

We evaluated the quality of the studies included in the meta-analysis with the NOS for case-control studies, which showed satisfactory scores of the trials with low or medium risk of bias (Tables 3 and S3).

The strength of our study is that standardized, well-defined, rigorous outcome measures were used to assess the role of lactose consumption-related disorders in IBS patients, and a sufficient number of articles were found to carry out a detailed statistical analysis. Only full-text papers were enrolled, where IBS patients with appropriate control groups were present. According to our results, more IBS patients reported themselves lactose intolerant before any objective tests compared to HCs, which can be highlighted with objective measures: significantly more maldigester IBS patients reported abdominal symptoms during or shortly after the diagnostic test (objective LI). However, except for the LBT with the highest lactose doses (40-50 g), the prevalence of LM was similar in the study groups. Our meta-analysis is the first to provide evidence for the connection between IBS and LI and our former[18] data suggest that a lactose-free or lactose-restricted diet (low-FODMAP) in the treatment of IBS could improve the therapeutic effect on IBS symptoms and might decrease health care-related and societal costs.

There are some limitations of our study. Firstly, we focused on the prevalence of LM and subjective/objective LI, and due to the lack of detailed, uniform, controlled, published data, we could not

perform a statistical analysis of individual symptoms. A uniform, consensus-based, well-comparable measurement of symptom severity, for example visual analog scale (VAS) is suggested for use in future studies. Because of the same reasons, we could not analyze the role of lactose-restricted diet or lactase replacement in this patient group; therefore, a network meta-analysis could be a useful future perspective to establish which treatment is better in IBS. Secondly, because of the lack of data in the different IBS subtypes, it is not clear which subgroup is mostly affected by LI. Moreover, the diagnostic criteria for IBS and the diagnostic thresholds of LBT and LTT were different in some studies which could influence the results. The sensitivity and specificity of these non-invasive tests are relatively high; however, false positive or negative results could have an effect on our findings. It should be taken into account that similar activity of lactase in two persons might result in different LBT results due to the different activity and composition of the intestinal microbiota and the lactase non-persistence allele is not always associated with LM.[21] Another difficulty is that it is hard to identify the food, responsible for the symptoms. The correlation between self-reported and objective LI increases with the ingested lactose dose.[26] Finally, we found significant heterogeneity in the analysis of the subjective and objective LI. We could not perform subgroup analysis with different amount of lactose in LI, however, it can influence the frequency and severity of the abdominal symptoms and therefore the prevalence of objective LI, as presented by Yang et al.[26]

More trials with standardized parameters are necessary in the future to provide the best quality of evidence regarding the correlation between IBS and LI. Only patients fulfilling the most recent diagnostic criteria for IBS (Rome IV) should be included in such studies. Outcomes should be reported for each IBS subtypes. Uniform outcome measures (eg VAS) regarding abdominal symptoms should be used to make the different studies scientifically comparable. More randomized controlled trials are needed to provide evidence about the role of lactose-free or restricted diet in IBS compared to placebo or lactase replacement. In these studies, a more accurate IBS-Symptom Severity Score (IBS-SSS) should be used in each IBS subtype, which measures not only the severity of the main symptoms, but also the quality of life. Clinical trials with different lactose doses are also suggested to test the role of IBS in LI among lactose maldigesters. Yao et al.[59] discuss the crucial points and difficulties of designing clinical trials in dietary interventions in patients with functional gastrointestinal disorders.

5 | CONCLUSION

This meta-analysis is the first to confirm that subjective and objective LI are more common in IBS patients compared to the healthy population, but LM has the same prevalence. Based on these findings and literature data, IBS can be a contributing factor of LI among people with LM. Further studies are needed to determine whether a confirmed diagnosis of IBS is an etiological factor in determining whether LM patients present with LI.

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CONFLICT OF INTEREST

No competing interests declared.

AUTHOR CONTRIBUTION

PV performed the systematic literature search, extracted data, conducted bias analysis, interpreted the results and drafted the manuscript; NG performed the statistical analysis; ZsSz, DP, MB, ÁV, and JT validated the extracted data, critically revised the manuscript for important intellectual content and approved the submitted draft; PH contributed at the bias assessment, critically revised the manuscript for important intellectual content and approved the submitted draft; IMC performed the bias assessment, critically revised the manuscript for important intellectual content and approved the submitted draft; AF, ZSz, DC, AG, ZR supervised, critically revised the manuscript for important intellectual content and approved the submitted draft; JC studied the concept and design, performed the literature search, contributed in data extraction, critically revised the manuscript for important intellectual content and approved the submitted draft. He is the guarantor for this paper.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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