

# Irritable Bowel Syndrome

From Diagnostic Criteria  
to Targeted Treatment

Zsa Zsa Weerts





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Targeted Treatment**

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# **Irritable Bowel Syndrome From Diagnostic Criteria to Targeted Treatment**

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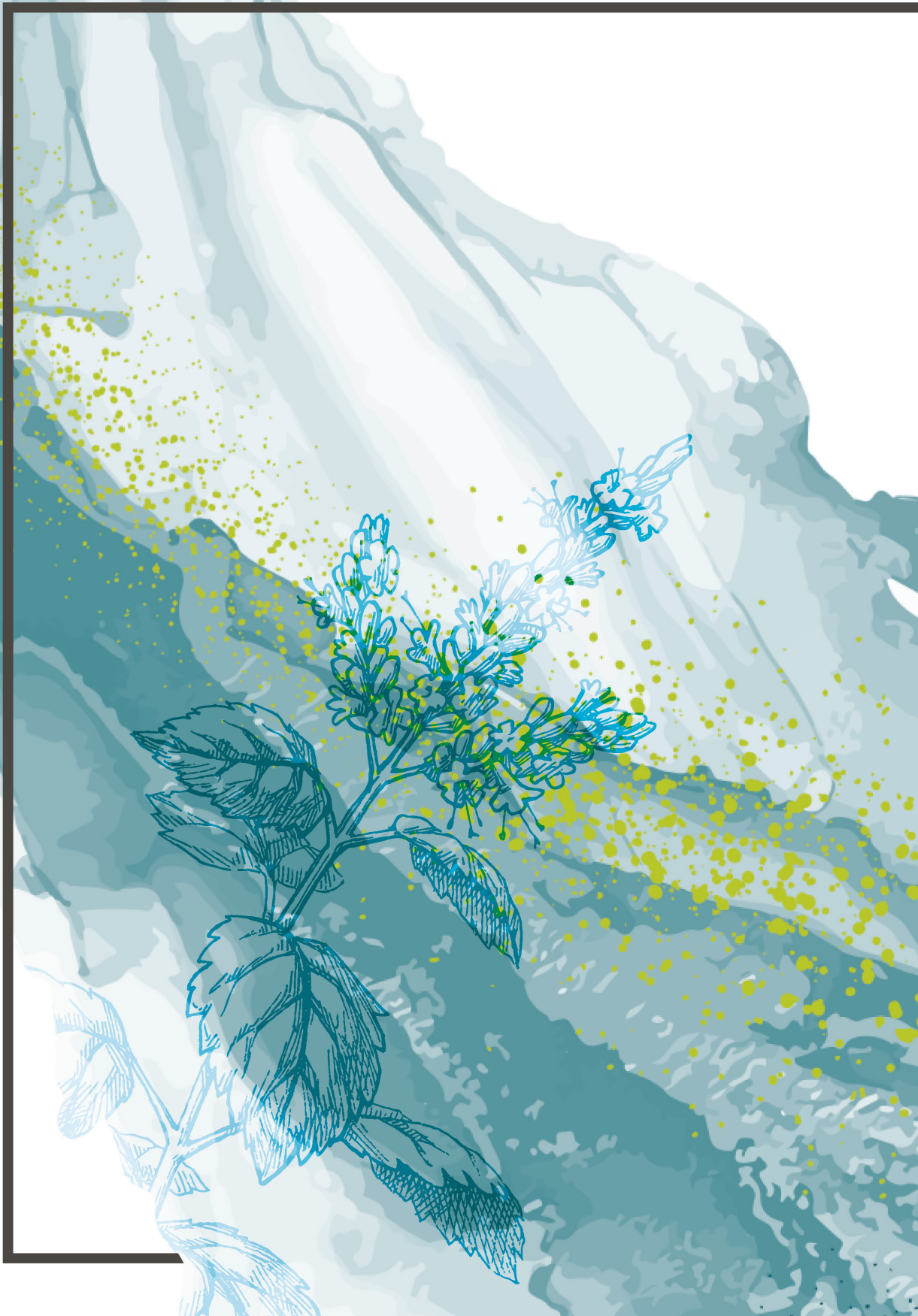
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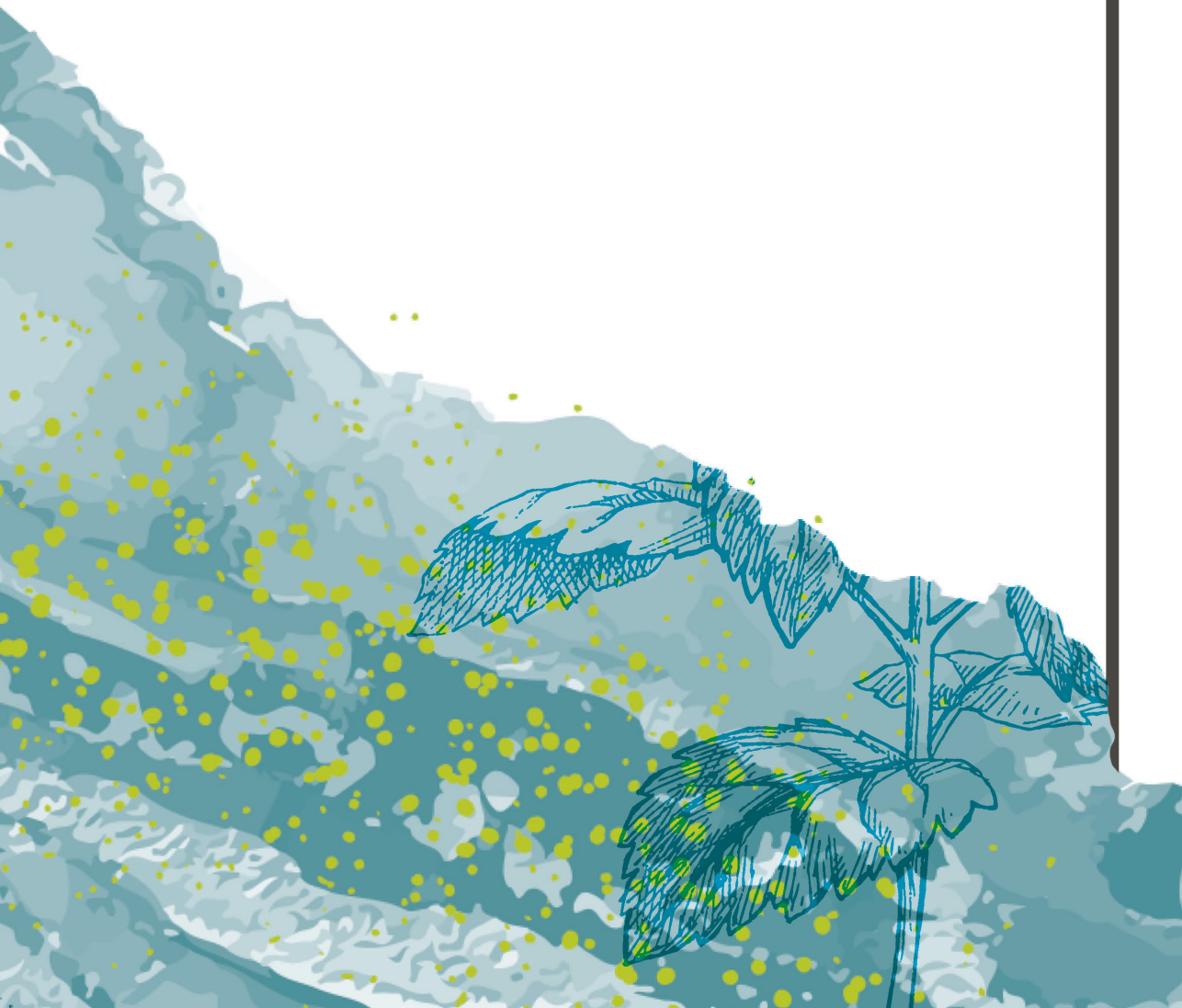
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# Chapter 1

General introduction







## General introduction

### Irritable bowel syndrome

Irritable bowel syndrome (IBS) is a highly prevalent disorder of brain-gut-interaction that is characterized by recurrent abdominal pain in combination with alterations in bowel habits.<sup>3</sup> Based on predominant stool pattern, IBS can be subtyped as diarrhea-predominant (IBS-D), constipation-predominant (IBS-C), mixed type (IBS-M), or unclassified IBS (IBS-U). Besides abdominal pain and disturbed defecation, many patients experience additional gastrointestinal (GI) symptoms such as abdominal cramping<sup>4</sup>, bloating (subjective sensation of abdominal fullness) and/or distention (objective increase in abdominal girth)<sup>5,6</sup>, flatulence<sup>7</sup>, and dyspepsia.<sup>8</sup> Symptoms are known to occur in episodes<sup>9</sup> with a chronic relapse remitting nature and vary widely between as well as within subjects, reflecting the complex, heterogeneous and multifactorial phenotype of IBS.<sup>10</sup>

IBS is part of a spectrum of functional GI disorders (FGID), '*classified by GI symptoms related to any combination of the following: motility disturbance, visceral hypersensitivity, altered mucosal and immune function, altered gut microbiota, and altered central nervous system (CNS) processing*', in absence of an identifiable organic cause. Other FGIDs include, but are not limited to functional heartburn, functional dyspepsia, belching, and functional constipation.<sup>11</sup> IBS often overlaps with these FGIDs<sup>12-16</sup> and is likely to have, at least to some extent, common underlying etiological mechanisms. In addition, IBS is also associated with various other intestinal, extra-intestinal, and psychiatric conditions, e.g. gastro-intestinal reflux disease (GERD), pelvic floor dyssynergia, chronic fatigue syndrome<sup>17</sup>, overactive bladder, fibromyalgia, increased somatization, depression, and anxiety.<sup>18</sup>

### Symptom-based diagnosis

At present, specific biomarkers to confirm IBS are not available and a diagnosis is made based on presence of typical symptoms.<sup>19,20</sup> Alarm symptoms that may require further investigations before diagnosing a patient with IBS are presented in *Box 1.1*.

**Alarm features for IBS\***

1. Blood in the stools, unless caused by fissures or hemorrhoids
2. Onset of symptoms after 50 years of age
3. Fever
4. Unintended weight loss
5. Nocturnal symptoms
6. Family history of coeliac disease, inflammatory bowel disease, or colon cancer
7. Abdominal mass
8. Appetite loss
9. Ascites
10. Anemia

**Box 1.1** Alarm features for IBS, presence of any of the following symptoms may warrant further investigation.

Since 1978, several criteria have been described to aid diagnostic accuracy of IBS both in research and clinical setting.<sup>21</sup> The Rome III diagnostic criteria (*Box 1.2*) were released in 2006<sup>2</sup> and were, until recently, the golden standard to diagnose IBS with an estimated sensitivity and specificity of 69.6% and 82.0%, respectively.<sup>22</sup>

**Rome III diagnostic criteria<sup>2</sup> for IBS\***

Recurrent abdominal pain or discomfort, on average:

At least 3 days per month in the last 3 months

Associated with 2 or more of the following:

1. Improvement with defecation
2. Onset associated with a change in frequency of stool
3. Onset associated with a change in form (appearance of stool)

\*Onset of symptoms at least 6 months prior to diagnosis

**Box 1.2** Rome III diagnostic criteria for IBS, published in 2006.

In 2016, the Rome IV criteria (*Box 1.3*) have been published<sup>1</sup> and have an estimated sensitivity and specificity of 62.7% and 97.1%, respectively.<sup>23</sup> The Rome IV criteria comprise two main updates when compared to Rome III: 1) The term abdominal discomfort has been removed, leaving the presence of abdominal pain the key requirement for IBS; 2) The frequency threshold of symptoms has been increased to at least 1 day per week.

**Rome IV diagnostic criteria<sup>1</sup> for IBS\***

Recurrent abdominal pain, on average:

At least 1 day per week in the last 3 months

Associated with 2 or more of the following:

4. Related to defecation
5. Associated with a change in frequency of stool
6. Associated with a change in form (appearance of stool)

\*Onset of symptoms at least 6 months prior to diagnosis

**Box 1.3** Rome IV diagnostic criteria for IBS, published in 2016.

## Prevalence and socioeconomic burden of IBS

IBS is highly prevalent and occurs in 5-20% of the population, with variations due to differences in diagnostic criteria used and geographical areas investigated. Furthermore, it has been shown that women and young people are more often affected.<sup>24</sup>

Although IBS is not associated with increased mortality, it has a major impact at societal level. The high prevalence, increased healthcare utilization, and loss in productivity in patients with IBS result in both high direct and indirect costs. IBS patients, for example, have more consultations with a medical professional regarding their IBS, receive more diagnostic investigations, and use more prescribed or over the counter drugs than patients without the IBS diagnosis. Total annual direct costs per patient were estimated to range between \$742-7547 (USA), €567-862 (France), and €791 (Germany).<sup>25,26</sup> Moreover, because IBS is associated with various comorbidities, studies have shown that the majority of healthcare charges in IBS patients was in fact for non-GI medical conditions and not for IBS.<sup>27,28</sup> In addition, IBS patients are more likely to be both absent from work (absenteeism) and impaired in productivity during work (presenteeism), compared to non-IBS patients.<sup>29</sup> Prior studies have estimated annual indirect costs per patient with IBS, being \$748 (in Canada), \$335 (in the United Kingdom), and €995 (in Germany).<sup>25,30</sup>

As healthcare systems differ between countries, findings from studies on healthcare costs are difficult to compare. In the Netherlands, the economic burden of IBS in the first three years after diagnosis was investigated by Flik *et al.*, using an insurance database.<sup>31</sup> They observed that direct costs increased with 486 and 2328 euro after IBS was diagnosed in primary versus secondary care, respectively. Further data from a societal perspective on costs for the Dutch situation are lacking.

It is well-recognized that IBS is associated with a pronounced interference with daily life activities and a decrease in quality of life (QoL)<sup>25,32,33</sup>, with QoL scores comparable to or even lower than in patients with GERD, diabetes, migraine, and asthma.<sup>34</sup> When applying the 'time-trade-off method', the mean reported QoL scores of an IBS patient correspond to a 35-year-old patient willing to sacrifice 10-15 years of the remaining 40 years (of life expectancy) in exchange for immediate perfect health.<sup>25,35</sup> Albeit hypothetical, this clearly demonstrates the considerable burden experienced by these patients.

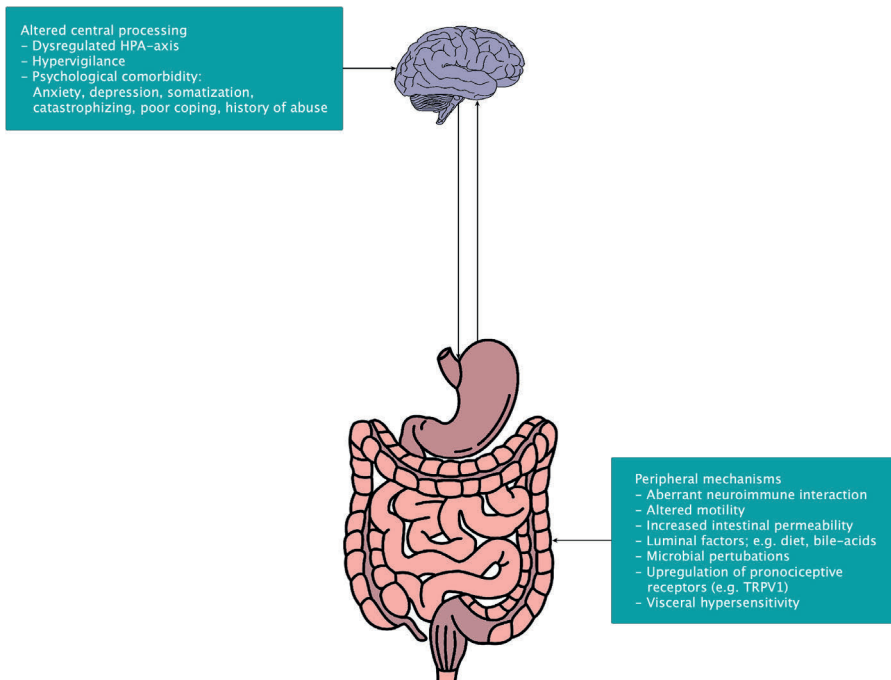
## **Pathophysiology of IBS**

Although the pathophysiology of IBS is complex, multifactorial, and incompletely understood, there is now substantial evidence for several mechanisms to be involved. Risk factors for IBS include female sex, a history of GI infections, previous abdominal surgery, use of antibiotics, somatic pain (e.g. migraine), endometriosis, and psychological factors, such as acute psychological stress, a history of stressful life events or sexual abuse, the presence of anxiety, depression, and somatization.<sup>18</sup> Furthermore, research data strongly suggests that an aberrant brain-gut-interaction has a central pathophysiologic role. Other factors implicated in IBS pathophysiology include visceral hypersensitivity, genetic susceptibility, disturbed intestinal motility, low grade immune activation, intestinal dysbiosis, excess fermentation by colonic microbiota, impaired gas tolerance, abdomino-phrenic dyssynergia, alterations in bile-acid metabolism, and psychological comorbidities.<sup>5,6,18</sup> It is assumed that several mechanisms, when present at the same time, lead to symptoms in the individual patient with IBS. These symptoms and underlying factors differ between subgroups of IBS.<sup>10</sup> The pathophysiology of IBS is depicted schematically in *Figure 1.1*. Some of the key-mechanisms involved will be described in the following paragraphs.

Altered brain-gut-interaction is a key factor that likely contributes to abdominal pain, which is a typical hallmark of IBS. Thresholds for abdominal pain are lowered (allodynia) and abdominal pain levels are higher (hyperalgesia) in patients with visceral hypersensitivity, which can be measured using the barostat procedure. The proportion of IBS patients with visceral hypersensitivity varies between studies and ranges to up to 60%.<sup>36</sup> Viscero-sensory function entails a bidirectional interaction between the enteric and spinal nerves and the central nervous system. From the gut, visceral stimuli travel from nerve endings located in all layers of the intestinal wall and mesentery<sup>37</sup> to afferent neurons residing in the dorsal root ganglia.<sup>38</sup> From the dorsal horn, afferent input is conveyed via spinal pathways to subcortical regions such as the nucleus of the solitary



tract in the brain stem, the primary relay station for vagal afferent information<sup>39,40</sup>, and then transduced to other parts of the brain. The vagal nerve carries afferents from mechano- and chemoreceptors throughout the entire GI tract, with the exception of the descending colon and rectum that are innervated by splanchnic nerves<sup>41</sup>, and is thereby a key nerve regarding viscerosensory function.<sup>42-44</sup> When visceral stimuli reach the brain, perception of the sensation can occur depending upon the type and fortitude of the stimulation, but also depending on central, supraspinal, and spinal bidirectional modulation, and local reflexes.



**Figure 1.1** Pathophysiological mechanisms in Irritable Bowel Syndrome.

Abdominal pain may originate from various anatomical levels: a) nerve endings and nerves can become abnormally activated or sensitized at the intestinal level; b) input can be altered at the spinal cord level; and c) stimuli can be modulated aberrantly at the spinal, supraspinal, and/or central level.

One of the mechanisms at the intestinal level that is associated with IBS, in at least a subset of patients, is immune activation. Several studies have shown increased numbers of mucosal mast cells, eosinophils, and T-lymphocytes in the small and large intestine of IBS patients versus healthy controls.<sup>18</sup> Furthermore, the mast cells found were located more closely to nerve endings and were more often in degranulating state in IBS patients, than in controls.<sup>18,38</sup> This intestinal immune activation may result in increased intestinal permeability, which leads to further low-grade immune cell infiltration.<sup>18</sup> Peripheral pro-inflammatory chemokines, cytokines, prostaglandins, substance P, calcitonin G-related peptide (CGRP), and several neurotrophins can subsequently activate and sensitize intestinal sensory nerve endings, thereby mediating nociceptive processes. This finding has been underscored by the fact that mucosal mediators derived from biopsy supernatants of patients with IBS provoked greater activation of visceral pain pathways when applied to intestinal preparations, compared with controls.<sup>18,38</sup>

Nociceptive neurons that play a role in the intestinal nerve sensitization associated with IBS can express Transient Receptor Potential (TRP) channels. The TRP family comprises numerous ligand-gated cation channels located in nociceptive neurons of vagal, spinal, and splanchnic primary afferents or in immune cells.<sup>45,46</sup> They are expressed in multiple visceral organs and have various chemo-, thermo-, and mechano-sensory functions.<sup>47,48</sup> Several types of TRP channels are present in the GI tract and have been implicated in visceral pain generation or inhibition, such as TRP Vanilloid 1 (TRPV1), TRP Ankyrin 1 (TRPA1), TRP Vanilloid 4 (TRPV4), and TRP Melastatin 8 (TRPM8). The most widely studied nociceptor, TRPV1, can be activated by high temperatures, low pH and exogenous stimulants, e.g. the pungent substance in hot chili peppers (capsaicin); mustard oil; and anandamide.<sup>48</sup> Activation results in release of several proinflammatory neurokinins such as substance P and CGRP. The role of TRPV1 modulation in sensory hyperalgesia has been established in animal models of visceral pain.<sup>49</sup> In addition, several studies demonstrated an increased colonic TRPV1 expression in IBS patients when compared to controls<sup>50,51</sup>, and elevated pain responses to capsaicin compared to controls.<sup>52</sup> TRPA1 is co-existing with TRPV1 on visceral afferents and likely functioning through interaction with TRPV1.<sup>45</sup> TRPA1 has mechano-sensory properties and in addition can be activated by low temperatures and various pungent compounds, e.g. cinnamon, garlic, and hydrogen peroxide. TRPV4 can be activated by osmosis, mechanical force, and slightly warmer temperatures than room temperature. Besides long-standing evidence from animal models that TRPA1 and TRPV4 are implicated in pain generation, a recent study has shown increased TRPA1 and TRPV4 mediated responses by neurons derived from biopsies of IBS patients, compared with healthy

controls. In addition, this study demonstrated that supernatants of rectal biopsies of IBS patients, but not healthy controls, sensitized TRPA1 and TRPV4 in murine sensory neurons.<sup>53</sup>

Whereas several TRP channels exhibit pro-nociceptive properties, there are indications that TRPM8 has anti-nociceptive effects. TRPM8 can be activated by several factors including low temperatures and cooling compounds, e.g. menthol, the main constituent of peppermint oil. TRPM8 has been suggested to exhibit antinociceptive properties by impeding the mechano- and sensory effects of TRPA1 and TRPV1 by cross-desensitization.<sup>54</sup> Moreover, results from an animal colitis model suggest that TRPM8 activation by agonist icilin can reduce cytokine and chemokine levels and as such, also has an anti-inflammatory effect.<sup>55</sup> Taken together, these results implicate that TRPM8 may be able to counteract abdominal pain and thereby may have therapeutic applications. However, more data on this topic, in particular from *in vivo* studies, is warranted to comprehend the exact role of TRP channels and their interaction in visceral nociceptive processing and immune function.

Intestinal dysbiosis is another factor that can contribute to sensitization of nerves and is associated with IBS, at least in subgroups of patients. A microbiota signature that has been confirmed by several comprehensive studies is the increased Firmicutes/Bacteroidetes ratio<sup>56</sup>, a relative depletion of Bifidobacteria and a general lower diversity of the microbiota.<sup>57</sup> It should however be noted that these findings could not be confirmed in all studies. In addition, changes in microbial metabolic activity<sup>58</sup> and a microbial signature associated with symptoms severity<sup>59</sup> have been found. The microbiome perturbations can impact the intestinal epithelium and its underlying immune system. Growing evidence moreover supports bidirectional interactions between the gut microbiome and the brain.<sup>60-63</sup> Data in support of this brain-gut-microbiome axis originate mainly from animal studies. For example, colonization of germfree mice with the bowel contents of IBS patients did result in immune activation, increased intestinal permeability, and anxious behavior that was not related to GI symptoms.<sup>63,64</sup> In recent years, evidence is also emerging from human data, such as brain-imaging studies, which found changes in brain activation patterns following administration of a probiotic.<sup>65,66</sup>

Another process that was found to be altered in a subset of IBS patients is central pain modulation. Intestinal nociceptive signals are transduced to the central nervous system where they are modulated extensively. This central pain modulation is a balance between inhibition and facilitation of the painful stimulus and a key determinant of

abdominal pain perception. In the 1990s, several research groups further investigated the brain-gut interactions by using functional Magnetic Resonance Imaging (fMRI) and positron emission tomography (PET).<sup>67</sup> These studies were important to elucidate brain areas and networks associated with visceral pain in healthy and disordered states, such as IBS. Since then, several studies have examined the complex role of the autonomic nervous system by applying a mechanical or thermal intestinal stimulus during fMRI. They demonstrated that painful rectal balloon distensions resulted in activation of various brain areas<sup>68</sup>; the primary and secondary somatosensory cortex, insula, cingulate cortex, prefrontal cortexes, and thalamus.<sup>67,69-72</sup> In addition, it was shown that individual factors such as coping mechanisms, depression, anxiety, cognitions, and emotions can bi-directionally modulate cortical, limbic, and brainstem nuclei. This can subsequently result in either amplification or subordination of noxious stimuli in healthy volunteers and IBS patients and thereby determines whether noxious stimuli are perceived as painful.<sup>73</sup> When compared to healthy controls, patients with IBS appeared to have different activation patterns of brain areas involved (in visceral pain processing and modulation).<sup>71,74</sup> Moreover, there are indications that the vagal efferent arm exhibits downward modulatory properties and can even affect low grade inflammation. Several studies have indicated that this efferent vagal activity can be impaired in IBS.<sup>42,43</sup> Altogether, these findings point to increased facilitation and/or decreased inhibition of pain signals in IBS.<sup>73,75</sup> In combination with peripheral alterations, this can contribute to abdominal pain. Further research is needed to investigate how all factors interact and why they lead to symptoms in some, but not all patients with IBS.

## **Treatment of IBS**

Currently, there is no effective cure for IBS. Available treatment modalities merely aim at symptom amelioration and mostly benefit only subgroups of patients. Regardless of the treatment chosen, pivotal for treatment success are a good patient-physician relationship and a timely discussion about treatment expectations.<sup>20</sup> Furthermore, as many psychiatric and extra-intestinal comorbidities interfere with patients' symptom severity as well as daily functioning, constructing a multidimensional clinical profile and assessing all factors that impede QoL, are needed to choose a patient-centered approach.<sup>76</sup>

Traditionally, treatment starts with reassurance and explaining the disorder. For a subgroup of patients with mild symptoms, this is sufficient and no further treatment is required.<sup>76</sup> Given that a large proportion of patients report their symptoms to be triggered by meals or specific foods<sup>57</sup>, dietary and lifestyle modifications can also relief



symptoms and are usually suggested next if further treatment is necessary.<sup>77</sup> General dietary and lifestyle modifications include less intake of e.g. alcohol, caffeine, spicy-, fat-, lactose containing or other foods that trigger symptoms, as well as decreasing meal portions and/or number of meals a day, modifying dietary fiber intake, and increasing physical activity.<sup>20,76</sup> In patients with bloating and or flatulence as predominant symptoms that respond inadequately to general dietary modifications, a diet low in FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) may be beneficial.<sup>78</sup> This diet ultimately aims at a balanced intake of FODMAP containing foods that do not, or solely to an acceptable extent, trigger symptoms, in combination with low intake or avoidance of FODMAP containing foods that trigger severe symptoms. If dietary and lifestyle modifications provide inadequate results, psychological and/or pharmacological treatment should be considered.

As psychosocial factors can influence GI symptoms and vice versa, various psychological treatments can be tried, such as cognitive behavioral therapy, gut-directed hypnotherapy, mindfulness-based therapy and psychodynamic therapy. When combined with medical and dietary therapies, gut-directed-psychotherapies collectively have a favorable number needed to treat of 4 and are at low risk for adverse events.<sup>79</sup> Nevertheless, the clinical implementation is still limited due to a lack of trained therapists in some geographical areas.<sup>80</sup>

If pharmacological treatment is preferred and diarrhea is the main predominant symptom, treatment options include the antidiarrheal drug loperamide<sup>76</sup>, the antibiotic rifaximin<sup>81</sup> and bile-acid binders.<sup>82</sup> In addition, recent pharmacological advancements have led to the development of eluxadolone, a mixed agonist of  $\mu$ -opioid and  $\kappa$ -opioid receptors and an antagonist of  $\delta$ -opioid receptors, for the treatment of diarrhea and to a lesser extent abdominal pain in IBS-D patients.<sup>83</sup> Eluxadolone treatment should, however, be closely monitored due to serious side effects (e.g. pancreatitis and sphincter of Oddi spasms).<sup>84</sup>

Pharmacological treatment of constipation often begins with the osmotic laxative polyethylene glycol.<sup>76</sup> Second-line options that have become available recently as a result of pharmacological developments include linaclotide<sup>85</sup>, and plecanatide<sup>86</sup>, and tenapanor.<sup>87</sup> Linaclotide and plecanatide are guanylate-cyclase-C agonists that increase fluid secretion into the intestinal lumen to hydrate stools and possibly also reduce abdominal pain. Tenapanor is a minimally absorbed, small molecule inhibitor of the gastrointestinal sodium/hydrogen exchanger isoform 3 that increases fluid and sodium into the intestinal lumen.<sup>88</sup> IBS patients that suffer from mixed type IBS should preferably

be managed by a tailored therapy that can be adapted when the predominant symptom switches.<sup>20</sup>

Drugs that can reduce symptoms of bloating include rifaximin, some probiotics, eluxadoline in case of accompanying diarrhea, and linaclotide, plecanatide, or tenapanor in case of accompanying constipation.<sup>5,87,89</sup> Pharmaceuticals that have shown some efficacy in treatment of abdominal pain, the most important hallmark of IBS, include tricyclic antidepressants, selective serotonin reuptake inhibitors, and antispasmodic drugs.<sup>76</sup>

Despite the large therapeutic array and advances in the management of IBS in recent years, a considerable proportion of patients remain refractory to currently licensed pharmacological therapies. Furthermore, the therapeutic gain over placebo is a mere 8 to 20% for most drugs available for IBS<sup>76</sup> and treatment efficacy of the most cardinal symptom in particular, abdominal pain, is often unsatisfactory. Factors that further complicate the search for better treatments are the generally high placebo response in patients with IBS in clinical trials (i.e. approximately 30%)<sup>90</sup>, and the chronic relapse-remitting and heterogeneous nature of IBS. In addition, it is difficult to capture treatment response since treatment effects cannot be monitored with biomarkers and end-of-day symptom diaries are often hindered by fake adherence.<sup>91</sup> More research into the underlying pathophysiological mechanisms, appropriate symptom assessment tools, but also new therapeutic compounds or alternative formulations of existing drugs are warranted to optimize future treatment of patients with IBS. In addition, because IBS imposes such a significant socioeconomic burden, it is important that therapies are not only effective, but associated costs should also be justifiable (i.e. cost-effectiveness). A drug of particular interest that is low in costs and was found to have a promising number needed to treat of 3-4 in meta-analyses is peppermint oil.<sup>22</sup>

Peppermint oil is a pharmacological compound of herbal origin that has been used to treat IBS for many years. Peppermint oil has an antispasmodic, smooth muscle relaxing effect by blocking calcium influx in the sarcolemma of the intestinal smooth muscle cells.<sup>92</sup> Furthermore, menthol, the main constituent of peppermint oil, is thought to have direct analgesic properties through interaction with previously described intestinal TRPM8 and TRPA1 receptors.<sup>93</sup> Other mechanisms of action include 5-hydroxytryptamine antagonism<sup>94,95</sup>, antimicrobial and antifungal effects<sup>96-99</sup>, and  $\kappa$ -opioid receptor agonism.<sup>100</sup> Currently, capsules that release the peppermint oil in the small intestine are available as an over-the-counter drug. Placebo controlled randomized trials using this small-intestinal release peppermint oil formulation in patients with IBS support

its usage and have demonstrated a reduction in abdominal pain and overall symptoms.<sup>101-104</sup> Similarly, meta-analyses indicate that peppermint oil has a treatment effect compared to placebo of approximately 30%.<sup>105,106</sup> Although these findings are highly favorable compared to other treatments<sup>107</sup>, the results are based on studies with methodological shortcomings, impeding the ability to draw firm conclusions about the effectiveness of peppermint oil in patients with IBS and limiting the incorporation in treatment guidelines. Therefore, there is a need for a well-designed randomized controlled trial conducted according to Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidelines on trials in IBS. Another factor hindering application in daily clinical practice is that conventional small-intestinal release peppermint oil is associated with bothersome upper GI adverse events such as heartburn, belching, and a peppermint oil taste<sup>101-103,108</sup>, which impair treatment adherence. It is likely that a colonic release and therefore more distal gastrointestinal exposure to peppermint oil would decrease these adverse events. This could also induce a more colonic anti-spasmodic effect as colonic application of peppermint oil has been found to inhibit colonic motor activity and peristalsis.<sup>93</sup> Furthermore, it can be envisaged that an ileocolonic release of peppermint oil enhances the local exposure of the ileocolonic nociceptive afferents to the oil and thereby increases its analgesic effects. Therefore, in addition to conducting a well-designed randomized controlled trial with small-intestinal release peppermint oil, it is worth exploring a colonic delivery system of peppermint oil as this may result in fewer adverse events and increased efficacy.

## Aims & outline of this thesis

IBS is a highly prevalent disorder of brain-gut interaction, of which the complex and multifactorial pathophysiology remains poorly understood. The socioeconomic burden associated with IBS is substantial and is likely caused by a combination of the challenging diagnostic process, the chronic relapse-remitting nature of IBS, and the overall meagre efficacy of the current therapeutic array. Further insight into these contributing factors may help prevent the drainage of precious health care costs and improve patients' quality of life.

We studied the change in diagnostic criteria and how this affects the IBS population, and its impact on quality of life in **part I** of the current thesis. As adequate treatment of the most cardinal symptom, abdominal pain, is often unsatisfactory, we addressed TRP channels as potential therapeutic targets and investigated the efficacy and cost-effectiveness of peppermint oil in **part II** of this thesis.

In 2016, the Rome diagnostic criteria for IBS were revised to the more stringent Rome IV criteria. Until then, most studies had been based on prior criteria, such as Rome III, and it was unknown which Rome III IBS patients were likely to meet the Rome IV criteria. This however, is important to know because the Rome defined IBS population is the target population for randomized clinical trials to investigate treatment response of novel treatment strategies. It is also meaningful to assess how the change in definition could affect clinical practice because the criteria are pivotal in the clinical diagnostic process and are meant to reduce the number of diagnostic investigations (and thus costs) by making a positive diagnosis. Therefore, in **chapter 2**, we investigated demographic, clinical, and psychosocial differences between Rome III and Rome IV IBS subjects, using diary-based surrogate Rome IV criteria in a large and well-defined patient cohort.

In most patients with IBS, symptoms fluctuate over time. Given that knowledge of factors affecting the natural disease course can help in the search for new patient tailored treatment strategies, it is pertinent to identify predictors for long-term symptom severity and quality of life. Therefore, we evaluated symptom evolution and characteristics that could predict the disease course over a five-year follow up period in patients participating in the Maastricht IBS cohort in **chapter 3**.

In the search for cost-effective treatments for patients with IBS, TRP channels are promising targets for therapeutic interventions. Growing evidence indicates that TRP

channels are involved in the propagation and processing of abdominal pain signals in IBS. In **chapter 4**, we reviewed current insights regarding TRP channels and their potential implications for treatment in IBS. As human data on the functional relevance of the TRPM8 channel is exceptionally limited, we investigated neuro-immune interactions in the human gut to elucidate the role of TRPM8 in nociceptive processes and gut health in **chapter 5**. Peppermint oil is a potent TRPM8 agonist and has been shown to ameliorate abdominal pain in IBS in prior studies. Most of these studies, however, suffered from methodological flaws or were conducted in small populations. In **chapter 6**, we assessed the pharmacokinetic profile of a newly developed ileocolonic release peppermint oil capsule in healthy volunteers. This formulation was produced to decrease upper GI adverse events associated with conventional, small-intestinal release peppermint oil and to increase efficacy by the enhancement of local colonic antinociception. Following the pharmacokinetic pilot study, we investigated the efficacy and safety of small-intestinal and ileocolonic release peppermint oil in a Rome IV IBS population in a randomized, placebo-controlled trial (RCT) with a highly qualitative experimental design. The results of this RCT have been described in **chapter 7**. Since IBS is associated with high healthcare related costs, it is increasingly important that therapeutic entities are also cost-effective. Therefore, in **chapter 8**, we determined the cost-effectiveness of peppermint oil alongside the previously mentioned RCT.

Due to the lack of validated biomarkers, end-of-day symptom diaries are important to assess treatment response in patients with IBS. The validity and reliability of paper diaries, however, is impeded by fake adherence and recall bias. Given that an accurate assessment of treatment response in RCTs is essential in the search for better treatments for patients with IBS, we implemented a framework for a digitalized data collection in our previously mentioned peppermint oil RCT. In **chapter 9**, we describe the smartphone application that was used as a digital symptom diary in addition to the overall data collection method. Furthermore, we assessed patient' adherence to the digital diary and investigated how patient characteristics associate with adherence.

Finally, in **chapter 10**, we summarize the main findings of this thesis, and discuss implications for future research in this area and everyday clinical practice.

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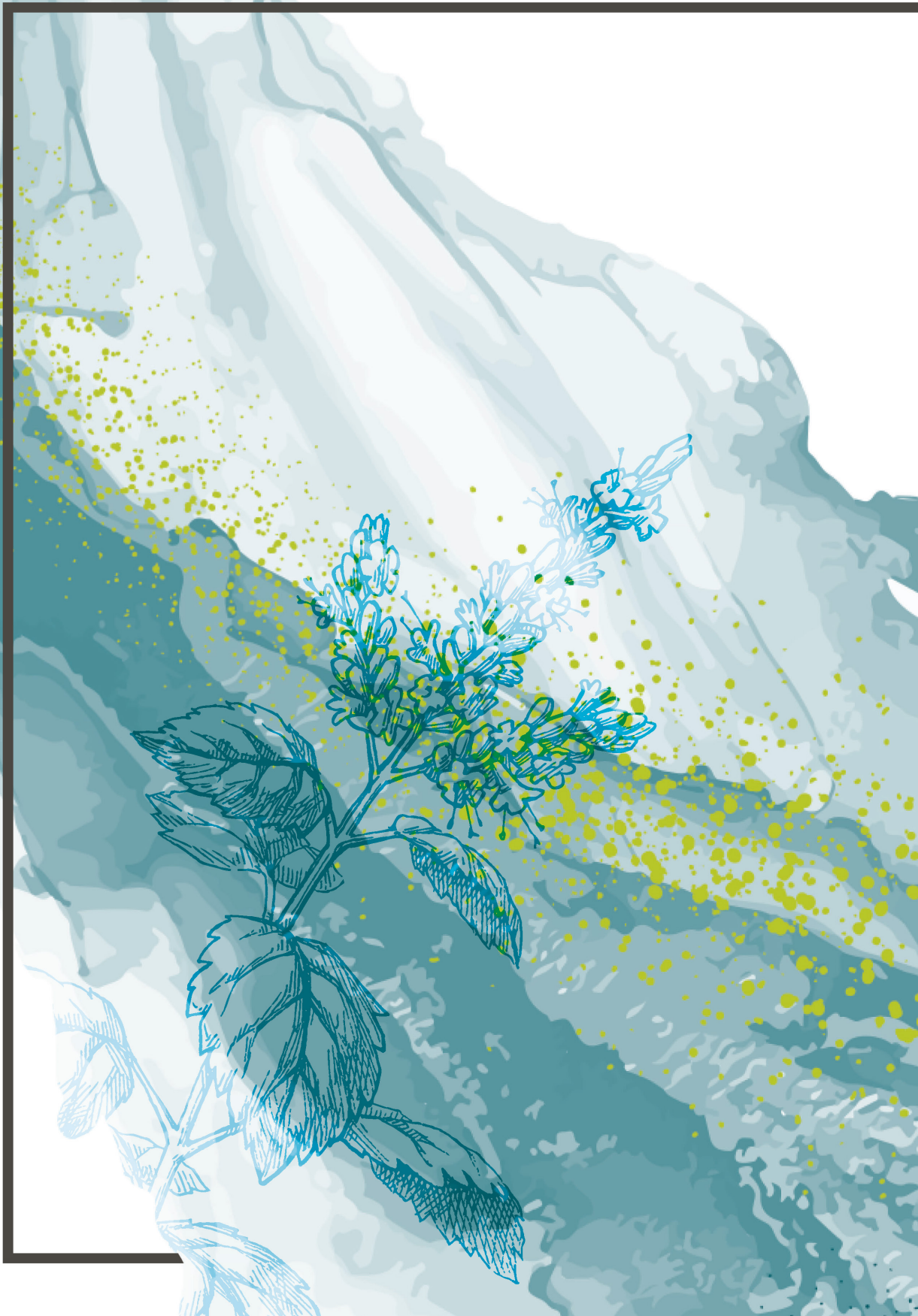






# Part I

Epidemiological aspects and clinical  
manifestations of irritable bowel syndrome





# Chapter 2

## Rome III versus Rome IV criteria for irritable bowel syndrome: A comparison of clinical characteristics in a large cohort study

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## Abstract

### Introduction

The Rome criteria for irritable bowel syndrome (IBS) have been revised and are expected to apply only to the subset of Rome III IBS subjects with abdominal pain as predominant symptom, occurring at least once a week. The aim of this study was to determine the percentage of Rome III IBS subjects that fulfills Rome IV criteria and to evaluate differences between Rome IV-positive and -negative subjects.

### Methods

404 Rome III IBS subjects completed a 14-day end-of-day symptom diary, the gastrointestinal symptom rating scale (GSRS), hospital anxiety and depression scale (HADS) and rand 36-item short-form health survey (SF-36). Diary-based surrogate Rome IV criteria were defined as occurrence of abdominal pain at least one day each week with a severity of  $\geq 2$  (*mild*; definition 1) or  $\geq 3$  (*considerable*; definition 2).

### Results

Using surrogate Rome IV criteria, 353 (87.4%, definition 1) and 249 (61.6%, definition 2) subjects were defined as Rome IV-positive. These patients were more often female, younger and recruited from secondary/tertiary care compared to Rome IV-negative subjects. They also presented with higher abdominal pain scores and gastrointestinal (GI) symptom severity on both end-of-day diary and GSRS, higher psychological symptom scores and lower quality of life compared to Rome IV-negative subjects.

### Conclusions

The Rome IV IBS population likely reflects a subgroup of Rome III IBS patients with more severe GI symptomatology, psychological comorbidities and lower quality of life. This implies that results from Rome III IBS studies may not be directly comparable to those from Rome IV IBS populations.



## Introduction

Irritable bowel syndrome (IBS) is a functional intestinal disorder characterized by abdominal pain associated with altered bowel habits. IBS has traditionally been subcategorized into four subtypes based on predominant stool pattern: diarrhea (IBS-D), constipation (IBS-C), a mix of diarrhea and constipation (IBS-M) or undefined predominant stool form (IBS-U). IBS is a prevalent disorder worldwide, with prevalence rates of 5-15% in the Western population.<sup>1,2</sup> Symptoms most likely result from complex interactions between several biological, psychological and social factors.<sup>3,4</sup> The exact underlying mechanisms of IBS pathophysiology are however not completely understood and as a consequence accurate non-invasive biomarkers for diagnosis, disease monitoring and treatment evaluation are not available.

At present, the diagnosis of IBS is symptom-based, using the Rome criteria. The Rome Foundation, a committee of international experts in the field of functional gastroenterology, has been working on the development and revision of diagnostic criteria for IBS, amongst other functional gastrointestinal (GI) disorders, since 1994. Recently, Rome III criteria (2006) have been updated to Rome IV criteria (2016). Major adjustments include removal of the term abdominal discomfort (Rome III), leaving only the occurrence of abdominal pain as the key requirement for Rome IV criteria. Furthermore, abdominal pain should be present on average at least one day per week in the Rome IV criteria (see *Box 2.1*).<sup>5,6</sup> This new frequency threshold was based on a summary report on the distribution of symptom occurrence rates for all the Rome III symptoms.<sup>7</sup> Most likely, fewer patients will fulfill the new Rome criteria compared to the previously set criteria. Indeed, using Rome IV criteria, a lower population prevalence of IBS has been reported by Whitehead and colleagues.<sup>8,9</sup> Furthermore, IBS subtype identification has been revised, by only taking into account symptomatic stools (*i.e.* loose/watery stools and hard/lumpy stools), which might result in a shift in IBS subtypes.<sup>3</sup>

Studies on previous editions of the Rome criteria have demonstrated varying IBS prevalence rates depending on the diagnostic criteria employed. Also, differences in patient characteristics and symptomatology have been reported between several criteria.<sup>10-13</sup> As a result of the requirement of weekly symptoms, Rome IV IBS patients are likely to be those with more severe symptomatology and possibly higher prevalence of psychiatric comorbidity and lower quality of life as compared to those fulfilling Rome III criteria. However, data comparing clinical features between Rome III- and Rome IV-IBS populations are still lacking.

**Rome III IBS**

- 1) Recurrent abdominal pain or discomfort\* at least 3 days per month in the last 3 months and associated with 2 or more of the following:
  - a. Improvement with defecation;
  - b. Onset associated with a change in frequency of stool;
  - c. Onset associated with a change in form (appearance) of stool.
- 2) Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis.

\* Discomfort means an uncomfortable sensation not described as pain.

**Rome IV IBS**

- 1) Recurrent abdominal pain, on average, at least 1 day per week in the last 3 months and associated with 2 or more of the following:
  - a. Related to defecation;
  - b. Associated with a change in frequency of stool;
  - c. Associated with a change in form (appearance) of stool.
- 2) Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis.

**Box 2.1** Definition of IBS according to Rome III and Rome IV criteria.

Rome criteria are widely used as a cornerstone for inclusion in IBS clinical trials and cohort studies. It is expected that in future studies only a subset of Rome III IBS patients, *i.e.* those with more severe abdominal pain, will be eligible for study participation by introducing Rome IV criteria. In order to generalize current data to Rome IV populations and to compare results from Rome III and Rome IV studies, it is important to evaluate which Rome III patients are likely to meet the Rome IV criteria. Therefore, the aim of this study was to determine the percentage of Rome III-positive IBS subjects that is also highly likely to fulfill Rome IV criteria, based on end-of-day symptom diaries, and to evaluate whether demographical, clinical and psychosocial differences exist between Rome IV-positive and Rome IV-negative subjects, in a well-defined Rome III IBS population.

## Materials and methods

### Study design

In the current analyses, data from a well-phenotyped Dutch cohort study, the Maastricht IBS (MIBS) Cohort, on the phenotypical and genotypical characterization of IBS patients<sup>14-16</sup>, were evaluated. The study protocol has been approved by the Maastricht University Medical Center + (Maastricht UMC+) Committee of Ethics in

February 2009 and was executed according to the revised Declaration of Helsinki (64<sup>th</sup> WMA General Assembly, Fortaleza, Brazil, October 2013). Furthermore, the study has been registered in the US National Library of Medicine (<http://www.clinicaltrials.gov>, NCT00775060).

## Study participants

Between July 2009 and May 2016, IBS patients aged 18-75 years were included in the Maastricht IBS cohort at the secondary/tertiary care outpatient department of Gastroenterology-Hepatology at the Maastricht UMC+ in Maastricht and via general practitioners practices in South-Limburg, the Netherlands. All subjects fulfilled the Rome III criteria (see Box 2.1) for IBS and were assigned the IBS subtype based on predominant bowel habit, *i.e.* diarrhea (IBS-D), constipation (IBS-C), a mix of diarrhea and constipation (IBS-M) or unspecified predominant bowel habit (IBS-U).<sup>3,17</sup> Rome III criteria were evaluated in a face-to-face interview by a trained clinical researcher. Medical history was taken by a gastroenterologist and if indicated, GI endoscopy, abdominal imaging and/or blood, breath or fecal analyses were performed to exclude organic disease. A history of abdominal surgery, except for uncomplicated appendectomy, cholecystectomy or hysterectomy, was reason for exclusion. All subjects gave their written informed consent before participation.

## Data collection

As subject inclusion was performed since 2009, Rome IV criteria were not collected in a face-to-face interview at the moment of inclusion. However, all participants completed an end-of-day diary on symptom severity and bowel habits, during 14 days, at the time of inclusion. Abdominal pain, amongst other symptoms, was scored using a 5-point Likert scale (1=not at all; 2=mild; 3=considerable; 4=severe; 5=extremely). Using this information, we retrospectively determined which subjects were highly likely to fulfill the Rome IV criteria, based on the presence of abdominal pain on at least one day in both the first and the second seven days (*i.e.* abdominal pain at least once a week). Rome IV criteria do not take into account abdominal pain severity, however, as we will use surrogate Rome IV criteria to evaluate differences between Rome IV-positive and -negative IBS patients, we will report on two definitions for those criteria: 'Definition 1: Abdominal pain score  $\geq 2$  once a week in each week' and 'Definition 2: Abdominal pain score  $\geq 3$  once a week in each week'. Only symptom diaries that were completed for at least 12 of the 14 days were considered eligible for analysis.

Information on demographics was collected using a predefined self-report questionnaire. Furthermore, subjects completed the gastrointestinal symptom rating scale (GSRs)<sup>18</sup>, hospital anxiety and depression scale (HADS)<sup>19,20</sup> and rand 36-item short-form health survey (SF-36)<sup>21,22</sup> for GI symptom severity, co-occurrence of depressive and/or anxiety symptoms and general quality of life, respectively.

In addition, in a subset of participants a rectal barostat procedure was performed. Measurement of rectal perception was performed using a standardized perception protocol, during which 17 pressure steps between 0 and 50 mm Hg, based on a semi-random staircase protocol, were applied. During each pressure step pain scores were reported on a 100 mm visual analogue scale (VAS). The cut-off value for visceral hypersensitivity was defined as a pain score  $\geq 20$  at pressure  $\leq 26$  mm Hg. A detailed description on the rectal barostat procedure was previously reported.<sup>23</sup>

The end-of-day diary data were additionally used to perform an exploratory analysis on whether Rome III IBS patients, that do not fulfill (surrogate) Rome IV criteria for IBS, are likely to fulfill Rome IV criteria for other functional bowel disorders. Definitions that were used for retrospective evaluation of Rome IV diagnoses for functional constipation (FC), functional diarrhea (FD) and functional abdominal bloating/distension (FAB/D) are shown in *Table S2.1*. Since the symptom diary was designed for IBS and not specifically for assessing those other disorders, not all criteria could be definitively checked.

## **Data and statistical analyses**

All analyses were performed using IBM SPSS Statistics, version 23 (IBM Statistics for Macintosh, Chicago, IL, USA).

The total study population is referred to as 'total'. Depending on whether subgroups are highly likely to fulfill Rome IV criteria based on the end-of-day diary or not they are referred to as 'Rome IV-positive' and 'Rome IV-negative', respectively. Results for 'Rome IV-positive' and 'Rome IV-negative' are presented separately for both definitions of fulfilling Rome IV criteria: 'Definition 1: Abdominal pain score  $\geq 2$  once a week in each week' and 'Definition 2: Abdominal pain score  $\geq 3$  once a week in each week'.

Categorical data are presented as proportions and differences between groups are tested using <sup>2</sup> or Fisher's exact test. Continuous data are presented as medians and interquartile ranges (IQR) and groups are compared using Mann-Whitney U test, taking

into account asymmetric distribution of the data. A  $P$ -value of  $<0.05$  was considered statistically significant.

## Results

### Study population

In total, 404 subjects that completed at least 12 days of the end-of-day symptom diary were included in the analyses: 293 (72.5%) were women and median age was 45 [IQR: 28-59] years. Seventy-two per cent ( $N=291$ ) was recruited from secondary/tertiary care and IBS subtypes (based on Rome III criteria) were distributed as follows: 140 (34.7%) IBS-D, 81 (20%) IBS-C, 159 (39.4%) IBS-M and 24 (5.9%) IBS-U. Further characteristics of the total study population are shown in *Table 2.1*.

### IBS according to Rome IV criteria – Definition I: Abdominal pain score $\geq 2$

Of the 404 IBS subjects diagnosed by Rome III criteria, 353 (87.4%) did meet the surrogate Rome IV criteria when assessed using the end-of-day symptom diary and the cut-off for abdominal pain severity of  $\geq 2$ .

Rome IV-positive subjects were more often female (74.5% vs. 58.8%,  $P<0.05$ ), younger (45 vs. 53 years,  $P<0.05$ ) and recruited from secondary/tertiary care (74.4% vs. 56.9%,  $P<0.05$ ) compared to Rome IV-negative subjects. Additionally, visceral hypersensitivity assessed by rectal barostat was present more often in Rome IV-positive subjects (47.5% vs. 11.8%,  $P<0.001$ ). Subtype distribution (*i.e.* based on Rome III criteria) was not different between both groups.

**Table 2.1** Demographical and clinical characteristics of the total study population and Rome IV-positive and -negative subjects, separated for definition 1 and 2 of surrogate Rome IV criteria, i.e. assessed using end-of-day symptom diaries.

	Definition 1: Abdominal pain score $\geq 2$		Definition 2: Abdominal pain score $\geq 3$	
	Rome IV-positive N=353 (87.4%)	Rome IV-negative N=51	Rome IV-positive N=249 (61.6%)	Rome IV-negative N=155
<b>Total</b>	N=404	N=51	N=249	N=155
<b>Female sex</b> , N (%)	293 (72.5)	30 (58.8) <sup>#</sup>	195 (78.3)	98 (63.2) <sup>*</sup>
<b>Age</b> , median [IQR]	45 [28 - 59]	53 [32 - 65] <sup>#</sup>	42 [26 - 57]	51 [36 - 63] <sup>*</sup>
<b>BMI</b> , median [IQR]	24.03 [21.41 - 27.63]	24.62 [21.58 - 27.52]	24.08 [21.40 - 27.47]	23.96 [21.45 - 27.98]
<b>Subtype Rome III</b> , N (%)				
IBS-D	140 (34.7)	22 (43.1)	85 (34.1)	55 (35.5)
IBS-C	81 (20.0)	8 (15.7)	51 (20.5)	30 (19.4)
IBS-M	159 (39.4)	17 (33.3)	101 (40.6)	58 (37.4)
IBS-U	24 (5.9)	4 (7.8)	12 (4.8)	12 (7.7)
<b>Secondary/tertiary care</b> , N (%)	291 (72.0)	29 (56.9) <sup>#</sup>	194 (78.2)	97 (62.6) <sup>*</sup>
<b>Hypersensitive</b> , N (%)	87 (44.4) <sup>a</sup>	2 (11.8) <sup>c*</sup>	72 (57.1) <sup>d</sup>	15 (21.4) <sup>**</sup>
<b>14-day mean score</b> , median [IQR]				
Abdominal pain	2.21 [1.57 - 2.92]	1.07 [1.00 - 1.21] <sup>*</sup>	2.71 [2.29 - 3.29]	1.43 [1.17 - 1.77] <sup>*</sup>
Abdominal discomfort	2.43 [1.93 - 2.93]	1.50 [1.07 - 1.86] <sup>*</sup>	2.72 [2.36 - 3.21]	1.79 [1.43 - 2.21] <sup>*</sup>
Abdominal bloating	2.07 [1.50 - 2.79]	1.43 [1.07 - 2.00] <sup>*</sup>	2.43 [1.93 - 3.18]	1.71 [1.14 - 2.07] <sup>*</sup>
Flatulence	2.21 [1.71 - 2.86]	1.71 [1.29 - 2.33] <sup>*</sup>	2.43 [1.93 - 3.07]	1.93 [1.36 - 2.36] <sup>*</sup>
Constipation	1.29 [1.00 - 1.86]	1.00 [1.00 - 1.29] <sup>*</sup>	1.50 [1.07 - 2.07]	1.08 [1.00 - 1.43] <sup>*</sup>
Diarrhea	1.21 [1.00 - 1.64]	1.07 [1.00 - 1.29] <sup>*</sup>	1.31 [1.07 - 1.98]	1.08 [1.00 - 1.43] <sup>*</sup>
<b>GSRs</b> , median [IQR]				
Abdominal Pain	3.33 [2.33 - 4.00]	2.67 [1.67 - 3.33] <sup>*</sup>	3.67 [2.67 - 4.33]	2.67 [2.00 - 3.67] <sup>*</sup>
Diarrhea Syndrome	3.33 [2.00 - 4.33]	2.67 [1.67 - 4.00] #	3.67 [2.33 - 4.67]	3.00 [2.00 - 4.00] <sup>*</sup>
Constipation Syndrome	3.00 [2.33 - 4.00]	2.00 [1.33 - 3.33] <sup>*</sup>	3.33 [2.33 - 4.33]	2.67 [1.67 - 3.67] <sup>*</sup>
Indigestion Syndrome	4.00 [3.25 - 5.00]	3.75 [2.75 - 4.50]	4.25 [3.25 - 5.25]	3.75 [2.75 - 4.50] <sup>*</sup>
Regurgitation Syndrome	1.50 [1.00 - 3.00]	1.00 [1.00 - 2.00] #	1.75 [1.00 - 3.50]	1.00 [1.00 - 2.50] <sup>*</sup>
<b>HADS</b> , N (%)				
Depression $\geq 8$	76 (19.0)	3 (5.9) <sup>*</sup>	59 (24.1)	17 (11.0) <sup>*</sup>
Anxiety $\geq 8$	142 (35.6)	11 (21.6) #	105 (42.9)	37 (24.0) <sup>*</sup>
<b>SF-36</b> , median [IQR]				
Physical Composite Score (PCS)	42.83 [33.98 - 50.28]	49.92 [41.42 - 53.89] <sup>*</sup>	39.23 [29.84 - 46.95]	48.99 [39.41 - 52.62] <sup>*</sup>
Mental Composite Score (MCS)	49.75 [41.23 - 55.82]	51.03 [41.98 - 57.73]	48.31 [40.76 - 54.58]	51.34 [42.16 - 57.19] #

Differences tested using Mann-Whitney U test for continuous data and  $\chi^2$ - or Fisher's exact test for categorical data. Significances are shown for both definitions of surrogate Rome IV criteria separately. <sup>#</sup> Significant difference between Rome IV-positive and Rome IV-negative subjects ( $P < 0.05$ ); <sup>\*</sup> Significant difference between Rome IV-positive and Rome IV-negative subjects ( $P < 0.001$ ); <sup>a-c</sup> Number of subjects that underwent rectal barostat; <sup>d</sup> 196; <sup>e</sup> 179; <sup>f</sup> 17; <sup>g</sup> 126; <sup>h</sup> 70.

With regards to GI symptoms, Rome IV-positive versus -negative subjects reported higher scores in the end-of-day diary for all symptoms assessed (*i.e.* abdominal pain, abdominal discomfort, abdominal bloating, flatulence, constipation and diarrhea) and higher symptom scores for all five GSRS-domains, however, this was not statistically significant for indigestion syndrome.

Furthermore, Rome IV-positive subjects showed a higher percentage of depressive (21.0% vs. 5.9%,  $P<0.001$ ) as well as anxiety (37.6% vs. 21.6%,  $P<0.05$ ) symptoms and lower physical composite scores (41.67 vs. 49.92,  $P<0.001$ ) with regards to quality of life. Mental composite scores of SF-36 did not show a significant difference between the groups. Results are shown in *Table 2.1*.

### **IBS according to Rome IV criteria – Definition 2: Abdominal pain score $\geq 3$**

When using the surrogate Rome IV criteria as defined by at least one day of abdominal pain each week using the cut-off for abdominal pain severity of  $\geq 3$ , of the 404 IBS subjects diagnosed by Rome III criteria, 249 (61.6%) were Rome IV-positive.

In line with the findings above, *i.e.* regarding the cut-off of abdominal pain severity of  $\geq 2$ , Rome IV-positive subjects were more often female, younger, recruited from secondary/tertiary care, more often hypersensitive on rectal barostat, showed higher symptom severity scores for all symptoms assessed in the end-of-day symptom diary and showed higher percentages of depressive as well as anxiety symptoms compared to Rome IV-negative subjects, when using the cut-off of  $\geq 3$ . Likewise, subtype distribution (*i.e.* based on Rome III criteria) did not differ between the groups.

Moreover, with regard to symptom severity on GSRS, Rome IV-positive versus -negative subjects scored significantly higher on all domains including indigestion syndrome when using the cut-off of  $\geq 3$ . Furthermore, Rome IV-positive subjects scored significantly lower on both physical composite score and mental composite score of SF-36 compared to Rome IV-negative subjects. Results are shown in *Table 2.1*.

### **Alternative Rome IV diagnoses in Rome IV-negative (IBS) subjects**

Of the Rome IV-negative (IBS) subjects, according to the surrogate Rome IV criteria using the cut-off for abdominal pain severity of  $\geq 3$ , 34 (23.61%) fulfilled surrogate Rome IV-criteria for functional constipation, 50 (34.25%) for functional diarrhea and 37 (25.69%) for functional abdominal bloating/distension. Results are shown in *Table S2.2*.

## Discussion

This study demonstrates that 61.6% to 87.4% of Rome III IBS patients is likely to also fulfill the new Rome IV criteria for IBS, depending on the cut-off for abdominal pain severity used, when applying surrogate Rome IV criteria based on end-of-day symptom diaries. Regardless of the cut-off chosen, Rome IV-positive subjects were more often female, younger and recruited from secondary/tertiary care than Rome IV-negative subjects. Not only did they present with higher abdominal pain scores, but overall symptom severity was higher in Rome IV-positive subjects, including a higher percentage of visceral hypersensitivity as assessed by rectal barostat. Additionally, higher percentages of comorbid psychological symptoms and lower quality of life were found for Rome IV-positive subjects. Taken together, the current findings imply that the Rome IV IBS population will likely reflect a subgroup of Rome III IBS patients with more severe overall gastrointestinal symptomatology, psychological comorbidities and lower quality of life.

To date, a recent study by Bai *et al.* investigated the agreement between Rome III and Rome IV criteria for IBS in a GI outpatient population in China. They found a moderate consistency between Rome III and Rome IV criteria, with prevalences of 12.4% and 6.1% based on Rome III and Rome IV criteria, respectively.<sup>24</sup> Similarly, Whitehead and colleagues reported prevalences of 10.7% and 5.7%, respectively, in a large population-based study.<sup>8,9</sup> Our current study confirms this decrease in prevalence by introducing Rome IV criteria, within a well-defined Rome III IBS population. Additionally, in line with these previously reported findings, our cut-off for abdominal pain severity of  $\geq 3$  may be most robust to define those subjects highly likely to fulfill Rome IV criteria, based on end-of-day diary.

We observed that Rome IV-positive subjects were more often of female gender than Rome IV-negative subjects. These differences were not demonstrated by Bai and colleagues.<sup>24</sup> Differences in symptom severity between men and women, however, have been reported previously, indicating possible differences in pathophysiological mechanisms as well as in pain perception and coping strategies. A meta-analysis by Adeyemo and colleagues demonstrated that women are more likely to report abdominal pain than men.<sup>25</sup> Furthermore, higher overall IBS symptom severity in female IBS patients has been reported by Bjorkman *et al.*<sup>26</sup> These previous findings could explain the higher percentage of women in our Rome IV-positive IBS population compared to the Rome IV-negative subjects.



With regards to clinical differences between Rome III and Rome IV IBS subjects, Bai *et al.* reported higher abdominal pain scores for Rome IV, but no differences in abdominal discomfort, abdominal bloating or demographic characteristics. In contrast, our study shows significantly higher scores for abdominal discomfort, bloating, flatulence, diarrhea and constipation, apart from abdominal pain. A possible explanation for the discrepancy between these studies is the method of data collection. Bai and colleagues retrospectively assessed the presence of above-mentioned symptoms at one time point, whereas in our study symptom severity scores were determined daily, using an end-of-day diary during 14 days.

As a study by Engsbro *et al.* demonstrated that IBS subtype classification differs depending on whether retrospective (*i.e.* Rome Diagnostic Questionnaire) or prospective methods (*i.e.* diary cards) are used<sup>27</sup>, we did not use the symptom diary to assess the IBS subtypes. However, we feel that the end-of-day diary provides an objective overview of present symptoms during a 14-day period and therefore can be used to identify patients highly likely to fulfill the Rome IV criteria. Furthermore, Rome IV criteria require abdominal pain to be present at least once a week on average during the last three months. The end-of-day symptom diary as used only provides us with information about the past two weeks. However, all subjects fulfilled Rome III criteria assessed using the Rome III Diagnostic Questionnaire, which also requires the abdominal complaints to be present during the previous three months. Therefore, we think that the surrogate Rome IV criteria are a reliable reflection of the Rome IV Diagnostic Questionnaire.

This is the first study focusing on clinical differences between Rome III and Rome IV IBS subjects. In conclusion, the current study underlines a decrease of IBS prevalence when using (surrogate) Rome IV compared to Rome III criteria. In addition to these findings, the question arises how to deal with the Rome IV-negative patient population presenting with IBS-symptoms in both primary and secondary/tertiary care and with regard to future clinical and mechanistic studies.

First, it might be interesting to explore whether alternative Rome IV disorders can now be diagnosed in these subjects, for example functional constipation, functional diarrhea or functional abdominal bloating/distension. An exploratory analysis in the current study demonstrates that 24% is likely to fulfill Rome IV-criteria for FC, 34% for FD and 26% for FAB/D. These results should, however, be interpreted with caution, since these diagnoses are based on surrogate criteria, using an IBS-specific end-of-day symptom diary. Nevertheless, this suggests that an additional  $\pm 16\%$  of Rome III IBS patients will

not fulfill any of these Rome IV diagnoses. Possibly, a small subset might be defined as Rome IV unspecified functional bowel disorder. However, it is very likely that patients reporting abdominal pain or bloating/distension less than once weekly (without predominant constipation or diarrhea), will not fulfill any Rome IV diagnosis due to the new frequency threshold.

Second, as the Rome criteria are not that universally used in routine clinical practice, in our opinion, patients with milder functional GI symptoms seeking health care, but not fulfilling one of the Rome IV diagnoses, should still be managed as functional GI disorders. With regard to future IBS research, patient inclusion should be based on the Rome IV criteria, in order to aim at agreement between studies.

Nevertheless, this study demonstrates that the Rome IV IBS population is represented by younger females with higher overall gastrointestinal symptom severity, including comorbid psychological symptoms and lower quality of life compared to the Rome III IBS population. Therefore, results from Rome III IBS studies may not be directly comparable to those from Rome IV IBS populations, which has implications for future IBS research in particular.

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## Supplementary Material

**Table S2.1** Definition of Rome IV criteria<sup>6</sup> and surrogate Rome IV criteria, based on 14-day end-of-day symptom diary, separately for functional constipation, functional diarrhea and functional abdominal bloating/distension.

Rome IV criteria	Surrogate Rome IV criteria
<b>FUNCTIONAL CONSTIPATION</b>	
1. Must fulfill two or more of the following: <ol style="list-style-type: none"> <li>Straining with defecation (at least 25% of stools)</li> <li>Lumpy or hard stools (BSFS 1-2) (at least 25% of stools)</li> <li>Sensation of incomplete evacuation (at least 25% of stools)</li> <li>Sensation of anorectal obstruction/blockage (at least 25% of stools)</li> <li>Manual maneuvers to facilitate defecation (at least 25% of stools)</li> <li>Fewer than three spontaneous stools per week</li> </ol>	1. <ol style="list-style-type: none"> <li>Not verifiable from diary data</li> <li><b>Checked in diary data:</b> Percentage number of BM's with BSFS 1-2 from total number of BM's over 14 days; fulfilled if <math>\geq 25\%</math></li> <li>Not verifiable from diary data</li> <li>Not verifiable from diary data</li> <li>Not verifiable from diary data</li> <li><b>Checked in diary data:</b> Number of BM's over 14 days; fulfilled if <math>\leq 6</math></li> </ol>
2. Loose stools are rarely present (BSFS 6-7)	2. <b>Checked in diary data:</b> Percentage number of BM's with BSFS 6-7 from total number of BM's over 14 days; fulfilled if $\leq 20\%$
3. Insufficient criteria for IBS or OIC	3. <b>Assumed true:</b> Additional analysis only considers subjects that fulfill Rome III for IBS (no OIC) and do not fulfill Rome IV for IBS
4. Symptom onset at least 6 months prior to diagnosis	4. <b>Assumed true:</b> Subjects fulfilled Rome III for IBS

Table S2.1 (continued)

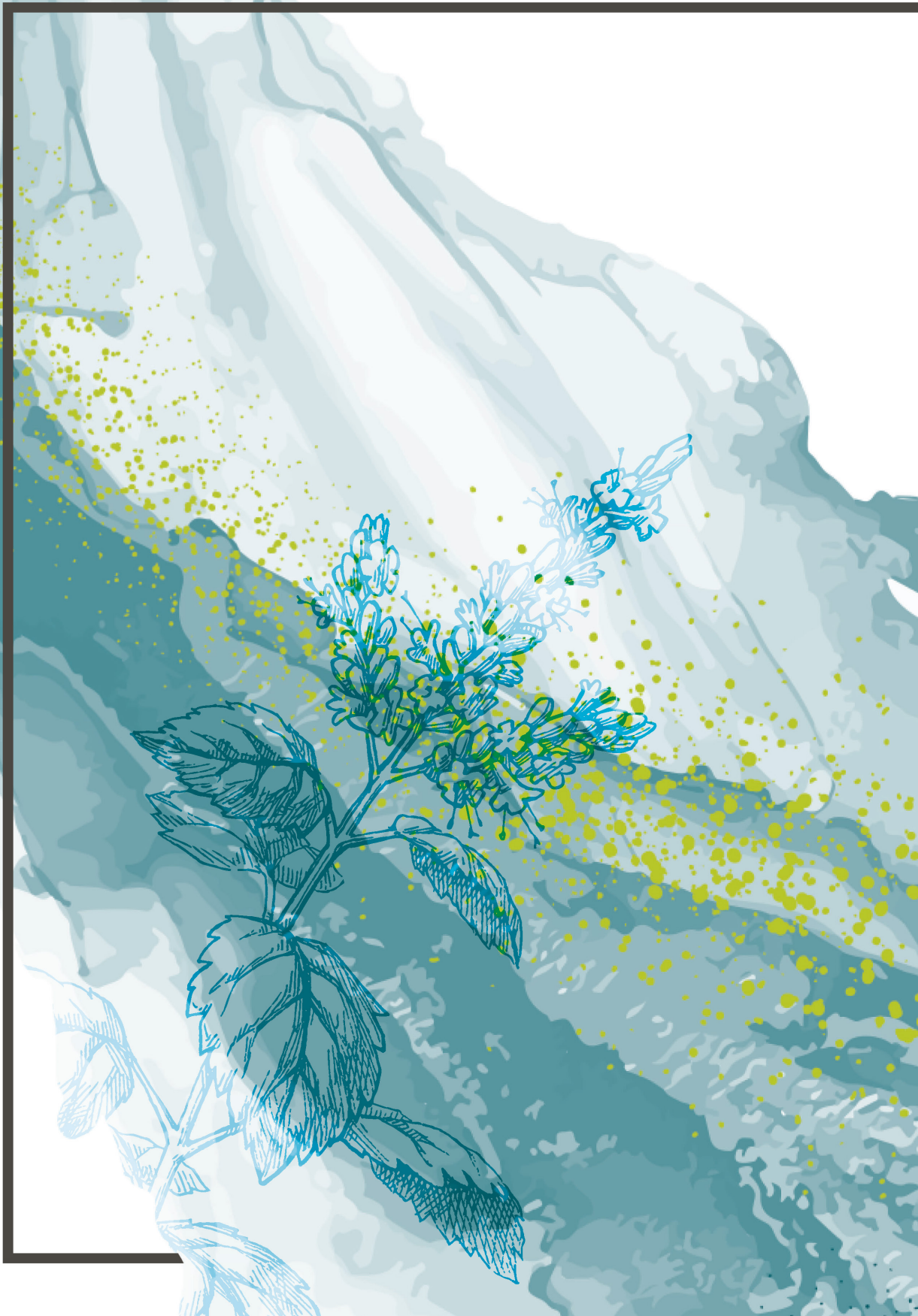
Rome IV criteria	Surrogate Rome IV criteria
<b>FUNCTIONAL DIARRHEA</b>	
<ol style="list-style-type: none"> <li>Loose or watery stools (BSFS 6-7) (at least 25% of stools)</li> <li>Without predominant abdominal pain or bloating</li> <li>Symptom onset at least 6 months prior to diagnosis</li> <li>Insufficient criteria for IBS-D</li> </ol>	<ol style="list-style-type: none"> <li><b>Checked in diary data:</b> Percentage number of BM's with BSFS 6-7 from total number of BM's over 14 days; fulfilled if <math>\geq 25\%</math></li> <li>Not verifiable from diary data</li> <li><b>Assumed true:</b> Subjects fulfilled Rome III for IBS</li> <li><b>Assumed true:</b> Additional analysis only considers subjects that do not fulfill Rome IV for IBS</li> </ol>
<b>FUNCTIONAL ABDOMINAL BLOATING/DISTENSION</b>	
<ol style="list-style-type: none"> <li>Recurrent bloating and/or distension (at least weekly)</li> <li>Abdominal bloating/distension predominates over other symptoms</li> <li>Insufficient criteria for IBS, FC, FD or PDS</li> <li>Symptom onset at least 6 months prior to diagnosis</li> </ol>	<ol style="list-style-type: none"> <li><b>Checked in diary data:</b> Symptom severity score for bloating <math>\geq 2</math> at least one day in both the first and the second seven days.</li> <li>Not verifiable from diary data</li> <li><b>Checked in diary data:</b> Additional analysis only considers subjects that do not fulfill Rome IV for IBS. Subjects fulfilling surrogate criteria for FC or FD were excluded from FAB/D analysis. Diagnosis PDS was not verifiable from diary data.</li> <li><b>Assumed true:</b> Subjects fulfilled Rome III for IBS</li> </ol>

BSFS: Bristol Stool Form Scale; FC: Functional Constipation; BM: Bowel movement; FD: Functional Diarrhea; OIC: Opioid induced constipation; FAB/D: Functional Abdominal Bloating/Distension; PDS: Post-prandial distress syndrome.

**Table S2.2** Distribution of alternative Rome IV diagnoses, based on surrogate Rome IV criteria, within Rome IV-negative (IBS) population, *i.e.* assessed using end-of-day symptom diaries\*.

	<b>Rome IV-negative</b> N=155
<b>Functional constipation</b> , N (%) (N=373)	34 (23.61) <sup>a</sup>
<b>Functional diarrhea</b> , N (%) (N=376)	50 (34.25) <sup>b</sup>
<b>Functional bloating/distension</b> , N (%) (N=370)	37 (25.69) <sup>a</sup>
<b>No alternative Rome IV diagnosis</b> , N (%) (N=370)	23 (15.97) <sup>a</sup>

<sup>a-d</sup> Number of subjects included in analyses: <sup>a</sup>144; <sup>b</sup>146. \* Rome IV IBS diagnosis according to 'Definition 2: Abdominal pain score  $\geq 3$  once a week in each week'.





# Chapter 3

Reduction in IBS symptom severity is not paralleled by improvement in quality of life in patients with irritable bowel syndrome

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## Abstract

### Introduction

Irritable Bowel Syndrome (IBS) is a brain-gut-disorder, of which the natural course varies between patients and is difficult to predict. This study aimed to evaluate symptom evolution over a five-year follow-up period and to identify baseline predictors for symptom severity and quality of life (QoL) at follow-up.

### Methods

Maastricht IBS Cohort participants completed questionnaires upon inclusion regarding demographics and lifestyle, gastrointestinal (GI) symptoms, anxiety and depression, and QoL. The same questionnaires, in addition to others, were completed after five years. Rome criteria were confirmed face-to-face at initial enrollment and through telephonic interviews at follow-up.

### Results

At a mean follow-up of 4.7 years, 379 patients were approached of whom 203 (53.7%) responded. Of these, 161 were reached by telephone and analyzed; 49 (30.4%) did not fulfill the Rome III criteria at follow-up and had lower levels of GI symptoms and GI-specific anxiety compared to those remaining Rome III-positive ( $P<0.001$ ). However, Rome III-negative patients had comparable levels of QoL and life satisfaction, comorbid anxiety and depression, work absenteeism and impaired productivity. No baseline predictors were found for being Rome III-positive or -negative. However, greater age and lower baseline physical QoL predicted lower physical QoL at follow-up ( $P<0.005$  and  $P<0.01$ , respectively), while lower baseline mental QoL predicted lower mental QoL at follow-up ( $P=0.005$ ). Additionally, higher anxiety and depression scores at follow-up were associated with lower QoL and life satisfaction at follow-up ( $P<0.001$ ).

### Conclusions

Long-term QoL and general well-being might depend on concurrent psychological symptoms, rather than GI symptom improvement.

## Introduction

Irritable Bowel Syndrome (IBS) is a brain-gut-disorder characterized by a chronic relapsing-remitting nature of symptoms, including abdominal pain and altered bowel habits. Global prevalence, based predominantly on the Rome III criteria, is estimated at 5-20%<sup>1</sup>, with varying rates according to geographical area and diagnostic criteria used.<sup>2</sup> Recent studies using the more restrictive Rome IV criteria point to lower prevalence rates of 5-6%.<sup>3,4</sup>

Although the exact pathophysiology of IBS remains incompletely understood, a multifactorial origin is generally recognized, in which dysregulation of the brain-gut-axis has a central role. Other factors include aberrant neuroimmune interactions, visceral hypersensitivity, genetic susceptibility, microbiome alterations, and psychosocial factors.<sup>5,6</sup> As interference of IBS with patients' everyday lives is extensive<sup>7</sup> and treatment results are often unsatisfactory, quality of life is low and comparable to chronic somatic diseases.<sup>8</sup>

Given the heterogeneous nature of the disorder, symptom patterns vary widely both between and within IBS patients and predicting individual disease courses remains challenging. IBS is known as a chronic, in many patients lifelong, condition with fluctuating gastrointestinal (GI) symptoms. Symptoms such as abdominal pain, constipation, and diarrhea are known to occur in episodes of several days followed by days without symptoms.<sup>9</sup> In addition, transitions from one predominant bowel habit type to the other are common and occur in up to 75% of patients.<sup>10-12</sup> Quality of life, on the other hand, has been shown to be relatively stable over a three-month period.<sup>13</sup> With regard to long-term symptom variability, several studies have investigated the disease course of IBS and have shown varying results with respect to symptom severity and quality of life. The majority of the prospective studies had a follow-up period of approximately one year, which is relatively short for a condition such as IBS. In addition, there is a lack of follow-up data for the Dutch population. As implications of IBS on quality of life have shown to vary considerably between different countries<sup>14</sup>, it is of added value to expand earlier findings and investigate the natural history of IBS in a Dutch, well-characterized population.

Finally, as IBS is a symptom-based diagnosis, it is of interest to assess how symptoms evolve over time and how these symptoms and long-term quality of life relate to the Rome diagnostic criteria for IBS, in particular when they are confirmed in a telephonic interview rather than a purely survey-based assessment at follow-up measurements.

Hence, the current follow-up study, which is part of a large prospective cohort study on the pheno- and genotypical characterization of IBS in the Netherlands, aimed 1) to gain further insight into symptom evolution and long-term quality of life over a five-year follow-up period; 2) to identify baseline predictors for higher symptom severity and greater quality of life impairment at follow-up; and 3) to assess how point 1 and 2 differ between patients that remain Rome III-positive and those who are Rome III-negative at follow-up.

## Materials and methods

### Irritable bowel syndrome cohort study

Since 2009, patients with IBS between 18 and 75 years of age who visit the outpatient clinic of the Gastroenterology-Hepatology division of the Maastricht University Medical Center+ (MUMC+), The Netherlands, are requested to enroll in the Maastricht IBS (MIBS) Cohort study. The MUMC+ is a university hospital with a combined secondary and tertiary care service in the area of South Limburg, The Netherlands. In addition, patients with IBS are recruited via general practitioners in the area of South Limburg. The MIBS Cohort is an extensively phenotyped cohort of IBS patients with a follow-up measurement five years after initial inclusion.<sup>15-17</sup> The research protocol had been approved by the MUMC+ Committee of Ethics and all study procedures were performed in compliance with Good Clinical Practice Guidelines and according to the revised Declaration of Helsinki.<sup>18</sup> The study had been registered in the US National Library of Medicine (*NCT00775060*). All subjects gave a written informed consent prior to participation.

### Subjects

Patients who had been included at least three years (five +/- two years) before the current study, *that is*, patients included in the MIBS cohort between September 2009 and September 2014, were eligible for participation in the current follow-up study. All patients fulfilled the Rome III criteria for IBS at the time of inclusion, which was confirmed by a trained clinical investigator in a face-to-face interview. Patients were assigned IBS subtypes based on their reported predominant stool-type, *i.e.* diarrhea (IBS-D), constipation (IBS-C), a mix of diarrhea and constipation (IBS-M), or unspecified predominant bowel habit (IBS-U). Their medical history was taken and, if any alarm symptoms were present or if deemed necessary by the gastroenterologist, GI endoscopy, abdominal imaging, and/or blood, breath and/or fecal analyses were

performed to exclude organic diseases. A history of abdominal surgery automatically led to exclusion, except for an uncomplicated appendectomy, hysterectomy, or cholecystectomy.

## Data collection

At time of enrollment in the MIBS Cohort, patients completed several questionnaires, *i.e.* on demographics and lifestyle, GI symptoms, symptoms of comorbid anxiety and depression, and general quality of life. The data were obtained administering a predefined self-report questionnaire on demographics and lifestyle, an end-of-day 14 days symptom diary, the gastrointestinal symptom rating scale (GSRS, scale 1-7, generates symptom scores for the following subdomains: abdominal pain, reflux, diarrhea, constipation, indigestion)<sup>19</sup>, the hospital anxiety and depression scale (HADS, scale 0-3, a screening tool for anxiety and depression)<sup>20,21</sup>, and the rand 36-item short-form health survey (SF-36, scale 1-6, generates a physical and a mental quality of life component summary).<sup>22,23</sup>

Approximately five years (+/- two years) after inclusion, patients were invited to complete several follow-up questionnaires. These included the same questionnaires that were filled out at baseline (with the exception of the end-of-day diary), with the addition of other questionnaires to further standardize the phenotyping of our population and to allow valid comparisons to other cohorts (*i.e.* international harmonization). Additional questionnaires included: the GSRS specific for IBS (GSRS-IBS, scale 1-7, generates symptom scores for the following subdomains: abdominal pain, bloating, constipation, diarrhea, satiety)<sup>24</sup>; the Visceral Sensitivity Index (VSI, scale 1-6, assesses GI-specific anxiety or, in other words, fear for GI symptoms)<sup>25</sup>; and the Satisfaction With Life Scale (SWLS, scale 1-6, assesses individual overall satisfaction with life).<sup>26-28</sup> As it is known that IBS can affect the ability to work<sup>29</sup>, a self-report questionnaire regarding productivity (Productivity Cost Questionnaire (PCQ), validated for the Dutch situation) was included as well.<sup>30,31</sup> Patients could opt for paper or digital web-based (invitation was sent via email) questionnaires to encourage participation. One reminder was sent when no response had been received within one month. A trained clinical investigator contacted all patients who did respond and confirmed the Rome III diagnostic criteria during a telephonic interview with them. Patients were reassigned IBS subtypes based on their predominant bowel habit, during the follow-up measurement. Reliable information on treatment history was not available for the majority of patients as this was not registered systematically during the follow-up period. Furthermore, many patients underwent self-treatment or treatment via their primary care physician, of

which data were not available and were therefore not included in the analysis of the current study.

## **Statistical analysis**

All statistical analyses were carried out using IBM SPSS statistics 23.0 (Chicago IL, USA) and GraphPad Prism 6.0 (La Jolla, CA, USA) for Macintosh. Continuous data are presented as medians and interquartile ranges (IQR) and categorical data as proportions (%). To compare (non-parametric) continuous data between subgroups, Mann-Whitney U tests were used. Wilcoxon-signed-rank tests were used to evaluate differences within subjects over time. For categorical data, groups were compared using the Chi-square or Fisher's exact test and differences over time were evaluated using the McNemar's test. Correlations were assessed according to Spearman. To decrease the false discovery error rate induced by multiple testing, a post-hoc correction was applied using the Benjamini-Hochberg step-up procedure.<sup>32</sup>

The patients participating in the follow-up were divided into two groups for comparison and further analyses, 1) Rome III-positive: patients who still fulfilled the Rome III criteria for IBS at follow-up; 2) Rome III-negative: patients who did not fulfill the Rome III criteria for IBS at follow-up. Multivariable logistic and linear regression models, respectively, were used to identify independent baseline predictors for 1) the presence of IBS according to the Rome III diagnostic criteria at follow-up; 2) quality of life at follow-up. We also used linear regression models to identify characteristics at follow-up that were associated with quality of life at follow-up. The  $\beta$  of the linear regression analyses signifies one-point change in the mental or physical quality of life component summary.

## **Results**

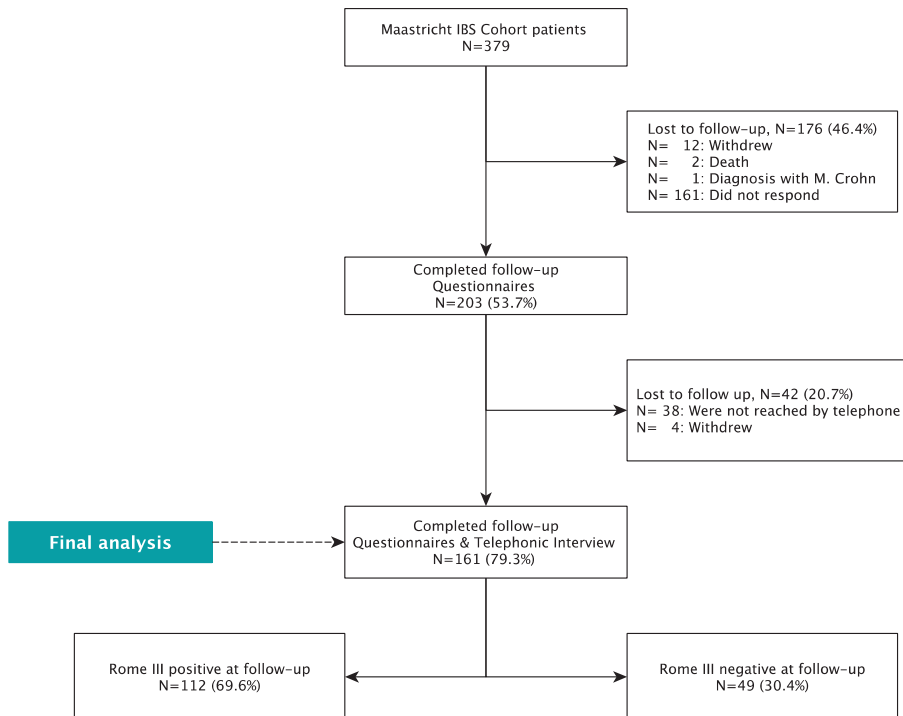
### **Study population for follow-up of the Rome III irritable bowel syndrome cohort**

At a mean follow-up time of 4.7 (SD 1.5) years, 379 patients with IBS, Rome III-positive at inclusion and participating at least three (five +/- two) years in the Maastricht IBS cohort, were approached for participation in the follow-up measurement. A total of 203 subjects (53.7%) responded by completing the electronic or paper questionnaires. Of these, 161 could be reached by telephone to confirm the Rome criteria, and these did not differ in demographics nor in symptom scores when compared to the 42 subjects that were not reached. A study flowchart is shown in *Figure 3.1*.

## Follow-up – Demographics, clinical characteristics, and symptoms of the Rome III-positive and –negative patients

The 161 patients in whom IBS criteria could be checked by phone were included in the final analysis. The majority of patients was female (74.5%) and median age was 53 years old (IQR=36-65).

In total, 49 out of the 161 (30.4%) did no longer fulfill the Rome III criteria for IBS at follow-up (Figure 3.1).



**Figure 3.1** Flowchart of follow-up of N=379 IBS patients included in the Maastricht IBS Cohort study. N; number, IBS; Irritable Bowel Syndrome.

Demographical and clinical characteristics at follow-up of the Rome III-negative and Rome III-positive patients are shown in Table 3.1. The groups did not differ significantly in age or gender, although the Rome III-positive group was slightly younger. As expected, Rome III-negative patients had significantly lower GI symptom scores compared to Rome III-positive

patients (assessed by both the GSRS and GSRS-IBS) at follow-up, which was shown on each of the following subdomains: abdominal pain, bloating, diarrhea, constipation, indigestion (all  $P<0.001$ ), and satiety ( $P<0.005$ ). Despite having less GI symptoms, they did not have significantly higher quality of life or more satisfaction with life. Although Rome III-negative patients had significantly less GI-specific anxiety at follow-up ( $P<0.001$ ), they did not have less symptoms of general anxiety and depression. Moreover, levels of impaired work productivity and absence from work did not differ between both groups.

**Table 3.1** Demographical and clinical characteristics, at a five-year follow-up period, of patients who still fulfilled the Rome III criteria for IBS at follow-up (FU Rome III-positive) and those who did not (FU Rome III-negative).

Characteristics at follow-up	FU Rome III-positive N=112	FU Rome III-negative N=49
<b>Age</b> , median years (IQR)	52 (34-65)	57 (45-67)
<b>Female gender</b> , N (%)	84 (75.00)	36 (73.47)
<b>BMI</b> , median kg m <sup>-2</sup> (IQR)	25.15 (21.88-28.70)	25.35 (21.62-28.37)
<b>GSRS</b> , median (IQR)		
Abdominal pain	3.33 (2.67-4.33)	2.33 (1.17-3.00)***
Reflux syndrome	1.50 (1.00-3.00)	1.50 (1.00-3.00)
Diarrhea syndrome	3.33 (2.00-4.67)	1.67 (1.00-3.00)***
Constipation syndrome	3.00 (2.00-4.00)	2.00 (1.33-3.00)***
Indigestion syndrome	3.75 (2.00-4.67)	3.00 (2.25-4.50)***
<b>GSRS-IBS</b> , median (IQR)		
Abdominal pain	4.00 (3.5-5.00)	2.50 (1.50-3.50)***
Bloating	4.33 (3.33-5.00)	3.00 (2.00-4.33)***
Constipation	2.50 (1.00-4.00)	2.00 (1.00-2.50)
Diarrhoea	3.25 (2.25-4.50)	2.00 (1.50-3.00)***
Satiety	3.00 (2.00-4.50)	2.50 (1.25-3.50)**
<b>Comorbid-FD</b> , N (%)	61 (55.00)	20 (41.67)
<b>Rome IV-positive IBS</b> , N (%)	69 (61.61)	-
<b>HADS, score &gt;8</b> , N (%)		
Depressive symptoms	26 (23.42)	8 (16.33)
General anxiety symptoms	33 (29.72)	8 (16.33)
<b>GI-specific anxiety</b> , median (IQR)	24.00 (13.00-37.00)	8.00 (4.00-23.00)***
<b>Quality of life</b> , median (IQR)		
PCS	45.10 (33.01-48.50)	48.96 (37.71-52.99)
MCS	51.27 (37.81-55.96)	54.75 (44.61-57.71)
<b>Satisfaction with life</b> , N (%)		
Very unsatisfied	3 (2.67)	0 (0.0)
Unsatisfied	7 (6.25)	2 (4.08)
Below average satisfaction	20 (17.86)	4 (8.16)
Averagely satisfied	25 (22.32)	14 (28.57)
Satisfied	37 (33.04)	20 (40.82)
Very satisfied	20 (17.86)	9 (18.37)
<b>Absence from work</b> , N (%)	18 (34.62)	6 (25.00)
<b>Impaired productivity at work</b> , N (%)	30 (61.22)	15 (62.50)

N, number of patients included in the analysis; IQR, Inter Quartile Range; BMI, Body Mass Index (kg m<sup>-2</sup>); GSRS, gastrointestinal symptom rating scale; FD, Functional Dyspepsia; HADS, hospital anxiety and depression scale; PCS, physical quality of life composite summary as assessed by SF-36; MCS, mental quality of life composite summary as assessed by SF-36; GI, gastrointestinal. Numbers may not add up to total due to missing. \*\*\* $P<0.001$  versus Rome III-positive; \*\* $P<0.005$  versus Rome III-positive.



## Baseline - Demographics, clinical characteristics, and symptoms of the Rome III-positive and –negative patients

Table 3.2 shows baseline demographics and clinical characteristics (at inclusion time into the cohort) of patients who were Rome III-positive and -negative at follow-up.

**Table 3.2** Baseline demographical and clinical characteristics, of patients still fulfilling the Rome III criteria for IBS at follow-up (FU Rome III-positive) and those who did not (FU Rome IV-negative).

Characteristics at inclusion	FU Rome III-positive N=112	FU Rome III-negative N=49
<b>Age</b> , median (IQR)	48 (29-61)	51 (39-61)
<b>Gender</b> , N female (%)	84 (75.0)	36 (73.47)
<b>BMI</b> , median kg m <sup>-2</sup> (IQR)	23.70 (21.50-28.70)	24.40 (21.50-28.05)
<b>Secondary/tertiary care</b> , N (%)	72 (64.29)	35 (71.43)
<b>IBS subtype</b> , N (%)		
IBS-D	40 (35.7)	19 (38.8)
IBS-C	23 (20.5)	8 (16.3)
IBS-M	47 (42.0)	16 (32.7)
IBS-U	2 (1.8)	6 (12.2)
<b>GSRs</b> , median (IQR)		
Abdominal pain	3.67 (2.33-4.00)	2.83 (2.00-3.67)
Reflux syndrome	1.50 (1.00-2.63)	1.00 (1.00-2.88)
Diarrhea syndrome	3.00 (2.33-4.67)	2.83 (1.67-4.25)
Constipation syndrome	3.17 (2.33-4.00)	1.49 (2.00-3.67)
Indigestion syndrome	4.25 (3.38-5.00)	3.88 (2.75-5.00)
<b>Diary</b> , median score (IQR)		
Discomfort	2.29 (2.00-2.72)	2.21 (1.64-2.57)
Pain	2.23 (1.64-2.79)	1.93 (1.27-2.50)
Constipation	1.29 (1.00-1.79)	1.14 (1.00-1.59)
Diarrhea	1.21 (1.07-1.64)	1.07 (1.00-1.38)
Bloating	2.00 (1.52-2.64)	2.00 (1.25-2.43)
Flatulence	2.29 (1.89-2.89)	2.23 (1.64-2.80)
Belching	1.36 (1.00-2.00)	1.18 (1.00-1.92)
Nausea	1.21 (1.00-1.81)	1.14 (1.00-1.57)
<b>HADS</b> , score >8, N (%)		
Depressive symptoms	20 (19.05)	6 (5.71)
General anxiety symptoms	41 (38.68)	17 (38.64)
<b>Quality of life</b> , median (IQR)		
PCS	41.71 (33.90-48.98)	45.51 (36.32-51.74)
MCS	50.20 (41.45-55.19)	50.67 (41.38-57.44)

N, number of patients included in the analysis; IQR, Inter Quartile Range; BMI, Body Mass Index (kg m<sup>-2</sup>); secondary/tertiary care as compared to primary care; GSRs, gastrointestinal symptom rating scale; HADS, hospital anxiety and depression scale; PCS, physical quality of life composite summary as assessed by SF-36; MCS, mental quality of life composite summary as assessed by SF-36. Numbers may not add up to total due to missing. Please note that several variables shown in Table 3.1 derive from questionnaires not administered at baseline and could therefore not be considered in the comparison of baseline variables in Table 3.2.

With regard to baseline levels of GI symptom severity (assessed by the GSRS, as baseline GSRS-IBS data were not available), it can be noted that Rome III-negative patients had slightly lower baseline scores for abdominal pain. Nevertheless, after correction for multiple comparisons, this did not reach statistical significance nor did other GI symptoms differ significantly between the groups at baseline. In terms of general anxiety, depression, and general quality of life scores, no baseline differences were observed between Rome III-positive and -negative patients (*Table 3.2*). Moreover, the proportion of patients that had been recruited for the cohort from the primary care setting did not differ between Rome III-positive and -negative patients (*Table 3.2*). In addition, the distribution in baseline IBS-subtypes did not differ between Rome III-positive and -negative patients (*Table 3.2*).

### **Symptom evolution over time**

*Table 3.3* shows the evolution of clinical characteristics and symptoms over time by pairwise comparisons between baseline and follow-up measurements. Overall, improvements in GI symptom severity (assessed by the GSRS, as baseline GSRS-IBS data were not available), general quality of life, and general anxiety and depression over a five-year period were small and did not reach significance in patients that still met the Rome III-diagnostic criteria at follow-up. Symptom severity for abdominal pain, diarrhea, constipation, and indigestion did improve significantly over time in the Rome III-negative patients, although they still had ‘low to moderate’ levels of symptoms after five years, with a median abdominal pain GSRS score of 2.33 (IQR=1.33-3.00), and a median indigestion GSRS score of 2.88 (IQR=2.25-3.94). However, similar to the Rome III-positive patients, the Rome III-negative patients did not improve significantly in quality of life during the follow-up period. In addition, their comorbid depression scores were relatively unaltered.

With regard to IBS subtype based on predominant bowel habit, *Supplementary Figure S3.1* illustrates the proportion of IBS subtypes at baseline and at follow-up. Sixty-two (55.4%) out of 112 Rome III-positive patients had changed IBS-subtype during the follow-up period. In *Supplementary Figure S3.2*, the within-patient subtype changes from baseline to follow-up are shown. IBS-D appeared the most stable subtype (72.5% of Rome III-positive unchanged), whereas IBS-M and IBS-C were least stable (76.6% and 60.9% of Rome III-positive changed respectively).

**Table 3.3** Clinical characteristics, at baseline and at time of follow-up, of patients who still fulfilled the Rome III criteria for IBS at follow-up (Rome III-positive) and patients who did not (Rome III-negative).

	Baseline	Follow-up
<b>FU Rome III-positive IBS patients, N=112</b>		
<b>BMI</b> , median kg m <sup>-2</sup> (IQR)	23.70 (21.45-28.70)	24.77 (21.92-28.60)**
<b>GSRs</b> , median (IQR)		
Abdominal pain	3.67 (2.33-4.00)	3.33 (2.50-4.33)
Reflux syndrome	1.50 (1.00-2.75)	1.50 (1.00-3.00)
Diarrhea syndrome	3.00 (2.33-4.67)	3.33 (1.83-4.67)
Constipation syndrome	3.00 (2.33-4.00)	3.00 (2.00-4.00)
Indigestion syndrome	4.00 (3.38-5.00)	3.75 (3.00-4.50)
<b>HADS</b> , score >8, N (%)		
Depressive symptoms	20 (19.05)	25 (23.81)
General anxiety symptoms	41 (39.05)	31 (29.52)
<b>Quality of life</b> , median (IQR)		
PCS	42.19 (34.36-48.90)	45.41 (33.44-48.78)
MCS	49.58 (37.87-54.96)	51.07 (37.57-55.93)
<b>FU Rome III-negative IBS patients, N=49</b>		
<b>BMI</b> , median kg m <sup>-2</sup> (IQR)	24.50 (21.30-28.08)	25.35 (21.68-28.73)
<b>GSRs</b> , median (IQR)		
Abdominal pain	2.83 (2.00-3.67)	2.33 (1.33-3.00)*
Reflux syndrome	1.00 (1.00-2.88)	1.50 (1.00-3.00)
Diarrhea syndrome	2.83 (1.67-4.25)	1.67 (1.08-3.00)**
Constipation syndrome	2.50 (2.00-3.67)	2.00 (1.33-3.00)***
Indigestion syndrome	3.88 (2.75-5.00)	2.88 (2.25-3.94)***
<b>HADS</b> , score >8, N (%)		
Depressive symptoms	6 (13.64)	7 (15.91)
General anxiety symptoms	17 (38.64)	7 (15.91)∞
<b>Quality of life</b> , median (IQR)		
PCS	45.51 (36.32-51.74)	48.85 (33.14-52.99)
MCS	50.68 (41.38-57.44)	53.38 (43.89-57.30)

N, number of patients included in the analysis; IQR, Inter Quartile Range; BMI, Body Mass Index (kg m<sup>-2</sup>); GSRs, gastrointestinal symptom rating scale; HADS, hospital anxiety and depression scale; PCS, physical quality of life composite summary as assessed by SF-36; MCS, mental quality of life composite summary as assessed by SF-36. Numbers may not add up to total due to missing. \*\*\**P*<0.001 vs. baseline; \*\**P*<0.005 vs. baseline; \**P*<0.01 vs. baseline; ∞*P*<0.05 vs. baseline.

## Multivariable regression model

No baseline predictors for fulfilling the Rome III diagnosis for IBS at follow-up could be identified with regression analyses. When looking for independent baseline predictors concerning quality of life at follow-up for both groups taken together (*N*=161), the combined regression model that included age, gender, baseline GI symptom severity, and baseline anxiety and depression, showed that only younger age ( -0.16, 95% CI -0.26; -0.06, *P*=0.002) and higher physical quality of life at baseline ( 0.59, 95% CI 0.41; 0.78, *P*<0.001) predicted higher physical quality of life at follow-up. Likewise, only higher

mental quality of life at baseline ( 0.46, 95% CI 0.15; 0.77,  $P=0.004$ ), but not baseline depression or anxiety scores, predicted higher mental quality of life at follow-up.

When looking for characteristics at follow-up that were associated with quality of life at follow-up, general anxiety ( -1.49, 95% CI -1.90; -1.07,  $P<0.001$ ), and depression levels ( -1.00, 95% CI -1.48; -0.53,  $P<0.001$ ) at follow-up were the only two parameters independently associated with mental quality of life at follow-up after correcting the model for age, gender, IBS-subtype, GI symptom severity, GI-specific anxiety, and general anxiety and depression. No characteristics at follow-up were found to independently associate with physical quality of life at follow-up. Baseline IBS-subtypes were not included in the models that looked for baseline predictors, as univariate analyses showed no significant differences in subtypes between groups, that is, the distribution in baseline subtypes did not differ significantly between Rome III-negative and -positive patients. Inclusion of these variables into the model would have decreased statistical validity and increased the probability of type I errors.

## Discussion

In the current prospective study, we evaluated the natural symptom evolution of patients with IBS in the Maastricht IBS cohort over time.<sup>33</sup> We demonstrated that 30.4% of patients did no longer fulfill the Rome III criteria after a five-year follow-up period. The most salient finding is that quality of life did not improve significantly in patients who showed a decrease in gastrointestinal symptom severity (*i.e.* being Rome III-negative at follow-up), compared to patients who had unaltered symptom severity over time (Rome III-positive). In addition, general wellbeing in terms of comorbid general anxiety and depression, work absenteeism and productivity, and life satisfaction were also comparable in those who still fulfilled the Rome III criteria at follow-up, when compared to those who did not.

Several studies have investigated IBS symptom evolution in relation to diagnostic criteria in patient cohorts, with varying results. A study by Williams *et al.* using the Rome II criteria reported that 52% had no IBS two years after web-based diagnosis<sup>12</sup>, Ford *et al.* found that 28% did not meet the Manning criteria after 10 years<sup>34</sup>, Mearin *et al.* reported that abdominal pain frequency decreased in 26% after one year<sup>35</sup>, and more recently, Card *et al.* reported that 27% of their large study population did not fulfill the Rome III criteria after one year.<sup>36</sup> Except for the study by Williams *et al.*, the proportion of

patients who do not meet the diagnostic criteria after a follow-up period is thus in line with the findings of this follow-up study in our Dutch cohort.

Given that clinical treatment decisions often rely on prognostic predictions of the disease course, we sought to identify baseline predictors for a less favorable disease course. This study, however, did not show any baseline characteristics associated with meeting the Rome III criteria at follow-up. As IBS is a highly heterogeneous disorder, larger study populations might be necessary to investigate what factors influence and thereby predict the natural disease course in (subgroups of) patients with IBS.

Rather unexpectedly, we found that a decrease in GI symptom severity did not lead to an improved quality of life in our study. Clevers *et al.* recently evaluated longitudinal symptom changes over time and, in contrast to our results, found that patients with lower GI symptom severity had significantly higher quality of life scores.<sup>37</sup> They also demonstrated that GI-specific anxiety is associated with an increase in GI symptom severity, which is in agreement with our results as GI-specific anxiety was significantly lower in the group that did not fulfill the Rome III criteria. The inconsistency between our data in terms of quality of life might be explained in part by the different questionnaires used. Clevers *et al.* used an IBS specific questionnaire, the IBS-QoL<sup>38</sup>, which assesses more disease specific changes in quality of life in contrast to generic quality of life instruments, such as the SF-36 that we used. Our data can therefore also be compared with other diseases and with the general population. Both the Rome III-positive and -negative group showed lower mean quality of life than the mean of a Dutch population sample<sup>39</sup> and of a USA based population without a functional GI disorder.<sup>40</sup> Additionally, we used the validated SWLS to score overall life satisfaction. This has been used in healthy persons and in patients with Crohn's disease<sup>41</sup>, but, to our knowledge, not in IBS patients. We found scores comparable to the ones reported by Crohn's disease patients for both the Rome III-positive and -negative group.

In contrast to GI symptom severity, we found that quality of life did not improve over time in those that were Rome III-negative at follow-up. The data reported here suggest that concurrent, but not baseline, psychological comorbidities are more predictive of this impaired long-term quality of life than GI symptom severity. Several studies in IBS have found similar results, in particular for the mental health related quality of life; *i.e.* Naliboff *et al.* reported that psychological distress had a stronger effect on health related quality of life than GI symptoms<sup>42</sup>, Koloski *et al.* reported that depression was independently associated with mental quality of life<sup>43</sup>, and Addante *et al.* reported that perceived stress, and anxiety and depression were significant predictors for mental

health related quality of life.<sup>44</sup> This raises the question whether current treatment goals for IBS in daily clinical practice should be revised. Gaining insight into which symptoms specifically affect a patient's quality of life can aid in reprioritizing personal treatment goals and increase the chance of a successful individualized treatment trajectory.<sup>45</sup> Currently, treatment is merely targeted towards the patient's predominant GI symptoms and in many cases, this entails primary treatment of pain, constipation, or diarrhea. Targeting GI symptom improvement without characterizing the full extent of the disorder and possible psychosocial modifiers may therefore contribute to treatment failure concerning quality of life over time. A helpful and pragmatic framework in this regard has been suggested by the Rome expert panel.<sup>45</sup> By following their five-step approach, clinicians can identify if and which psychological factors contribute to the disease burden in the individual patient and consider psychological treatments. In some patients, this might include the use of pharmacological neuromodulators<sup>46</sup>, but other therapy options include cognitive behavioral therapy (CBT), gut-directed hypnotherapy<sup>47</sup>, and dynamic psychotherapy.<sup>48</sup> Due to the relative lack in therapist availability, clinical implementation of these therapies is still limited. Recent attempts to reduce resource use without compromising effectiveness may assist in making psychological treatment more widely available; e.g. group hypnotherapy<sup>49</sup>, internet-delivered exposure-based CBT<sup>50</sup>, and home-based CBT.<sup>51</sup> In that light, it will be interesting to explore whether these developments can affect long-term quality of life and well-being in patients with IBS.

Several limitations of the current study should be noted. Only two time-points within the five-year period were assessed; no further data were available about the period in between the baseline and follow-up measurement, including data on treatment received in this period. Therefore, we can neither comment about the frequency and duration of IBS flares within these five years, nor about the effect of treatment on symptoms, nor on the causal order of the impaired quality of life and psychological comorbidities. Moreover, selection bias cannot be excluded. Follow-up cohort studies depend on long-term dedication of their participants and the proportion of non-responders (loss to follow) in this study was 46.4%. To assess for potential selection bias, we tested for differences in baseline characteristics between responders and non-responders at follow-up. The groups did not differ in baseline demographic and lifestyle characteristics, nor in baseline symptom scores and subtypes (data not shown). Hence, there is no clear reason to assume that the non-responders would have had different outcomes at follow-up. Finally, a larger sample size would have been desirable. Due to a relatively small Rome III-negative group, small effects concerning quality of life or other parameters could have been missed, especially since we have corrected for multiple

comparisons. Although we argue that this adjustment improves the reproducibility of our results, an unnecessarily high false negative rate cannot be ruled out. Because of the small sample size, it is important that our findings are corroborated in other cohorts of patients with IBS. Until then, findings should be considered exploratory and appropriate caution should be taken when interpreting the results of the current study.

Strengths of this prospective follow-up study include the heterogeneous and well-characterized Maastricht IBS cohort population, *i.e.* in-depth phenotyping enables comparison to other large cohorts and future studies should explore possibilities of pooling data from different centers to cluster phenotypes and validate the robustness of findings such as the ones reported here. Moreover, since we have recruited patients from both primary and secondary/tertiary care settings (*Table 3.1*), our population is representative for the general Dutch IBS population. Another strength is the evaluation of the Rome criteria in a telephonic follow-up interview with patients. Due to the current lack of validated biomarkers, IBS remains a symptom-based diagnosis for which the Rome criteria are used to aid diagnostic accuracy, both in research and clinical settings.<sup>52</sup> Since the Rome criteria have not been developed nor validated as a self-administered questionnaire, asking patients to complete the Rome criteria without supervision by a trained researcher may lead to bias and over- or under-estimation of the actual diagnosis, as previously demonstrated by Lovell *et al.*<sup>2</sup> In contrast to earlier studies on follow-up of IBS, our study method implied a complete reevaluation of the diagnosis in a telephonic interview and, thus, allows for a more valid and less biased interpretation of Rome diagnosed IBS prevalence over time.

In conclusion, the current prospective study contributes to existing insight regarding symptom evolution over time in IBS patients and showed that 30% of patients did no longer fulfill the Rome III criteria after a five-year follow-up period. However, the decrease in GI symptom severity (*i.e.* being Rome III-negative at follow-up), did not impact quality of life nor life satisfaction. Our results indicate that long-term quality of life and general well-being might depend on comorbid psychological symptoms, *i.e.* affective states, rather than gastrointestinal symptom severity.

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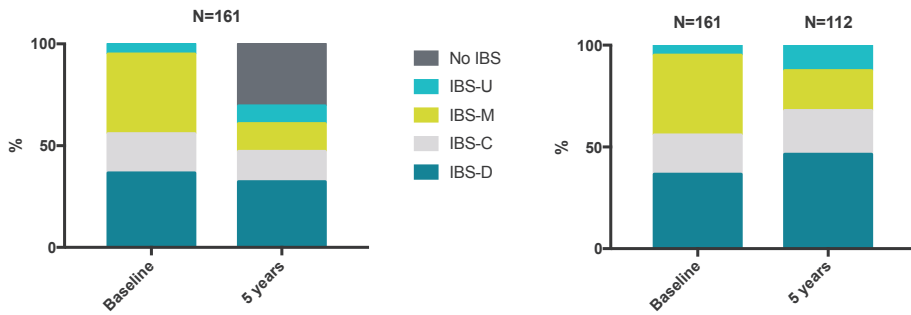
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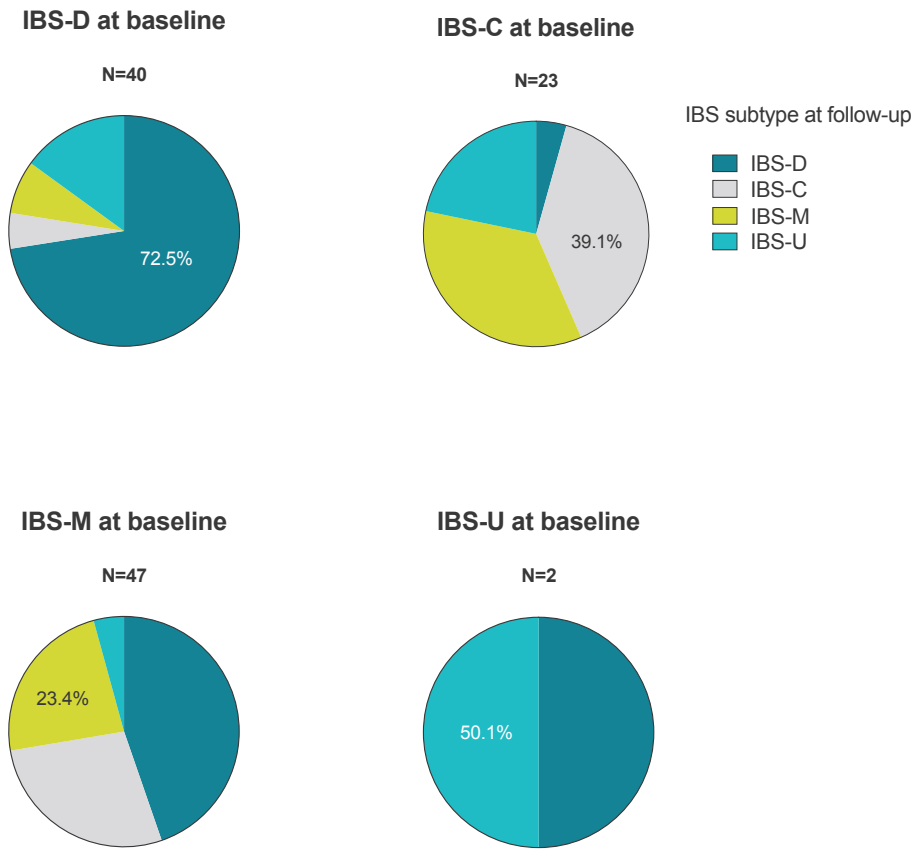
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## Supplementary Material



**Figure S3.1** Proportion of patients with IBS-D, IBS-C, IBS-M, IBS-U, and No IBS (at follow-up) in the total population analyzed,  $N=161$ , and the proportion of patients with IBS-D, IBS-C, IBS-M, IBS-U in the population that was Rome III-positive at follow-up,  $N=112$ . The figure does not account for transitions of individual patients to different subtypes. N; number, IBS; Irritable Bowel Syndrome. IBS-D; Irritable Bowel Syndrome, diarrhea predominant subtype. IBS-C; Irritable Bowel Syndrome, constipation predominant subtype. IBS-M; Irritable Bowel Syndrome, mixed stool type subtype. IBS-U; Irritable Bowel Syndrome, undefined subtype.



**Figure S3.2** Pie slices represent the proportion of patients with a particular IBS subtype at follow-up (please refer to legend for information on which subtype). The percentage depicted within the pie represents the proportion of patients that were diagnosed as having the same IBS subtype at follow-up, as at baseline. IBS-D; Irritable Bowel Syndrome, diarrhea predominant subtype. IBS-C; Irritable Bowel Syndrome, constipation predominant subtype. IBS-M; Irritable Bowel Syndrome, mixed stool type subtype. IBS-U; Irritable Bowel Syndrome, undefined subtype.

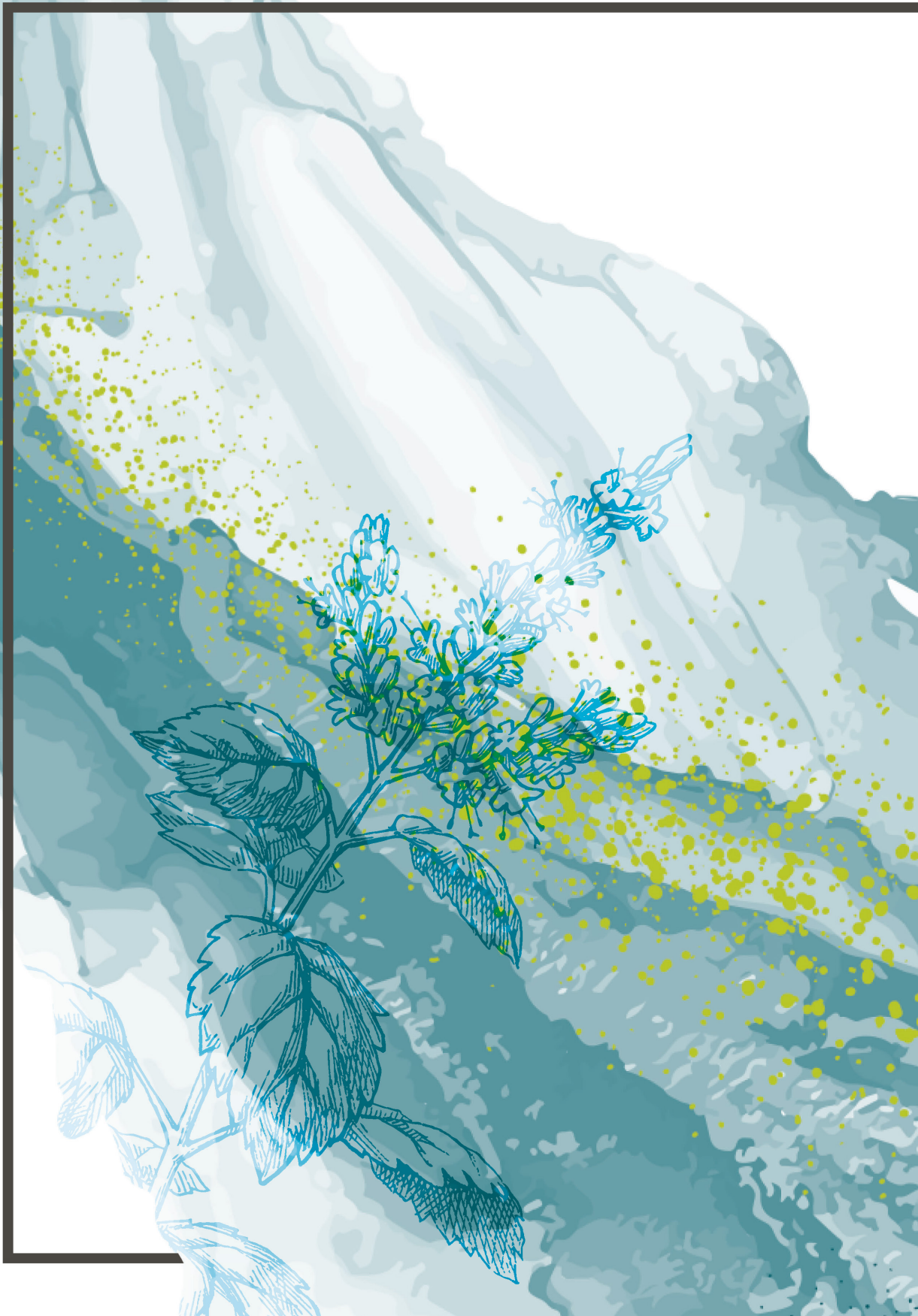






## Part II

Transient receptor potential channels  
as therapeutic target





# Chapter 4

Review article: transient receptor potential potential channels as possible therapeutic targets in irritable bowel syndrome

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## Abstract

### Background

Abdominal pain in irritable bowel syndrome (IBS) remains challenging to treat effectively. Researchers have attempted to elucidate visceral nociceptive processes in order to guide treatment development. Transient Receptor Potential (TRP) channels have been implicated in the generation (TRPV1, TRPV4, TRPA1) and inhibition (TRPM8) of visceral pain signals. Pathological changes in their functioning have been demonstrated in inflammatory conditions, and appear to be present in IBS as well. Our aim was to provide a comprehensive review of the current literature on TRP channels involved in visceral nociception. In particular, we emphasise the clinical implications of these nociceptors in the treatment of IBS.

### Methods

Evidence to support this review was obtained from an electronic database search via PubMed using the search terms “*visceral nociception*”, “*visceral hypersensitivity*”, “*irritable bowel syndrome*” and “*transient receptor potential channels*”. After screening the abstracts the articles deemed relevant were cross-referenced for additional manuscripts.

### Results

Recent studies have resulted in significant advances in our understanding of TRP channel mediated visceral nociception. The diversity of TRP channel sensitization pathways is increasingly recognised. Endogenous TRP agonists, including poly-unsaturated fatty acid metabolites and hydrogen sulphide, have been implicated in augmented visceral pain generation in IBS. New potential targets for treatment development have been identified (TRPA1 and TRPV4), and alternative means of affecting TRP channel signalling (partial antagonists, downstream targeting and RNA-based therapy) are currently being explored.

### Conclusions

The improved understanding of mechanisms involved in visceral nociception provides a solid basis for the development of new treatment strategies for abdominal pain in IBS.

## Introduction

Irritable bowel syndrome (IBS) is a gastrointestinal disorder characterized by chronic recurrent abdominal pain and alterations in bowel habit. The pathophysiology of IBS is incompletely understood, which poses obstacles in the search for effective therapeutic approaches. While the defecation pattern can generally be managed adequately with pharmacotherapy, abdominal pain tends to be difficult to treat effectively in IBS patients. In the search for new therapeutic strategies, accumulating interest has been given to peripheral mechanisms of nociception as a key target to develop novel analgesics for IBS-related pain. It is now widely accepted that an altered visceral sensitivity through abnormal endogenous pain processing plays an important role in the pathogenesis. This can result both from peripheral and central sensitization processes.<sup>1</sup> By virtue of peripheral sensitization of nociceptive afferents, increased nociceptive discharge can result in the generation of pain symptoms.<sup>2</sup> The responsiveness of these nociceptive afferents or nociceptors, is determined by the expression of specific channels sensing noxious stimuli.<sup>3</sup> The discovery of sensory transducer molecules, including the transient receptor potential (TRP) channel family has opened a new horizon in understanding peripheral nociceptive processes. TRP channels constitute a family of nonselective cation channels. Several members of this family, of which the Vanilloid I capsaicin receptor (TRPV1) has been studied most extensively, have been identified to function as integrators and transducers of nociceptive signals in both somatic and visceral pain. However, as nearly all sensory neurons and several non-neuronal cell types express TRPV1, its role is not limited to nociception. TRP channels indeed appear to have a broad spectrum of functions in the human body, a topic that has been reviewed in detail, elsewhere.<sup>4</sup> This review will focus on current knowledge with regards to the potential role of TRP channels in the pathogenesis of pain symptoms in IBS, with particular emphasis on visceral nociception. Specifically, we will summarise their clinical implications and discuss the future of TRP channel targeted therapy.

## Methods

Evidence to support this review was obtained from an electronic database search via PubMed by two of the authors (AB & ZW) using the search terms “*visceral nociception*”, “*visceral hypersensitivity*”, “*irritable bowel syndrome*” and “*transient receptor potential channels*”. The last date of the search was 21<sup>st</sup> of July 2017. After screening the abstracts the articles deemed relevant were cross-referenced for additional manuscripts.

## **Irritable bowel syndrome pathophysiology**

Several mechanisms have been hypothesized to play a role in the pathogenesis of IBS, including disturbances in microbiota, low-grade inflammation, immune activation, intestinal barrier dysfunction and altered bile salt absorption. Discussing these mechanisms separately is beyond the scope of this article. A thorough overview is provided in a recent review article.<sup>5</sup> We would like to emphasise that IBS is a heterogeneous disease. Even identical symptoms are likely caused by different processes.<sup>5</sup> Grouping IBS patients on the basis of stool pattern thus promotes heterogeneity, resulting in varying results with different cohorts. This aspect is also relevant when studying the role of TRP channels in visceral pain generation in IBS. Indeed, low-grade mucosal inflammation has been proposed as an important pathophysiological factor in IBS.<sup>6</sup> Researchers have since demonstrated a sensitizing effect of inflammatory mediators on various TRP channels, as will be discussed below. It is important to note that inflammation does not seem to be required to maintain visceral sensitization, as two recent clinical studies investigating the effects of mesalazine in IBS failed to demonstrate any benefits.<sup>7,8</sup> On the other hand, post-inflammatory sensitization can provide a theoretical explanation for IBS-like symptoms after gastroenteritis, known as post-infectious IBS, and after achieving endoscopic and biochemical remission in inflammatory bowel disease (IBD). However, it would be inadequate to assume inflammation as the sole driving factor of visceral hypersensitivity. Shortcomings of our current knowledge on TRP channel sensitization should be recognized.

## **Sensory innervation of the intestine**

Nociceptive signalling from the oesophagus to the proximal colon is conducted through the vagal nerve. Information from mid to distal colon and rectosigmoid is carried by the lumbar splanchnic and sacral pelvic nerves. The vagal nerve contains the peripheral terminals of pseudo-unipolar neurons with their cell bodies located in the nodose ganglia. Visceral sensory information from the vagal nerve supply is transduced into the solitary nuclei located in the medulla oblongata. The splanchnic and pelvic nerves contain axons of pseudo-unipolar neurons arising from a dorsal root ganglion (DRG). Peripheral sensory information from this supply is transduced into the dorsal horn of the spinal cord and ascends via the spinothalamic tract.<sup>9</sup> Peripheral nociceptive signalling is extensively modulated by the central nervous system, resulting in suppression or augmentation of the nociceptive input. These central processes determine whether nociceptive signalling (sensing and transmitting noxious stimuli) is perceived as pain (unpleasant experience).<sup>10</sup>

Sensory afferents of the vagal and spinal nerves have previously been divided in different subclasses.<sup>11</sup> Based on their sensitivity to mechanical stimuli, afferents were divided in mucosal, muscular, serosal and mesenteric fibre classes.<sup>2</sup> Mucosal afferents were defined as responsive to fine tactile and chemical stimuli, whereas serosal and mesenteric afferents respond to noxious mechanical stimuli.<sup>1</sup> Sensing of intermediate (physiological) distension was attributed to muscular afferents. An additional class specific to the pelvic pathway, the muscular-mucosal class, responds to tactile and distension stimuli. It should be noted that evidence supporting the suspected anatomical distribution described above is currently lacking. Song *et al.* have attempted to morphologically identify specialised afferent axonal structures in the guinea pig intestine.<sup>12</sup> They were able to demonstrate mechanosensitive fibres in the mesenteries and preparations of isolated mucosa/submucosa of the ileum and colon. However, no mechanosensitive fibres were observed in preparations of isolated muscle layers (with intact myenteric ganglia and serosa). Afferent functioning therefore appears to depend on molecular characteristics rather than the location within the gut wall.<sup>1,13</sup> Functional differences in nociceptor transducer molecules and their divergent expression along sensory afferents determine the physiological role of these afferents.<sup>13</sup> Understanding the functioning of individual TRP channels may provide further insights into nociceptive processes and sensitization mechanisms. Below we will discuss in detail the TRP channels that have been identified as key players in visceral nociception. These include TRPV1, TRPV4, TRPA1 and TRPM8. An overview of current data on these channels and their implications in IBS is provided in *Table 4.1*.

### **TRP channel regulation**

In order to understand TRP channel functioning, one must be aware of the complex molecular modulation that these channels are subjected to. Modulatory processes can either result in sensitization or desensitization of the respective afferent.<sup>14</sup> While desensitization prevents nociceptive signaling, sensitization of nociceptors enhances their discharge (*i.e.* potentiates the nociceptor response to a second stimulus).

**Table 4.1** Data on TRP channels in IBS

<b>Channel</b>	<b>Implications in IBS</b>	<b>Study type</b>	<b>Reference</b>
<b>TRPV1</b>	Sensitized and/or upregulated in colonic tissue samples of IBS patients, resulting in enhanced capsaicin sensitivity.	Human studies	19, 20, 26-28
	Sensitized by inflammatory mediators.	Animal study Combined study	32 34
	Expression profiles regulated epigenetically, likely influenced by psychological stress through glucocorticoids and/or catecholamines.	Human study Animal studies	27 36, 37
	Indirectly involved in mechanosensation.	Animal study	21, 48
<b>TRPV4</b>	Elevated levels of endogenous agonist 5,6-EET in the supernatant in of colonic biopsies from IBS-D patients.	Human study	31
	Sensitized by inflammatory mediators.	Animal studies	42-44
	Putative direct mechanosensitive nociceptor in humans.	Human study	41
<b>TRPA1</b>	Functional coupling with TRPV1.	Animal study	52
	Activated by hydrogen sulphide, present in IBS-D patients with small bacterial overgrowth.	Human study	57
	Sensitized by inflammatory mediators.	Animal studies	53, 54
	Likely to act as a directly mechanosensitive nociceptor in hyperalgesia.	Animal studies Human study	48, 49 50
<b>TRPM8</b>	Inhibits chemo- and mechanosensory responses of TRPA1 and TRPV1.	Animal study	61
	Potentially protective against nociceptor sensitization through anti-inflammatory effects.	Animal study	62
	TRPM8 polymorphisms are associated with slower colonic transit and an increased risk of IBS-C and IBS-M in humans.	Human study	58

Several mechanisms are involved in TRP channel regulation.<sup>14</sup> First, gene expression can be altered through DNA methylation, resulting in gene silencing. Second, posttranslational modifications (e.g. phosphorylation and dephosphorylation) affect channel functioning. Phosphorylation cascades can be initiated by various sensitizing agents (discussed below). Depending on the agent, different phosphorylation pathways

are involved (e.g. protein kinase A, protein kinase C, calmodulin-dependent kinase).<sup>15</sup> In contrast, dephosphorylation reduces TRP channel sensitivity to stimuli. Finally, TRP channels can be degraded by movement to intracellular lysosomes,<sup>16</sup> or mobilized from intracellular pools to the cell membrane (translocation).<sup>15</sup> All of these processes are kept in balance in physiological conditions, but can be disrupted in disease. Currently, we are only beginning to understand the role of these modulatory processes in IBS. Considering our growing knowledge on TRP channels in visceral pain, future studies may focus on this unexplored field to guide treatment development.

## TRPV1

Of all TRP channels, TRPV1 has been studied most extensively. Studies investigating the expression patterns of TRPV1 in mice have demonstrated the channel's presence along the entire gastrointestinal tract.<sup>17</sup> Although human studies are more scarce, the expression of TRPV1 in the oesophagus and colon is now well documented, and the channel is suspected to be present in the human small intestine as well.<sup>18</sup> Immunostaining of human colon biopsies has demonstrated TRPV1-positive fibres throughout the mucosa, with a particular abundance in the submucosal plexus.<sup>19,20</sup> Activation of these fibres by noxious stimuli results in action potential generation and pain sensation. TRPV1 is activated by noxious heat ( $>42^{\circ}\text{C}$ ), protons ( $\text{pH} < 6$ ) and the vanilloid capsaicin, the pungent principle in hot peppers.<sup>20</sup> In addition, several compounds have been identified as endogenous agonists. These include inflammatory mediators such as lipoxygenase products and prostaglandins, and endocannabinoids such as anandamide (see *Table 4.2*). Furthermore, TRPV1 seems to be involved in afferent signalling of mechanical stimuli,<sup>3</sup> but its exact mechanism is still poorly understood. Whether the mechanosensory properties of TRPV1 are related to indirect effects on neuronal excitability or interactions with other TRP channels, remains to be established.<sup>21</sup>

The use of potent chemical activators such as capsaicin has provided valuable information on the functioning of TRPV1-expressing afferents. Upon activation, in addition to the generation of an action potential, these sensory afferents release pro-inflammatory sensory neuropeptides; calcitonin-gene related peptide (CGRP) produces local vasodilation and substance P (SP) increases venular and capillary permeability leading to plasma protein extravasation and oedema formation, collectively referred to as neurogenic inflammation.<sup>22</sup> TRPV1-expressing sensory neurons can therefore influence GI vascular, immune and smooth muscle function, as well as sensitize surrounding nociceptors.<sup>3</sup> Under physiological conditions, these effects are

counteracted by the anti-inflammatory effects of somatostatin, which has also been shown to be released by capsaicin-sensitive afferents.<sup>23</sup> Sustained disruptions in the balance of these pro- and anti-inflammatory neuropeptides may result in the pathological sensitization of nociceptive afferents, as well as local tissue inflammation (see *Figure 4.1*). Importantly, these processes do not seem to be limited to TRPV1, but also apply to the other TRP channels discussed in this review.

Sensitization of TRPV1-expressing afferents has been demonstrated in IBS patients by increased perceptive responses to capsaicin in multiple studies.<sup>20,24,25</sup> Gonlachanvit *et al.* demonstrated that diarrhoea predominant IBS (IBS-D) patients experience greater abdominal burning after a single ingestion of a spicy meal or standard meal in combination with a capsaicin capsule, compared to healthy controls.<sup>24</sup> As symptoms developed within one hour after ingestion, proximal gut hypersensitivity to capsaicin was suggested to exist in these patients. Schmulson *et al.* showed a significantly decreased rectal pain threshold in IBS patients after a 7-day chilli rich diet compared to a diet without chilli, suggesting TRPV1-induced visceral hyperalgesia.<sup>25</sup>

More recently, van Wanrooij *et al.* studied the effects of rectal capsaicin application in IBS patients.<sup>20</sup> Patients reported increased pain intensity, a similar effect lacking in healthy volunteers. Furthermore, the pain response appeared to be independent of anticipatory anxiety, suggesting a direct capsaicin effect on nociceptive mucosal afferents.

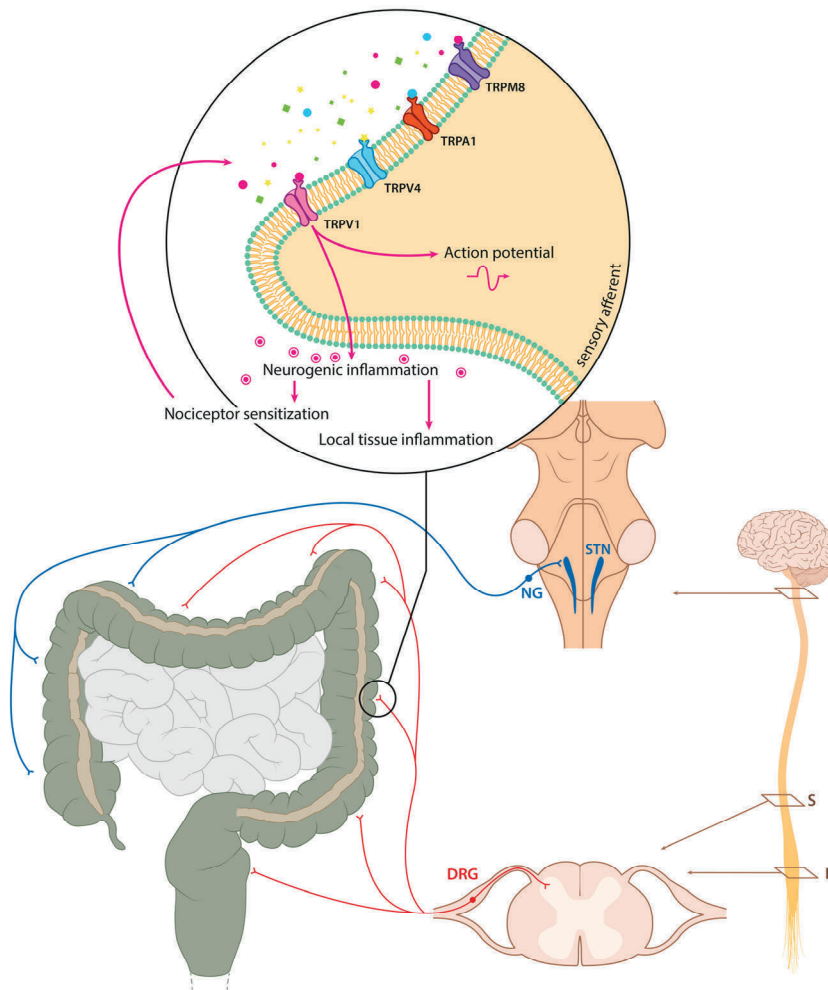
Mechanisms underlying the increased capsaicin sensitivity in IBS patients have been studied extensively. Akbar *et al.* demonstrated upregulation in sigmoid mucosal samples of IBS patients.<sup>19</sup> This increase correlated with symptom severity, suggesting that an increase in afferent discharge through TRPV1 activation might be directly related to pain symptom generation. Our earlier study corroborated these findings, demonstrating increased transcription of TRPV1 in IBS patients, which also strongly correlated with symptom severity.<sup>26</sup> More recently, two studies confirmed the augmented expression of TRPV1 in colonic biopsies of IBS-D patients.<sup>27,28</sup> It should be noted that the overall density of innervation has been shown to be increased in IBS patients. Increased TRPV1 sensitivity may therefore be due to axonal sprouting rather than isolated TRPV1 upregulation, possibly as a result of increased nerve growth factor expression.<sup>28,29</sup> On the other hand, Van Wanrooij *et al.* were unable to objectify increased numbers of mucosal TRPV1 in colonic biopsies of IBS patients, as compared to healthy controls.<sup>20</sup> Even when the IBS patient group with visceral hypersensitivity (defined by decreased discomfort threshold during rectal distension) was analysed separately, no significant



upregulation of TRPV1 was found. The question whether increased capsaicin responsiveness in IBS relates to individual TRPV1 sensitivity or TRPV1 expression thus remains without a decisive answer.

**Table 4.2** Identified agonists and physical stimuli of TRP channels discussed in this paper.<sup>16,55,56,110-112</sup>

Channel	Exogenous agonists	Physical stimuli	Endogenous agonists
<b>TRPV1</b>	Capsaicin (red pepper), polygodial (mountain pepper), piperine (black pepper), gingerol (ginger), olvanil, resiniferatoxin, camphor, diphenylboronic anhydride, double-knot toxin (DkTx), vanillotoxin (tarantula toxin), phenylacetylirvanil, 2-aminoethoxydiphenyl borate (2-APB), evodiamine, cannabidiol, cannabigerol	(Thermal >42°C)	Acid (pH < 6), lipoxygenase products (e.g. 12-(S)-hydroperoxyeicosatetraenoic acid (12S-HPETE), 15-(S)-hydroperoxyeicosatetraenoic acid (15S-HPETE), leukotriene B4 (LTB4), 5-(S)-hydroxyeicosatetraenoic acid (5S-HETE)), reactive oxygen species (ROS), adenosine, ATP, lysophosphatidic acid, polyamines (e.g. spermine, spermidine, and putrescine) and conjugates of biogenic amines (e.g. N-arachidonylethanolamine (anandamide), N-arachidonoyldopamine (NADA), N-oleoyldopamine, N-oleylethanolamine (OLEA), N-arachidonolylserine, N-hexadecanamide, and various N-acyltaurines and N-acylsalsolinols)
<b>TRPV4</b>	Bisandrographolide A (BAA), alpha-phorbol 12,13-didecanoate (4 $\alpha$ -PDD), phorbol 12-myristate 13-acetate (4 $\alpha$ -PDH), apigenin, GSK1016790A and RN1747	Mechanical and thermal (>24°C)	Citric acid, dimethylallyl pyrophosphate, anandamide, arachidonic acids and epoxyeicosatrienoic acid metabolites (e.g. 5,6-epoxyeicosatrienoic acid (5,6-EET) and 8,9-EET, which also mediate TRPV4 activation by cell swelling)
<b>TRPA1</b>	Allyl isothiocyanate (AITC), cinnamaldehyde (cinnamon), allicin (garlic), carvacrol and thymol (oregano), curcumin (turmeric), capsiate (capsinoid), acrolein, menthol, icilin, nicotine, URB597, chlorobenzylidene malononitrile (tear gas), formalin, $\alpha,\beta$ -unsaturated aldehydes, auranofin, PF-4840154, cannabichromene, cannabidiol (CBD), tetrahydrocannabinol (THC) and apomorphine (agonist in low micromolar range and antagonist in higher concentration)	Mechanical and thermal (<15°C and >25°C)	Prostaglandins (e.g. prostaglandin A1, 8-iso-prostaglandin A2, and 15-deoxy- $\Delta$ -prostaglandin J2), 4-Hydroxynonenal (4-HNE, a lipid peroxidation product), 4-oxononenal (4-ONE), methylglyoxal, reactive oxygen species (ROS), cytokines (e.g. TNF- $\alpha$ and IL-6), bradykinin and hydrogen sulphide
<b>TRPM8</b>	Menthol, icilin, linalool, geraniol, WS-3, WS-12, WS-23, PMD38, hydroxycitronellal, FrescolatMGA, FrescolatML, CoolactP, Cooling Agent 10, cis- and trans-p-menthane3 and CPS-36	Thermal (<28°C)	Unknown



**Figure 4.1** Schematic depiction of nociceptive afferent innervation of the intestine. Proximal (blue) neurons travel through the vagal nerve. These neurons transduce sensory information through the nodose ganglia into the solitary tract nuclei. Distal (red) neurons travel through the splanchnic and pelvic nerves (two distinct systems). Both the splanchnic and pelvic nerves' somata reside in the dorsal root ganglia. In addition, splanchnic nerves travel through prevertebral ganglia (not shown). The nociceptive afferents within these neurons presumably have their nerve endings (top inset) in the mucosa/submucosa and mesenteries.<sup>12</sup> Various stimuli (shown at the top) can activate the nociceptors, depending on the expressed TRP channels (see Table 4.2). Stimuli include exogenous agonists (e.g. capsaicin or menthol), physical stimuli (e.g. mechanical or thermal) and endogenous agonists (e.g. prostaglandins or lipoxygenase products). Activation of nociceptors by these stimuli results in action potential generation and pain sensation. In addition, inflammatory mediators are released (neurogenic inflammation), which can result in TRP channel sensitization and local tissue inflammation. NG= nodose ganglion, STN= solitary tract nucleus, DRG= dorsal root ganglion, S= splanchnic nerves, P= pelvic nerves.

### *TRPV1 functioning in (post-)inflammatory conditions*

As discussed above, the mediators of neurogenic inflammation are known to sensitize TRPV1. In addition, systemic inflammatory mediators have been shown to be involved in both sensitization and activation of TRPV1 (see *Table 4.2*).<sup>30</sup> Their potential relevance to IBS pathophysiology is evident, as many have postulated a role for subclinical inflammation in IBS.<sup>5</sup> However, inflammation has been argued to be within the physiological range in IBS.<sup>6</sup> Moreover, a study measuring poly-unsaturated fatty acids in colon biopsy material from IBS-D patients and healthy controls, failed to demonstrate differences in concentrations of lipoxygenase products (TRPV1 agonists).<sup>31</sup> It is possible that the role of inflammatory mediators in TRP channel sensitization is limited to post-inflammatory hyperalgesia, as encountered in post-infectious IBS and IBD in remission. Animal studies have provided evidence for TRPV1 mediated post-inflammatory hyperalgesia, using experimental colitis models induced by dextran sodium sulphate. After recovery from colitis, TRPV1 deficient mice showed no pain-related behavioural responses or increased visceromotor responses to colorectal distension, whereas these responses were readily observed in wildtype mice.<sup>32</sup>

Another explanation for inflammation mediated hyperalgesia in IBS could be related to histamine. Barbara *et al.* observed increased numbers of mucosal mast cells in close proximity to sensory nerves in colon biopsies of IBS patients,<sup>33</sup> and these findings have been confirmed in a more recent study.<sup>28</sup> Moreover, Wouters *et al.* demonstrated an increased Ca<sup>2+</sup> response and increased number of responding neurons to capsaicin in histamine pre-treated biopsy specimens of healthy volunteers.<sup>34</sup> Immunostaining showed co-expression of histamine receptor H1 (HRH1) and TRPV1 on submucosal neurons in both IBS patients and healthy controls. A functional coupling of these receptors therefore appears likely. In a proof-of-concept trial, the same study group demonstrated a significant decrease in abdominal pain scores in IBS patients after 12 weeks of treatment with the HRH1 antagonist ebastine, as compared to placebo. Unfortunately, not all patients reported pain relief, emphasising the heterogeneity of the IBS patient population.

### *Chronic stress and epigenetics*

IBS is often described to be a disorder of the brain-gut axis. In this model, psychological stress is generally accepted as a key factor influencing GI symptoms and vice versa. Importantly, animal models have suggested both glucocorticoid- and catecholamine-mediated TRPV1 upregulation.<sup>35,36</sup> In addition, epigenetic mechanisms may regulate the effects of chronic stress on TRPV1 expression. Increased histone acetylation of the

TRPV1 promoter has been demonstrated in chronic stress models in rats, resulting in TRPV1 upregulation in DRG derived neurons.<sup>37</sup> Furthermore, the epigenetics of visceral pain perception have been investigated in diarrhoea predominant IBS patients.<sup>27</sup> Two miRNAs known to decrease TRPV1 expression, miR-199a and miR-199b, were found to be significantly downregulated and shown to correlate with pain scores. Taken together, these results indicate epigenetic alterations, possibly under the influence of psychological stress, modulate TRPV1 functioning in IBS.

#### *Activation of sensitized nociceptors*

Currently identified endogenous agonists of TRPV1, as well as the other TRP channels discussed in this review, are primarily related to inflammation (see *Table 4.2*). As already noted, (subclinical) inflammation is not the sole underlying mechanism in IBS. Furthermore, it is unknown whether the concentrations of endocannabinoids known to activate TRPV1 *in vitro* are high enough *in vivo* in order to achieve activation.<sup>38</sup> This constitutes a significant gap in our knowledge of peripheral nociception in IBS, as it remains unclear what stimuli ultimately activate sensitized nociceptors *in vivo*. Although capsaicin is a common dietary constituent, it is unlikely to be a major factor in abdominal pain generation in IBS. Current understanding of intestinal signalling suggests that nociceptive signals are generated by exciting sensitized nociceptors as a result of mechanical stimulation or distension. These mechanical stimuli could be related to physiological motor responses of the intestine.<sup>6</sup> High amplitude colonic contractions have been shown to be of a magnitude above nociceptive thresholds in visceral hypersensitivity. Therefore, mechanical stimuli generated by the gut itself may be responsible for the generation of pain symptoms through sensitized nociceptors.

## **TRPV4**

Studies investigating expression patterns of Transient Receptor Potential Vanilloid 4 (TRPV4) in the human colon have demonstrated immunoreactivity in the submucosa and serosa.<sup>39</sup> Initially termed vanilloid receptor-related osmotically activated channel (VR-OAC), this channel has been implicated in the detection of osmolarity changes.<sup>40</sup> In addition, TRPV4 is now known to sense strong acidosis, temperatures >24°C and, among others, the synthetic phorbol ester alpha-phorbol 12,13-didecanoate (4 PDD) (see *Table 4.2*). Currently identified endogenous agonists include anandamide and the poly-unsaturated fatty acid metabolites 5,6-epoxyeicosatrienoic acid (5,6-EET) and 8,9-EET.

Accumulating evidence points toward a role of TRPV4 in mechanosensation.<sup>3,41</sup> Under basal conditions, TRPV4 is thought to primarily sense high threshold mechanical stimuli.<sup>42</sup> Comparing TRPV4 knockout and wildtype mice, responses to noxious distension pressures were diminished in TRPV4 knockouts. In contrast, responses did not differ at innocuous pressures. TRPV4 is however considered to play a major role in visceral hypersensitivity.<sup>42,43</sup> Intracolonic administration of 4 PDD has been shown to induce hyperalgesia in mice.<sup>42</sup> In IBS, several pathways have been proposed to result in visceral hypersensitivity through TRPV4 sensitization. The effects on TRPV4 of known mediators of visceral hypersensitivity, serotonin and histamine, were investigated in one study.<sup>44</sup> Serotonin and histamine administration was demonstrated to result in potentiated TRPV4 responses to 4 PDD. The same research group postulated a role for proteases in mediating visceral hypersensitivity.<sup>45</sup> Subsequent studies have supported this theory.<sup>42,43</sup> Activation of Protease-Activated Receptor (PAR<sub>2</sub>), a channel that is also co-expressed with TRPV4 in afferents innervating the colon, resulted in visceral hyperalgesia in wildtype mice. Because hyperalgesia was lacking in TRPV4 knockout mice, this channel was suspected to be the downstream effector of PAR<sub>2</sub> mediated visceral hypersensitivity.<sup>42</sup>

Human studies on visceral TRPV4 functioning are currently limited. In one study researchers acquired supernatant of colonic biopsies from IBS-D patients.<sup>31</sup> Intracolonic administration of the supernatant resulted in visceral hypersensitivity in mice. This was concluded to be TRPV4 mediated, as injection of TRPV4 targeted small interfering RNA prevented the effect. Subsequently, potential TRPV4 agonists in the supernatant were quantified. The concentration of 5,6-EET was found to be significantly elevated and correlated with patients' abdominal pain severity and frequency. Interestingly, 5,6-EET production was linked to PAR<sub>2</sub> activation, as PAR<sub>2</sub> agonist peptide induced 5,6-EET synthesis in sensory neurons. The authors therefore suggested 5,6-EET to be an endogenous TRPV4 agonist with a major role in visceral hypersensitivity in IBS-D patients. Again, the heterogeneity of IBS should be emphasised as the above solely relates to IBS-D patients. No significant differences in poly-unsaturated fatty acid metabolite concentrations in supernatants have been observed in constipation predominant IBS (IBS-C) patients, mixed bowel habit IBS (IBS-M) patients and healthy volunteers.

## TRPA1

To date, our knowledge on Transient Receptor Potential Ankyrin 1 (TRPA1) functions in visceral nociception is mostly limited to animal models. TRPA1 is thought to primarily

act as a chemosensor, responding to various irritants and spices, among others cinnamaldehyde (cinnamon) and allyl isothiocyanate (AITC), the pungent compound in mustard oil, horseradish and wasabi.<sup>3</sup> Currently identified endogenous agonists include prostaglandins and products of oxidative stress (see *Table 4.2*). Furthermore, TRPA1 has been implicated in temperature sensation, although its responsiveness has long been debated. Recently, a U-shaped temperature-activation curve was demonstrated using human TRPA1 in lipid bilayer and whole-cell patch-clamp recordings.<sup>46</sup> This study group observed TRPA1 activation in temperatures below 15 degrees Celsius, as well as temperatures above 25 degrees, but little activation to temperatures in between.

In addition to the above, TRPA1 has been studied intensively for its suspected mechanosensitive properties. It appears to have no role in sensing high pressure distension under basal conditions.<sup>47</sup> However, TRPA1 has been demonstrated to be an important mediator of visceral hyperalgesia.<sup>48,49</sup> Several animal studies have indicated that the mechanical stimulus threshold can be decreased upon chemical activation with mustard oil. These findings have recently been confirmed in an *ex vivo* study using human colonic tissue.<sup>50</sup> Moreover, TRPA1 can be sensitized via PAR<sub>2</sub> activation. Similar to TRPV4, TRPA1 may therefore be one of the effectors of protease mediated visceral hypersensitivity.

Several characteristics of TRPA1 add to the complexity of its functioning. TRPA1 is almost exclusively expressed in TRPV1-positive neurons. Both channels have been shown to interact with each other.<sup>51</sup> Similar to TRPV1, TRPA1 can be desensitized upon repeated stimulation.<sup>52</sup> In addition, TRPA1 activation can be reduced upon repeated capsaicin application, a process referred to as cross-desensitization.<sup>52</sup> Indeed, Brierley *et al.* showed that TRPA1 knockout mice were responsive to capsaicin, but lacked mechanical desensitization that normally follows afterwards.<sup>48</sup> Thus, whereas TRPV1 is responsible for the direct response to capsaicin, the subsequently reduced mechanosensory function appears to be TRPA1-mediated. These results are in line with the general belief that TRPV1 itself is not directly mechanically gated,<sup>21</sup> as the reduced response to mechanical stimuli upon chemical desensitization of TRPV1 relies on TRPA1.

#### *TRPA1 functioning in (post-)inflammatory conditions*

TRPA1 expression has previously been investigated in inflamed human colonic tissue, showing upregulation in IBD patients with active inflammation.<sup>51</sup> Evidence explaining the role of TRPA1 in inflammation however remains contradictory, with reports of both pro- and anti-inflammatory effects. Although its effect on inflammation is enigmatic,

TRPA1 itself is undoubtedly affected by inflammatory mediators. Indeed, the endogenous agonists of TRPA1 are related to inflammation (see *Table 4.2*). Furthermore, studies investigating the effects of chemically induced colitis in mice demonstrated sensitization of visceral afferents to mechanical stimuli. These effects were observed in wildtype mice, but not in TRPA1 deficient mice.<sup>47,53,54</sup>

Since TRPV1, TRPV4 and TRPA1 have all been shown to be involved in inflammation induced visceral hyperalgesia, one could assume that combined sensitization of these channels provides a particularly potent mechanism to induce hypersensitivity. Synergistic effects of TRPV1 and TRPA1 inhibition have indeed been demonstrated in the attenuation of colorectal distension-associated pain behaviour at high pressures in rats.<sup>54</sup> Unfortunately, this concept has not yet been proven in IBS. Cenac *et al.* have measured endogenous agonists of TRPA1, TRPV1 and TRPV4 (primarily inflammatory mediators) in the supernatant of colon biopsy material of IBS patients.<sup>31</sup> They demonstrated elevated levels of endogenous agonists of TRPV4, but not TRPA1 or TRPV1. Another study involved peripheral blood mononuclear cell supernatants from IBS-D patients.<sup>55</sup> The supernatants were shown to induce mechanical hypersensitivity *in vitro* in colonic afferent neurons. Cytokine concentrations in the supernatants were subsequently measured, showing elevated levels of TNF- $\alpha$ , soluble IL-2, IL-6, IL-10, IL-1 and the chemokines CCL3 and CCL4. Combined with the expression profiles of the receptors of these signalling molecules in colonic nerves, the authors proposed TNF- $\alpha$ , IL-6, IL-10 and IL-1 as possible mediators of mechanical hypersensitivity in IBS-D. The mechanism of action of TNF- $\alpha$  was demonstrated to be TRPA1 dependent, as its sensitizing effect was abolished in the presence of a TRPA1 antagonist. In contrast, the selective inhibition of TRPV1 using low doses of capsazepine had no effects on mechanical hypersensitivity induced by TNF- $\alpha$ . In addition, a more recent study by the same group demonstrated IL-6 mediated mechanical hypersensitivity to be TRPA1 dependent as well.<sup>56</sup> Whether an interaction of sensitized TRP channels plays an important role in IBS thus remains to be elucidated.

Future studies should include different approaches covering the diversity of potential pathophysiological mechanisms in IBS. For example, elevated levels of hydrogen sulphide, an endogenous TRPA1 agonist, were recently demonstrated in IBS-D patients with small bacterial overgrowth.<sup>57</sup> These findings demonstrate that mechanisms of TRP channel sensitization may vary among patients.

## TRPM8

Transient Receptor Potential Melastatin 8 (TRPM8) appears to be one of the least studied TRP channels in humans. Only very recently TRPM8 polymorphisms have been demonstrated to be associated with slower colonic transit and an increased risk of IBS-C and IBS-M in humans.<sup>58</sup> However, data on TRPM8 functioning is mainly based on animal studies, limiting our understanding of its role in visceral pain generation. Our current knowledge of TRPM8 is that it has a role in thermosensation (primarily low temperatures).<sup>59</sup> Several chemical compounds are able to activate TRPM8, among others menthol and icilin. Many will acknowledge that the sensation of mentholated liniments is difficult to describe. Cold and burning perceptions alternate upon application. Because of these opposing sensory inputs, menthol is thought to also activate channels other than TRPM8. Indeed, some authors have pointed to menthol-induced TRPA1 activation in order to explain the diverse psychophysical sensations after topical application of menthol.<sup>60</sup> Moreover, coupling of TRPM8 to both TRPV1 and TRPA1 has been demonstrated previously.<sup>61</sup> As mentioned above, AITC is able to cause mechanical hypersensitivity through TRPA1 activation. This effect, however, does not occur after pre-treatment with icilin. In contrast, icilin-induced mechanical desensitization is absent in TRPA1 deficient mice, indicating that the effect was TRPA1 mediated. Likewise, capsaicin is able to cause mechanical desensitization, but not after icilin pre-treatment.<sup>61</sup> TRPM8 is therefore thought to inhibit chemo- and mechanosensory responses of TRPA1 and TRPV1, and thus provide antinociceptive effects through cross-desensitization.

In addition, visceral TRPM8 is thought to have a role in inflammation. Human studies have revealed increased TRPM8 expression in colonic biopsy material from IBD patients as compared to healthy controls.<sup>62</sup> Several experimental colitis models in mice have suggested protective effects of TRPM8 activation. In these studies, icilin treatment significantly attenuated induced colitis in wildtype mice, but not in TRPM8 deficient ones.<sup>62-64</sup> The protective effects of TRPM8 activation have been linked to CGRP, which co-localizes with TRPM8 in the human colon.<sup>64</sup> Although the pro-inflammatory effects of CGRP in neurogenic inflammation are evident, primarily consisting of vasodilation, anti-inflammatory effects of the neuropeptide have been observed as well.<sup>65,66</sup> De Jong *et al.* demonstrated expression of the components of the CGRP receptor, calcitonin receptor-like receptor (CLR) and receptor activity modifying protein-1 (RAMPI), on CD11c+ dendritic cells in the murine spleen.<sup>64</sup> CGRP knockout mice were shown to have higher levels of pro-inflammatory cytokines (including TNF- and IL-6) in chemically induced acute colitis, as compared to wildtype mice. Moreover, CD11c+ dendritic cells



were found to be co-localized with CGRP positive fibres in the murine colon. CGRP was therefore thought to exert a protective role in colitis via inhibition of the release of pro-inflammatory cytokines, through interaction with local dendritic cells. Indeed, TRPM8 deficient mice showed significant improvement in disease activity after treatment with recombinant CGRP, whereas no effect of treatment was observed in wildtype mice with induced colitis. Paradoxically, enhanced CGRP expression levels have been observed in mucosal fibres of TRPM8 deficient mice.<sup>62</sup> This discrepancy has yet to be clarified, although it is likely that a disrupted colonic CGRP release prevents the neuropeptide from reaching its effector. Taken together, these results suggest that TRPM8 upregulation is a protective mechanism aimed at mitigating tissue inflammation. Therefore, TRPM8 could theoretically protect against nociceptor sensitization by inflammatory mediators.

### **Non-neuronal TRP channel expression**

As already mentioned, TRP channels are expressed by a multitude of cell types including those of non-neuronal origin (both intestinal and extra-intestinal).<sup>67</sup> Examples of non-neuronal cells expressing TRP channels include vascular smooth muscle cells, endothelial cells, keratinocytes and intestinal epithelial cells. The possible involvement of the latter in IBS pathophysiology should not be overlooked. Increased intestinal permeability has been demonstrated in IBS-D patients,<sup>68</sup> and has been associated with visceral hypersensitivity.<sup>69</sup> In two studies, TRPV4 activation with 5,6-EET and 4 PDD resulted in increased intestinal permeability.<sup>70,71</sup> One of the proposed mechanisms was via downregulation of tight junction proteins. However, conflicting results have been obtained for the role of TRPV1 in regulating intestinal permeability. Capsaicin has previously been shown to increase permeability. In contrast, the endocannabinoid-like compound oleylethanolamine has been shown to be able to both increase and decrease intestinal permeability via TRPV1.<sup>72</sup> Similarly, the role of TRPA1 in the regulation of intestinal permeability remains controversial. Fothergil *et al.* demonstrated decreased trans-mucosal resistance in colon tissue from mice after AITC and cinnamaldehyde administration.<sup>73</sup> We demonstrated no effects on small intestinal permeability with the administration of cinnamaldehyde in twelve healthy controls.<sup>74</sup> It therefore remains unclear to what extent TRP channels contribute to IBS pathophysiology via altering intestinal permeability.

### **Motility effects of TRP channel activation**

Although we here focus on the role in visceral nociception, it should be noted that TRP channels are known to affect gut motor function as well.<sup>21</sup> The effects of TRP channel

activation on motility are not only channel dependent, but also location dependent. For example, TRPV1-positive fibres located in the lower oesophageal sphincter that are exposed to gastric acid cause a local inhibitory reflex, lowering the intraluminal pressure.<sup>75</sup> On the other hand, application of capsaicin in the distal colon and rectum in mice has been shown to cause fast transient colonic contractions followed by a delayed sustained contraction.<sup>76</sup> These results indicate that different effector pathways are involved depending on the location. Suggested effectors are the tachykinin receptors (mainly NK1 and NK2), which respond to neuropeptides released upon TRP channel activation, as discussed above.<sup>77</sup> Indeed, the contractility lowering effect in the oesophagus was demonstrated to be NK1 dependent via local substance P release, whereas NK2 activation was shown to be responsible for long lasting contractility in the distal colon.<sup>75,76</sup> Additional pathways are likely to be involved however, as fast transient colonic contractions were shown to be inhibited by NK1 antagonists. Other TRP channels have also been implicated in motility. Whereas TRPV4 has previously been demonstrated to inhibit colonic motility in mice via reduced NO-dependent calcium release, TRPA1 has been implicated in both reduced and increased motor activity.<sup>39,78,79</sup> Taken together, the effects on gut motility emphasise the involvement of TRP channels in IBS pathophysiology, as they may account for both the altered defecation pattern as well as pain symptoms encountered in the syndrome.

## Clinical implications

Although all of the TRP channels discussed above are suggested to have a role in visceral pain generation, only two (TRPV1 and TRPM8) have been implied in the treatment of IBS. Generally, two strategies are exploited in blocking TRP channels. One is the direct inhibition by administration of antagonists, the other one by repeated stimulation in order to desensitize the channel and its respective nerve terminal. In addition, several alternative techniques for TRP channel targeted therapy have been developed, that will be discussed hereafter. A summary of the optional therapeutic strategies related to each TRP channel is provided in *Table 4.3*.

### *TRP antagonists*

The discovery of TRPV1 as a key player in nociception led to the development of TRPV1 antagonists as novel therapeutics in pain control. However, investigators soon encountered a major hurdle, as the first compounds interfered with thermoregulation. Several first-generation compounds were stranded in pre-clinical trials as they elicited marked hyperthermia.<sup>80,81</sup> Moreover, interspecies differences in TRPV1 functioning further complicated research. While one TRPV1 antagonist appeared to be safe in

animals, its first human clinical trial was prematurely halted as hyperthermia was observed in three out of four patients.<sup>82</sup> Interestingly, while TRPV1 has been identified as a thermosensor, its temperature threshold is well above physiological body temperature. It is possible that adverse effects are not TRPV1 related and represent an off-target effect. Additionally, classical TRPV1 antagonists impair the noxious heat sensation and may therefore increase burn risk. Together, these results disfavoured the development of non-selective TRPV1 antagonists. Therefore, new strategies are being explored in order to tackle thermoregulation related adverse events in TRPV1 targeted therapy. Several second generation modality specific antagonists have been developed, as it has been observed that hyperthermia is less severe with compounds that are full antagonists for capsaicin, but not for protons.<sup>83</sup> Two recent phase-I trials reported no side effects of two modality-selective TRPV1 antagonists.<sup>84,85</sup> Their potency as analgesics however remains to be established in future studies.

**Table 4.3** Summary of therapeutic strategies in relation to the TRP channels discussed in this paper.

	<b>TRPV1</b>	<b>TRPV4</b>	<b>TRPA1</b>
<b>Antagonists</b>	Potent analgesics, but thermoregulation interference with first generation compounds <sup>80-82</sup>  Modality-selective antagonists currently being developed <sup>108</sup>	Reduction of human nociceptor mechanosensitivity <i>ex vivo</i> <sup>41</sup>	Tested in neuropathies with good safety profiles <sup>105</sup>  Several antagonists currently evaluated in clinical trials <sup>105</sup>
<b>Desensitization</b>	Six weeks of chilli treatment effective in IBS-D patients, but short-term adverse effects may limit adherence <sup>87</sup>	Evidence currently lacking	Repeated TRPA1 stimulation results in desensitization <sup>52</sup>
<b>Cross-desensitization</b>	Inhibition of chemo- and mechanosensory responses with peppermint oil*	Inhibition of chemo- and mechanosensory responses with peppermint oil*	Reduced mechanosensitivity through repeated capsaicin administration <sup>52</sup>
<b>Downstream targets</b>	NK1 antagonists may provide anti-hyperalgesic effects <sup>96</sup>  Improved abdominal pain and stool pattern in female IBS-D patients with NK2 antagonist ibodutant <sup>94</sup>  Somatostatin analogues may provide anti-hyperalgesic effects <sup>98-101</sup>  Improved abdominal pain scores in IBS patients with HRH1 antagonist ebastine <sup>34</sup>	Evidence currently lacking	Evidence currently lacking
<b>RNA-based therapy</b>	Plausible <sup>102</sup>	Plausible <sup>102</sup>	Plausible <sup>102</sup>

\*Effects mediated by TRPM8 activation<sup>61</sup>

### *Desensitization*

The desensitizing properties of TRPV1 agonists such as capsaicin have been exploited for many years by topical preparations in the treatment of neuropathic pain.<sup>86</sup>

At this moment, only one small randomized crossover study investigated the effects of six weeks of chilli treatment in IBS-D patients.<sup>87</sup> At the end of treatment, patients reported significantly decreased post-prandial abdominal burning sensations. Similar positive effects have been demonstrated in epigastric pain in patients with functional dyspepsia.<sup>88</sup> An important drawback of capsaicin therapy however, is its short-term aggravating effects, possibly limiting adherence in the clinical setting. Therefore, more studies are needed to evaluate efficacy and feasibility in a larger IBS population. In addition, it remains to be established at which dose, frequency and length of administration repeated capsaicin is able to, if all, induce desensitization of TRPV1-positive nerve endings in the gut.

Ultra-rapid desensitization using potent agonists offer a theoretical background for achieving a fast analgesic response. One of these ultra-potent TRPV1 agonists is resiniferatoxin, which causes sustained calcium influx, resulting in a cytotoxic intracellular free calcium concentration and consequent axonal damage of TRPV1-positive neurons.<sup>89</sup> Unfortunately, its potency also poses challenges. Similar to first generation TRPV1 antagonists, resiniferatoxin increases the heat pain threshold. Moreover, high or repeated systemic doses of resiniferatoxin induce long-lasting damage to TRPV1-positive neurons,<sup>90</sup> rendering it not suitable for therapeutic applications in IBS.

### *Cross-desensitization*

Another mechanism aimed at analgesia is related to TRPV1 channel cross-desensitization. As mentioned above, TRPM8 activation is thought to provide antinociceptive effects through subsequent inhibition of TRPV1 and TRPA1. Peppermint oil, containing the TRPM8 agonist menthol, exploits these beneficial effects and is registered for the use in IBS in several countries.<sup>91</sup> It should be noted that the exact mechanism of action remains to be elucidated. Moreover, its effects appear to reach beyond TRP channel signalling as calcium channel mediated smooth muscle relaxation has been observed in vitro in human colon tissue without the involvement of TRPM8.<sup>92</sup>

Multiple small trials have evaluated the efficacy of peppermint oil in IBS. Side effects of this herbal therapy are rather mild, with heartburn being the most common.<sup>91</sup> One meta-analysis reported that 75% of IBS patients experience improvement in abdominal pain compared with only 27% in patients receiving placebo.<sup>91</sup> In a more recent trial, patients received either a sustained release peppermint oil preparation ensuring drug release in the small intestine, or a placebo. Patients receiving peppermint oil reported a significantly greater reduction in abdominal pain or discomfort compared with patients receiving placebo after a treatment duration of 28 days.<sup>93</sup> New preparations have been developed recently ensuring peppermint oil release in the colon, trials are ongoing and data on the efficacy of these new formulations will be reported in the near future.

#### *Downstream targets of therapy*

Drugs that target molecules downstream of TRPV1 provide an attractive pharmacological alternative to direct TRPV1 inhibition. As TRPV1 activation is accompanied by the release of sensory neuropeptides inducing neurogenic inflammation and sensitization of surrounding nociceptors, it makes sense to target mediators (or their respective receptors) of this process. Indeed, antagonists of the tachykinin NK1 and NK2 receptors of substance P and neurokinin A respectively, are being developed for various purposes, among others for symptom relief in IBS. For example, ibodutant, a neurokinin-2 receptor antagonist, has been shown to improve abdominal pain and stool pattern in female IBS-D patients.<sup>94</sup> Moreover, neurokinin-1 receptor antagonists such as aprepitant are currently being used as anti-emetics and have been suggested as analgesics as well.<sup>95,96</sup> Unfortunately, several pre-clinical studies demonstrated that NK1 antagonists lacked analgesic effects.<sup>97</sup> It should be noted that animal models studying the effects of NK1 antagonists simulated *somatic* pain, whereas the NK1 receptors are more relevant in *visceral* pain.<sup>96</sup> Moreover, NK1 antagonists are thought to function as anti-hyperalgesic agents rather than analgesics as they do not affect baseline nociception, but attenuate nociceptive responses sensitized through inflammation.<sup>97</sup> Although at first sight this does not appear favourable, inhibiting the sensitized state rather than the normal resting state may actually make NK1 antagonists excellent candidates for treating visceral pain. The most important advantage would be that physiological functions may remain unaltered. In fact, similar mechanisms likely explain the beneficial effects of the HRH1 antagonist ebastine in IBS patients, which has been discussed above.<sup>34</sup> As TRPV1 sensitization after histamine pre-treatment has been shown to be HRH1 mediated, the effects of ebastine on abdominal pain are thought to be related to the inhibition of the sensitized state of TRPV1.

One additional mediator of neurogenic inflammation has been implicated as a potential target in the development of analgesic therapies. Somatostatin, originating from capsaicin-sensitive sensory afferents, counteracts pro-inflammatory neuropeptides CGRP and substance P. Indeed, the potent analgesic effects of somatostatin have long been recognized.<sup>98</sup> Early studies using the somatostatin analogue octreotide have demonstrated increased thresholds of visceral sensory perception in IBS patients.<sup>99,100</sup> Moreover, anti-hyperalgesic effects of selective agonists of somatostatin receptor 1 and 2 have been reported in induced visceral hypersensitivity in mice.<sup>101</sup> Therefore, somatostatin analogues or receptor agonists may provide additional therapeutic strategies in visceral pain. Clinical studies will need to substantiate their applicability in IBS patients.

#### *RNA-based therapy*

The observation of decreased expression of several TRPV1 targeting miRNAs in IBS patients suggests an opportunity for RNA-based therapy. The therapeutic potential of post-transcriptional gene silencing by RNA interference is increasingly being recognized.<sup>102</sup> The major benefit of synthetic siRNAs is their high target specificity, preventing the suppression of unrelated genes. Unfortunately, several challenges have yet to be overcome before RNA-based therapy can become a therapeutic option in IBS. The oral bioavailability of oligonucleotides is limited, confining the possible modes of administration to more invasive approaches. Furthermore, the effects are only transient, demanding repeated treatment. Therefore, there is a need for stable TRPV1 targeting siRNAs that are readily absorbed in the GI tract.

#### *Exploiting new targets*

Our current knowledge on the role of visceral TRPV4 and TRPA1 in humans is limited. Nonetheless, data from animal studies, discussed above, suggest that these channels are viable targets for IBS therapy. The previous discovery of elevated levels of the endogenous TRPV4 agonist 5,6-EET in IBS-D patients and the putative implication of TRPV4 in mechanical hypersensitivity justify the need for further research. TRPA1-targeted therapy has been explored in animal models of visceral pain. However, contrasting evidence was found demonstrating attenuated visceral nociception in rodents with both TRPA1 antagonists as agonists.<sup>54,103,104</sup> It is unclear whether the mechanism of action of the latter was based on desensitization. Arguing against such a mechanism is the observation that reduced abdominal contractions were observed in mice after a single oral dose of a TRPA1 agonist. Furthermore, one TRPA1 antagonist has recently shown efficacy in patients with painful diabetic neuropathy, and several

other TRPA1 antagonists have entered the phase of testing in clinical trials.<sup>105</sup> Although the true potential of these drugs will need to be explored, their safety profile appears to be more favourable than that of early TRPV1 antagonists. Given this advantage, we expect a boost in TRPA1 targeted therapy development in the near future.

New targets may continue to be identified. TRP channel regulatory processes in IBS constitute relatively unexplored terrain (see “TRP channel regulation”). Improved understanding of the underlying molecular processes may help identify targets that modulate TRP channel functioning.

### **Challenges of TRP channel targeted therapy**

Although the possibilities of TRP channel targeted therapy appear endless, treatment development has been plagued by many challenges over the past years.<sup>16</sup> As mentioned above, the first TRPV1 antagonists were associated with thermo-regulatory side effects. To date, the mechanisms by which these side effects arise are unknown. New selective agents may provide a solution to this issue. However, other challenges are to be expected.<sup>16</sup> This is mainly provoked by two factors. First, TRP channels are expressed in a wide range of tissues (see section “Non-neuronal TRP channel expression”). Targeting TRP channels consequently affects systems other than nociceptors. For example, a systemically administered TRPV4 agonist resulted in endothelial dysfunction and cardiovascular collapse in one study.<sup>106</sup> TRPV1 expressed in the central nervous system is thought to have a role in mood disorders. Although conflicting data exists, TRPV1 antagonism may exacerbate depressive symptoms.<sup>38</sup> Regulatory relations between organ systems further complicate the matter. TRPV1-expressing neurons are thought to regulate immunological functions, and interference with this function could prove detrimental in systemic inflammatory conditions.<sup>107</sup> The other major issue of TRP targeted treatment development is related to the large diversity of possible stimuli of each channel. Even selective agents will not immediately overcome this hurdle. For example, a new TRPV1 antagonist that does not cause hyperthermia, was shown to potentiate proton-induced calcium influx in one study.<sup>108</sup> This unforeseen effect could prove problematic in the upper gastrointestinal tract, as it may result in excessive TRPV1 activation by gastric acid (e.g. physiological gastro-oesophageal reflux).

In addition, several limitations currently exist in the study of TRP channels and their role in nociception. Basic research is complicated by the lack of quality reagents. Antibodies used to investigate expression patterns possess poor specificity. A similar issue is encountered with agonists/antagonists used to investigate TRP channel functioning, which presents a major problem in understanding TRP biology.<sup>109</sup> Furthermore, studies

regarding TRP channel involvement in visceral pain generation mostly focus on inflammation. Although animal colitis models have provided a wealth of information, alternative mechanisms of TRP channel sensitization should be explored. As discussed above, IBS is highly heterogeneous. Further insights in the mechanisms underlying IBS are needed in order to expand our knowledge of peripheral nociception and ultimately guide IBS treatment development.

## Conclusions

In summary, TRPV1, TRPV4, TRPA1 and TRPM8 have been shown to play important roles in visceral pain generation and inhibition, making them potential targets in the treatment of IBS. TRPV1 antagonists have proven to be potent analgesics, but it remains difficult to produce compounds with an acceptable safety profile. Different strategies targeting TRPV1 (*i.e.* modality-selective antagonism) or downstream molecules (NK1 or NK2 antagonists or somatostatin agonists) may solve this issue. TRPV1 desensitization strategies may provide suitable alternatives, yet short-term adverse effects may limit treatment adherence. In contrast, TRPV1 cross-desensitization with peppermint oil is attractive because of the low prevalence of adverse effects. The mechanism of action of peppermint oil however appears to reach beyond TRP channel signalling. In addition, TRPV4 and TRPA1 provide promising new targets. In our opinion, TRPA1 represents an important candidate for the development of new treatments of visceral pain. Its putative implication in mechanosensation in hyperalgesia but apparent lack of such function under basal conditions suggests a major role in IBS. Moreover, early TRPA1 antagonists have proven to be safe in clinical trials, rendering TRPA1 targeted therapy less of a pharmaceutical challenge than TRPV1 inhibition.



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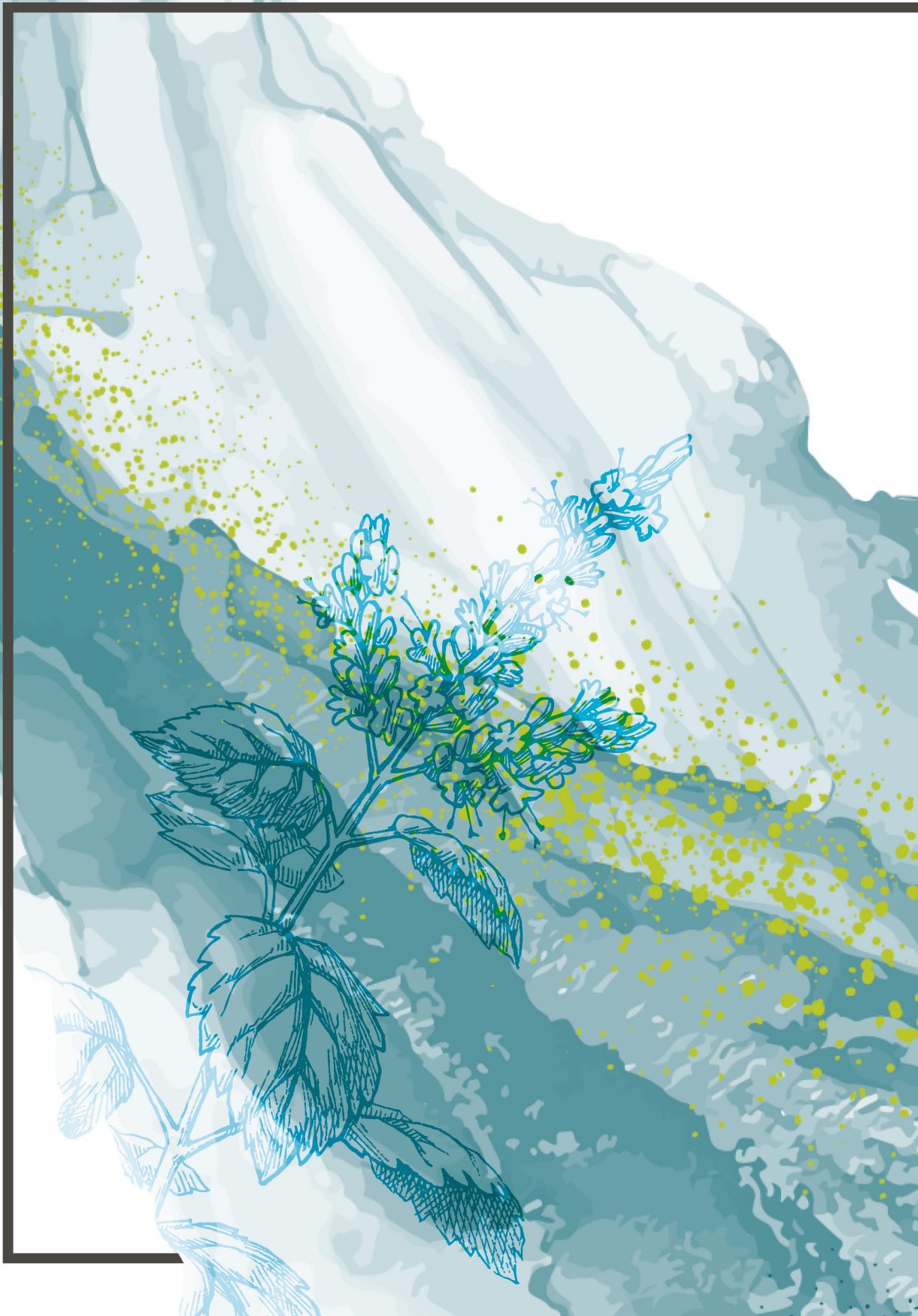
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# Chapter 5

A putative anti-inflammatory role  
for TRPM8 in irritable bowel syndrome  
- an exploratory study

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## Abstract

### Introduction

Chronic and recurring pain is a characteristic symptom in irritable bowel syndrome (IBS). Altered signalling between immune cells and sensory neurons within the gut may promote generation of pain symptoms. As transient receptor potential melastatin 8 (TRPM8) agonists, such as L-menthol in peppermint oil, have shown to attenuate IBS pain symptoms, we began investigating potential molecular mechanisms.

### Methods

Colonic biopsy tissues were collected from patients with IBS and controls, in two separate cohorts. Immunohistochemistry was performed to identify TRPM8 localization. Quantitative PCR was performed to measure mucosal mRNA levels of TRPM8. In addition, functional experiments with the TRPM8 agonist icilin were performed *ex vivo* to examine cytokine release from biopsies. Daily diaries were collected to ascertain pain symptoms.

### Results

In biopsy tissue from IBS patients, we showed that TRPM8-immunoreactivity is colocalized with immune cells predominantly of the dendritic cell lineage, in close approximation to nerve endings, and TRPM8 protein and mRNA expression was increased in IBS patients compared to controls ( $P < 0.001$ ). TRPM8 mRNA expression showed a significant positive association with abdominal pain scores ( $P = 0.015$ ). Treatment of IBS patient biopsies with icilin reduced release of inflammatory cytokines IL-1 $\beta$ , IL-6 and TNF- $\alpha$  ( $P < 0.050$ ).

### Conclusions

These data indicate TRPM8 may have important anti-inflammatory properties and by this virtue can impact neuro-immune disease mechanisms in IBS.



## Introduction

Irritable bowel syndrome (IBS) is a disorder of gut–brain interaction characterized by chronic recurrent pain and altered bowel habits thought to arise from disturbances in the neuro-immune regulatory balance of afferent signal processing.<sup>1</sup> IBS is highly prevalent with an estimated prevalence in the general population of ~5%.<sup>2</sup> In addition, IBS has a profound negative impact on quality of life and carries a substantial socioeconomic burden.<sup>3</sup> Patients are diagnosed based on their symptoms using the Rome criteria.<sup>4</sup> Importantly, these symptoms occur in the absence of gross structural abnormalities. The sensory symptoms of pain are the most debilitating aspects to patients yet are the least responsive to pharmacological treatment, highlighting the lack of understanding of the mediators and mechanisms involved in pain sensing of the lower gastrointestinal tract.

We recently demonstrated that peppermint oil<sup>5</sup>, a commonly used therapeutic in IBS, was able to improve pain outcomes. The beneficial effects of peppermint oil have also been confirmed in recent meta-analyses.<sup>6,7</sup> However, the mechanism of action remains somewhat obscure. Traditionally, peppermint oil is considered an anti-spasmodic due to the inhibition of calcium influx into the sarcolemma of the intestinal smooth muscle cells. On the other hand, the primary constituent of peppermint oil L-menthol, is also an agonist for the transient receptor potential melastatin 8 (TRPM8) channel. TRPM8 has an established role as an ion channel that responds to cold temperatures (<30°C), and the aforementioned menthol, a cyclic terpene alcohol found in mint leaves.<sup>8</sup> Expression of TRPM8 has been described in a distinct population of sensory nerves including trigeminal ganglia (TG), dorsal root ganglia (DRGs) and in epithelial cells of the prostate and bladder.<sup>9</sup> In the murine gastrointestinal tract, TRPM8 is expressed on colonic primary afferent neurons and activation of the channel with the agonist icilin inhibits chemo- and mechanosensory responses of pro-nociceptive TRP channels (TRPV1, TRPA1).<sup>10</sup> The activation of these pro-nociceptive channels also leads to the release of pro-inflammatory mediators from afferent nerve endings, resulting in a phenomenon collectively referred to as neurogenic inflammation.<sup>11</sup> Interestingly, in mouse models of colonic inflammation, TRPM8 knock-out mice have a more severe disease phenotype<sup>12,13</sup> and treatment with TRPM8 agonist icilin attenuates colonic inflammation.<sup>14</sup> Specifically, activation of TRPM8 expressed on calcitonin-gene related peptide (CGRP) containing sensory nerves reduces mucosal inflammation by inducing CGRP release, which then inhibits release of pro-inflammatory cytokines IL-6 and tumour necrosis factor (TNF)- $\alpha$  by CD11c+ dendritic cells.<sup>13</sup> Furthermore, murine macrophages expressing TRPM8 regulate pro- and anti-inflammatory mechanisms via

modulating release of TNF- $\alpha$  and IL-10.<sup>12</sup> Human data on the role of intestinal TRPM8 is limited to a single study showing upregulation of TRPM8 mRNA in biopsies from Crohn's disease patients, in both inflamed and non-inflamed mucosa.<sup>14</sup> In IBS, a single brief report describes an association of TRPM8 gene polymorphism with colonic transit.<sup>15</sup> Collectively, these studies show that there is a potential role for TRPM8-mediated reduction of gut symptoms. We sought to shed light on the putative role of TRPM8 in IBS. We hypothesized, in line with findings from previous animal studies, that TRPM8 has an anti-inflammatory effect and by this virtue represents a key factor in the pathophysiology of IBS.

## Materials and methods

### Patient population

IBS patients (age 18-75 years) were recruited from 2 centres: the Maastricht University Medical Center+ (MUMC), The Netherlands, and The Royal London Hospital, in London, UK.

Medical history was taken by a (trainee) gastroenterologist or research nurse, and if indicated, abdominal imaging and/or blood, fecal analyses were performed to exclude organic disease. A history of abdominal surgery, except for uncomplicated appendectomy, cholecystectomy, or hysterectomy, was reason for exclusion. The diagnosis of IBS was confirmed based on the Rome III criteria by an investigator independent from the treating physician.

Healthy controls for the Maastricht cohort were recruited from the general population using advertisements. Controls from the London cohort were attending the Endoscopy unit for anaemia investigation and did not have macroscopic inflammation or any organic abnormality other than polyps and/or hemorrhoids. Controls from both the Maastricht and London cohort were requested to complete the Rome III questionnaire to rule out the presence of IBS. Demographic characteristics of all study patients are presented in *Table 5.1*. The study protocols had been approved by the respective local Ethics Committees, and all study procedures were performed in compliance with Good Clinical Practice Guidelines and according to the revised Declaration of Helsinki. All subjects gave a written informed consent prior to participation.

**Table 5.1** Demographic characteristics of the study population.

	<b>Patients with IBS</b>	<b>Controls</b>
	N=30 (Maastricht) + 26 (London)	N=23 (Maastricht) + 6 (London)
<b>Age</b> , median (IQR)	37.5 (27.0-53.8)	23.2 (20.8-30.9)
<b>Female</b> , N (%)	35 (62.5)	13 (44.8)
<b>BMI</b> , median (IQR)	25.2 (20.9-29.4)	22.9 (20.5-24.4)*
<b>GSRs</b> , median (IQR)		
Abdominal pain	3.7 (2.7-4.7)	1.3 (1.0-2.0)***
Reflux syndrome	2.0 (1.0-3.5)	1.0 (1.0-1.0)**
Diarrhea syndrome	3.7 (1.7-4.3)	1.7 (1.3-2.7)*
Constipation syndrome	3.3 (2.3-4.7)	1.3 (1.0-2.3)***
Indigestion syndrome	4.3 (4.0-5.3)	1.8 (1.5-3.3)***
<b>HADS</b> , score >8, N (%)#		
Depressive symptoms	6 (20.0)	-
General anxiety symptoms	9 (30.0)	-
<b>IBS-subtype</b> , N (%)		N.A.
Diarrhea	14 (16.5)	
Constipation	11 (19.6)	
Mixed	15 (26.8)	
Unspecified	16 (28.6)	

GSRs: gastrointestinal symptom reporting scale. HADS: Hospital anxiety and depression scale. #data only available for the Maastricht cohort. Differences between groups were analysed using Chi square and Mann-Whitney U test. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  versus control.

## Symptom diaries

A representative subset of patients with IBS from the Maastricht cohort (N=15) filled in a 14-day end-of-day symptom diary 2 weeks prior to colonoscopy in order to quantify symptom severity. Symptoms were scored based on a 0-5 Likert scale. In addition, the validated questionnaires Gastrointestinal Symptom Rating Scale (GSRs) and Hospital Anxiety and Depression Score (HADS) were collected for characterization of the patient population.

## Tissue collection

For TRPM8 IHC experiments, full thickness colon samples were obtained from patients undergoing surgery for colon cancer (N=3) or IBD resection (N=4) at The Royal London Hospital (Barts Health NHS Trust) with approval of the East London and The City HA Local Research Ethics Committee and after written patient consent. Specimens were taken following macroscopic examination and were a minimum of 10 cm away from tumor, resection margins or lymphatic drainage field.

Colonic biopsies (right-sided proximal and sigmoid colon) were collected from IBS patients and controls in both Maastricht and London during routine colonoscopy examination under conscious sedation using midazolam and fentanyl or pethidine, from macroscopically normal mucosa. A standard biopsy forceps with a diameter of 2.8 mm (Boston Scientific) was used to obtain mucosal biopsies.

Full thickness tissue and biopsies collected in London were placed in cold 4% paraformaldehyde, and fixed for 16-18h or 2h at 4°C, respectively. Biopsies collected in Maastricht were directly placed in Eppendorf tubes and snap frozen in liquid nitrogen and stored at -80°C. Biopsy samples collected in Maastricht that were used for IHC were thawed and subsequently fixed in 2% PFA for 2h.

### **RNA isolation and quantitative PCR**

Total RNA was isolated from the frozen biopsies using TRIzol reagent (Invitrogen, Carlsbad, USA) and purified with the RNeasy Plus Mini Kit (Qiagen, the Netherlands). Quantity and purity of the RNA samples was determined using a Nanodrop spectrophotometer (NanoDrop Technologies, Wilmington, USA). A concentration from 5 µg/µl total RNA was used. Finally, the cDNA was diluted 100× with RNase free water and amplified: each reaction contained 12.5 µl iQ Sybr Green Supermix, 1 µl of 10 µM gene-specific forward and reverse primers, 4 µl diluted cDNA template and 5.5 µl sterile water. Reactions were run on the CFX 96 Real-time qPCR Detection System (Bio-Rad). PCR conditions used were 3 min at 95°C, followed by 40 amplification cycles of 10 sec at 95°C and 45 sec at 60°C. Data were expressed as normalized expression ratios.

Primers: GAPDH F: TGCACCACCAACTGCTTAGC; R: GGCATGGACTGTGGTCATGAG; TRPM8 F: GCTGTACAAAGCCTTCAGCAC, R: CTCATCACTGGCAAGGTCCA. Quantitative PCR experiments were performed in 30 sigmoid biopsies and 24 right-sided colon biopsies from 30 IBS patients and 23 healthy controls (all obtained from the Maastricht cohort).

#### *Immunofluorescent staining and tissue analysis*

Prior to analyses, biopsy samples were cryoprotected in 30% sucrose overnight, followed by an incubation in 50% sucrose and 50% OCT cryostat sectioning medium, and finally embedded in a cryomold with OCT. Tissues were stained as described.<sup>13</sup> In brief, cryoprotected tissues were cut into 10 µm sections and incubated with Trident Universal Protein Blocking Reagent (Gene Tex, GTX30963) for 2 h before primary

antibody TRPM8 (1:200, Alomone: ACC-049) with either CGRP (1:400, Thermo Fisher, ABS026 - 05 - 02), CD103 (1:400, DAKO, R7188) and CD11C (1:200, Abcam, ab33483), CD64 (1:400, DAKO, R7129), CD68 (1:400, DAKO, M0876) and mast cell tryptase (1:400, DAKO, MC052) was applied overnight (4°C). Tissues were then washed and incubated (60 min, room temperature) with species-specific Alexa Fluor conjugated secondary antibodies (1:400, A-11001, A-21451, A-11036, Carlsbad, CA). A Leica DM4000 epifluorescence microscope was used to visualize TRPM8+ immunoreactivity (IR). Immunoreactive cells for each target were counted manually by two independent observers in each section and averaged over five fields of view – all taken at 40x magnification. Observers were blinded to the source of tissue, *i.e.* IBS subtype, as tissue was assigned a collection number until data analysis. Immunohistochemistry experiments were performed in biopsies from 28 IBS patients (5/28 obtained from the Maastricht cohort) and 7 controls (all obtained from the London cohort). No primary controls were performed to eliminate non-specific binding of secondary antibodies, and TRPM8 control antigen was used to ensure binding specificity (*Supplementary Figure S5.4*).

## Cytokine experiments

IBS biopsies were placed in 200µL Krebs solution (control), or in icilin (treatment) (1 µM, Tocris, product code: 1531) in 200 µL Krebs solution, and were maintained at 37°C with 95% CO<sub>2</sub>/5% O<sub>2</sub> overnight (16-18 h) in 96 well plates. The supernatant was then removed and aliquots stored at -20°C. Quantification of cytokines the IL-1β, IL-6, TNF-α, IL-10, and IL-8 was performed using a customized human cytokine/chemokine/growth factor assay panel (Milliplex MAP Multiplex assay, Merck Millipore, product code HCYTOMAG-60K), and analyzed on Luminex MAGPIX Instrument with xPONENT 4.3 (Luminex Corporation). Cytokine experiments were performed using sigmoid biopsies from 12 IBS patients (all obtained from the London cohort).

## Statistical analysis

Statistical analysis was performed using SPSS 26 (IBM Corporation) or GraphPad Prism, V.5.02. (GraphPad Software, Inc). Statistical analysis for cell counting was performed using the Mann-Whitney test for unpaired data. For the comparison of mRNA expression between IBS patients and controls, a multivariable linear regression was performed correcting for age and gender. Wilcoxon signed-rank test was performed to compare sigmoid mRNA with proximal colon mRNA levels (within subjects). Relation to symptoms was also assessed using multivariate linear regression correcting for age and gender. For cytokine release assays, paired analysis with statistical significance using

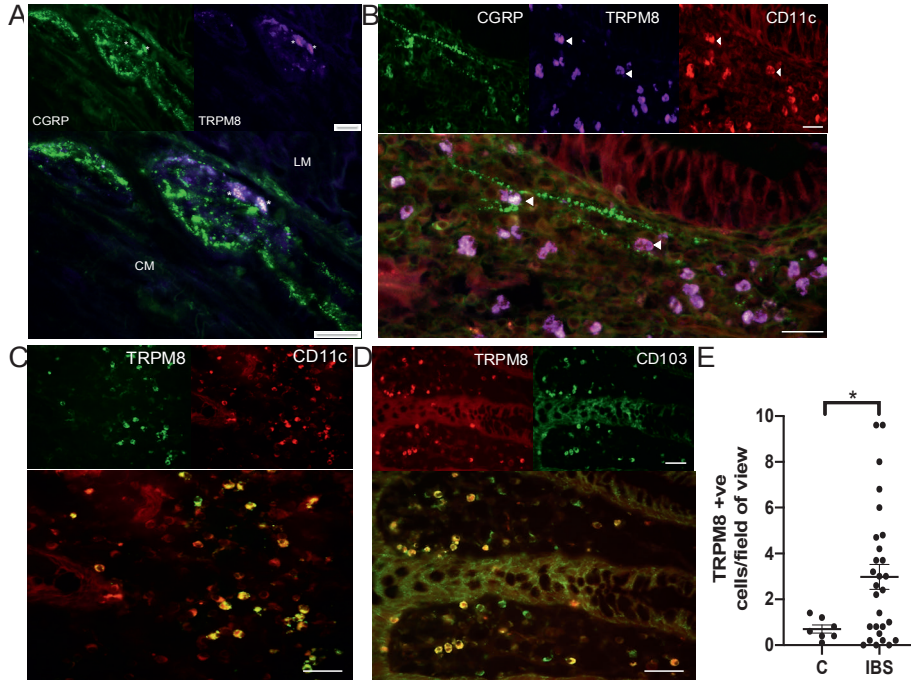
Wilcoxon matched-pairs signed rank test was used. Significance was defined as  $P < 0.05$ . Considering the exploratory nature of the experiments, no formal sample size calculation or correction for multiple testing was applied. All data is presented as median and interquartile range (IQR) and  $P < 0.05$  was deemed significant.

## Results

In human, uninflamed colonic tissue obtained from surgical resection, TRPM8 immunoreactivity (IR) was observed to colocalize with cell bodies within the myenteric plexus and co-labelled with CGRP (*Figure 5.1A*), corroborating findings in mouse myenteric ganglia.<sup>10</sup> Majority of TRPM8-IR in the inflamed mucosa (of Crohn's disease patients) was found to colocalize with CD11c+ immune cells with a small population colocalizing with CD45+ (expressed in all differentiated haematopoietic cells) immune cells (*Supplementary Figure S5.1*), and not with CGRP-IR mucosal nerve endings as previously reported in mice<sup>13</sup> (*Figure 5.1B*). TRPM8-IR CD11c+ cells were however, in close apposition to mucosal CGRP-IR nerve fibers (*Figure 5.1B*). As CD11c identifies a sub-population of dendritic cells (DC) in addition to monocytes<sup>16</sup>, we further explored the DC phenotype using CD103, a marker of a DC subset termed 'conventional DC1' commonly found in gastrointestinal mucosa.<sup>17</sup> In a representative IBS patient biopsy, TRPM8-IR was colocalized on both CD11c+ and CD103+ cells in colonic mucosa (*Figure 5.1C & 5.1D*). We also assessed TRPM8-IR in relation to mast cells and macrophage/monocytes and found absence of colocalization on these cell types (*Supplementary Figure S5.3*). These findings suggested a possible pathway mediating IBS symptom relief following TRPM8 agonism considering the apparent colocalization of TRPM8-IR with dendritic cells in close proximity to CGRP-IR mucosal nerve fibers.

We therefore examined TRPM8 expression in IBS patient and control biopsies from cohorts at Maastricht University Medical Centre+ (Netherlands) and The Royal London Hospital (U.K.) (56 IBS patients and 30 controls in total, demographic details in *Table 5.1*). mRNA expression of TRPM8 in the sigmoid colon was significantly higher in IBS patients compared to healthy controls ( $P < 0.001$ ) (*Figure 5.2A*, examined in 30 IBS patients, 23 controls, all obtained from the Maastricht Cohort). This increased expression was also found when counting TRPM-IR cells within the mucosa of IBS patients compared to controls ( $P = 0.045$ ) (*Figure 5.1E*, examined in 28 IBS patients (5/28 obtained from the Maastricht Cohort) and 7 controls (all obtained from the London Cohort)). IBS subtype analysis showed no differences in sigmoid TRPM8 mRNA expression between IBS subtypes. When comparing immunoreactivity, TRPM8-IR cells

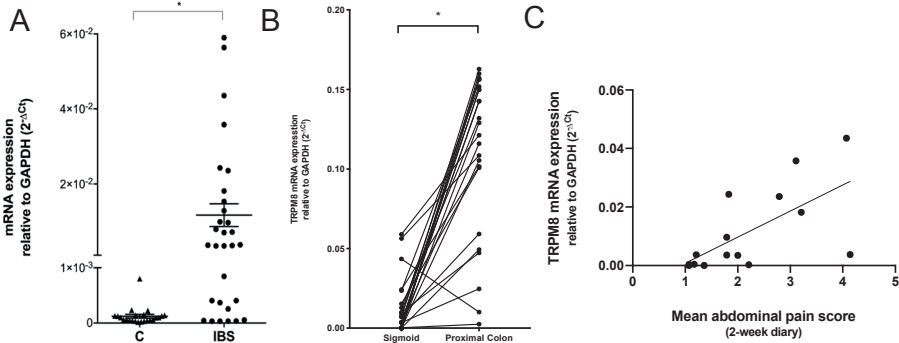
were increased in the IBS-D and IBS-M groups compared to IBS-C and IBS-undefined (Supplementary Figure S5.2).



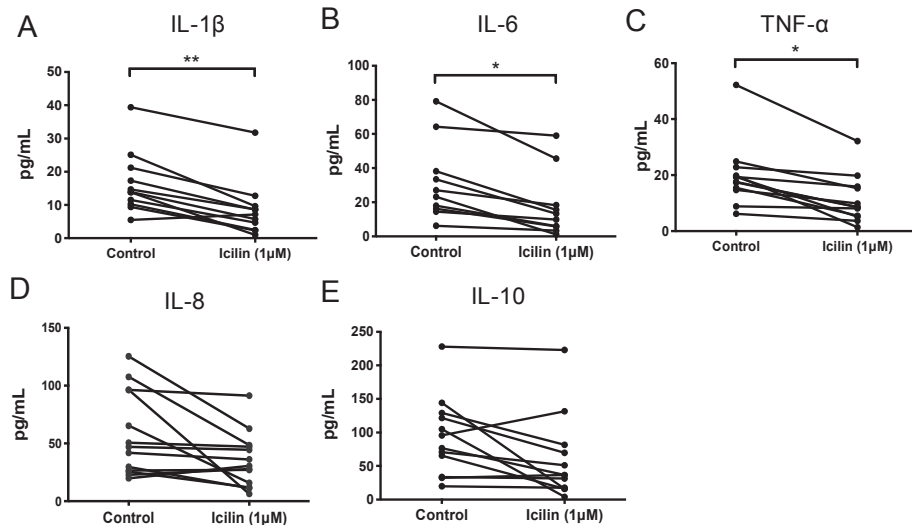
**Figure 5.1** TRPM8 expressed on human immune cell populations is increased in IBS patients and TRPM8 agonism reduces inflammatory cytokine release. **A:** In human colon (uninflamed), TRPM8 is expressed on CGRP immunoreactive fibers within the myenteric plexus. **B:** In inflamed colonic mucosa (Crohn's disease), TRPM8 (purple) is expressed on CD11c (red) positive cells that are in close apposition to CGRP immunoreactive nerve endings. **C & D:** In IBS patient biopsies, high levels of TRPM8 are observed in the mucosa which co-label with CD11c+ and CD103+ abundantly. **E:** IBS patients (IBS) had significantly higher levels of TRPM8-immunoreactive cells compared to controls (C) ( $N=28$  IBS,  $N=7$  C,  $*P=0.045$ ). Data shown as median and IQR, with  $P<0.05$  deemed significant. Circular (CM) and longitudinal muscular (LM) layers. Scale bar=10  $\mu$ m.

These results should be interpreted with caution due to the relatively small number of patients in each IBS-subtype. Interestingly, in IBS patients, mRNA expression of TRPM8 in the right-sided proximal colon was comparatively higher to that of the sigmoid colon ( $N=24$ ,  $P<0.001$ ) (Figure 5.2B). In addition, sigmoid TRPM8 mRNA expression showed a significant positive association with abdominal pain scores as determined using a 2-week diary ( $\beta=48.2$ , 95% CI 11.5;85.0,  $P=0.015$ , Figure 5.2C).

Based on the experiments performed on the localization of TRPM8-IR, these data suggested that stimulation of TRPM8 may influence cytokine release by mucosal immune cells. Incubation of paired IBS biopsies with TRPM8 agonist icilin ( $1 \mu\text{M}$ ) compared to buffer control, significantly reduced release of cytokines IL-1 $\beta$ , IL-6, and TNF- $\alpha$  but not IL-10 or IL-8 (Figure 5.3A-5.3E).



**Figure 5.2** Colonic TRPM8 mRNA expression is increased in IBS patients. **A:** mRNA expression of TRPM8 in sigmoid biopsy tissue is significantly increased in IBS patients (IBS) compared to healthy controls (C) ( $N=30$  IBS,  $N=23$  C,  $*P<0.001$ ). Data shown as median and IQR, corrected for age and gender, with  $P<0.05$  deemed significant. **B:** Differential expression of TRPM8 mRNA in right-sided colon (proximal) vs. sigmoid (distal) colon in mucosal samples from IBS patients ( $N=24$ ,  $*P<0.001$ ). **C:** TRPM8 mRNA expression in sigmoid biopsies is significantly associated to pain symptom scores in IBS ( $N=15$ ,  $P=0.015$ , corrected for age and gender, with  $P<0.05$  deemed significant).



**Figure 5.3** TRPM8 agonism reduces inflammatory cytokine release from IBS patient biopsies. Stimulation of IBS patient biopsies (paired) with the selective TRPM8 agonist icilin ( $1 \mu\text{M}$ ) reduced release of inflammatory cytokines IL-1 $\beta$ , IL-6, and TNF $\alpha$  but not IL-8 or anti-inflammatory cytokine IL-10.  $P<0.05$  is deemed significant.



## Discussion

Here, we provide preliminary findings in support for TRPM8 as a potential anti-inflammatory mediator in IBS patients. Increased TRPM8-IR on dendritic cells within the colonic mucosa coupled with decreased release of cytokines begins to delineate the potential cellular mechanisms underlying the therapeutic benefit of TRPM8 agonists, such as L-menthol in peppermint oil. Decreased release of cytokines IL-1 $\beta$ , IL-6, and TNF- $\alpha$  from DCs by TRPM8 stimulation is important as their respective receptors are expressed on colonic visceral afferents that mediate pain generation.<sup>18</sup> Indeed, peripheral blood mononuclear cells (PBMC) isolated from IBS patients secrete increased levels of these same cytokines, IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , compared to healthy controls.<sup>18</sup> In addition, TRPM8-IR CD103+ DCs may also interact with CGRP-IR sensory neurons that are in close apposition, possibly by neuro-immune communications where CGRP inhibits cytokine release from dendritic cells as demonstrated in mice.<sup>13</sup> Considering that we observed increased mRNA expression and TRPM8-IR (which appears to colocalize with immune cells) in IBS, we postulate that this represents an inducible counterregulatory anti-inflammatory mechanism. This mechanism is potentially related to the severity of pain symptoms, as we also observed a positive association between TRPM8 mRNA expression and pain scores. This is supported by data demonstrating increased pain scores associated with elevated levels of inflammatory cytokines in IBS-diarrhoea patients.<sup>18</sup> There is growing (albeit inconclusive) evidence that an inflammatory response is likely to contribute to pain symptoms in IBS patients through low-grade inflammation in a similar manner to IBD patients suggesting a commonality in pain mechanisms via neuro-immune mechanisms, with IBS-D patients having increased levels of IL-8 and IL-1.<sup>19-22</sup> In mice, colonic visceral afferents express receptors for the cytokines, IL-1 $\beta$ , IL-6, TNF- $\alpha$  and IL-10, and visceral hypersensitivity is induced by these cytokines in electrophysiology experiments.<sup>18-22</sup> Indeed, peripheral blood mononuclear cells (PBMC) isolated from IBS patients secrete increased levels of these same cytokines, IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , compared to healthy controls.<sup>18</sup> Human visceral afferents have the capacity to respond to numerous inflammatory mediators<sup>23-24</sup> and are likely similarly responsive directly to cytokines. This is a mechanism that requires further detailed exploration but is supported by the studies presented here demonstrating close apposition of sensory nerve endings and immune cells. In addition, the observed regional differences in TRPM8 mRNA expression (proximal colon higher compared to distal colon) may also provide an explanation for the phenomenon that the clinical efficacy of peppermint oil also depends on the release profile of its formulation.<sup>5</sup>

Certain limitations apply to our findings. First, we did not have the statistical power to differentiate findings according to IBS-subtype. Subtype-specific changes in neuro-immune activation have indeed been shown previously.<sup>18</sup> Second, patients were not specifically matched to controls. This resulted in an older IBS population, in particular in the Maastricht cohort (presumably due to a bias towards older patients undergoing colonoscopy). There were also more females in the IBS group compared to the control group, which was not significantly different in the aggregate cohort. We corrected for these factors by using multivariate linear regression corrected for age and gender. Third, diary scores were only completed by a subset of patients (15/30 of the Maastricht cohort). Although the subset was representative for the Maastricht IBS cohort and a significant association was shown between pain score and TRPM8 mRNA expression, the found association does not necessarily reflect a causal relationship. Fourth, the immunoreactivity identified need not necessarily reflect the exact expression of the TRPM8 protein molecule. The finding of potential TRPM8 expression on immune cells is a novel finding and should therefore warrant the necessary confirmation from future studies. Nevertheless, our previous experiments using this same antibody supports the specificity of our findings.<sup>25</sup> Aside from the TRPM8-specific agonist, no additional antagonist was applied to further confirm TRPM8 specificity of the effects observed in the functional experiments. Therefore, future experiments with healthy controls and TRPM8 antagonists will need to be performed to corroborate findings. Finally, due to limited availability of biopsies from controls, cytokine release experiments were only conducted on IBS patient biopsies. Although the number of TRPM8-IR cells was significantly higher in IBS compared to healthy controls, release of inflammatory cytokines may be reduced in control biopsy tissue.

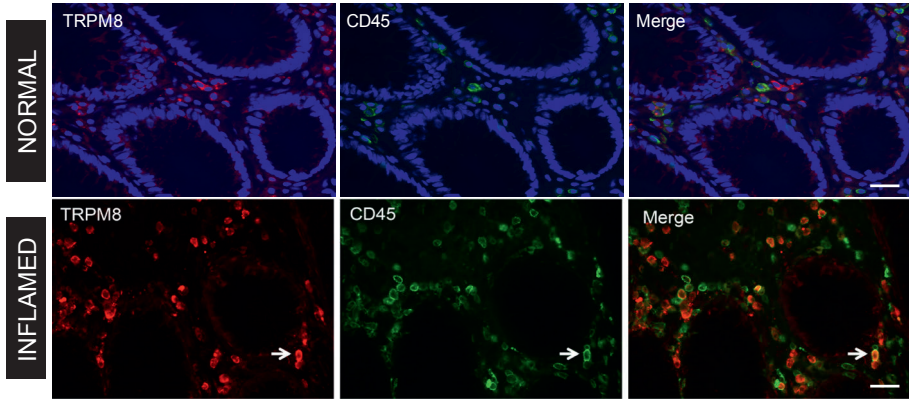
The mechanism by which TRPM8 influences IBS symptom generation therefore remains to be established. We were not able to directly ascertain the expression and function of TRPM8 on dendritic cells, the presumed neuro-immune interaction is therefore primarily based on surrogate parameters derived from the current experimental setup and therefore have an exploratory rather than a confirmatory nature. As this was a small, preliminary study, findings will need to be substantiated in a larger IBS patient cohort. Nevertheless, fundamental understanding of the role of TRPM8 in the regulation of neuro-immune interactions, and in particular the identification of the endogenous agonist(s) of TRPM8, may provide further mechanistic insight and improve therapeutic targeting in IBS and other disorders characterized by chronic abdominal pain.

## References

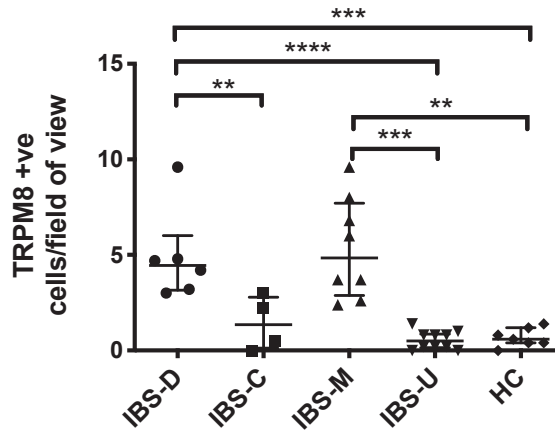
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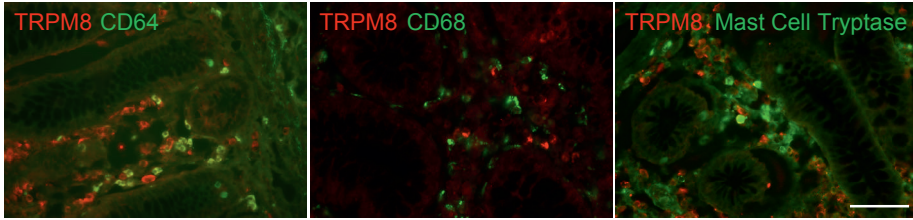
## Supplementary Material



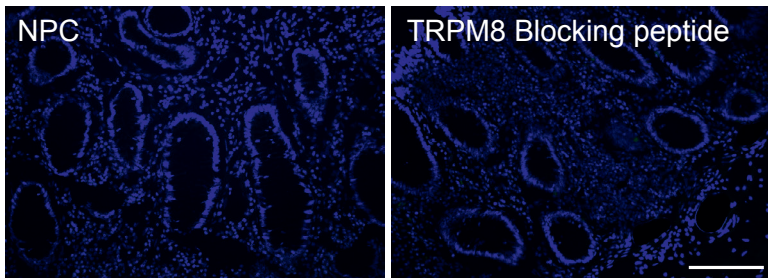
**Figure S5.1** Expression of TRPM8 on CD45+ cells in normal, uninfamed and inflamed (Crohn's disease) mucosa. Arrow points to CD45+ cell that co-expresses TRPM8 Scale bar = 10  $\mu$ m.



**Figure S5.2** TRPM8-immunoreactive (IR) cells/field of view classified by IBS sub-types. Data is presented as median and interquartile range (N: IBS-D=6, IBS-C=4, IBS-Mixed=8, IBS (unclassified)=10 & Healthy controls=7).



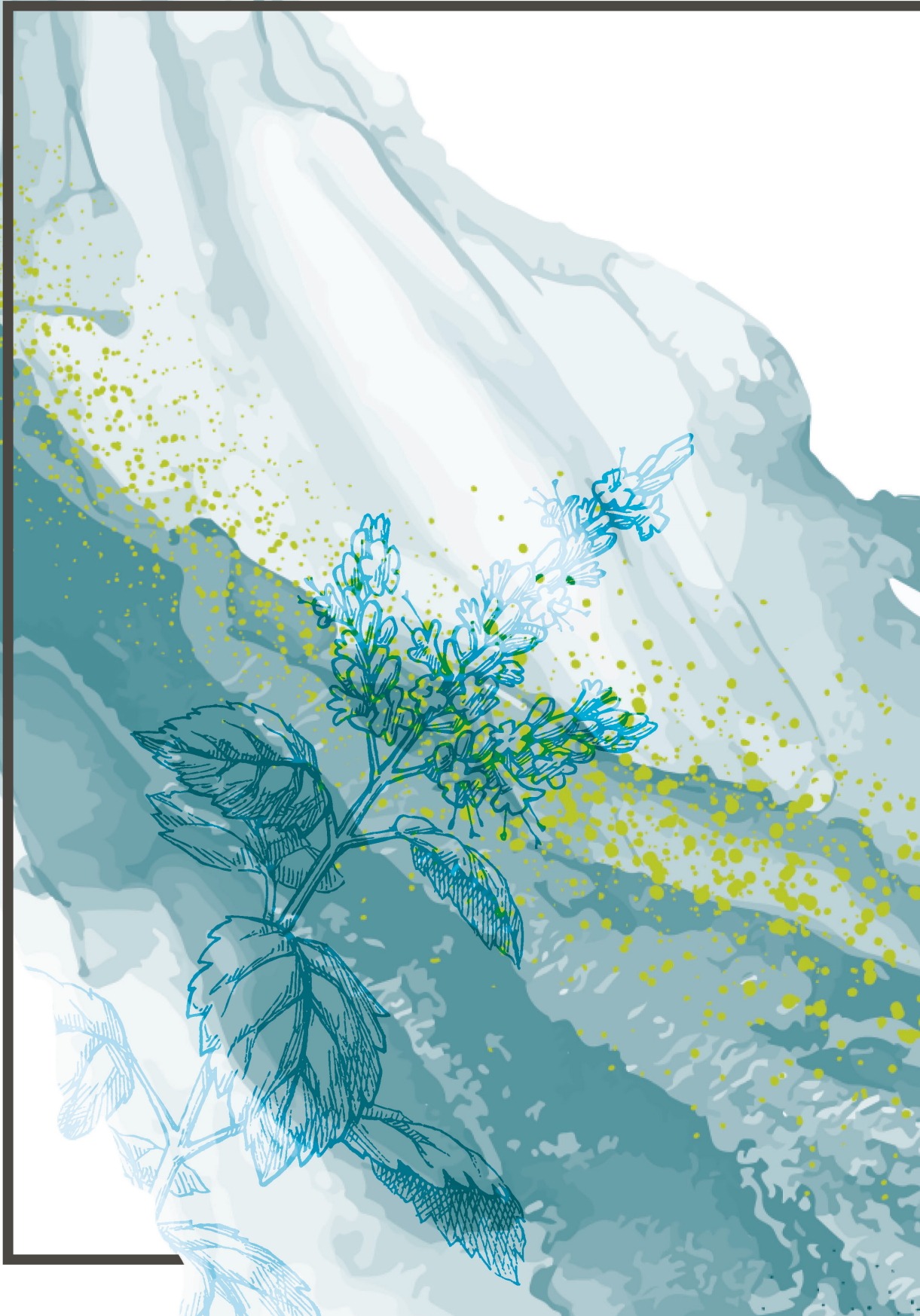
**Figure S5.3** TRPM8 does not co-label with monocyte/macrophage expressing CD64 and CD68, or with mast cells expressing mast cell tryptase, in human colon tissue. Scale bar=50  $\mu$ M.



**Figure S5.4** Assessment of TRPM8 antibody specificity using a) no primary control (NPC) to eliminate non-specific secondary antibody staining and b) blocking peptide to quench TRPM8 binding sites. Scale bar=50  $\mu$ M.









# Chapter 6

A novel ileocolonic release peppermint oil capsule for treatment in irritable bowel syndrome: a phase I study in healthy volunteers

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## Abstract

### Introduction

Peppermint oil (PO) has been shown to reduce abdominal pain in patients with Irritable Bowel Syndrome (IBS). PO is assumed to induce intestinal smooth muscle relaxation and desensitization of nociceptive nerve afferents. To increase colonic PO concentration, an ileocolonic release peppermint oil (IC-PO) capsule has been developed. The aim of this study was to compare pharmacokinetic parameters of the currently available small-intestinal release PO (SI-PO) and the novel IC-PO.

### Methods

In this randomized, double-blind, crossover study, subjects received 182 mg of either SI-PO or IC-PO in a crossover design with a >14 days washout period. Blood samples were collected to determine menthol-glucuronide concentrations.

### Results

Eight healthy volunteers (50% female, median age 22) were included. The time to reach the maximum concentration ( $T_{max}$ ) of IC-PO was significantly longer compared to SI-PO with a median (IQR) of 360 (360-405) versus 180 (120-180) min. The lag time ( $T_{lag}$ ) was significantly longer with a median (IQR) of 225 (204-284) for IC-PO compared to 37 (6-65) for SI-PO. The Area Under the menthol-glucuronide plasma concentration time Curves were significantly smaller with a median (IQR) of 2331  $\mu\text{g}^*\text{h}/\text{L}$  (2006-2510) for IC-PO compared to 2623  $\mu\text{g}^*\text{h}/\text{L}$  (2471-2920) for SI-PO. No significant differences were found in peak concentrations and elimination half-lives.

### Conclusions

IC-PO has a significantly delayed peak menthol-glucuronide concentration and  $T_{lag}$ , both pointing to the release of PO in the more distal part of the intestine. This may enhance therapeutic efficacy as it results in increased exposure of colonic mucosal afferents to the PO. A randomized controlled trial investigating the efficacy of SI and IC-PO in IBS is currently ongoing.

## Introduction

Irritable bowel syndrome (IBS) is a complex functional bowel disorder affecting up to 10-20% of the population in developed countries.<sup>1,2</sup> It is characterized by recurrent abdominal pain associated with changes in bowel habits.<sup>1</sup> Of the currently available treatment entities, peppermint oil released in the upper small intestine has been shown to be effective in reducing complaints of abdominal pain and inducing global symptom improvement<sup>3,4</sup> with a reported Number Needed to Treat (NNT) of 2-3.<sup>5,6</sup>

The main constituent of peppermint oil is L-menthol, which is rapidly metabolized to menthol-glucuronide and excreted in urine when taken orally. The exact mechanism of how peppermint oil acts remains to be elucidated, but is most likely multifactorial.<sup>7</sup> What is known however, is its dose-related relaxational effect of intestinal smooth muscle through the inhibition of calcium influx into the sarcolemma of smooth muscle cells<sup>8-10</sup> and thereby potentially decreasing abdominal cramps. Furthermore, there are indications that peppermint oil has a direct local anti-nociceptive effect in the colon through an interaction of L-menthol with transient receptor potential (TRPM8 and/or TRPA1) channels, channels known to play a role in visceral hypersensitivity and pain generation.<sup>11-14</sup> Other studies have reported inhibition of serotonin type 3 receptors (5HT<sub>3</sub>) in the human colon<sup>15</sup>, antimicrobial<sup>16,17</sup>, and carminative effects.<sup>18</sup> Capsules containing peppermint oil are available as an over the counter drug on the European market<sup>19</sup> and capsules containing peppermint oil microspheres are available as a medical food product in the USA and Canada.<sup>20</sup> All these formulations release peppermint oil in the small intestine.

The use of small-intestinal release peppermint oil is associated with upper gastrointestinal side effects, such as an altered sensation in the mouth in up to 11% and dyspeptic symptoms including heartburn, reflux and belching in up to 24% of patients.<sup>21-24</sup> These burdensome symptoms negatively affect therapy adherence. To decrease these side effects, it could be argued that an ileocolonic release of peppermint oil is beneficial. In addition, a colonic release may increase efficacy in IBS patients by enhancing local colonic relaxation and TRP stimulation. Therefore, a new peppermint oil soft capsule formulation with a predominant distal ileocolonic release has been developed using a previously described ileocolonic delivery technology to coat existing peppermint oil capsules.<sup>25-27</sup>

This study aimed to determine the pharmacokinetic differences and safety of both the small-intestinal release peppermint oil and the ileocolonic release peppermint oil in an *in*

*vitro* model and in healthy volunteers. Because the ileocolonic release formulation will release the peppermint oil in the ileocolonic region as opposed to the upper small intestine, we hypothesized that this will result in a delayed and possibly lower peak menthol-glucuronide concentration in the plasma compared to plasma concentrations found after administration of small-intestinal release capsules.

## Materials and methods

### In vitro GISS experiment

The novel ileocolonic release capsules were tested in the gastro-intestinal simulation system (GISS). The GISS is an *in vitro* model based on the pharmacopoeial dissolution test and has been described in detail elsewhere.<sup>28</sup> In summary, the model simulates pH and transit times through the human gastro-intestinal tract and enables variation of these and other parameters such as osmolality and agitation.<sup>28,29</sup> Table 6.1 shows the four GISS test phases.

### Phase I human trial

The research protocol was approved by the Maastricht University Medical Center+ Committee of Ethics and all study procedures were performed in compliance with Good Clinical Practice Guidelines and according to the revised Declaration of Helsinki. The study has been registered in the US National Library of Medicine (NCT 02291445) and the EudraCT database (2014-004195-32) and all subjects gave a written informed consent prior to participation.

**Table 6.1** Specifications of the four phases of the dissolution test (GISS).

Phase	Gastrointestinal Segment	Volume (mL)	Residence time (h)	pH	Osmolality (mOsmol/kg)
I	Stomach	500	2.0	1.2 ± 0.10	150 ± 25
II	Jejunum	629	2.0	6.8 ± 0.20	250 ± 50
III	Ileum (distal)	940	0.5	7.5 ± 0.25	250 ± 50
IV	Colon (proximal)	1000	1.5	6.0 ± 0.25	250 ± 60

GISS; gastro-intestinal simulation system. Adapted from Schellekens *et al.*, 2007.<sup>28</sup>

### Subjects

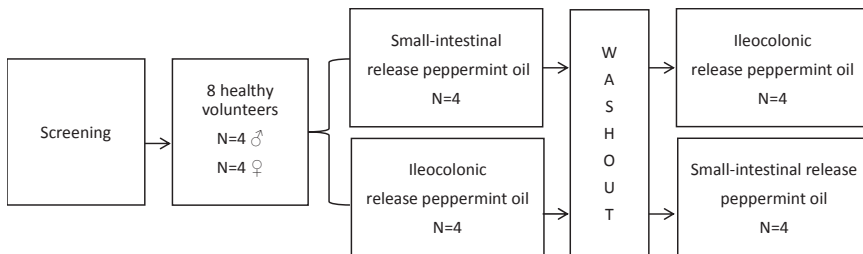
All subjects were healthy, non-smoking volunteers between 18 and 65 years old, with a BMI between 18 and 25 kg/m<sup>2</sup> and no history of gastrointestinal or other chronic

disease (as assessed by screening in which medical history, physical examination, and vital signs were checked). Participants were recruited via local advertisements and a national recruitment website. Exclusion criteria included high intake of alcohol (>15 consumptions per week) or caffeine (>8 cups coffee a day). Women of fertile age underwent a standard pregnancy test and were instructed to continue their contraception throughout the study.

Medication use (except for contraception) was prohibited in the 14 days prior to the test period and volunteers were instructed to abstain from alcohol, caffeine, grapefruit, and menthol- or peppermint oil containing products such as toothpaste, candy, and mouthwash for 48 h before each test day. Menthol-free toothpaste was provided beforehand.

## Study design

This study is designed according to US Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidelines for bioequivalence studies<sup>30,31</sup>; a randomized, double blind, two-treatment, single dose, crossover pharmacokinetic study with a wash-out period of at least 14, but no longer than 21 days (Figure 6.1).



**Figure 6.1** Study design; after screening, participants (healthy volunteers) were randomized and received small-intestinal release peppermint oil (PO) or ileocolonic release PO in a crossover design with a wash-out period of <21 days but >14 days.

The study consisted of two identical test periods of 24 hours in which all participants received 182 mg of ileocolonic and small-intestinal release peppermint oil capsules. In line with the European Pharmacopoeia entry for peppermint oil<sup>32</sup>, both 182 mg formulations contained between 51 mg and 105 mg of L-menthol (normal variance between capsules, no differences between small-intestinal versus ileocolonic release). As the estimated half-life of menthol-glucuronide in both preparations was between 3 and

10 h<sup>33,34</sup>, no carry-over effects were anticipated as a result of the chosen wash-out period.

Randomization, preparation, and labeling of the study medication were performed by an independent third party (Tiofarma BV, Oud Beijerland, The Netherlands). Half of the subjects received ileocolonic release capsules in the first test period (and small-intestinal release capsules in the second test period) and half of the subjects received small-intestinal release capsules in the first test period (and ileocolonic release capsules in the second test period), on the basis of on a randomized pre-selection using web-based randomization software. All capsules were packaged in identical, sealed containers and subject numbers and whether it was the first or second test day were mentioned on the label, ensuring allocation concealment.

On both test days, subjects arrived at the hospital after fasting overnight. Upon arrival, the subject had an intravenous catheter inserted for blood sampling. Prior to the administration of the peppermint oil capsule, several baseline measurements were taken; a venous blood sample was taken to determine baseline plasma menthol-glucuronide, an evaluation of baseline side effects was conducted and blood pressure/heart rate were measured. At  $t=0$ , the study medication was administered with a 200 mL glass of water. Consequently, at  $t= 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14,$  and 24 h, venous blood sampling, side effect evaluation and blood pressure/heart rate measurements were repeated. Two hours after capsule intake, a standardized breakfast was provided (two sandwiches with cheese and cucumber, glass of milk, 384 calories in total). Lunch and dinner were subsequently provided at  $t=6$  h and  $t=10$  h after capsule intake, respectively. The last measurement before midnight took place at  $t=14$  h, after which the intravenous cannula was removed and the subject was allowed to return home. Participants returned to the hospital for the last measurements at  $t=24$  h. Throughout the complete study, volunteers were instructed to report any side effects. A telephonic evaluation took place between both test periods.

### **Primary and secondary outcomes**

The primary outcome was  $T_{max}$ : time to reach peak menthol-glucuronide concentration in plasma. The secondary outcomes were;  $T_{lag}$ , time to reach a menthol-glucuronide concentration of 45  $\mu\text{g/L}$ ; AUC, Area Under the plasma concentration Curve;  $C_{max}$ , peak menthol-glucuronide concentrations;  $T_{1/2}$ , elimination half-life; side effects and tolerability, as determined by a side effect questionnaire and blood pressure/heart rate measurement.

## Pharmacokinetic analysis

The pharmacokinetic profile of small-intestinal and ileocolonic release peppermint oil capsules was determined by menthol-glucuronide analysis in the blood. In total, 14 venous blood samples (+/-8 mL per time point) were collected in heparinized tubes at the time points mentioned above. Within two hours after collection, samples were centrifuged at 3120 rpm for 10 min at 4°C and plasma supernatants were stored at -80°C until assayed. Samples were analyzed for menthol-glucuronide concentration, the primary metabolite of L-menthol, by a 15 h incubation at 37°C with beta-glucuronidase and using Gas Chromatography Mass Spectrometry (GC/MS); the method has been described in detail elsewhere.<sup>35,36</sup> A detection limit of 5 µg/L applied. Menthol-glucuronide concentrations are expressed in micrograms per liter.

## Statistical analysis

A power calculation was performed (two-sided=0.05; power=0.80; Sd=109; minimal detectable difference in means=150min); at least 7 subjects needed to complete the study to reliably demonstrate a significant difference in Time to reach peak menthol-glucuronide concentration in the plasma ( $T_{max}$ ), between the small-intestinal versus the novel ileocolonic release peppermint oil capsules. Anticipating 1 dropout, the aim was to include 8 participants.

Statistical analyses were carried out using IBM SPSS statistics 23.0 (Chicago IL, USA) and GraphPad Prism 6.0 (La Jolla, CA, USA) for Macintosh. As the sample size was small ( $N=8$ ), data were analyzed using non-parametric tests.  $T_{max}$ ,  $T_{lag}$ ,  $C_{max}$  values were determined directly from the plasma concentration-time profiles for each subject and were analyzed for comparison by the Wilcoxon-signed rank test. AUCs and  $T_{1/2}$  were calculated by pharmacokinetic software MWPharm 3.80 (Mediware) using the log-linear trapezoidal rule (non-compartmental analysis). AUCs and  $C_{max}$  were logarithmically transformed and a 90% CI interval was calculated of the log-transformed parameter ratios (small-intestinal/ileocolonic release) to assess bioequivalence. A  $P$  value of less than 0.05 was considered statistically significant. There were no missing data.

## Safety and tolerability analysis

Subjects were monitored for adverse events by direct observation during the first 14 h after peppermint oil administration and again for 1 h after 24 h. During both test periods, participants completed a questionnaire regarding adverse events and tolerability at the 14 time points mentioned above. In addition, vital signs were reviewed

at these 14 time points. In between test periods and after the last test period, volunteers were telephoned to inquire after possible adverse events.

## Results

### In vitro GISS experiment

*In vitro* analysis of the newly produced ileocolonic release peppermint oil capsules in the GISS showed that the actual release of the peppermint oil was postponed until the last phase (colon). Part of the coating remained intact and contained a small residual amount of peppermint oil (Table 6.2).

**Table 6.2** Results of ileocolonic release peppermint oil capsules in *in vitro* dissolution test (GISS).

Phase	Gastrointestinal segment	Capsule appearance	Capsule location in dissolution vessel	Oil observed on surface buffer solution
I	Stomach	Intact	Bottom	None
II	Jejunum	Intact, minor cracks in coating	Bottom	Slight amount
III	Ileum (distal)	Intact, small cracks in coating	Bottom	Small amount
IV	Colon (proximal)	Open - small residual amount of oil inside	Floating on surface buffer solution	Large amount

GISS; gastro-intestinal simulation system, PO; peppermint oil.

### Phase I human trial

Eight healthy volunteers were screened, included, and randomly assigned to a specific treatment order. All participants were between 20 and 65 years old (median 22.2, IQR 20.8-28.8). See Table 6.3 for baseline characteristics. All participants completed the study.

**Table 6.3** Summary of participant demographic and baseline characteristics.

	Total N=8	Small-intestinal release PO on first test day N=4	Ileocolonic release PO on first test day N=4
<b>Female sex</b> , N (%)	4 (50)	1 (25)	3 (75) *
<b>Age</b> , years (median + IQR)	22.2 (20.8-28.8)	24.1 (21.2-55.0)	21.7 (20.6-27.9)
<b>BMI</b> , kg/m <sup>2</sup> (median + IQR)	21.5 (20.2-23.0)	21.7 (18.5-23.8)	21.5 (20.6-22.2)
<b>Height</b> , cm (median + IQR)	177 (171-183)	181 (177-192)	173 (168-177)
<b>Weight</b> , kg (median + IQR)	65.5 (64.0-72.0)	71.0 (65.3-79.8)	64.0 (61.8-66.3)

Differences were tested using Mann-Whitney *U* test for non-parametric data and chi-square or Fisher's exact for parametric data. \* Significant difference in gender between the group receiving small-intestinal peppermint oil (PO) on the first test day and the group receiving ileocolonic release PO on the first test day ( $P < 0.05$ ). N=8 (total group).



## Menthol-glucuronide levels

Baseline menthol-glucuronide concentrations were all below the detection limit, except for one subject on the first test day who had a concentration of 5.79 µg/L. These low levels were considered evidence that the instructions given regarding avoidance of menthol-containing products were sufficient.

The pharmacokinetic parameters of 182 mg small-intestinal and 182 mg ileocolonic release peppermint oil are shown in *Table 6.4*. The plasma concentration time curve is given in *Figure 6.2*. For an overview of plasma concentration time curves per individual subject, please see *Figure S6.1* in the *Supplementary Material*.

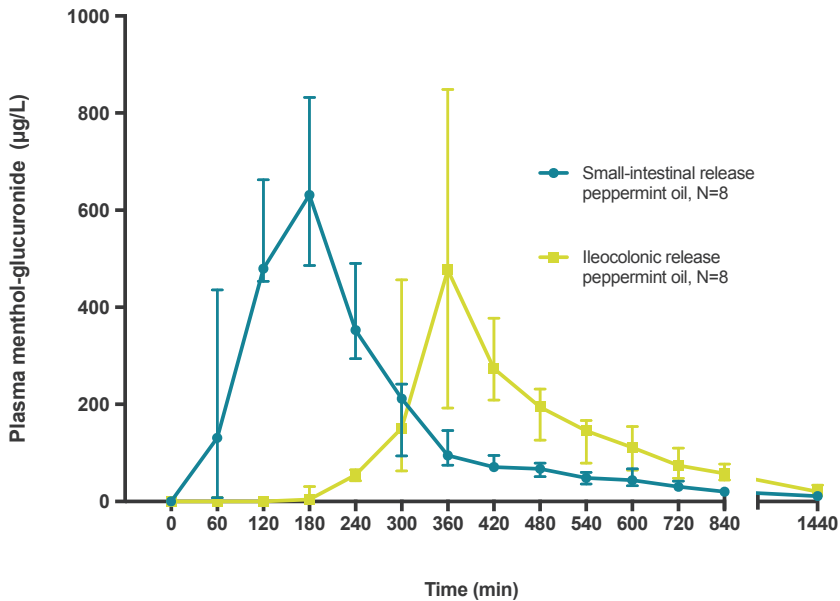
**Table 6.4** Pharmacokinetic parameter results; small-intestinal release and ileocolonic release peppermint oil.

	Small-intestinal release peppermint oil	Ileocolonic release peppermint oil	Ratio LN- transformed parameter (90% CI)
<b>T<sub>max</sub> (min)</b>			N.A.
Arithmetic Mean (SEM)	165 (15)	375 (19)*	
Median (IQR)	180 (120-180)	360 (360-405)*	
Geometric mean (SEM)	160 (15)	372 (19)*	
<b>T<sub>lag</sub> (min)</b>			N.A.
Arithmetic Mean (SEM)	38 (12)	241 (18)*	
Median (IQR)	37 (6-65)	225 (204-284)*	
Geometric mean (SEM)	22 (12)	237 (18)*	
<b>AUC<sub>0-24</sub> (µg*h/L)</b>			1.02 (1.01-1.04)
Arithmetic Mean (SEM)	2664 (84)	2246 (118)*	
Median (IQR)	2623 (2471-2920)	2331 (2006-2510)*	
Geometric mean (SEM)	2655 (84)	2222 (118)*	
<b>C<sub>max</sub> (µg/L)</b>			1.02 (1.01-1.04)
Arithmetic Mean (SEM)	817.9 (90)	558.0 (100)	
Median (IQR)	702 (644-1020)	563.6 (268-849)	
Geometric mean (SEM)	788.2 (90)	487 (100)	
<b>T<sub>1/2</sub> (hours)</b>			N.A.
Arithmetic Mean (SEM)	9.2 (1.4)	6.4 (0.6)	
Median (IQR)	7.7 (7.0-10.8)	6.1 (5.1-7.4)	
Geometric mean (SEM)	8.6 (1.4)	6.2 (0.6)	

Differences between both peppermint oil capsules were tested using Wilcoxon-signed-rank test. \* Significant difference between small-intestinal release and ileocolonic release peppermint oil ( $P < 0.05$ ). LN; natural log, CI; confidence interval, T<sub>max</sub>; time to reach maximum plasma concentration, SEM; standard error of the mean, IQR; interquartile range, T<sub>lag</sub>; time to reach a menthol-glucuronide concentration of 45 µg/L, AUC; area under the concentration time curve, C<sub>max</sub>; maximum concentration, T<sub>1/2</sub>; Time required for the plasma concentration to reach half of its original value (Elimination half-life), N.A.; non-applicable.

T<sub>max</sub> of the ileocolonic release peppermint oil capsules was significantly longer in all participants compared to T<sub>max</sub> of small-intestinal release peppermint oil with a median (IQR) of 360 minutes (360-405) versus 180 minutes (120-180) respectively,  $P < 0.05$

( $P=0.010$ ). Median difference in  $T_{max}$  between both formulations in individual participants was 180 minutes, (IQR 180-240, 95% CI 180-140).  $T_{lag}$  was significantly delayed in ileocolonic release peppermint oil, with a median (IQR) of 225 (204-284) versus 37 (6-65) minutes,  $P<0,05$  ( $P=0.012$ ). In addition,  $AUC_{0-24hrs}$  differed significantly between the ileocolonic and the small-intestinal release capsules, with a median (IQR) of 2331  $\mu\text{g}^*\text{h/L}$  (2006-2510) and 2623  $\mu\text{g}^*\text{h/L}$  (2471-2920) respectively,  $P<0.05$  ( $P=0.017$ ). No significant differences were found in  $C_{max}$  ( $P=0.28$ ) and  $T_{1/2}$  ( $P=0.16$ ) of either capsule. Mean ratios of log-transformed AUCs and  $C_{max}$  of 1.02 (90% CI 1.01-1.04) were found.



**Figure 6.2** Concentration-time curve, menthol-glucuronide concentration was measured in 8 healthy volunteers ( $N=8$ ) in  $\mu\text{g/mL}$  on 14 time points after both peppermint oil (PO) capsule administrations. The ileocolonic release PO has a significantly elongated time to reach the maximum plasma concentration. Circles and squares represent median plasma glucuronide levels + Interquartile Range (+IQR).

## Adverse events and tolerability

No serious adverse events occurred during the study nor during one week follow up. Adverse events were reported in five subjects, but were all mild and transient. Adverse events are shown in *Table 6.5*.

**Table 6.5** Adverse events occurring after administration of a single 182 mg peppermint oil dose.

	Small-intestinal release PO N=8	Ileocolonic release PO N=8
<b>Acid regurgitation</b> , N (%)	1 (12.5)	0 (0)
<b>Fecal urgency</b> , N (%)	1 (12.5)	0 (0)
<b>Headache</b> , N (%)		
Mild	0 (0)	2 (25)
Moderate/severe	0 (0)	0 (0)
<b>Altered fecal odor</b> , N (%)	0 (0)	2 (25)
<b>Vital sign abnormalities</b> , N (%)	0 (0)	0 (0)

PO; peppermint oil, N; number. N=8 (total group). Altered fecal odor implied a peppermint oil odor.

## Discussion

This is the first study to investigate a novel ileocolonic release peppermint oil formulation and to compare its pharmacokinetic parameters, safety, and tolerability with the small-intestinal release peppermint oil formulation currently available. Our results demonstrate that ileocolonic release peppermint oil soft capsules have a significantly delayed lag time ( $T_{lag}$ ) and reach their peak concentration significantly later ( $T_{max}$ ) than small-intestinal release peppermint oil capsules. These findings point to the release of peppermint oil in a more distal part of the human gastrointestinal tract, the colon most presumably. Although different in release kinetics, a single dose of both formulations of peppermint oil can be considered bioequivalent regarding exposure in healthy volunteers, because the 90% confidence interval (CI) for the log-transformed parameter ratios (small-intestinal/ileocolonic release) is between the 80-125% confidence interval, the standard bioequivalence criterion as stated in the FDA and EMA guidelines on the investigation of bioequivalence.<sup>30,31</sup>

Several authors have previously reported on the pharmacokinetic parameters of small-intestinal release peppermint oil, often referred to as enteric-coated capsules: White *et al.* found a mean  $T_{max}$  of 5 and 2.8 hours for menthol-glucuronide after three Colpermin® or Mintec® capsules were taken, containing 0.2 ml peppermint oil each and a mean L-menthol concentration of 110 mg and 117 mg respectively.<sup>19</sup> Mascher *et al.* found a mean  $T_{max}$  of 3 hours and a mean menthol  $C_{max}$  of 1196 ng/ml after two Enteroplant® capsules were taken, containing 90 mg of peppermint oil each.<sup>34</sup> Our findings for the small-intestinal release peppermint oil capsules are in line with both White *et al.* and Masher *et al.* in terms of  $T_{max}$  found, although the  $C_{max}$  differed slightly. A possible explanation for this modest discrepancy is that different small-intestinal

release formulations of peppermint oil, produced by different manufacturers, produced from a different harvest of mint leaves, were used. L-Menthol concentrations in peppermint oil are known to vary between 30% and 55%.<sup>32</sup> When taken orally, L-menthol is rapidly metabolized to menthol-glucuronide, which can be measured successfully in blood-plasma by Gas Chromatography Mass Spectrometry.<sup>35</sup> Consequently, the menthol-glucuronide is excreted in urine.<sup>9</sup>

The menthol-glucuronide concentration was not measured directly in the small intestine and colon because of the practical difficulties of an ileocolonic intubation. Unlike other studies that have compared peppermint oil formulations, however, we think pharmacokinetic parameters could be compared more reliably as both formulations used here contained the same amount of L-menthol; the ileocolonic release peppermint oil capsules were created by overencapsulation of small-intestinal release peppermint oil capsules from the same manufacturer.<sup>19</sup> We presume that the difference in  $T_{max}$  found between the small-intestinal and ileocolonic release peppermint oil reflects the longer transit needed to reach the colon.

It should be also noted that our study showed large interquartile ranges in plasma menthol-glucuronide levels, indicating high inter-subject variability. Measures taken to decrease any variability were standardized meals and snacks, an overnight fast, and the complete abstinence from caffeine, alcohol, smoking, and medicines affecting gastrointestinal function in a predefined period prior to drug administration. The variability found can therefore probably be explained by normal biological variation in gastrointestinal transit times, and polymorphic variation in cytochromes P450 (CYP) enzymes, which are known to facilitate L-menthol metabolism.<sup>37-40</sup>

The delayed peak concentration of the novel ileocolonic release peppermint oil and, thus, more distal exposure to peppermint oil are not only expected to increase therapeutic efficacy, but it is also expected to lead to a different and possibly milder spectrum of adverse events, as there is less upper gastrointestinal but more distal gastrointestinal exposure to peppermint oil. This pilot study has examined possible adverse events after only a single dose of peppermint oil and was not powered to draw any conclusions regarding adverse events since it only included eight volunteers. In addition to evaluating short term adverse events, future studies should also evaluate the long-term adverse events occurring when peppermint oil capsules of 182 mg are taken three times daily, the dosage identified by previous studies as being effective in IBS patients and the industry norm.<sup>20,22,24,41</sup>

As discussed above, there are indications that peppermint oil has a direct local anti-nociceptive effect in the intestine through an interaction with transient receptor potential (TRP) channels. When peppermint oil makes contact with the skin or oral membranes, a general cooling sensation is induced through stimulation of the TRPM8 (transient receptor potential melastain 8) receptor.<sup>42</sup> Interestingly, experimental research in murine models suggests that L-menthol, through stimulation of the TRPM8 receptor, may be able to cross-desensitize the TRPV1 (transient receptor potential vanilloid 1) receptor. TRPV1 is a pro-nociceptive receptor, well-known for its involvement in animal models of visceral hypersensitivity and its up-regulation in the colon-mucosa of IBS patients<sup>13,43</sup>, indicating a role in pain generation in IBS. Together with its role in thermo-sensation, TRPM8 is believed to play a role in protective mechanisms in states of intestinal inflammation.<sup>44</sup> Similar to the stimulation of TRPM8, menthol may also be able to stimulate the TRPA1 (transient receptor potential ankyrin 1) receptor, another TRP channel suggested to contribute to visceral pain. TRPV1, TRPA1, and TRPM8 receptors are probably co-expressed on ileocolonic mucosal afferents<sup>11,45</sup>, suggesting a complex and incomprehensively understood interaction between these receptors that leads to the analgesic effect of peppermint oil. We hypothesize that the ileocolonic release of peppermint oil enhances the therapeutic efficacy as the application specifically results in increased exposure of the ileocolonic mucosal afferents described. Moreover, the anti-spasmodic effect and, thereby, alleviating effect of peppermint oil performs equally well, if not better, when peppermint oil is applied to the colon locally; it has been applied intraluminally in endoscopic practice to decrease pain caused by the procedure and to enhance the field of view during the endoscopy through the suppression of peristalsis.<sup>46-48</sup> This pharmacokinetic study served as proof of concept study and we are currently conducting a randomized, placebo-controlled trial in IBS patients to compare the efficacy of small-intestinal and ileocolonic release peppermint oil in IBS patients (*NCT 02716285*). This study should confirm whether peppermint oil capsules with an ileocolonic release do indeed attenuate abdominal pain in IBS patients.

A potential limitation of this study is that L-menthol was the only ingredient of peppermint oil taken into account; other constituents of peppermint oil include menthone, cineole, menthyl acetate, isomenthone, menthofurane, limonene, pulegone, carvone, and isopulegol.<sup>32,49</sup> These could potentially contribute to clinical effects, but were not measured in the plasma samples. For example, in addition to the anti-spasmodic effect, peppermint oil has been shown to inhibit serotonin type 3 receptors (5HT<sub>3</sub>) in the human colon.<sup>15</sup> This inhibitory effect could only be partly accounted for by L-menthol in another experimental study<sup>50</sup>, suggesting the involvement of one or more

of the other ingredients mentioned above. Furthermore, the toxic compounds known to be present in peppermint oil in very low quantities – in equal dosages for ileocolonic release and small-intestinal release peppermint oil - were also not measured. Pulegone and menthofuran normally appear in peppermint oil in doses of between 1-9% and less than 4.0%<sup>32</sup> and could potentially harm chronic peppermint oil users. Some animal studies reported toxicity due to pulegone and menthofuran; high dosages in rats were associated with hepatocyte vacuolization, liver necrosis, and possibly cyst-like spaces in the cerebellum.<sup>7</sup> Nevertheless, when taken in the recommended dosages, the EMA and the FDA consider peppermint oil to be generally safe. Even if the EMA states that no confirmed cases of liver damage due to peppermint oil usage have been reported<sup>51</sup>, they do advise against the continuous use of peppermint oil for longer than three months.<sup>52</sup> It remains to be elucidated whether this advice is substantiated – no studies have assessed the long-term effect, thus no studies have revealed damage occurring after three months – but further research into this topic is certainly desired.

Of note is that half of the participants were male, whereas the male/female ratio in IBS patients is usually estimated to be 1:2.<sup>53</sup> Ideally, future research should include IBS patients who may experience altered motility and/or altered transit times and should preferably also investigate relatively more females. Although large effects of sex on pharmacokinetic parameters are not expected, factors such as menstrual cycle and lower body weights, and thereby smaller distribution volumes, higher body fat percentages etc. could be of influence.<sup>54</sup>

## Conclusions

A novel ileocolonic release peppermint oil formulation has been developed to decrease upper gastro-intestinal side effects associated with small-intestinal release peppermint oil. This study provides evidence that the ileocolonic release peppermint oil has a significantly delayed peak menthol-glucuronide concentration, pointing to a more delayed and therefore more distal intestinal release of peppermint oil. The ileocolonic release may enhance therapeutic efficacy as it results in increased exposure to the colonic mucosal afferents and decrease adverse events. A randomized placebo-controlled trial (RCT) investigating the efficacy of small-intestinal and ileocolonic release peppermint oil in IBS patients has been initiated and is currently ongoing. This RCT is based on the pharmacokinetic data from the present study.

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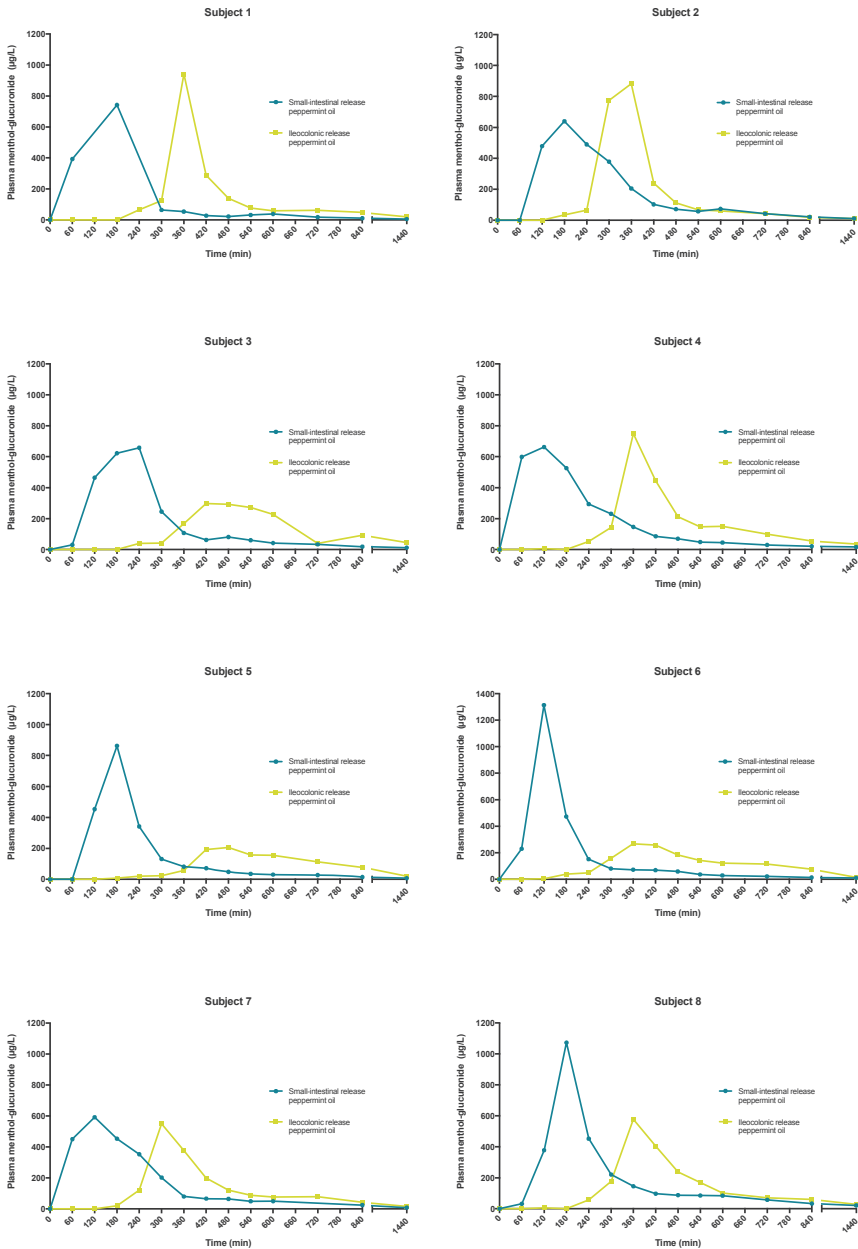
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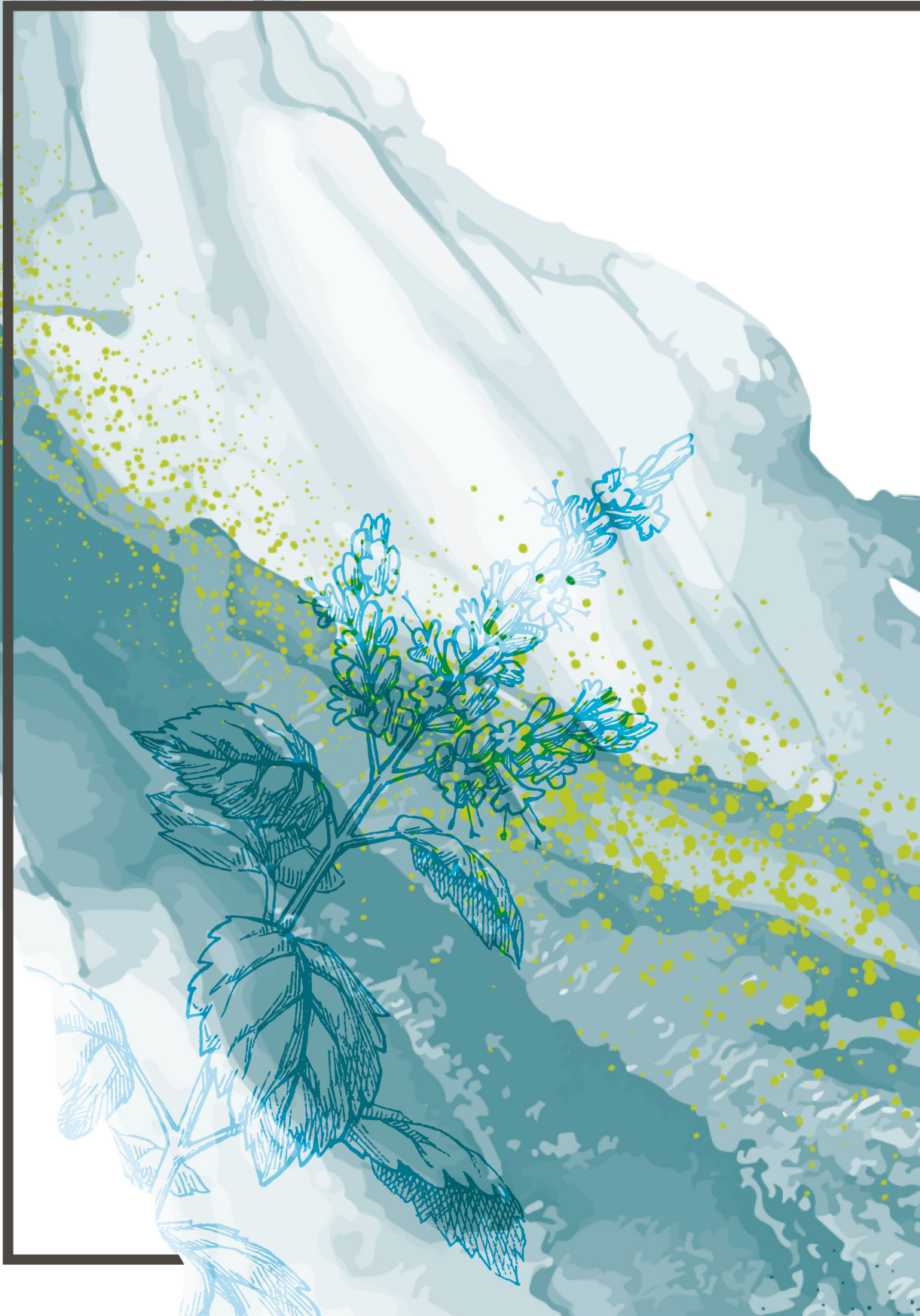
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# Supplementary Material



**Figure S6.1** Concentration-time curve, menthol-glucuronide concentration was measured in healthy volunteers (N=8) in µg/mL on 14 time points after both peppermint oil (PO) capsule administrations.





# Chapter 7

Efficacy and safety of peppermint oil in a randomized, double-blind trial of patients with irritable bowel syndrome

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## Abstract

### Introduction

Peppermint oil is frequently used to treat irritable bowel syndrome (IBS), despite a lack of evidence for efficacy from high-quality controlled trials. We studied the efficacy and safety of small-intestinal release peppermint oil in patients with IBS and explored the effects of targeted ileocolonic release peppermint oil.

### Methods

We performed a double-blind trial of 190 patients with IBS (Rome IV criteria) at 4 hospitals in The Netherlands from August 2016 through March 2018; 189 patients were included in the intent-to-treat analysis (mean age, 34.0 years; 77.8% female; 57.7% in primary care), and 178 completed the study. Patients were randomly assigned to groups given 182 mg small-intestinal release peppermint oil, 182 mg ileocolonic release peppermint oil, or placebo for 8 weeks. The primary endpoint was abdominal pain response, as defined by the US Food and Drug Administration (FDA): at least a 30% decrease in the weekly average of worst daily abdominal pain compared with baseline in at least 4 weeks. The co-primary endpoint was overall relief of IBS symptoms, as defined by the European Medicines Agency (EMA). Secondary endpoints included abdominal pain, discomfort, symptom severity, and adverse events (AEs).

### Results

Abdominal pain response did not differ significantly between groups: 29 of 62 patients in the small-intestinal release peppermint oil group had a response (46.8%,  $P=0.170$  vs placebo), 26 of 63 patients in the ileocolonic release peppermint oil group had a response (41.3%,  $P=0.385$  vs placebo), and 22 of 64 patients in the placebo group had a response (34.4%). We did not find differences among the groups in overall relief (9.7%,  $P=0.317$  and 1.6%,  $P=0.351$  vs 4.7% for placebo). The small-intestinal peppermint oil did, however, produce greater improvements than placebo in secondary outcomes of abdominal pain ( $P=0.016$ ), discomfort ( $P=0.020$ ), and IBS severity ( $P=0.020$ ). AEs, although mild, were more common in both peppermint oil groups ( $P<0.005$ ).

### Conclusions

We found that neither small-intestinal release nor ileocolonic release peppermint oil (8 weeks) produced statistically significant reductions in abdominal pain response or overall symptom relief, when using FDA/EMA recommended endpoints. The small-intestinal release peppermint oil did, however, significantly reduce abdominal pain, discomfort, and IBS severity. These findings do not support further development of ileocolonic release peppermint oil for treatment of IBS.

## Introduction

Irritable bowel syndrome (IBS) is a disorder of the gut-brain axis characterized by recurrent chronic abdominal pain and altered bowel habits.<sup>2</sup> IBS is highly prevalent with an estimated prevalence in the general population of 5-6% according to Rome IV criteria.<sup>3,4</sup> IBS has a profound negative impact on quality of life and carries a substantial socioeconomic burden.<sup>5</sup> Although the number of therapeutic options has grown recently<sup>6</sup>, treatment of abdominal pain remains challenging and is often unsatisfactory. One of the pharmacotherapeutic entities currently used is peppermint oil. This agent of herbal origin has menthol as its main constituent and is presumed to have several mechanisms of action including intestinal smooth muscle relaxation<sup>7</sup>, modulation of transient receptor potential (TRP) channel mediated visceral nociception<sup>8-10</sup>, 5-hydroxytryptamine antagonism<sup>11</sup>, antimicrobial and antifungal effects<sup>12-14</sup>, and  $\kappa$ -opioid receptor agonism.<sup>15</sup> Enteric-coated capsules that release peppermint oil in the small intestine are currently available as an over-the-counter (OTC) drug in Europe<sup>16</sup> and as a medical food labeled product in the USA and Canada.<sup>17</sup>

Guideline recommendations<sup>18</sup> regarding the use of (small-intestinal release) peppermint oil in IBS treatment are currently based on prior studies showing highly favorable results in terms of abdominal pain reduction and global improvement of symptoms.<sup>17,19-23</sup> Most of these studies, however, were hampered by significant methodological shortcomings that impede the ability to draw firm conclusions. Moreover, the Food and Drug Administration (FDA)<sup>24</sup> and the European Medicines Agency (EMA)<sup>25</sup> have defined robust, albeit provisional, endpoints for IBS trials since 2012, and the Rome diagnostic criteria for IBS have been updated in 2016. Taken together, there is a need for a well-designed trial in Rome IV-defined IBS patients that investigates efficacy according to these stringent endpoints to refute or validate earlier findings. The primary objective of this multicenter, randomized, placebo-controlled study was thus to determine the efficacy and safety of small-intestinal release peppermint oil in a Rome IV IBS population according to FDA and EMA guidelines. We hypothesized that, in Rome IV IBS patients, conventional small-intestinal release peppermint oil would be more effective compared to placebo.

A secondary aim was to explore the efficacy and safety of a novel soft gel peppermint oil capsule with a predominant distal ileocolonic release. The pharmacokinetic profile of this formulation has been described recently.<sup>1</sup> The rationale for using ileocolonic release was based on experimental findings that peppermint oil has a direct local antinociceptive effect in the colon through an interaction of menthol with TRPM8 and/or TRPA1



channels on sensory afferents.<sup>8</sup> We therefore hypothesized that a higher exposure of the colonic afferents through targeted ileocolonic delivery of peppermint oil would enhance antinociceptive effects and thereby improve efficacy. In addition, small-intestinal release peppermint oil therapy is often discontinued due to mild, but burdensome upper gastrointestinal (GI) adverse events (AEs) that are assumed to be related to the relaxation of the lower esophageal sphincter<sup>26</sup> and can hamper therapy adherence. We therefore also postulated that the ileocolonic release formulation would decrease these AEs.

## Materials and methods

### Study design, setting, and patients

The **PEppeR**mint Oil for the treatment of Irritable Bowel **S**ndrome: optimizing therape**U**tic str**A**tegies using targeted **DE**livery (PERSUADE) study was a randomized, double-blind, placebo-controlled trial and was performed in four Dutch hospitals: one academic with a combined secondary and tertiary care function (Maastricht University Medical Center+ (MUMC+)), and three secondary care (Hospital Gelderse Vallei, Ede; Alrijne Hospital, Leiden; Medical Center Leeuwarden). The study protocol had been approved by the MUMC+ Ethics Committee (applicable to all centers). All study procedures were performed in compliance with Good Clinical Practice Guidelines and according to the revised Declaration of Helsinki. All subjects gave written informed consent prior to participation. All authors had access to the study data and reviewed and approved the final manuscript.

Patients, between 18 and 75 years of age, fulfilling the Rome IV criteria for IBS, without alarm symptoms, were recruited via primary care, via the outpatient clinics of the above-mentioned hospitals, or via self-referral through public advertisements, social media, and the Dutch IBS patient federation. Detailed in- and exclusion criteria are given in the *Supplementary Material*. Patients were screened for eligibility in a prescreening (telephone interview) and a medical screening that included history taking and a physical examination. After the screening, eligible patients entered a 14-days pre-treatment period during which they scored their daily worst abdominal pain in a digital symptom diary (scored on an 11-point numerical-rating-scale (NRS), 0=no pain, 10=worst possible pain). Subsequently, those with a mean worst abdominal score of at least 3 were then randomized to 182 mg of small-intestinal release peppermint oil (Tempocol, WillPharma S.A.), 182 mg of ileocolonic release peppermint oil (Tempocol, core-



capsules, coated with a Colopulse coating layer<sup>1,27</sup>), or placebo (microcrystalline cellulose) intake orally. Randomization was done with ALEA (Abcoude, The Netherlands) Screening and Enrolment Application Software using the minimization method, accounted for inclusion center, IBS subtypes (diarrhea, mixed, constipation, undefined), sex, and age. All study medication was over-encapsulated with identical hard gelatin capsules and packaged in identical blisters to ensure allocation concealment by Tiofarma S.A. (Oud-Beijerland, the Netherlands). Patients were instructed to self-administer three capsules daily, 30 min before breakfast, lunch, and dinner, during eight weeks. An eight-week treatment period was chosen as we expected the clinical effect to occur within this period based on previous studies.<sup>17,21</sup> This treatment duration was also selected to mitigate potential hazardous effects of long-term peppermint administration related to certain constituents.<sup>26</sup> Nevertheless, safety issues were later refuted by the EMA.<sup>28</sup> To decrease possible AEs, in particular heartburn and belching, a gradual titration schedule was followed in the first week of 1-1-2-2-2-3-3 capsules per day, respectively. Patients, investigators and health care providers were blinded to treatment allocation.

Patients were instructed to refrain from lifestyle changes (e.g. a change in diet or exercise routine) throughout the study. Rescue medication, *i.e.* acetaminophen alone or a combination with NSAIDs, PPIs, antacids, Histamine H<sub>2</sub>-receptor antagonists, loperamide, polyethylene glycol and psyllium, were allowed after consultation with the investigator (ZW). All rescue medication had to be documented in the digital diary.

Study visits were conducted at the start of the pre-treatment period (screening), at randomization, and at the end of the treatment period (end-visit). Throughout the pre-treatment and eight-week treatment periods, patients had to complete daily questions on worst abdominal pain (scored on an 11-point NRS, 0=no pain, 10=worst possible pain), stool evacuation frequency and consistency assessed by the Bristol Stool Form Scale (BSFS), and presence of AEs in a digital diary. Relief of IBS symptoms (scored on a 7-point NRS, 1=no relief, 7=completely relieved), and abdominal discomfort, abdominal bloating, abdominal cramping, belching, nausea, and urgency (all scored on an 11-point NRS, 0=no symptoms, 10=worst possible symptoms) were assessed once weekly. In addition, at week 1, 2, 4, 6, and 8, and at month 3 and 6 of follow-up after the treatment period, patients were asked to complete several web-based questionnaires, including the IBS Severity Scoring System (IBS-SSS)<sup>29</sup>, the Irritable Bowel Syndrome Quality of Life (IBS-QoL)<sup>30</sup>, the EuroQoL-5D (EQ-5D-5L)<sup>31,32</sup>, the Generalized Anxiety Disorder-7 (GAD-7)<sup>33</sup>, and the Patient Health Questionnaire-9 (PHQ-9).<sup>34</sup> At the beginning of week 2, 4, and 6, patients were contacted by telephone for follow-up and

safety assessment. The treatment period was followed by a six months follow-up period in which no treatment was given. An overview of the study design and timing of the questionnaires is given in *Supplementary Figure S7.1*.

## **Electronic data capture and data storage**

Investigators documented all research findings in an Electronic Case Report File (eCRF). An electronic smartphone application was developed for the digital symptom diary in which entering data from previous days was impossible. The eCRF, web-based questionnaires, and diary all featured built-in routing, data validation, and response requirements to stimulate data quality and completeness.

## **Primary efficacy endpoints**

The primary endpoint was the percentage of abdominal pain responders, according to FDA definition<sup>24</sup>, with a responder being a patient with at least 30% decrease in the weekly average of worst daily abdominal pain (scored on an 11-point NRS) compared to baseline, in at least 50% of the treatment period, *i.e.* four weeks.

In line with EMA recommendations to use a global improvement outcome in trials treating two or more IBS-subtypes<sup>25</sup>, response to global relief of IBS symptoms was included as a co-primary endpoint, using a 7-point NRS. A global relief responder was defined as a patient with a weekly relief of threshold 6 or 7 on the NRS in at least 50% of the treatment period, *i.e.* four weeks.

We expected that peppermint oil would not influence bowel habit substantially. Therefore, improvements in bowel movements and stool consistency were not included into a combined primary efficacy endpoint<sup>24</sup>, but were analyzed separately as secondary outcome measures.

## **Secondary endpoints**

Secondary endpoints included symptom improvements of abdominal pain, abdominal discomfort, abdominal bloating, abdominal cramping, belching, nausea, and urgency. IBS symptom severity, stool frequency and consistency (based on the BSFS), use of rescue medication, quality of life, and comorbid anxiety and depression scores were also assessed. Another secondary endpoint was defined as moderate relief of IBS symptoms, with a patient being a responder if they had a symptom relief of threshold 5 or higher on the 7-point NRS in at least four of the treatment weeks. In addition, a different

threshold for the abdominal pain response was included, with a responder being a patient with at least 50% decrease in worst daily abdominal pain in at least four weeks. Primary efficacy outcomes were also evaluated according to IBS subtype as secondary outcomes.

Treatment adherence was quantified by counting returned capsules at the study end-visit. Patients were deemed adherent if at least 80% of study medication was taken during the treatment period or until discontinuation of the study. Compliance rate to the digital diary was defined by percentage of entry days completed during the treatment period or until discontinuation with the study.

### **Safety assessment**

Safety was assessed by the incidence, nature, and severity of AEs occurring during the treatment period. Researchers documented AEs during all telephone follow-up moments (week 2, 4, and 6) and during the end-visit at week 8. In addition, participants were asked to report AEs in the digital symptom diary.

### **Statistical analysis**

The sample size calculation was based on the most recent meta-analysis<sup>35</sup> available at the time of study design, indicating that 57% of the peppermint oil group had abdominal pain improvement (versus no improvement), compared with 27% in the placebo group. A sample size of 42 in both the placebo and the small-intestinal release peppermint oil group was required to detect a 30% efficacy difference between groups, with a power of 80% at the two-sided 0.05 -level. Anticipating that ileocolonic release would increase efficacy, the same sample of 42 was chosen to compare this group with placebo. To account for heterogeneity, an inflation factor of 1.23 was applied.<sup>36</sup> To account for a 13% dropout, an additional 1.15 inflation factor was applied. Therefore, 60 patients per group were required.

All analyses were based on the intention-to-treat (ITT) principle, with correction for the minimization variables sex, inclusion center, IBS-subtype, and age. The responder outcomes were analyzed using multiple logistic regression. Odds Ratios (OR), two-sided 95% confidence intervals (CI), and corresponding *P*-values are reported. Patients with fewer than four weekly diary entries were considered “non-responders” for that week, regardless of their score. To account for multiple comparisons (both intervention groups with placebo and two primary outcomes), two-sided *P*-values of  $\leq 0.05/4=0.0125$  were considered statistically significant for the primary outcomes.

Additionally, a per-protocol (PP) analysis was performed. The PP-population included all randomized patients who had at least 80% adherence to treatment and had completed the treatment period. A detailed description of the statistical analysis of secondary outcomes, for which a multiplicity correction was applied resulting in a significance level of  $<0.025$ , is given in the *Supplementary Material*. Statistical analyses were carried out using IBM (Armonk, NY, USA) SPSS statistics 25.0 for Macintosh.

## Results

### Patient disposition, demographics, and baseline characteristics

Between August 2016 and March 2018, 622 patients were screened for participation in this study of whom 190 were randomized (*Supplementary Figure S7.2*). One patient was erroneously randomized, i.e. without having a mean worst abdominal score of more than 3 during the pre-treatment period, and excluded from further analyses. Therefore, the modified ITT-population consisted of 189 patients. Baseline characteristics are shown in *Table 7.1* and were balanced across treatment groups (mean overall age 34.0 years old, standard deviation 13.3, 77.8% female, 95.8% Caucasian, 57.7% primary care). In total, 11 patients withdrew from the study: nine discontinued as a result of adverse events, one because of insufficient therapeutic response, and one for personal reasons.

Of the small-intestinal release peppermint oil group, 90.3% was adherent to study treatment during the complete treatment period or until discontinuation, compared with 92.1% of the ileocolonic release peppermint oil group, and 96.9% of the placebo group ( $P=0.330$  between groups, *Supplementary Table S7.1*).

Overall compliance to the digital diary was high and did not differ significantly between small-intestinal release peppermint oil, ileocolonic release peppermint oil, and placebo, being 88.3% ( $P=0.561$ ), 85.3% ( $P=0.357$ ), and 87.2% during the complete treatment period or until discontinuation (*Supplementary Table S7.1*). Compliance to the web-based questionnaires was also high: only a single patient did not complete the questionnaires at the end of the treatment period. All other patients completed the symptom questionnaires with no missing values until the end of the study or until discontinuation.

**Table 7.1** Summary of patient demographic and baseline characteristics (ITT-population).

	Placebo N=64	Small-intestinal release Peppermint oil N=62	Ileocolonic release Peppermint oil N=63
<b>Demographic data</b>			
<b>Age, years</b>			
Mean (SD)	35.5 (15.2)	32.0 (11.1)	34.4 (13.1)
Range	19-70	18-66	18-64
<b>Sex, N (%)</b>			
Female	49 (76.6)	51 (82.3)	47 (74.6)
Male	15 (23.4)	11 (17.7)	16 (25.4)
<b>Race, N (%)</b>			
Caucasian	63 (98.4)	60 (96.8)	58 (92.1)
Mixed <sup>¶</sup>	1 (1.6)	2 (3.2)	5 (7.9)
<b>BMI, mean (SD)</b>	24.6 (5.2)	25.6 (5.7)	26.5 (5.1)
<b>Educational level, N (%)</b>			
No education	0	0	1 (1.6)
Low	0	4 (6.5)	11 (17.5)
Moderate	32 (50.0)	23 (37.1)	25 (39.7)
High	32 (50.0)	35 (56.5)	26 (41.3)
<b>Employment status, N (%)</b>			
Currently studying	12 (18.8)	10 (16.1)	10 (15.9)
Employed, full- or part-time	41 (64.1)	40 (64.6)	40 (63.5)
Unemployed	3 (4.7)	3 (4.8)	4 (6.3)
Incapacitated for work	2 (3.1)	4 (6.5)	7 (11.1)
Homemaker	1 (1.6)	4 (6.5)	2 (3.2)
Retired	5 (7.8)	1 (1.6)	0
<b>Setting, N (%)</b>			
Primary care	39 (60.9)	36 (58.1)	34 (54.0)
Secondary care	16 (25.0)	14 (22.6)	11 (17.5)
Combined secondary & tertiary care	9 (14.1)	12 (19.4)	18 (28.6)
<b>IBS-subtype, N (%)‡</b>			
Diarrhea	29 (45.3)	25 (40.3)	29 (46.0)
Constipation	14 (21.9)	12 (19.4)	16 (25.4)
Mixed	12 (18.8)	15 (24.2)	13 (20.6)
Undefined	9 (14.1)	10 (16.1)	5 (9.7)
<b>Abdominal symptoms, mean (SD)</b>			
Abdominal pain <sup>§</sup>	5.3 (1.3)	5.5 (1.2)	5.4 (1.4)
Abdominal discomfort <sup>±</sup>	6.3 (1.4)	6.4 (1.3)	6.5 (1.2)
Abdominal bloating <sup>±</sup>	6.4 (1.8)	6.4 (2.0)	6.7 (1.9)
Abdominal cramping <sup>±</sup>	6.2 (1.8)	6.0 (2.1)	6.3 (1.6)
Belching <sup>±</sup>	3.3 (2.5)	3.5 (2.4)	3.3 (2.7)
Nausea <sup>±</sup>	3.0 (2.4)	3.5 (2.7)	3.7 (2.5)
<b>Bowel symptoms, mean (SD)</b>			
Urgency <sup>±</sup>	6.2 (1.7)	6.3 (1.7)	6.6 (1.6)
<b>IBS severity<sup>†</sup></b>			
Mean score (SD)	270.8 (74.2)	277.0 (73.6)	281.8 (68.7)
Mild, N (%)	7 (10.9)	3 (4.8)	5 (7.9)
Moderate, N (%)	34 (53.1)	35 (56.5)	31 (49.2)
Severe, N (%)	23 (35.9)	24 (38.7)	27 (42.9)

**Table 7.1** (continued)

	Placebo N=64	Small-intestinal release Peppermint oil N=62	Ileocolonic release Peppermint oil N=63
<b>IBS Quality of Life</b> , mean score (SD) <sup>μ</sup>	74.0 (14.2)	72.2 (14.7)	72.8 (16.6)
<b>EQ-5D-5L</b> , mean utility score (SD) <sup>‡</sup>	0.72 (0.2)	0.74 (0.2)	0.74 (0.2)
<b>Psychological comorbidities<sup>#</sup></b>			
Anxiety, mean (SD)	6.0 (4.4)	4.5 (3.9)	5.7 (4.6)
Depression, mean (SD)	7.0 (4.7)	6.6 (4.4)	6.7 (4.6)

BMI body mass index in kg/m<sup>2</sup>; IBS Irritable Bowel Syndrome. ¶ Self-reported race; placebo, N=1 mixed race is 1/4<sup>th</sup> Asian; small-intestinal release peppermint oil, N=1 mixed race is 1/4<sup>th</sup> Asian, and N=1 mixed race is 1/2<sup>nd</sup> unknown; ileocolonic release peppermint oil, N=4 mixed race is 1/4<sup>th</sup> Asian, and N=1 mixed race was 1/2<sup>nd</sup> Asian; ‡ Determined in a face-to-face interview (Rome IV); § Assessed daily during the pre-treatment period using an 11-point NRS in the digital diary: 0=no symptoms, 10=worst possible pain; ± Assessed weekly during the pre-treatment period using an 11-point NRS in the digital diary: 0=no symptoms, 10=worst imaginable symptoms; † The IBS-SSS consists of 5-items with a maximum score of 100, higher scores indicate more severe symptoms; μ The IBS-QoL consists of 34-items with a 5-point Likert scale: 1=good, 5=worst quality of life; ‡ The EQ-5D-5L measures 5-dimensions of QoL. Raw scores are transformed to utility scores<sup>31</sup>, which vary from 1 (perfect health) to 0 (death); # Anxiety, the GAD-7 consists of 7-items, and depression, the PHQ-9 consists of 9-items, both with a 4-point response scale, 0=not at all, 3=almost every day.

## Primary efficacy outcomes

The proportion of abdominal pain responders did not differ significantly between groups: 46.8% in small-intestinal release peppermint oil (OR 1.68; 95% CI 0.80, 3.51;  $P=0.170$ ; number needed to treat (NNT) 8.1) and 41.3% in ileocolonic release peppermint oil (OR 1.39; 95% CI 0.66, 2.90;  $P=0.385$ ; NNT 14.5), compared with 34.4% in placebo (Table 7.2, Supplementary Table S7.2, Figure 7.1).

The proportion of global relief responders did also not differ significantly between groups: 9.7% in small-intestinal release peppermint oil (OR 2.12; 95% CI 0.49, 9.17;  $P=0.317$ ), and 1.6% in ileocolonic release peppermint oil (OR 0.33; 95% CI 0.03, 3.35;  $P=0.351$ ), compared with 4.7% in placebo (Table 7.2, Figure 7.1).

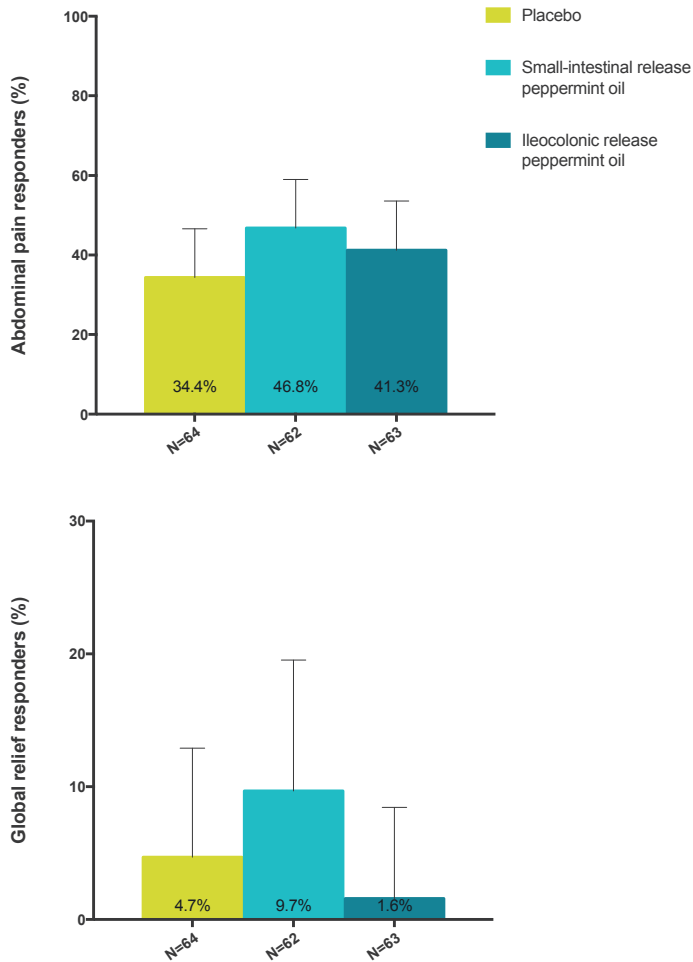
In the PP-analysis, the primary endpoints did not differ significantly between groups (Supplementary Table S7.3).

No significant differences in primary efficacy outcomes were observed for each IBS-subtype separately (Supplementary Table S7.9).

**Table 7.2** Responder endpoints (ITT-population).

	Placebo			Small-intestinal release Peppermint oil			Ileocolonic release Peppermint oil		
	No. responders (%)	N=64		No. responders (%)	N=62		No. responders (%)	N=63	
<b>Primary endpoints</b>									
Abdominal pain, 30%¶	22 (34.4)		0.170	29 (46.8)		1.68 (0.80–3.51)	26 (41.3)		1.39 (0.66–2.90)
Global relief†	3 (4.7)		0.317	6 (9.7)		2.12 (0.49–9.17)	1 (1.6)		0.33 (0.03–3.35)
<b>Secondary endpoints</b>									
Moderate relief§	13 (20.3)		0.030	24 (38.7)		2.47 (1.09–5.56)	13 (20.6)		0.99 (0.41–2.38)
Abdominal pain, 50%#	8 (12.5)		0.062	16 (25.8)		2.51 (0.96–6.59)	13 (20.6)		1.85 (0.69–4.96)

P-values, ORs and corresponding two-sided 95% confidence intervals were calculated using multiple logistic regression adjusted for minimization variables. ¶ A responder was a patient with at least 30% decrease in mean worst daily abdominal pain in at least 4 out of 8 weeks (FDA-recommendation); † A responder was a patient with at least a global relief score of 6 or 7 (on a 7-point NRS) in at least 4 out of 8 weeks (EMA-recommendation); § A responder was a patient with at least a global relief score of 5, 6, or 7 (on a 7-point NRS) in at least 4 out of 8 weeks; # A responder was a patient with at least 50% decrease in mean worst daily abdominal pain in at least 4 out of 8 weeks.



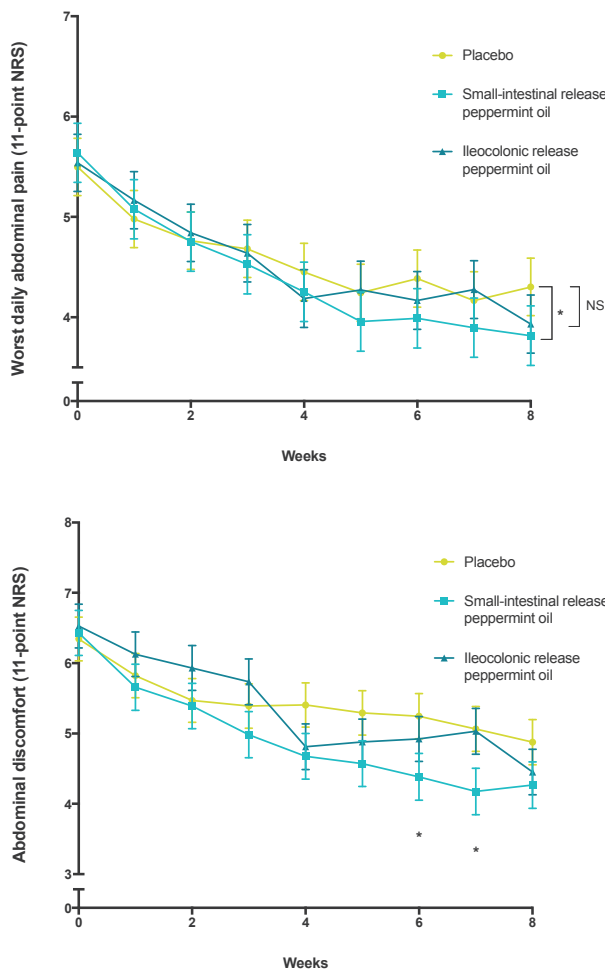
**Figure 7.1** Percentage of patients who were abdominal pain responders (a) and global relief responders (b) in the ITT-population. (a) Abdominal pain responder: a patient with at least 30% decrease in mean worst daily abdominal pain in at least 4 out of 8 weeks. (b) Global relief responder: a patient with at least a relief score of 6 or 7 (on a 7-point NRS) in at least 4 out of 8 weeks. Values are percentages, bars represent standard errors.

## Secondary efficacy outcomes

Results of exploratory secondary outcomes are presented in *Table 7.2* and *Supplementary Table S7.4*. The small-intestinal release peppermint oil resulted in significantly more reduction in daily worst abdominal pain at week eight, with a corrected difference in change from baseline on an 11-point NRS, compared with placebo, of  $-0.63$  (95%CI,  $-1.14, -0.12$ ;  $P=0.016$ ) (*Supplementary Table S7.4*).

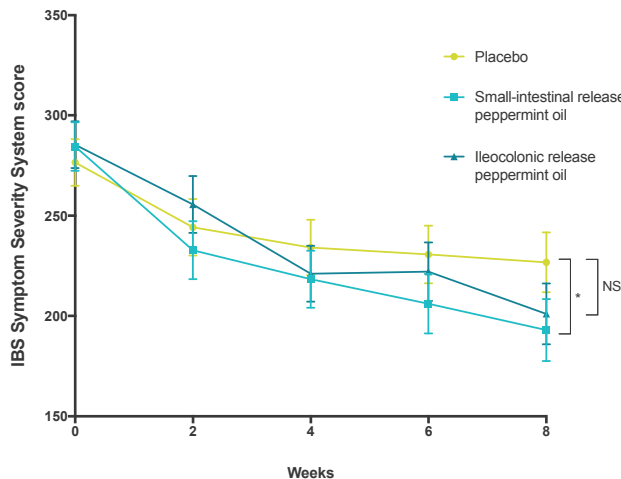


The small-intestinal release peppermint oil was also superior over placebo with respect to abdominal discomfort. This effect appeared at week six of treatment, with corrected differences in change from baseline on an 11-point NRS, when compared with placebo, of -0.95 (95%CI -1.74, -0.15;  $P=0.020$ ) at six weeks, -0.97 (95%CI -1.71, -0.24;  $P=0.009$ ) at seven weeks, and -0.69 (95% CI -1.36, -0.03;  $P=0.041$ , non-significant as =0.025) at eight weeks (Figure 7.2, Supplementary Table S7.4).



**Figure 7.2** Abdominal pain and discomfort scores in the ITT-population ( $N=189$ ). Values are adjusted estimated marginal means derived from the linear mixed model, bars represent standard errors. The small-intestinal peppermint oil group had significantly more reduction in mean daily worst abdominal pain compared with placebo at week 8 ( $P=0.016$ ). The small-intestinal peppermint oil group also had significantly more reduction in abdominal discomfort compared with placebo, ( $P=0.020$ ,  $P=0.009$ , and  $P=0.041$  at week 6, 7 and 8 of treatment, respectively). The ileocolonic release peppermint oil group did not differ significantly in reduction in abdominal pain and discomfort compared with placebo. Assessed weekly using an 11-point NRS in the digital diary.

A significantly greater improvement in IBS symptom severity was found among those treated with small-intestinal release peppermint oil, with a corrected difference in change from baseline of -41.8 on the IBS-SSS total score (-91.5 versus -49.8 for small intestinal release versus placebo; 95%CI for difference -76.88, -6.70;  $P=0.020$ ) at week eight (Figure 7.3, Supplementary Table S7.4). A greater percentage of the small-intestinal release peppermint oil group reported a symptom relief score of at least 5 (moderate relief) in at least four treatment weeks (38.7%,  $P=0.030$ , non-significant), compared with placebo (20.3%) (Table 7.2, Supplementary Figure S7.3). In addition, both peppermint oil groups reported using rescue medication for pain fewer times than the placebo group, *i.e.* on average 3.71 ( $P=0.087$ ), 3.16 ( $P=0.039$ ), and 5.16 times for small-intestinal release, ileocolonic release peppermint oil, and placebo, respectively (Supplementary Table S7.8). However, this did not reach the pre-specified level of significance ( $=0.025$ ).



**Figure 7.3** IBS-SSS in the ITT-population ( $N=189$ ). Values are adjusted estimated marginal means derived from the linear mixed model, bars represent standard errors. The small-intestinal peppermint oil group had significantly more reduction in IBS severity at the end of the eight-week treatment period. \* $P=0.020$ . The absolute change from baseline in small-intestinal release peppermint oil was -91.53 points. The ileocolonic release peppermint oil group did not differ significantly in severity reduction compared with placebo ( $P=0.053$ ). Assessed using the IBS-SSS questionnaire consisting of 5-items with each a maximum score of 100.

Ileocolonic release peppermint oil did not yield significantly more relief, reduction in abdominal discomfort or abdominal pain, nor improvement in IBS severity over placebo (Supplementary Table S7.4). When using a larger abdominal pain decrease threshold, *i.e.* 50 instead of 30%, the proportion of abdominal pain responders did not differ

significantly between groups (Table 7.2). Apart from a few significant changes at single time-points, there were no sustained differences between groups with regard to nausea, abdominal bloating, urgency, or comorbid anxiety and depression (Supplementary Table S7.4). All treatment groups showed improvements in quality of life that persisted over time, without a significant difference between groups (Supplementary Table S7.4). No significantly different changes were observed in stool consistency and frequency across treatment groups apart from a single time point in stool consistency (week 6, Supplementary Table S7.5). When analyzing consistency and frequency for each IBS subtype separately, no significant changes were found apart from an increased stool consistency in IBS-D at a single time point (week 6 in the small intestinal peppermint oil group, week 3 in the ileocolonic release peppermint oil group, Supplementary Table S7.6-7). Efficacy outcomes did not differ significantly between primary and secondary/tertiary care patients (Supplementary Table S7.10, Supplementary Material).

Follow-up measurements until six-months after cessation of treatment also showed no significant differences between placebo and both forms of peppermint oil (Supplementary Table S7.4).

### **Adverse events/safety results**

Table 7.3 summarizes the AEs reported during the treatment period. No serious adverse events or deaths were reported. In both peppermint oil groups, the total number of AEs was significantly higher compared with placebo (mean (SE) 4.26 (0.37) for small-intestinal release ( $P=0.012$ ) and 4.54 (0.45) for ileocolonic release peppermint oil ( $P=0.001$ ), versus 2.78 (0.34) for placebo).

The most common adverse events were heartburn or GERD symptoms, belching (with and without a minty taste), and headache in small-intestinal release peppermint oil and an altered anal sensation or sensitive urethra, headache and abdominal cramps in ileocolonic release peppermint oil. Concerning belching, in the first two weeks of treatment, the small-intestinal release peppermint oil group had a larger increase in belching from baseline, compared to placebo ( $P<0.001$  at week one,  $P=0.023$ , at week two). Severity of this symptom, however, returned to pre-treatment level after three weeks until the end of treatment, (Supplementary Figure S7.6). More patients on peppermint oil versus placebo discontinued treatment due to adverse events (three in the small-intestinal peppermint oil group (4.8%) and five in the ileocolonic release peppermint oil group (7.9%), compared with one in the placebo group (1.6%).

**Table 7.3** Summary of treatment emerging adverse events (ITT-population):

	Placebo N=64 2.78 (0.34)	Small-intestinal release Peppermint oil N=62 4.26 (0.37)*	Ileocolonic release Peppermint oil N=63 4.45 (0.45)†
<b>Total different AEs, mean (SE)</b>			
<b>AEs<sup>‡</sup>, mean frequency (SE) / N (%)</b>			
Headache	1.56 (0.40) / 21 (32.8)	2.34 (0.59) / 25 (40.3)	2.17 (0.65) / 26 (41.3)
Heartburn/GERD symptoms	0.61 (0.16) / 18 (28.1)	2.84 (0.88) / 31 (50.0)	1.81 (0.60) / 23 (36.5)
Nausea	1.91 (0.78) / 23 (35.9)	1.45 (0.78) / 16 (25.8)	2.21 (0.77) / 18 (28.6)
Belching	1.03 (0.36) / 15 (23.4)	3.71 (1.04) / 28 (45.2)	0.56 (0.21) / 12 (19.0)
Belching with/or minty taste	0.02 (0.02) / 1 (1.6)	4.68 (0.99) / 36 (58.1)	0.51 (0.16) / 14 (22.2)
Abdominal cramps	0.55 (0.22) / 12 (18.8)	1.42 (0.51) / 13 (21.0)	3.76 (0.99) / 29 (46.0)
Altered anal sensation and/or sensitive urethra	0.55 (0.27) / 9 (14.1)	1.48 (0.45) / 22 (35.5)	3.60 (0.95) / 39 (61.9)
Peppermint oil scent stool	0.02 (0.02) / 1 (1.6)	0.69 (0.22) / 18 (29.0)	2.02 (0.83) / 18 (28.6)
<b>AEs leading to discontinuation, total N</b>	1	3	5
Headache, N	0	1	0
Palpitations, N	1	0	0
Diarrhea and abdominal cramps, N	0	0	1
Combination <sup>¶</sup> , N	0	1	2
Combination <sup>‡</sup> , N	0	1	0
Combination <sup>§</sup> , N	0	0	2

AE adverse event; SE standard error; GERD Gastroesophageal Reflux Disease. ± Occurrence of AEs was self-reported in the daily symptom diary. The total number of different AEs for small-intestinal release compared with placebo was significantly higher \* $P=0.01$  †, as well as for ileocolonic release peppermint oil compared with placebo # $P=0.001$ ; ¶ Combination of i.e. flatulence, bloating, abdominal pain; ‡ Combination of i.e. headache, tightness of the chest, belching, bloating, muscle cramp; § Combination of i.e. diarrhea, abdominal cramps, altered anal sensation, belching, altered taste.

## Discussion

To our knowledge, this is the first randomized, double-blind, placebo-controlled clinical trial of peppermint oil in patients with Rome IV-defined IBS. It showed that neither small-intestinal release nor ileocolonic release peppermint oil led to a statistically significant reduction in abdominal pain or increase in global relief based on the pre-specified primary outcome measures as defined by FDA and EMA guidelines. Small-intestinal release peppermint oil, but not ileocolonic, however, did yield statistically significant improvements in exploratory secondary outcomes of IBS symptom severity, abdominal pain, and abdominal discomfort. AEs occurred more often in both peppermint oil groups compared to placebo, but were all mild and transient.

The treatment effect of small-intestinal peppermint oil was not as pronounced as anticipated based on the results of previous meta-analyses<sup>35,37</sup>, which indicated a difference in dichotomous overall abdominal pain improvement of 30% between placebo and peppermint oil.<sup>35</sup> This discrepancy may relate to the more stringent criteria used in the current study, as our primary outcome measure required an abdominal pain reduction compared to baseline of at least 30% in at least four out of eight weeks treatment. In contrast to our study, none of the earlier trials investigating peppermint oil reported this endpoint. The most recent randomized trial investigated a sustained small-intestinal release peppermint formulation (182 mg) of which the pharmacokinetics are comparable to the one used in the current study, in 72 IBS (Rome III) patients. They used the change from baseline in the Total IBS Symptom Score as a primary endpoint and found a significantly greater reduction of 15.7% in the peppermint oil group compared to placebo.<sup>17</sup> In the current study, the placebo response rate according to the stringent FDA definition was 33%, which is similar to previous studies using this outcome measure.<sup>38-40</sup> The therapeutic gain of small-intestinal peppermint oil over placebo was 12.4%, corresponding to a NNT of 8. Albeit non-significant, this difference in response rate is numerically comparable to the previous studies in IBS reporting statistically significant differences between linaclotide<sup>38</sup>, and plecanatide<sup>39</sup> and placebo. Of note is that the recent ACG Monograph<sup>18</sup> mentions a NNT of 4 for peppermint oil (using the data hitherto available), which is considerable better than the NNT that we found, but also than the NNT for linaclotide (6), plecanatide (10), or eluxadoline (12.5). Since we powered the study for an expected 30% difference<sup>35</sup>, it seems plausible that a type II error may exist and a statistical significant difference between groups would have been identified had we included a larger number of patients. Another reason for the discrepancy may be differences in baseline characteristics of our study population compared with populations previously investigated. In contrast with earlier work, a large

part of our population was recruited from primary care, patients had to fulfill the Rome IV diagnostic criteria for IBS<sup>2</sup>, and had to have an objectified mean worst abdominal score of at least 3 (on an 11-point NRS). Finally, the overall quality of evidence achieved thus far could explain the conflicting findings throughout the literature. Peppermint oil was evaluated in numerous clinical trials that were hindered by methodological limitations including lack of description of allocation concealment or of randomization method used, no description of how blinding was handled, no usage of validated endpoints, or treatment periods of one month or shorter.<sup>37,41</sup> As such, treatment effects may have been biased or overestimated, complicating the ability to draw firm conclusions.

Since measuring treatment response in IBS patients is based on self-reported symptoms, defining optimal outcome measures in IBS trials has been subject of ongoing debate. It has been postulated that the current recommended provisional FDA/EMA endpoints are limited in their ability to capture all multidimensional aspects of IBS symptoms and treatment response due to the over-focus on certain main symptoms and the dichotomization of continuous responses.<sup>42,43</sup> It is therefore important to take into account various appropriate endpoints to distinguish between clinically relevant and non-relevant responses, in particular when these are used for clinical decision-making. For instance, the small-intestinal, but not ileocolonic release peppermint oil group had a significantly greater reduction in abdominal pain, discomfort, and IBS symptom severity scores, compared to placebo. Furthermore, adherence to study treatment was excellent and discontinuation due to headache, belching, or other AEs was low (6.4%). In addition, all AEs were mild and transient and the most common one, *i.e.* belching, subsided after the second week of treatment. This indicates a rather good tolerability of peppermint oil when administered with a gradual titration schedule for the first week. Thereby, the current results show, in our opinion, that small-intestinal release peppermint oil does have a moderate efficacy in patients with IBS and should not be ignored as a treatment option in everyday practice.

We had hypothesized that a targeted ileocolonic release of peppermint oil would have led to an augmented efficacy of treatment owing to a more local colonic anti-nociceptive effect based on recent experimental evidence suggesting the involvement of TRP channels on colonic sensory afferents.<sup>8</sup> In the current study, however, we found no evidence of symptomatic benefits of ileocolonic release peppermint oil over placebo. In addition, although upper GI adverse events were indeed diminished compared with the small-intestinal release peppermint oil, the novel formulation resulted in more severe abdominal cramping in the beginning of the treatment period. Our findings therefore,

taken together, do not support the use or further development of this formulation for treatment in patients with IBS. The reason for increased reporting of abdominal cramps upon administration of ileocolonic release peppermint oil is unclear and unexpected given the smooth muscle relaxatory effects of the agent. As far as the effects of peppermint oil are concerned and on the basis of these findings, however, we speculate that the small intestine could be of superior importance compared to the colon with regards to pain symptom generation and relief in IBS. In addition, considering the late onset of beneficial effects, we further postulate the involvement of TRP channels on intestinal sensory afferents rather than a primarily antispasmodic effect that is assumed to occur more rapidly.

Currently, treatment of IBS is often tailored towards improvement of patient's most predominant symptom. If initial treatment fails to achieve satisfactory results, linaclotide and eluxadoline are examples of recent pharmacological advancements that have led to novel drug development and can be used to treat constipation- and diarrheal-type IBS, respectively. Despite high quality evidence, their somewhat less favorable adverse event profile should be considered and may limit applicability.<sup>38,40</sup> Of the therapeutic entities available for IBS, none has been able to cure or alter the disorder on the long-term. This reflects our incomplete pathophysiological understanding of IBS, which leads to the inability to target specific disease mechanisms. In this perspective and in view of our findings, peppermint oil appears to be a favorable initial treatment entity in IBS owing to the following reasons: 1) peppermint oil is readily available as a low-cost OTC drug; 2) adverse events are at most mild and transient of nature; 3) using a pharmacological agent of herbal origin without the risk of serious adverse events could be attractive for patients. In fact, in the Netherlands, peppermint oil was the most preferred treatment option when given the choice of ten treatment options (education on IBS, other antispasmodics, antidepressants, and elimination/FODMAP diet included).<sup>44</sup> It is worth noting that because improvements in exploratory secondary outcomes were observed rather towards the end of the treatment period, and belching arises at the beginning of treatment, but normalizes soon after, patients should be encouraged to continue treatment. Finally, to avoid disappointment, providers could communicate that there is little evidence for long-term beneficial effects after discontinuing with peppermint oil treatment. Future research should investigate the safety and effect of longer treatment periods.

This study has several limitations. First, the population was relatively young, female, and predominantly of Caucasian origin; therefore, data may not necessarily be generalizable to more diverse IBS populations. We speculate that the use of social media as a

recruitment strategy may have contributed to this relatively young study population. Nevertheless, the subtype distribution was in line with epidemiological findings in IBS.<sup>45</sup> Future studies are required to ascertain the effect in populations from different geographical regions; a current trial in the USA investigating placebo responses uses a peppermint oil comparator.<sup>46</sup> However, because we have recruited IBS patients from primary, secondary and tertiary care, and via social media accounts of the participating centers, we argue that the current study population is representative for the Dutch IBS population seeking help for their symptoms. Caution is, however, necessary when applying these results to clinical practice as they might only apply to patients who have a certain level of pain symptoms, corresponding to both the Rome IV and the FDA pain entry criteria. Second, blinding of the patients may not have been entirely successful due to the smell and taste of peppermint oil and other recognizable adverse events. We tried to limit a confounding effect through the identical appearance of capsules by over-encapsulation. Third, due to possible power limitations and increase in type I error (multiple testing), secondary endpoint analyses should be considered exploratory. Fourth, the treatment period was relatively short in comparison to other IBS trials, therefore potential benefits from a longer treatment period (*i.e.* 12-26 weeks) could not be ascertained.

Strengths of the current study include the soundness of the experimental design with compliance to recent guidelines on IBS drug trials and as such, reporting on stringent primary outcomes according to FDA and EMA guidelines and intention-to-treat analyses; the meticulous use of state-of-the-art electronic data capture ensuring data quality and completeness; and a well-characterized patient population comprised of both primary and secondary/tertiary care patients diagnosed according to Rome IV diagnostic criteria for IBS with a low drop-out rate.

In summary, peppermint oil compared to placebo was not superior in patients with IBS, when using the pre-specified outcome measures abdominal pain response and global relief of IBS symptoms based on recommendations by the FDA and EMA. We found no benefits of a targeted ileocolonic release peppermint oil formulation for treatment in IBS. Conventional small-intestinal release peppermint oil did, however, improve secondary outcomes such as abdominal pain, abdominal discomfort, and IBS symptom severity with a minimal adverse event profile and high tolerability. Peppermint oil may thus be considered as a worthwhile treatment option for symptom management in IBS.



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## Detailed inclusion and exclusion criteria

Patients had to be between 18 and 75 years of age and needed to fulfill the Rome IV diagnostic criteria for IBS. If alarm symptoms were present (e.g. unexplained rectal blood loss or weight loss), a colonoscopy or other relevant tests were performed to exclude organic disease. Exclusion criteria were inability to read or understand Dutch, history of GI disorders such as inflammatory bowel disease, celiac disease, or thyroid dysfunction (if not well-regulated), history of major abdominal surgery or radiotherapy interfering with GI function. An uncomplicated appendectomy, cholecystectomy, or hysterectomy were allowed unless within six months prior to screening. Other exclusion criteria were use of peppermint oil capsules in the three months prior to screening, a known allergic reaction to peppermint oil, current drug abuse, and a history of liver or gallbladder/biliary disease. Women had to use contraceptives and have a negative urine pregnancy test, or be postmenopausal for at least two years. The use of one antidepressant or one PPI was allowed, if a patient had been and would stay on a stable dose. Prohibited concomitant medications included opioids, prokinetics, stimulant laxatives (i.e. bisacodyl), linaclotide, prucalopride, and anti-spasmodic drugs. Regular use of NSAIDs, antibiotics, osmotic laxatives, and antidiarrheal drugs was prohibited.

## Treatment allocation

Randomization was done with ALEA software using the minimization method, accounted for inclusion center, IBS subtypes, gender, and age. A random element was incorporated into each step of the minimization to ensure allocation concealment. As such, when an imbalance of more than two subjects per treatment group existed (in a specific inclusion center), there was a 10% chance that the subsequent randomization would overrule this already existing imbalance.

## Statistical analysis of secondary outcomes

For secondary continuous outcomes, treatment effects were analyzed at different time-points after correction for baseline using linear mixed models with treatment group, minimization variables, time, and time\*group interaction as fixed-effects. A likelihood-based approach was used to deal with missing values. Different covariance structures (unstructured, autoregressive moving average 1.1, heterogeneous Toeplitz, heterogeneous first-order-autoregressive) were explored to choose the best based on

the Schwarz's Bayesian Criterion (smaller values indicate a better fit). Estimated means (standard error, SE) per time-point, *P*-values, and 95% CIs are reported.

A multiplicity correction was applied according to the following principle: for each secondary outcome measure, two comparisons are made to placebo (one for small-intestinal release peppermint oil and one for ileocolonic release peppermint oil). Assuming the chance of 5% for a type I error for a single comparison, a two-sided *P*-value  $\leq 0.025$  was considered statistically significant for secondary outcome analyses. Secondary outcomes were exploratory in nature.

**Table S7.1** Adherence to study medication and compliance to the digital symptom diary (ITT-population).

	Placebo N=64	Small-intestinal release Peppermint oil N=62	Ileocolonic release Peppermint oil N=63
<b>Adherent to study medication<sup>¶</sup></b>			
Number of patients (%)	62 (96.9)	56 (90.3)	58 (92.1)
<b>Compliance rate to the digital symptom diary<sup>‡</sup></b>			
Mean % (SE)	87.2 (1.47)	88.3 (1.09)	85.3 (1.48)

¶ Adherence to study medication was quantified by counting returned capsules at the study end-visit. Patients were deemed adherent if at least 80% of study medication was taken during the complete treatment period or until discontinuation. There were no significant differences in adherence between placebo and small-intestinal, or ileocolonic release peppermint oil,  $P=0.212$  and  $P=0.333$ , respectively; <sup>‡</sup> Compliance rate to the digital diary was defined by the percentage of entry days completed during the complete treatment period or until discontinuation. There were no significant differences between placebo and small-intestinal release, or ileocolonic release peppermint oil,  $P=0.405$  and  $P=0.285$ , respectively.

**Table S7.2** Number needed to treat and number needed to harm (ITT-population).

	Small-intestinal release Peppermint oil N=62	Ileocolonic release Peppermint oil N=63
<b>Number needed to treat</b>		
Based on primary abdominal response outcome <sup>¶</sup>	8.1	14.5
Based on moderate global relief outcome <sup>§</sup>	5.4	N.A.
<b>Number needed to harm</b>		
Based on AE prompting discontinuation	30	15

¶ A responder was defined as a patient with at least 30% decrease in mean worst daily abdominal pain in at least 50% of weeks in which treatment was given; § A responder was defined as a patient with at least a global relief score of 5, 6, or 7 (on a 7-point NRS) in at least 50% of weeks in which treatment was given. AE Adverse Event.



Table S7.4 Other secondary efficacy endpoints (ITT-population).

Measurement	Placebo N=64		Small-intestinal release Peppermint oil N=62		Ileocolonic release Peppermint oil N=63	
	Estimated means (SE)	P-value	Estimated means (SE)	Treatment effect (95% CI)	Estimated means (SE)	Treatment effect (95% CI)
<b>Mean worst abdominal pain<sup>§</sup></b>						
Baseline	5.50 (0.29)	-	5.64 (0.29)	-	5.54 (0.29)	-
Week 1	4.98 (0.29)	0.905	5.08 (0.29)	-0.04 (-0.77; 0.68)	5.17 (0.29)	0.15 (-0.58; 0.87)
Week 2	4.76 (0.29)	0.674	4.75 (0.29)	-0.15 (-0.85; 0.55)	4.84 (0.29)	0.04 (-0.66; 0.74)
Week 3	4.68 (0.29)	0.394	4.53 (0.30)	-0.30 (-0.98; 0.39)	4.64 (0.29)	-0.08 (-0.76; 0.59)
Week 4	4.45 (0.29)	0.310	4.25 (0.30)	-0.34 (-0.99; 0.32)	4.19 (0.29)	-0.31 (-0.96; 0.35)
Week 5	4.24 (0.29)	0.180	3.96 (0.30)	-0.42 (-1.05; 0.19)	4.27 (0.29)	-0.01 (-0.64; 0.61)
Week 6	4.39 (0.29)	0.074	3.99 (0.30)	-0.54 (-1.13; 0.05)	4.17 (0.29)	-0.25 (-0.85; 0.33)
Week 7	4.17 (0.29)	0.144	3.89 (0.30)	-0.41 (-0.97; 0.14)	4.28 (0.29)	0.07 (-0.49; 0.63)
Week 8	4.30 (0.29)	0.016	3.82 (0.30)	-0.63 (-1.14; 0.12)	3.93 (0.29)	-0.41 (-0.92; 0.10)
<b>Abdominal discomfort<sup>‡</sup></b>						
Baseline	6.35 (0.31)	-	6.43 (0.32)	-	6.53 (0.31)	-
Week 1	5.82 (0.31)	0.601	5.66 (0.33)	-0.25 (-1.19; 0.69)	6.13 (0.32)	0.12 (-0.81; 1.06)
Week 2	5.47 (0.31)	0.772	5.39 (0.32)	-0.16 (-1.07; 0.74)	5.93 (0.32)	0.28 (-0.63; 1.19)
Week 3	5.39 (0.32)	0.282	4.98 (0.33)	-0.49 (-1.39; 0.40)	5.74 (0.33)	0.16 (-0.74; 1.07)
Week 4	5.41 (0.32)	0.064	4.68 (0.33)	-0.81 (-1.68; 0.05)	4.81 (0.33)	-0.77 (-1.65; 0.10)
Week 5	5.29 (0.32)	0.056	4.57 (0.32)	-0.80 (-1.63; 0.02)	4.88 (0.32)	-0.59 (-1.42; 0.24)
Week 6	5.25 (0.32)	0.020	4.38 (0.33)	-0.95 (-1.74; -0.15)	4.92 (0.32)	-0.50 (-1.30; 0.29)
Week 7	5.07 (0.32)	0.009	4.18 (0.33)	-0.97 (-1.71; -0.24)	5.03 (0.33)	-0.21 (-0.95; 0.53)
Week 8	4.88 (0.32)	0.041	4.27 (0.33)	-0.69 (-1.36; -0.03)	4.45 (0.33)	-0.60 (-1.27; 0.06)



Table S7.4 (continued)

Measurement	Placebo N=64		Small-intestinal release Peppermint oil N=62		Ileocolonic release Peppermint oil N=63	
	Estimated means (SE)	P-value	Estimated means (SE)	Treatment effect (95% CI)	Estimated means (SE)	Treatment effect (95% CI)
<b>Abdominal bloating<sup>‡</sup></b>						
Baseline	6.53 (0.38)	-	6.44 (0.39)	-	6.68 (0.38)	-
Week 1	5.51 (0.38)	0.594	5.15 (0.40)	-0.27 (-1.28; 0.73)	5.52 (0.38)	-0.15 (-1.15; 0.86)
Week 2	5.06 (0.38)	0.994	4.97 (0.39)	-0.00 (-0.98; 0.97)	5.45 (0.39)	0.23 (-0.75; 1.21)
Week 3	5.21 (0.38)	0.316	4.63 (0.40)	-0.49 (-1.45; 0.47)	5.11 (0.39)	-0.25 (-1.22; 0.72)
Week 4	5.03 (0.38)	0.109	4.19 (0.40)	-0.75 (-1.67; 0.17)	4.58 (0.39)	-0.61 (-1.54; 0.33)
Week 5	4.74 (0.38)	0.328	4.22 (0.40)	-0.44 (-1.32; 0.44)	4.77 (0.39)	-0.13 (-1.02; 0.77)
Week 6	5.04 (0.39)	0.051	4.10 (0.40)	-0.86 (-1.72; 0.00)	4.51 (0.39)	-0.69 (-1.55; 0.17)
Week 7	4.96 (0.39)	0.009	3.81 (0.40)	-1.07 (-1.87; -0.26)	4.06 (0.40)	-1.06 (-1.87; -0.24)
Week 8	4.52 (0.39)	0.269	4.03 (0.40)	-0.43 (-1.19; 0.33)	4.18 (0.39)	-0.52 (-1.28; 0.24)
<b>Abdominal cramping<sup>‡</sup></b>						
Baseline	6.10 (0.33)	-	5.91 (0.34)	-	6.15 (0.33)	-
Week 1	4.87 (0.35)	0.322	5.07 (0.36)	0.38 (-0.38; 1.15)	5.93 (0.35)	1.01 (0.25; 1.77)
Week 2	4.87 (0.37)	0.929	4.73 (0.39)	0.04 (-0.85; 0.93)	5.56 (0.38)	0.64 (-0.26; 1.54)
Week 3	4.76 (0.39)	0.984	4.57 (0.40)	-0.01 (-0.96; 0.94)	5.12 (0.40)	0.31 (-0.66; 1.27)
Week 4	4.86 (0.37)	0.648	4.46 (0.38)	-0.21 (-1.10; 0.69)	4.86 (0.39)	-0.05 (-0.97; 0.86)
Week 5	4.97 (0.38)	0.345	4.36 (0.39)	-0.42 (-1.29; 0.45)	4.83 (0.39)	-0.19 (-1.08; 0.69)
Week 6	5.03 (0.39)	0.140	4.18 (0.40)	-0.66 (-1.54; 0.22)	4.85 (0.39)	-0.24 (-1.11; 0.64)
Week 7	4.40 (0.39)	0.218	3.69 (0.40)	-0.52 (-1.36; 0.31)	4.88 (0.40)	0.43 (-0.42; 1.27)
Week 8	4.36 (0.42)	0.419	3.83 (0.43)	-0.34 (-1.17; 0.49)	4.23 (0.42)	-0.18 (-1.02; 0.66)

Table S7.4 (continued)

Measurement	Placebo N=64			Small-intestinal release Peppermint oil <sup>a</sup> N=62			Ileocolonic release Peppermint oil N=63		
	Estimated means (SE)	Estimated means (SE)	P-value	Estimated means (SE)	Treatment effect (95% CI)	P-value	Estimated means (SE)	Treatment effect (95% CI)	P-value
<b>Belching<sup>‡</sup></b>									
Baseline	3.41 (0.35)	3.65 (0.36)	-	3.41 (0.36)	-	-	3.41 (0.36)	-	-
Week 1	2.92 (0.35)	4.87 (0.36)	0.0003	4.87 (0.36)	1.85 (0.86; 2.83)	0.0003	2.98 (0.35)	0.25 (-0.73; 1.24)	0.615
Week 2	3.10 (0.34)	4.42 (0.34)	0.023	4.42 (0.34)	1.10 (0.15; 2.05)	0.023	3.00 (0.36)	0.06 (-0.90; 1.02)	0.898
Week 3	2.95 (0.38)	3.70 (0.35)	0.276	3.70 (0.35)	0.52 (-0.42; 1.45)	0.276	2.84 (0.40)	0.03 (-0.92; 0.98)	0.952
Week 4	2.75 (0.37)	3.21 (0.33)	0.605	3.21 (0.33)	0.24 (-0.66; 1.13)	0.605	2.18 (0.30)	-0.31 (-1.22; 0.60)	0.506
Week 5	2.68 (0.36)	2.59 (0.29)	0.534	2.59 (0.29)	-0.27 (-1.13; 0.59)	0.534	2.43 (0.38)	-0.18 (-1.05; 0.69)	0.683
Week 6	2.65 (0.38)	2.96 (0.36)	0.493	2.96 (0.36)	0.29 (-0.55; 1.14)	0.493	2.19 (0.33)	-0.33 (-1.17; 0.51)	0.440
Week 7	2.31 (0.34)	2.90 (0.38)	0.627	2.90 (0.38)	0.20 (-0.59; 0.99)	0.627	2.15 (0.36)	-0.13 (-0.93; 0.67)	0.754
Week 8	2.49 (0.36)	2.88 (0.34)	0.655	2.88 (0.34)	0.17 (-0.58; 0.92)	0.655	1.74 (0.28)	-0.53 (-1.28; 0.23)	0.169
<b>Nausea<sup>‡</sup></b>									
Baseline	2.89 (0.39)	3.29 (0.40)	-	3.29 (0.40)	-	-	3.56 (0.39)	-	-
Week 1	3.82 (0.40)	2.71 (0.41)	0.327	2.71 (0.41)	-0.50 (-1.51; 0.50)	0.327	3.08 (0.40)	-0.40 (-1.40; 0.61)	0.437
Week 2	2.70 (0.40)	2.58 (0.41)	0.300	2.58 (0.41)	-0.52 (-1.49; 0.46)	0.300	2.87 (0.40)	-0.49 (-1.48; 0.50)	0.329
Week 3	2.86 (0.40)	2.15 (0.41)	0.026	2.15 (0.41)	-1.10 (-2.07; -0.13)	0.026	2.69 (0.41)	-0.83 (-1.81; 0.15)	0.097
Week 4	2.76 (0.40)	2.07 (0.41)	0.024	2.07 (0.41)	-1.08 (-2.02; -0.14)	0.024	2.00 (0.41)	-1.42 (-2.38; -0.46)	0.004
Week 5	2.63 (0.40)	2.12 (0.41)	0.051	2.12 (0.41)	-0.91 (-1.82; 0.00)	0.051	2.21 (0.41)	-1.08 (-2.01; -0.16)	0.051
Week 6	2.33 (0.40)	2.06 (0.42)	0.151	2.06 (0.42)	-0.66 (-1.56; 0.24)	0.151	2.64 (0.40)	-0.35 (-1.25; 0.55)	0.447
Week 7	2.33 (0.40)	2.02 (0.42)	0.108	2.02 (0.42)	-0.71 (-1.57; 0.15)	0.108	2.15 (0.41)	-0.84 (-1.71; 0.04)	0.060
Week 8	2.20 (0.41)	1.92 (0.42)	0.115	1.92 (0.42)	-0.67 (-1.50; 0.16)	0.115	1.81 (0.41)	-1.05 (-1.89; -0.21)	0.015

Table S7.4 (continued)

Measurement	Placebo N=64		Small-intestinal release Peppermint oil N=62		Ileocolonic release Peppermint oil N=63		P-value
	Estimated means (SE)	Treatment effect (95% CI)	Estimated means (SE)	Treatment effect (95% CI)	Estimated means (SE)	Treatment effect (95% CI)	
<b>Urgency*</b>							
Baseline	6.41 (0.32)	-	6.55 (0.33)	-	6.73 (0.32)	-	-
Week 1	5.56 (0.33)	0.04 (-0.87; 0.96)	5.74 (0.34)	0.04 (-0.87; 0.96)	5.88 (0.33)	0.01 (-0.91; 0.92)	0.988
Week 2	5.34 (0.33)	0.25 (-0.64; 1.14)	5.73 (0.34)	0.25 (-0.64; 1.14)	5.93 (0.33)	0.28 (-0.62; 1.18)	0.541
Week 3	5.56 (0.33)	-0.45 (-1.33; 0.43)	5.25 (0.34)	-0.45 (-1.33; 0.43)	5.70 (0.34)	-0.18 (-1.07; 0.71)	0.695
Week 4	5.42 (0.33)	-0.41 (-1.26; 0.43)	5.15 (0.34)	-0.41 (-1.26; 0.43)	5.36 (0.34)	-0.38 (-1.23; 0.49)	0.395
Week 5	5.12 (0.33)	-0.20 (-1.02; 0.62)	5.06 (0.34)	-0.20 (-1.02; 0.62)	5.41 (0.34)	-0.03 (-0.86; 0.81)	0.949
Week 6	4.90 (0.33)	-0.36 (-1.17; 0.45)	4.68 (0.35)	-0.36 (-1.17; 0.45)	5.24 (0.33)	0.04 (-0.77; 0.84)	0.930
Week 7	5.09 (0.33)	-0.61 (-1.37; 0.15)	4.61 (0.34)	-0.61 (-1.37; 0.15)	5.22 (0.34)	-0.18 (-0.96; 0.59)	0.645
Week 8	5.06 (0.33)	-0.63 (-1.35; 0.10)	4.57 (0.35)	-0.63 (-1.35; 0.10)	4.97 (0.34)	-0.40 (-1.13; 0.33)	0.282
<b>IBS symptom severity†</b>							
Baseline	276.53 (11.68)	-	284.52 (12.10)	-	285.42 (11.65)	-	-
Week 2	244.21 (14.10)	-19.38 (-48.01; 9.25)	232.82 (14.50)	-19.38 (-48.01; 9.25)	255.65 (14.15)	2.56 (-26.06; 31.18)	0.860
Week 4	234.16 (13.77)	-23.81 (-54.61; 6.98)	218.33 (14.21)	-23.81 (-54.61; 6.98)	221.10 (13.94)	-21.95 (-52.85; 8.95)	0.163
Week 6	230.68 (14.42)	-32.52 (-66.07; 1.03)	206.13 (14.90)	-32.52 (-66.07; 1.03)	222.10 (14.58)	-17.45 (-15.01; 16.11)	0.306
Week 8	226.80 (14.97)	-41.79 (-76.88; -6.70)	192.99 (15.45)	-41.79 (-76.88; -6.70)	201.05 (15.14)	-34.64 (-69.75; 0.48)	0.053
Month 6*	215.66 (13.97)	-8.86 (-42.03; 24.31)	213.31 (14.42)	-8.86 (-42.03; 24.31)	224.28 (14.11)	-0.02 (-33.27; 33.23)	0.999
<b>IBS quality of life score‡</b>							
Baseline	70.47 (2.47)	-	68.75 (2.56)	-	69.77 (2.47)	-	-
Week 4	73.66 (2.47)	0.93 (-1.75; 3.60)	72.87 (2.56)	0.93 (-1.75; 3.60)	74.01 (2.47)	1.05 (-1.65; 3.75)	0.443
Week 8	75.01 (2.50)	2.56 (-0.17; 5.29)	75.85 (2.59)	2.56 (-0.17; 5.29)	75.54 (2.50)	1.24 (-1.50; 3.97)	0.374
Month 6*	74.70 (2.52)	-0.31 (-3.34; 2.71)	72.79 (2.61)	-0.31 (-3.34; 2.71)	73.92 (2.52)	-0.13 (-3.17; 2.90)	0.931
<b>EQ-5D-5L‡</b>							
Baseline	0.70 (0.03)	-	0.71 (0.03)	-	0.72 (0.03)	-	-
Week 4	0.71 (0.03)	0.04 (-0.01; 0.10)	0.76 (0.03)	0.04 (-0.01; 0.10)	0.73 (0.03)	0.00 (-0.05; 0.06)	0.943
Week 8	0.72 (0.03)	0.04 (-0.01; 0.10)	0.77 (0.03)	0.04 (-0.01; 0.10)	0.74 (0.03)	0.00 (-0.05; 0.06)	0.914
Month 6*	0.79 (0.03)	-0.02 (-0.75; 0.04)	0.78 (0.03)	-0.02 (-0.75; 0.04)	0.77 (0.03)	-0.04 (-0.09; 0.02)	0.174

Table S7.4 (continued)

Measurement	Placebo	Small-intestinal release Peppermint oil	Ileocolonic release Peppermint oil				
	N=64 Estimated means (SE)	N=62 Estimated means (SE)	Treatment effect (95% CI)	P-value	Estimated means (SE)	Treatment effect (95% CI)	P-value
<b>Anxiety<sup>#</sup></b>							
Baseline	6.83 (0.69)	5.33 (0.71)	-	-	6.54 (0.69)	-	-
Week 8	6.48 (0.68)	4.45 (0.71)	-0.53 (-1.55; 0.49)	0.304	5.07 (0.69)	-1.12 (-2.14; -0.10)	0.031
Month 6*	6.57 (0.69)	5.81 (0.71)	0.71 (-0.50; 1.91)	0.250	6.00 (0.69)	-2.66 (-1.48; 0.94)	0.664
<b>Depression<sup>#</sup></b>							
Baseline	8.05 (0.71)	7.58 (0.73)	-	-	7.63 (0.71)	-	-
Week 8	6.11 (0.71)	5.78 (0.73)	-0.73 (-1.78; 0.32)	0.170	6.11 (0.71)	-0.46 (-1.51; 0.59)	0.391
Month 6*	7.05 (0.76)	7.39 (0.79)	0.79 (-0.50; 2.09)	0.228	7.28 (0.77)	0.68 (-0.62; 1.98)	0.303

Treatment effect is the corrected difference in change from baseline between small-intestinal release peppermint oil and placebo, or ileocolonic release peppermint oil and placebo, obtained from linear mixed modelling. The absolute uncorrected change from baseline within treatment groups, i.e. not the difference in change compared to placebo, can be calculated using the given estimated means. P-value is level of significance of comparison between small-intestinal release peppermint oil, or ileocolonic release peppermint oil and placebo. The treatment period consisted of eight weeks (week 1 to week 8). The follow-up period consisted of six months without treatment. § Assessed daily during the pre-treatment and treatment period using an 11-point NRS in the digital diary; 0 = no symptoms, 10 = worst possible pain; ± Assessed weekly during the pre-treatment period and treatment period using an 11-point NRS in the digital diary; 0 = no symptoms, 10 = worst imaginable symptoms; † Assessed using the IBS-SSS questionnaire consisting of 5-items with each a maximum score of 100 (scored on a visual analogue scale); severity of pain, duration of pain, severity of abdominal distention, dissatisfaction with bowel habits, and disruption in quality of life; μ Assessed using the IBS-QoL questionnaire consisting of 34-items with a 5-point Likert scale: 1 = good quality of life, 5 = worse quality of life; ¶ Assessed using the EQ-5D-5L questionnaire that measures 5 dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Dutch social tariffs were used to transform raw EQ-5D-5L scores to utility scores<sup>37</sup>, which vary from a completely healthy state (1) to a state of death (0); # Anxiety was assessed using the GAD-7 questionnaire consisting of 7-items with a 4-point response scale: 0 = not at all, 3 = almost every day. Depression was assessed using the PHQ-9 questionnaire consisting of 9-items with a 4-point response scale: 0 = not at all, 3 = almost every day; \* Values for the six months follow-up were obtained from the corrected model for the treatment period including six months follow-up, all other values are obtained from the corrected model for the eight-week treatment period.

**Table S7.5** Stool frequency and stool consistency (ITT-population).

Measurement	Placebo N=64			Small-intestinal release Peppermint oil N=62			Ileocolonic release Peppermint oil N=63		
	Estimated means (SE)	Treatment effect (95% CI)	P-value	Estimated means (SE)	Treatment effect (95% CI)	P-value	Estimated means (SE)	Treatment effect (95% CI)	P-value
<b>Frequency scores<sup>§</sup></b>									
Baseline	9.10 (0.75)	-	-	9.38 (0.76)	-	-	8.55 (0.75)	-	-
Week 1	9.23 (0.67)	0.30 (-1.11; 1.71)	0.675	9.81 (0.68)	0.30 (-1.11; 1.71)	0.675	9.00 (0.68)	0.32 (-1.08; 1.74)	0.649
Week 2	7.69 (0.66)	0.48 (-1.36; 2.33)	0.607	8.45 (0.66)	0.48 (-1.36; 2.33)	0.607	6.63 (0.66)	-0.50 (-2.35; 1.34)	0.591
Week 3	6.80 (0.58)	0.83 (-1.21; 2.88)	0.423	7.91 (0.59)	0.83 (-1.21; 2.88)	0.423	6.50 (0.58)	0.25 (-1.78; 2.28)	0.807
Week 4	6.75 (0.66)	0.23 (-2.09; 2.54)	0.847	7.26 (0.67)	0.23 (-2.09; 2.54)	0.847	6.48 (0.67)	0.28 (-2.03; 2.60)	0.810
Week 5	6.50 (0.59)	-0.04 (-2.38; 2.31)	0.975	6.74 (0.61)	-0.04 (-2.38; 2.31)	0.975	5.60 (0.61)	-0.34 (-2.68; 2.00)	0.776
Week 6	5.55 (0.66)	0.12 (-2.40; 2.65)	0.923	5.95 (0.66)	0.12 (-2.40; 2.65)	0.923	5.56 (0.68)	0.56 (-1.98; 3.10)	0.664
Week 7	5.49 (0.52)	-0.75 (-3.14; 1.64)	0.538	5.02 (0.53)	-0.75 (-3.14; 1.64)	0.538	4.58 (0.56)	-0.36 (-2.76; 2.05)	0.770
Week 8	4.06 (0.42)	0.32 (-1.97; 2.62)	0.783	4.66 (0.42)	0.32 (-1.97; 2.62)	0.783	4.00 (0.45)	0.49 (-1.82; 2.80)	0.678
<b>Stool consistency<sup>‡</sup></b>									
Baseline	4.10 (0.16)	-	-	3.97 (0.16)	-	-	4.30 (0.16)	-	-
Week 1	4.06 (0.16)	0.23 (-0.13; 0.60)	0.212	4.15 (0.16)	0.23 (-0.13; 0.60)	0.212	4.49 (0.16)	0.24 (-0.13; 0.60)	0.202
Week 2	4.15 (0.16)	0.18 (-0.21; 0.56)	0.366	4.20 (0.16)	0.18 (-0.21; 0.56)	0.366	4.47 (0.16)	0.12 (-0.27; 0.50)	0.551
Week 3	4.17 (0.16)	0.22 (-0.19; 0.62)	0.291	4.25 (0.17)	0.22 (-0.19; 0.62)	0.291	4.44 (0.17)	0.08 (-0.32; 0.48)	0.704
Week 4	4.08 (0.17)	0.21 (-0.21; 0.62)	0.335	4.15 (0.17)	0.21 (-0.21; 0.62)	0.335	4.55 (0.17)	0.28 (-0.14; 0.70)	0.197
Week 5	4.14 (0.16)	0.33 (-0.09; 0.76)	0.124	4.34 (0.17)	0.33 (-0.09; 0.76)	0.124	4.66 (0.17)	0.33 (-0.10; 0.75)	0.129
Week 6	4.14 (0.16)	0.52 (0.09; 0.95)	<b>0.018</b>	4.53 (0.16)	0.52 (0.09; 0.95)	<b>0.018</b>	4.61 (0.17)	0.27 (-0.16; 0.71)	0.219
Week 7	4.22 (0.16)	0.23 (-0.22; 0.68)	0.325	4.31 (0.17)	0.23 (-0.22; 0.68)	0.325	4.85 (0.18)	0.43 (-0.03; 0.89)	0.065
Week 8	4.27 (0.17)	0.16 (-0.32; 0.63)	0.520	4.29 (0.17)	0.16 (-0.32; 0.63)	0.520	4.62 (0.18)	0.16 (-0.32; 0.64)	0.517

Treatment effect is the corrected difference in change from baseline between small-intestinal release peppermint oil and placebo, or ileocolonic release peppermint oil and placebo, obtained from linear mixed modelling. P-value is level of significance of comparison between small-intestinal release peppermint oil, or ileocolonic release peppermint oil and placebo. The treatment period consisted of eight weeks (week 1 to week 8). § Frequency scores: Defined as Spontaneous Bowel Movements (SBMs) per week. A general decrease in SBMs was found over time (Baseline 9.10 - Week 8 4.06, F30.03, P<0.000). This reduction in registration of bowel movements was seen among all subtypes and we hypothesize that this is due to logging fatigability rather than an actual decrease in stool frequency. ‡ Stool consistency: Weekly average score assessed using the Bristol Stool Form Scale. Scale: 1=separate hard lumps, like nuts (difficult to pass); 2=sausage-shaped, but lumpy; 3=like a sausage but with cracks on its surface; 4=like a sausage or snake, smooth and soft; 5= soft blobs with clear-cut edges (passed easily); 6=fluffy pieces with ragged edges, a mushy stool; 7=watery, no solid pieces, entirely liquid.

**Table S7.6** Stool frequency and stool consistency in patients with IBS subtype diarrhea.

Measurement	Placebo N=29		Small-intestinal release Peppermint oil N=25		Ileocolonic release Peppermint oil N=29		P-value
	Estimated means (SE)	Treatment effect (95% CI)	Estimated means (SE)	Treatment effect (95% CI)	Estimated means (SE)	Treatment effect (95% CI)	
<b>Frequency scores in patients with IBS subtype diarrhea<sup>§</sup></b>							
Baseline	11.31 (1.20)	-	11.80 (1.29)	-	9.79 (1.20)	-	-
Week 1	11.90 (1.17)	-1.23 (-3.49; 1.03)	11.16 (1.26)	0.285	11.07 (1.17)	0.69 (-1.48; 2.86)	0.532
Week 2	9.46 (1.24)	1.01 (-2.12; 4.13)	10.96 (1.32)	0.525	8.65 (1.24)	0.71 (-2.32; 3.74)	0.644
Week 3	7.96 (1.05)	1.42 (-1.99; 4.82)	9.87 (1.15)	0.414	7.92 (1.05)	1.47 (-1.78; 4.73)	0.374
Week 4	8.01 (1.28)	1.41 (-2.67; 5.49)	9.91 (1.39)	0.498	8.46 (1.29)	1.97 (-1.95; 5.89)	0.323
Week 5	7.43 (1.06)	-0.76 (-4.70; 3.18)	7.16 (1.17)	0.704	6.75 (1.11)	0.83 (-2.98; 4.65)	0.667
Week 6	6.46 (1.23)	-0.28 (-4.63; 4.08)	6.67 (1.30)	0.901	7.05 (1.26)	2.11 (-2.15; 6.37)	0.330
Week 7	6.17 (0.86)	-1.50 (-5.43; 2.43)	5.16 (0.96)	0.452	5.11 (0.91)	0.46 (-3.34; 4.26)	0.813
Week 8	4.53 (0.63)	-0.40 (-4.13; 3.33)	4.62 (0.71)	0.832	4.72 (0.68)	1.71 (-1.89; 5.31)	0.350
<b>Stool consistency in patients with IBS subtype diarrhea<sup>‡</sup></b>							
Baseline	4.64 (0.19)	-	4.81 (0.21)	-	5.10 (0.19)	-	-
Week 1	4.47 (0.19)	0.14 (-0.28; 0.56)	4.78 (0.21)	0.518	5.24 (0.19)	0.31 (-0.10; 0.72)	0.141
Week 2	4.49 (0.20)	0.20 (-0.26; 0.67)	4.87 (0.21)	0.385	5.10 (0.20)	0.15 (-0.30; 0.60)	0.521
Week 3	4.25 (0.20)	0.56 (0.06; 1.06)	4.98 (0.22)	0.028	5.26 (0.20)	0.55 (0.07; 1.02)	<b>0.023</b>
Week 4	4.27 (0.20)	0.42 (-0.11; 0.95)	4.87 (0.22)	0.118	5.28 (0.20)	0.54 (0.04; 1.05)	0.036
Week 5	4.75 (0.20)	0.26 (-0.27; 0.80)	5.19 (0.22)	0.333	5.39 (0.20)	0.18 (-0.34; 0.70)	0.496
Week 6	4.51 (0.20)	0.69 (0.14; 1.24)	5.37 (0.21)	<b>0.014</b>	5.40 (0.20)	0.43 (-0.11; 0.97)	0.119
Week 7	4.54 (0.20)	0.34 (-0.24; 0.92)	5.05 (0.23)	0.255	5.62 (0.21)	0.62 (0.06; 1.18)	0.030
Week 8	4.62 (0.21)	0.28 (-0.33; 0.89)	5.07 (0.23)	0.366	5.44 (0.22)	0.36 (-0.23; 0.95)	0.232

Treatment effect is the corrected difference in change from baseline between small-intestinal release peppermint oil and placebo, or ileocolonic release peppermint oil and placebo, obtained from linear mixed modelling. P-value is level of significance of comparison between small-intestinal release peppermint oil, or ileocolonic release peppermint oil and placebo. The treatment period consisted of eight weeks (week 1 to week 8). § Frequency scores: Defined as Spontaneous Bowel Movements per week; ‡ Stool consistency: Weekly average score assessed using the Bristol Stool Form Scale. Scale: 1=separate hard lumps, like nuts (difficult to pass); 2=sausage-shaped, but lumpy; 3=like a sausage but with cracks on its surface; 4=like a sausage or snake, smooth and soft; 5= soft blobs with clear-cut edges (passed easily); 6=fluffy pieces with ragged edges, a mushy stool; 7=watery, no solid pieces, entirely liquid.

**Table S7.7** Stool frequency and stool consistency in patients with IBS subtype constipation.

Measurement	Placebo		Small-intestinal release Peppermint oil		Ileocolonic release Peppermint oil	
	Estimated means (SE)	P-value	Estimated means (SE)	Treatment effect (95% CI)	Estimated means (SE)	Treatment effect (95% CI)
	N=14		N=12		N=16	
<b>Frequency scores in patients with IBS subtype constipation<sup>§</sup></b>						
Baseline	6.79 (0.82)	-	5.96 (0.88)	-	5.56 (0.76)	-
Week 1	7.14 (0.83)	0.796	6.58 (0.90)	0.27 (-1.79; 2.32)	5.56 (0.78)	-0.36 (-2.27; 1.56)
Week 2	5.86 (0.70)	0.753	5.42 (0.76)	0.39 (-2.05; 2.82)	3.81 (0.65)	-0.82 (-3.08; 1.44)
Week 3	5.57 (0.72)	0.969	4.80 (0.80)	0.05 (-2.71; 2.82)	4.57 (0.70)	0.22 (-2.36; 2.81)
Week 4	5.86 (0.79)	0.370	3.67 (0.82)	-1.36 (-4.36; 1.64)	4.58 (0.74)	-0.05 (-2.88; 2.77)
Week 5	6.18 (1.01)	0.630	4.47 (1.10)	-0.88 (-4.49; 2.73)	4.59 (0.96)	-0.37 (-3.74; 3.00)
Week 6	5.19 (0.80)	0.778	3.90 (0.86)	-0.46 (-3.68; 2.76)	4.24 (0.76)	0.27 (-2.73; 3.28)
Week 7	5.86 (0.89)	0.454	3.72 (0.96)	-1.31 (-4.77; 2.16)	4.12 (0.89)	-0.52 (-3.80; 2.77)
Week 8	3.81 (0.78)	0.478	4.15 (0.83)	1.17 (-2.09; 4.42)	3.69 (0.78)	1.10 (-1.99; 4.19)
<b>Stool consistency in patients with IBS subtype constipation<sup>‡</sup></b>						
Baseline	3.23 (0.32)	-	2.99 (0.34)	-	3.08 (0.30)	-
Week 1	3.07 (0.32)	0.202	3.47 (0.34)	0.64 (-0.35; 1.63)	3.57 (0.30)	0.65 (-0.27; 1.57)
Week 2	2.82 (0.32)	0.579	2.87 (0.34)	0.29 (-0.74; 1.31)	3.53 (0.30)	0.85 (-0.10; 1.80)
Week 3	3.73 (0.32)	0.903	3.43 (0.35)	-0.07 (-1.13; 1.00)	3.66 (0.31)	0.07 (-0.93; 1.07)
Week 4	3.35 (0.33)	0.945	3.15 (0.34)	0.04 (-1.06; 1.14)	3.68 (0.31)	0.48 (-0.57; 1.52)
Week 5	3.01 (0.33)	0.855	2.88 (0.35)	0.10 (-1.02; 1.23)	3.54 (0.31)	0.68 (-0.38; 1.74)
Week 6	3.10 (0.33)	0.337	3.42 (0.35)	0.56 (-0.59; 1.71)	3.55 (0.31)	0.59 (0.49; 1.67)
Week 7	3.17 (0.33)	0.142	3.81 (0.35)	0.87 (-0.30; 2.05)	3.92 (0.33)	0.90 (-0.22; 2.02)
Week 8	3.55 (0.35)	0.860	3.42 (0.37)	0.11 (-1.12; 1.34)	3.83 (0.35)	0.42 (-0.75; 1.60)

Treatment effect is the corrected difference in change from baseline between small-intestinal release peppermint oil and placebo, or ileocolonic release peppermint oil and placebo, obtained from linear mixed modelling. P-value is level of significance of comparison between small-intestinal release peppermint oil, or ileocolonic release peppermint oil and placebo. The treatment period consisted of eight weeks (week 1 to week 8). § Frequency scores: Defined as Spontaneous Bowel Movements per week; ‡ Stool consistency: Weekly average score assessed using the Bristol Stool Form Scale. Scale: 1=separate hard lumps, like nuts (difficult to pass); 2=sausage-shaped, but lumpy; 3=like a sausage but with cracks on its surface; 4=like a sausage or snake, smooth and soft; 5= soft blobs with clear-cut edges (passed easily); 6=fluffy pieces with ragged edges, a mushy stool; 7=watery, no solid pieces, entirely liquid.

**Table S7.8** Use of rescue medication (ITT-population).

	Placebo N=64	Small-intestinal release Peppermint oil N=62	Ileocolonic release Peppermint oil N=63
<b>Pain medication</b>			
Mean frequency of use (SE) <sup>¶</sup>	5.16 (0.82)	3.71 (0.59)	3.16 (0.48)
Number of patients (%) <sup>‡</sup>	50 (78.1)	44 (71.0)	46 (73.0)
<b>GI-medication<sup>‡</sup></b>			
Mean frequency of use (SE) <sup>¶</sup>	5.05 (0.81)	4.42 (0.63)	3.17 (0.46)
Number of patients (%) <sup>‡</sup>	53 (82.8)	48 (77.4)	45 (71.4)

<sup>¶</sup> Mean frequency of use per patient during the eight-week treatment period; <sup>‡</sup> GI-medication comprises of i.e. antacids, laxatives, and anti-diarrheal drugs; <sup>‡</sup> Number of patients and percentage of patients that used the medication at least once during the eight-week treatment period. The differences in mean frequency between small-intestinal release peppermint oil and placebo ( $P=0.087$  for pain medication,  $P=0.457$  for GI-medication) and ileocolonic release peppermint oil and placebo ( $P=0.039$  for pain medication,  $P=0.044$  for GI medication) did not reach statistical significance ( $\alpha=0.025$ ).



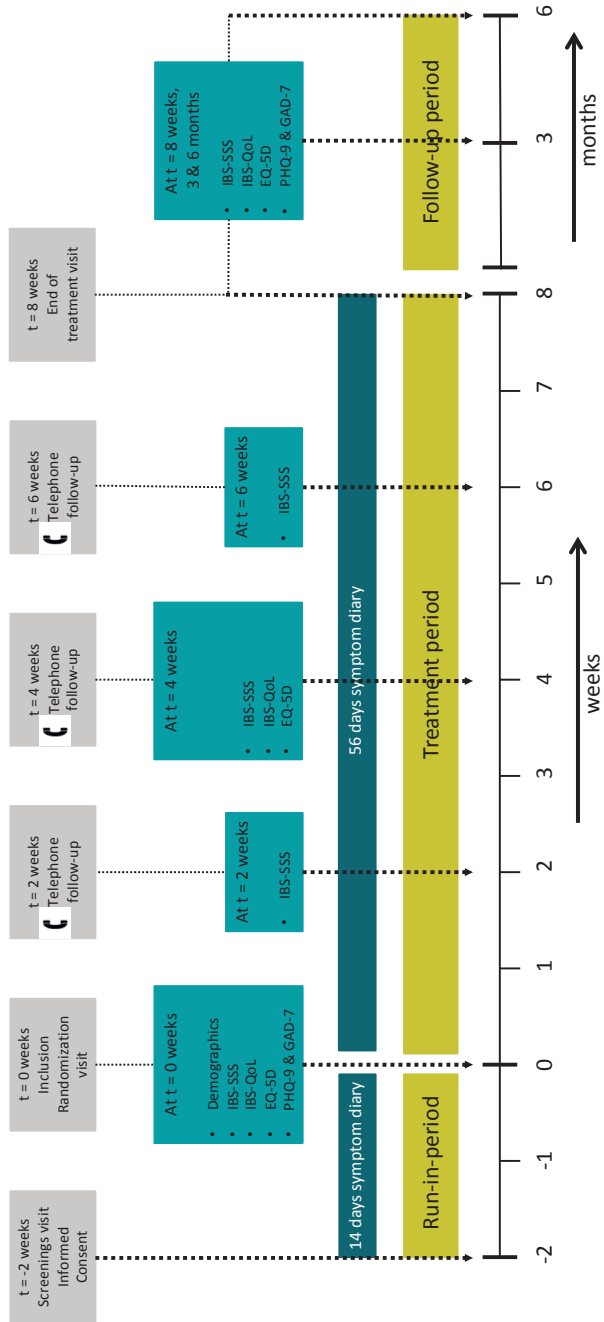
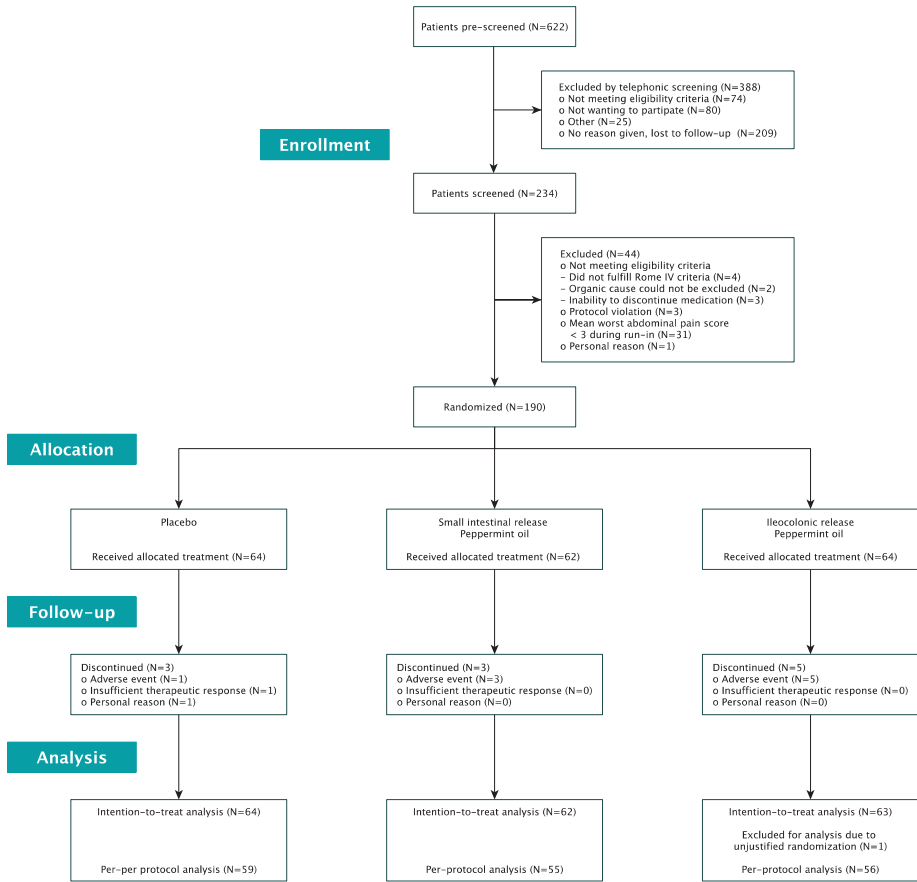
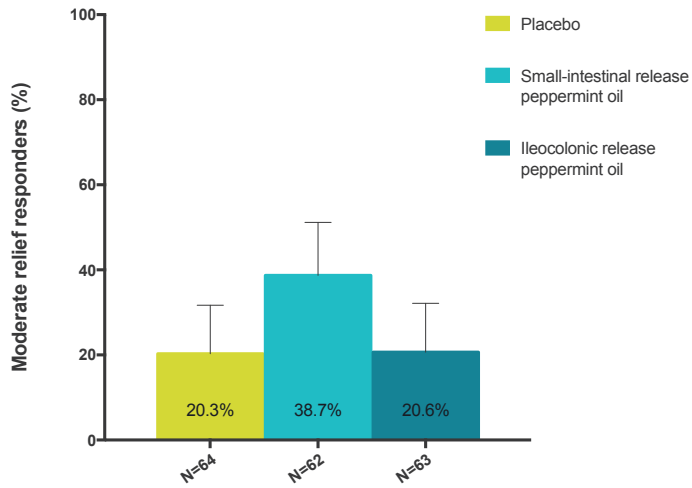



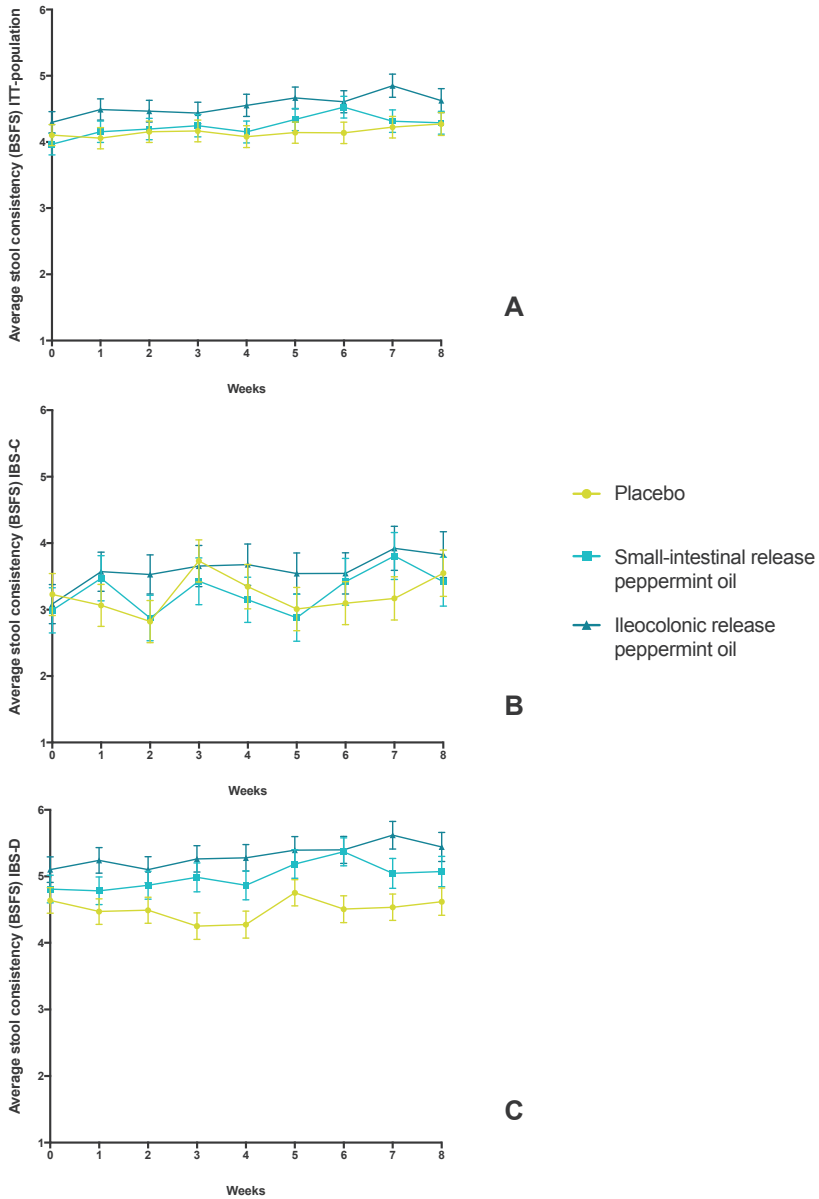
Figure S7.1 Study design of the PERSUADE study.



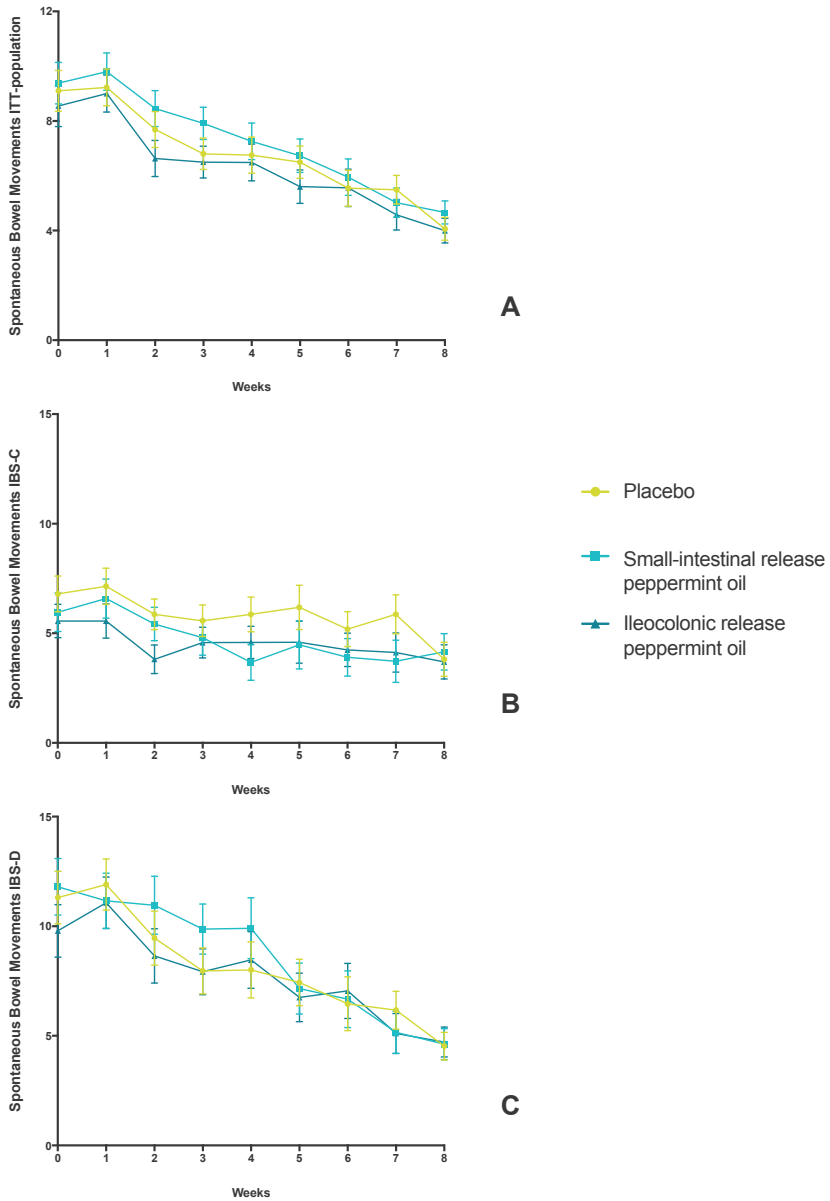
**Figure S7.2** Flowchart of patients included in the PERSUADE study. IBS; Irritable Bowel Syndrome.



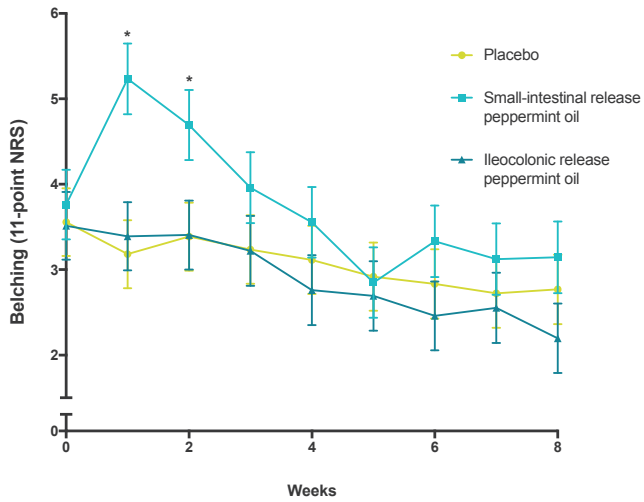
**Figure S7.3** Percentage of patients who were moderate relief responders in the ITT-population. A responder was a patient with at least a relief score of 5, 6 or 7 (on a 7-point NRS) in at least 4 weeks out of 8 weeks. Values are percentages, bars represent standard errors.  $P=0.030$  for small-intestinal release peppermint oil,  $P=0.980$  for ileocolonic release peppermint oil, both compared with placebo.



**Figure S7.4** **A.** Stool consistency in the ITT-population ( $N=189$ ), **B.** Stool consistency in the IBS-C population ( $N=42$ ), **C.** Stool consistency in the IBS-D population ( $N=83$ ). Values are adjusted estimated marginal means derived from the linear mixed model, bars represent standard errors. The small-intestinal peppermint oil group had significantly more increase in stool consistency at week 6 compared to placebo ( $P=0.018$ ). Assessed daily using the Bristol stool form scale (BSFS) in the digital diary.



**Figure S7.5** **A.** Stool frequency in the ITT-population ( $N=189$ ), **B.** Stool frequency in the IBS-C population ( $N=42$ ), **C.** Stool frequency in the IBS-D population ( $N=83$ ). Values are adjusted estimated marginal means derived from the linear mixed model, bars represent standard errors. Assessed daily in the digital diary.



**Figure S7.6** Belching scores in the ITT-population ( $N=189$ ). Values are adjusted estimated marginal means derived from the linear mixed model, bars represent standard errors. The small-intestinal peppermint oil group had significantly more increase in belching at the week 1 and 2,  $*p=0.0003$ , and  $*p=0.023$ , respectively. The ileocolonic release peppermint oil group did not differ significantly in belching compared with placebo. Assessed weekly on an 11-point NRS in the digital symptom diary.

**Table S7.9** Primary endpoints per IBS-subtype (ITT-population).

	Placebo		Small-intestinal release Peppermint oil		Ileocolonic release Peppermint oil				
	N=64		N=62		N=63				
	No. responders (%)	No. responders (%)	No. responders (%)	P-value	Odds Ratio (95% CI)	No. responders (%)	P-value	Odds Ratio (95% CI)	
<b>Primary endpoints</b>									
Abdominal pain <sup>¶</sup>									
IBS-D	11/29 (37.9)	10/25 (40.0)	0.985	0.985	0.99 (0.32 – 3.08)	9/29 (31.0)	0.444	0.65 (0.65 – 1.98)	
IBS-C	6/14 (42.9)	5/12 (41.7)	0.905	0.905	0.90 (0.17 – 4.81)	10/16 (62.5)	0.114	3.80 (0.73 – 19.90)	
IBS-M	3/12 (25.0)	7/15 (46.7)	0.278	0.278	2.62 (0.46 – 14.87)	4/13 (30.8)	0.931	0.92 (0.12 – 6.76)	
IBS-U	2/9 (22.2)	7/10 (70.0)	0.070	0.070	8.06 (0.84 – 77.12)	3/5 (60.0)	0.135	7.28 (0.54 – 98.64)	
Global relief <sup>¶</sup>									
IBS-D	1/19 (3.4)	1/25 (4.0)	0.887	0.887	1.24 (0.07 – 23.75)	1/29 (3.4)	0.958	0.93 (0.05 – 16.70)	
IBS-C	1/14 (7.1)	1/12 (8.3)	0.986	0.986	1.03 (0.05 – 21.25)	0/16 (0)	0.998	N.A.	
IBS-M	1/12 (8.3)	2/15 (13.3)	0.798	0.798	1.44 (0.09 – 23.43)	0/13 (0)	0.999	N.A.	
IBS-U	0/9 (0)	2/10 (20.0)	0.999	0.999	N.A.	0/5 (0)	1.000	N.A.	

<sup>¶</sup> A responder was defined as a patient with at least 30% decrease in mean worst daily abdominal pain in at least 50% of weeks in which treatment was given (FDA-recommendation); ‡ A responder was defined as a patient with at least a global relief score of 6 or 7 (on a 7-point NRS) in at least 50% of weeks in which treatment was given (EMA-recommendation).

**Table S7.10** Primary endpoints per healthcare setting (ITT-population).

	Small-intestinal release Peppermint oil N=62			Ileocolonic release Peppermint oil N=63			
	No. responders (%)	No. responders (%)	P-value	Odds Ratio (95% CI)	No. responders (%)	P-value	Odds Ratio (95% CI)
	Placebo N=64						
<b>Primary endpoints</b>							
Abdominal pain <sup>¶</sup>							
Primary care	12/39 (30.8)	15/36 (41.7)	0.393	1.53 (0.58 – 4.08)	16/34 (47.1)	0.206	1.89 (0.71 – 5.07)
Secondary/tertiary care	10/25 (40.0)	14/26 (53.8)	0.333	1.83 (0.54 – 6.25)	10/29 (34.5)	0.772	0.83 (0.23 – 3.01)
Global relief <sup>¶</sup>							
Primary care	1/39 (2.6)	3/36 (8.3)	0.213	5.15 (0.39 – 68.0)	1/34 (2.9)	0.695	1.83 (0.09 – 37.23)
Secondary/tertiary care	2/25 (8.0)	3/26 (11.5)	0.946	1.08 (0.12 – 9.50)	0/29 (0)	N.A.	N.A.

¶ A responder was defined as a patient with at least 30% decrease in mean worst daily abdominal pain in at least 50% of weeks in which treatment was given (FDA-recommendation); † A responder was defined as a patient with at least a global relief score of 6 or 7 (on a 7-point NRS) in at least 50% of weeks in which treatment was given (EMA-recommendation).



## Exploratory supplementary analyses of effect modification

### Effect modification gender

To explore possible effect modifiers in a supplementary exploratory analysis, we added the interaction of treatment group with the possible effect modifier to the model. This explorative post hoc analysis showed that gender was an effect modifier of treatment group and the primary abdominal pain outcome, likelihood ratio (LR) test for interaction term:  $P=0.016$ ). For men ( $N=42$ ), the small-intestinal release peppermint oil did have a significant treatment effect on the primary outcome with 81.8% of men being a responder (OR 9.14, 95% CI 1.36; 61.54,  $P=0.02$ ), compared with 33.3% in the placebo group. For women ( $N=147$ ), however, no significant differences were found in abdominal pain response rate between small-intestinal release peppermint oil, with 39.2% of women being a responder, (OR of 1.20, 95% CI 0.53; 2.76,  $P=0.67$ ), compared with 34.7% in the placebo group. The relatively low number of included men implies that the effect found should be interpreted with appropriate caution.

### Effect modification primary care versus secondary/tertiary care

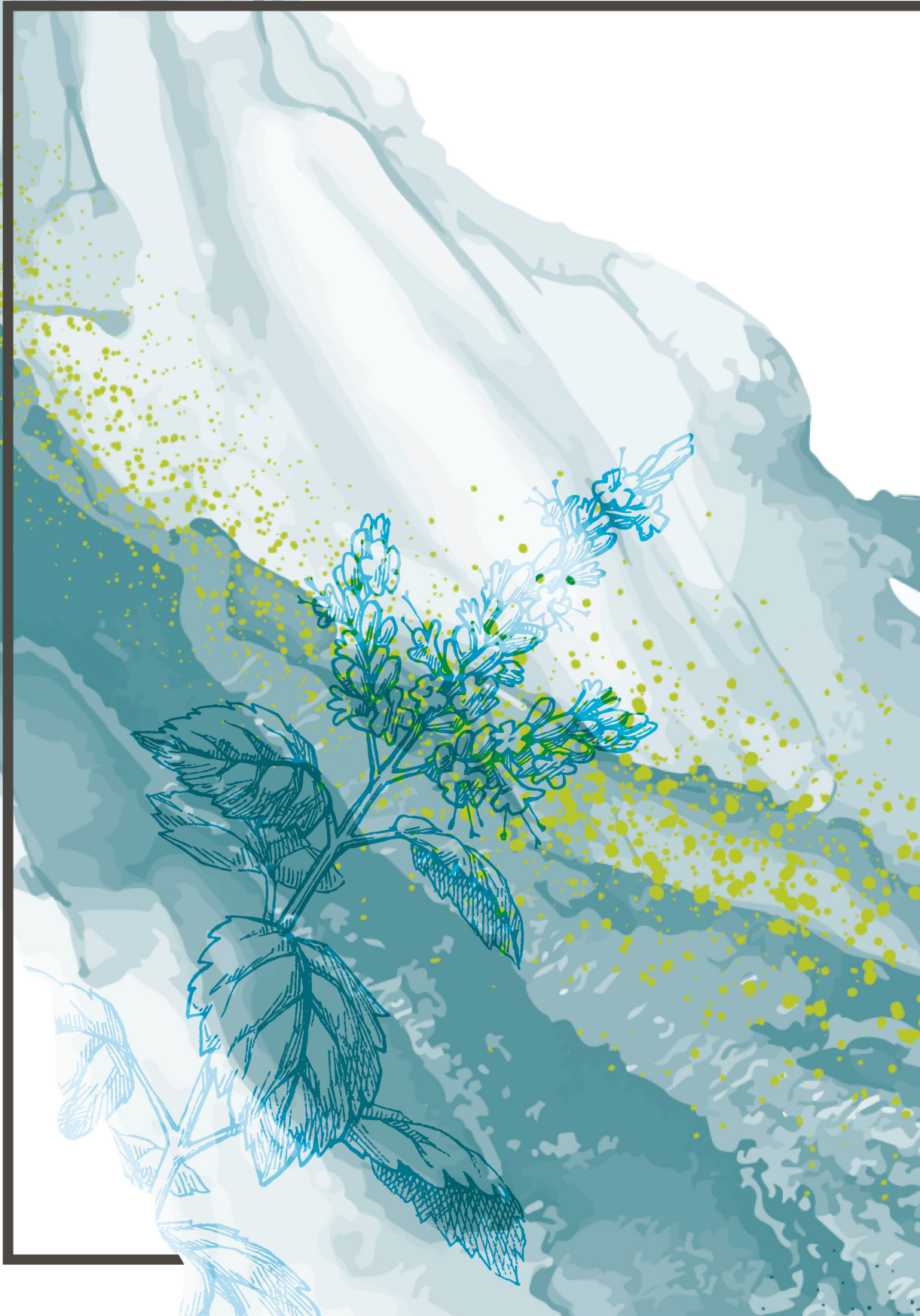
We explored a potential effect modification of being a primary care patient versus secondary/tertiary care patient in a supplementary exploratory analysis. The proportion of abdominal pain responders according to FDA definition (30% decrease in worst abdominal pain, in at least 50% of treatment weeks) did not differ significantly between primary and secondary/tertiary care patients, *i.e.* 43/109 (39.4%) primary care patients were responders, compared with 34/80 (42.5%) secondary/tertiary care patients ( $P=0.793$ ). To double-check however, we added the interaction of treatment group with the categorical variable of being a primary care patient (or not) to the model that was corrected for minimization variables age, gender, IBS-subtype, and inclusion center. This explorative post hoc analysis showed that being a primary care patient was not a significant effect modifier of treatment group and the primary abdominal pain response outcome (likelihood ratio (LR) test for interaction term:  $P=0.398$ ).

### Effect modification baseline abdominal pain scores

To assess potential effect modification of baseline abdominal pain on the primary outcome abdominal pain response, we added the interaction of treatment group with baseline mean worst abdominal pain to the model that was corrected for minimization variables age, gender, IBS-subtype, and inclusion center. This explorative post hoc

analysis showed that baseline mean worst abdominal pain was not a significant effect modifier of treatment group and the primary abdominal pain response outcome (LR test  $P=0.322$ ). Similarly, when dividing patients into two groups based on baseline mean worst abdominal pain, *i.e.* a group with the lowest 2/3 of baseline abdominal pain and a group with the highest 1/3 of baseline abdominal pain, the proportion of abdominal pain responders did not differ significantly between groups ( $P=0.086$ ).





# Chapter 8

A trial-based economic evaluation of peppermint oil for the treatment of irritable bowel syndrome

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# Abstract

## Background

Irritable Bowel Syndrome (IBS) is a prevalent, chronic gastrointestinal disorder that imposes a substantial socioeconomic burden. Peppermint oil is a frequently used treatment for IBS, but evidence about cost-effectiveness is lacking. The objective of this trial-based economic evaluation was to assess the cost-effectiveness of small-intestinal release peppermint oil versus placebo in patients with IBS.

## Methods

In a multicenter randomized placebo-controlled trial, cost-effectiveness was evaluated from a societal perspective. The incremental cost-effectiveness ratios were expressed as 1) incremental costs per Quality Adjusted Life Years (QALY), and 2) incremental costs per successfully treated patient, *i.e.* per abdominal pain responder (according to FDA definitions), both after an eight-week treatment period with placebo versus peppermint oil. Cost-utility and uncertainty were estimated using non-parametric bootstrapping. Sensitivity analyses were performed to examine parameter uncertainty.

## Results

The analysis comprised 126 patients ( $N=64$  placebo,  $N=62$  small-intestinal release peppermint oil). Peppermint oil was a dominant treatment compared to placebo in 46% of bootstrap replications. Peppermint oil was also more effective but at higher cost in 31% of replications. The net benefit acceptability curve showed that peppermint oil has a 56% probability of being cost-effective at a conservative willingness-to-pay threshold of €10.000 per QALY. Peppermint oil was also a dominant treatment per additional successfully treated patient according to FDA definitions, *i.e.* in 51% of the bootstrap replications. In this case, the acceptability curve showed an 89% probability of being cost-effective

## Conclusion

In patients with IBS, small-intestinal release peppermint oil appears to be a cost-effective treatment although there is uncertainty surrounding the ICER. When using abdominal pain responder as outcome measure for the ICER, peppermint oil has a high probability of being cost-effective. The use of peppermint oil, which is a low-cost treatment, can be justified by the modest QALY gains and slightly higher proportion of abdominal pain responders. More research and long-term data are necessary to confirm the cost-effectiveness of peppermint oil. NCT02716285

## Introduction

Irritable Bowel Syndrome (IBS) is a prevalent and chronic disorder of brain-gut-interaction characterized by chronic abdominal pain and altered bowel habits.<sup>1</sup> IBS has a large negative impact on quality of life (QoL)<sup>2</sup> and is associated with considerable costs for patients, healthcare systems and society.<sup>3-5</sup>

Regarding direct costs to healthcare systems, IBS patients are reported to have increased numbers of consultations, emergency room visits, hospitalizations, and prescribed medications, when compared to patients without IBS.<sup>2,6</sup> Additionally, a large proportion of patients use over-the-counter (OTC) drugs or complementary medicine leading to high out of pocket costs.<sup>6</sup> Regarding indirect costs<sup>3,4</sup>, IBS patients are more likely to be both absent from work (absenteeism) and impaired during work (presenteeism) when compared to non-IBS patients.<sup>6</sup> Summed with the reduced QoL in patients, IBS leads to a high socioeconomic disease burden.<sup>2,7,8</sup>

Effective therapies are therefore crucial to decrease this burden. Generally, symptom improvement should result in a better health-related QoL, less resource use and less productivity loss. Peppermint oil is a frequently used treatment for IBS and we previously reported the results of the largest randomized clinical trial (RCT) with peppermint oil to date.<sup>9</sup> A recent meta-analysis, including data from this trial, confirmed the therapeutic superiority of small-intestinal peppermint oil over placebo in IBS.<sup>10</sup> Trial based data on the cost-effectiveness of peppermint oil however, are lacking so far. The objective of this trial-based economic evaluation was therefore to assess the cost-effectiveness of peppermint oil compared with placebo, in patients with IBS.

## Materials and methods

This economic evaluation was performed in a multicenter, placebo-controlled, double-blind RCT on the clinical efficacy of peppermint oil as a secondary outcome. The study was performed in four Dutch hospitals, one academic with a combined secondary/tertiary care function (Maastricht University Medical Center+, MUMC+), and three secondary care (Hospital Gelderse Vallei, Ede; Alrijne Hospital, Leiden; Medical Center Leeuwarden, Leeuwarden). The research protocol had been approved by the MUMC+ Committee of Ethics and has been registered in the US National Library of Medicine (Clinicaltrials.gov, NCT02716285). All study procedures were performed in compliance with GCP and the Declaration of Helsinki. All subjects had given a written



informed consent prior to participation. Full details of the clinical trial have been published elsewhere<sup>9</sup> and are briefly summarized below. All authors had access to the study data and reviewed and approved the final manuscript.

### **Patients, setting and interventions**

IBS patients, between 18 and 75 years of age were eligible for inclusion if they fulfilled the ROME IV criteria.<sup>11</sup> Patients had to have a mean daily worst abdominal pain score  $\geq 3$  during a two-week run-in period (on an eleven-point numerical rating scale (NRS)). Randomization was done with ALEA Screening and Enrollment software using the minimization method accounted for inclusion center, IBS subtypes, gender, and age. Patients were assigned to 182 mg of small-intestinal release peppermint oil in enteric-coated soft gel capsules (Tempocol, WillPharma S.A.), ileocolonic release peppermint oil (Tempocol core capsules, coated with ColoPulse coating layer<sup>12</sup>), or placebo. The study consisted of a two-week run-in period, an eight-week treatment period, followed by a six-month follow-up period in which no study medication was given. Patients were asked to refrain from lifestyle changes and new treatments. Standard care however, could be continued in a stable manner. Patient inclusion took place between August 2016 and March 2018.

### **Economic evaluation**

The economic evaluation was performed in accordance with the Dutch guidelines for cost-calculations.<sup>13,14</sup> the CHEERS checklist and The Professional Society for Health Economics and Outcomes Research (ISPOR) guidelines, based on intention-to-treat analysis. Costs were calculated from the societal perspective and expressed in 2017 euros. The primary outcome of the current study is the incremental cost-effectiveness ratio (ICER), calculated as difference in costs between peppermint oil and placebo divided by the difference in QALY between peppermint oil and placebo, over the eight-week treatment period. As the newly formulated ileocolonic release peppermint oil did not yield any benefits over small-intestinal release peppermint oil<sup>9</sup>, this formulation will not be developed further and will not be available to patients. Consequently, this formulation was not taken into account in this economic evaluation.

Costs included all IBS-related direct costs (*i.e.* outpatient consultations, general practice consultation, dietician, and mental healthcare) and indirect costs (*i.e.* absenteeism, presenteeism, and impaired unpaid work). Additionally, cost of treatment assigned was included (small-intestinal release peppermint oil or placebo). An overview of treatment and other costs per unit is given in *Table 8.1*.



Health-related resource use was assessed using the iMTA Medical Consumption Questionnaire (MCQ), which is designed to measure costs in the Dutch healthcare system (see <https://www.imta.nl>). The MCQ was completed at baseline and after eight-weeks of treatment. As the recall-period of the MCQ is three months, the recall period was adjusted to eight weeks for assessment at the end of the eight-week treatment period. After all data had been collected, a distinction was made between IBS-unrelated and potentially related costs based on expert opinion; e.g. drug treatment for comorbid cardiovascular disease was not included, whereas visits to a gastroenterologist or GP, or mental healthcare were. Direct costs were calculated by multiplying resource use by the cost price per resource unit, adopting reference prices derived from the Dutch costing manual (*Table 8.1*). Medication costs were obtained from the Dutch Pharmacotherapeutic Compass (Healthcare Insurance Board, 2017). Additionally, for each visit to a care provider, travel expenses were calculated using a standard cost of €0.19 per average kilometer.

Indirect health-related costs were measured using the iMTA Productivity Cost Questionnaire (PCQ), which was designed and validated for the Dutch situation (see <https://www.imta.nl>).<sup>15</sup> The PCQ was completed at baseline and at four and eight-weeks of treatment. The PCQ includes questions on productivity loss of paid work (absenteeism and presenteeism) and productivity loss of unpaid work (e.g. voluntary work, or homemaking and caregiving). Indirect costs were calculated by multiplying the hours absent (using self-reported dates of sick leave) or impaired (using a self-reported inefficiency score) by average wage rates per hour. Since the majority of costs and outcomes were measured within one year, no discounting was applied and all costs were indexed for inflation to 2017. A detailed description of the calculation of indirect costs is given in the *Supplementary Material*.

**Table 8.1** Overview of costs per unit of resource use.

Resource use	Unit	Cost (euro)	Reference
<b>Study treatment</b>			
Placebo	168 capsules	0.00	Manufacturer
Small-intestinal release peppermint oil	168 capsules	41.86	Manufacturer
<b>General practitioner consultation</b>	consultation/visit	34.00	Dutch costing manual
<b>Gastroenterologist consultation</b>	consultation/visit	93.00	Dutch costing manual
<b>Social work consultation</b>	consultation/visit	67.00	Dutch costing manual
<b>Mental healthcare consultation</b>	consultation/visit	94.60	Dutch costing manual
<b>Travel cost car or public transport</b>	kilometer	0.19	Dutch costing manual
<b>Parking cost</b>	visit	3.07	Dutch costing manual
<b>Average wage women</b>	hour	32.36	Dutch costing manual
<b>Average wage men</b>	hour	38.82	Dutch costing manual
<b>Productivity cost unpaid work</b>	hour	14.34	Dutch costing manual

The EuroQol-5D (EQ-5D-5L) was used to measure patients' health-related QoL at baseline, four and eight weeks of treatment. The EQ-5D-5L measures five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression and has shown good performance in IBS patients.<sup>16</sup> Dutch social tariffs were used to transform raw EQ-5D-5L scores to utility scores.<sup>17</sup> The IBS-QoL questionnaire consists of 34 items with a 5-point Likert scale, and was used to determine the impact of IBS and treatment on QoL.<sup>18</sup>

All data was collected electronically using web-based questionnaires and a smartphone-based symptom diary.

### Statistical analysis

Statistical analyses were performed using SPSS 25.0 (Armonk, NY, USA) and Microsoft Excel 16.16.7. The proportion of missing data and the missing data pattern were investigated using descriptives and patterns function in SPSS, and associations between missingness and baseline and outcome variables were investigated with logistic regression, to inform on the missing data mechanism. Missing values missing (completely) at random were handled by multivariate imputation by chained equations using predictive mean matching<sup>19</sup> with gender, IBS-subtype, age, baseline IBS severity, baseline utility, and treatment group. QALYs were calculated by the area under the curve, in which the time in a certain health state was multiplied by the utility of this health state. The time horizon was the eight-week treatment period. For the QALY calculation, utility values were corrected for baseline differences between groups with the mean absolute difference method.<sup>20</sup> ICER was calculated as the difference in costs divided by the difference in QALYs between peppermint oil and placebo. Nonparametric bootstrapping with 10.000 and 1.000 simulations was used to calculate the difference in costs between groups and to examine the uncertainty surrounding the ICER, respectively. This method requires resampling and derives a cost-effectiveness ratio from each of the generated repeated samples<sup>21-23</sup>, thereby increasing the robustness of the results and accounting for within and between imputation variability.<sup>19</sup> Results of the bootstrap analysis are presented in cost-effectiveness planes and net benefit acceptability curves. A cost-effectiveness plane is a scatterplot of simulated ICERs and presents the four situations of additional costs and additional QALY's of peppermint oil compared to placebo. If the majority of the ICERs appear in the southeast quadrant, this indicates higher effectiveness at lower costs, *i.e.* peppermint oil dominates placebo. The northwest quadrant on the other hand indicates lower effectiveness at higher costs, *i.e.* peppermint oil is inferior compared to placebo. With

regard to the other two quadrants (lower effectiveness at lower costs, and higher effectiveness at higher costs), the choice for peppermint oil depends on the threshold value, *i.e.* the maximum amount society/decision maker is willing to pay (WTP) for a particular health gain. The net benefit acceptability curve shows the probability that the new treatment is cost-effective compared to placebo over various willingness to pay (WTP) values. The net monetary benefit (NMB) is calculated with the following formula:  $WTP * \text{difference in QALY} - \text{difference in costs}$ . Commonly used WTP thresholds per QALY (one additional year in perfect health) in the Netherlands are €20.000 for mild, €50.000 for moderately severe and €80.000 for a severe condition.<sup>24</sup> As IBS does not increase mortality and our study includes a relatively short time horizon (eight weeks), a WTP threshold of €10.000 (estimated €65.000 per year) was chosen for the calculation of the net monetary benefit. Prior studies investigating cost-effectiveness in IBS have applied thresholds varying between £30.000<sup>25</sup> and \$80.000<sup>26</sup> per QALY with the last study covering a treatment period of ten weeks.

### Sensitivity analyses

To assess the impact of different parameters on the results, several univariate sensitivity analyses were performed. First, the main clinical effectiveness outcome, *i.e.* the proportion of responders instead of QALY, was used. According to the Food and Drug Administration (FDA) definition a responder had at least a 30% decrease in the mean weekly worst daily abdominal pain (measured daily, on an 11-point NRS) compared to baseline, in at least four (out of eight) weeks. As this endpoint does not capture a generic health related QoL, a lower WTP threshold was chosen to calculate the net monetary benefit, *i.e.* €5.000. For the second sensitivity analysis, unadjusted QALYs were used, *i.e.* no correction for baseline differences in QALYs between groups was calculated. The final sensitivity analysis was a cost-effectiveness analysis from the healthcare perspective, *i.e.* considering only direct costs.

## Results

Overall, the intention-to-treat population of the clinical trial with three treatment arms consisted of 189 patients.<sup>9</sup> Of these 189, 126 patients were included in this cost-effectiveness study ( $N=64$  in the placebo group,  $N=62$  in the small-intestinal release peppermint), of whom 120 completed the study. Baseline characteristics are presented

in Table 8.2. Compliance and missing data are described in the *Supplementary Material* and *Supplementary Table S8.1*.

**Table 8.2** Summary of patient demographic, baseline characteristics, and baseline quality of life (ITT population).

	Placebo N=64	Small-intestinal release Peppermint oil N=62
<b>Demographic data</b>		
<b>Age, years</b>		
Mean (SD)	35.5 (15.2)	32.0 (11.1)
Range	19-70	18-66
<b>Gender, N (%)</b>		
Female	49 (76.6)	51 (82.3)
<b>Setting, N (%)</b>		
Primary care	39 (60.9)	36 (58.1)
Secondary care	16 (25.0)	14 (22.6)
Combined secondary & tertiary care	9 (14.1)	12 (19.4)
<b>Employment status, N (%)</b>		
Currently studying	12 (18.8)	10 (16.1)
Employed, full- or part-time	41 (64.1)	40 (64.6)
Unemployed	2 (3.1)	3 (4.8)
Incapacitated for work	2 (3.1)	4 (6.5)
Homemaker	1 (1.6)	4 (6.5)
Retired	5 (7.8)	1 (1.6)
Missing	1 (1.6)	0
<b>IBS Quality of Life, mean score (SD) on IBS-QoL</b>	74.0 (14.2)	72.2 (14.7)
<b>Psychological comorbidities</b>		
Anxiety, mean (SD)	6.0 (4.4)	4.5 (3.9)
Minimal anxiety, N (%)	26 (40.6)	36 (58.1)
Mild anxiety, N (%)	29 (45.3)	18 (29.0)
Moderate anxiety, N (%)	4 (6.3)	6 (9.7)
Severe anxiety, N (%)	5 (7.8)	2 (3.2)
Depression, mean (SD)	7.0 (4.7)	6.6 (4.4)
Minimal depression, N (%)	22 (34.4)	27 (43.5)
Mild depression, N (%)	27 (42.2)	24 (38.7)
Moderate depression, N (%)	8 (12.5)	7 (11.3)
Moderately severe depression, N (%)	6 (9.4)	3 (4.8)
Severe depression, N (%)	1 (1.6)	1 (1.6)

Overall, a somewhat greater improvement in QoL was found in the small-intestinal release peppermint oil group compared to placebo, although these differences did not reach statistical significance (*Supplementary Table S8.2*).

Mean costs per category are presented in Table 8.3. During the eight-week treatment period, direct healthcare costs differed with the peppermint oil group showing significantly lower costs compared to placebo. Differences were mainly caused by mental healthcare utilization (Table 8.3). There were no significant differences between

groups in indirect costs during the treatment period, except for a higher productivity loss in unpaid work in the small-intestinal peppermint oil group (Table 8.3).

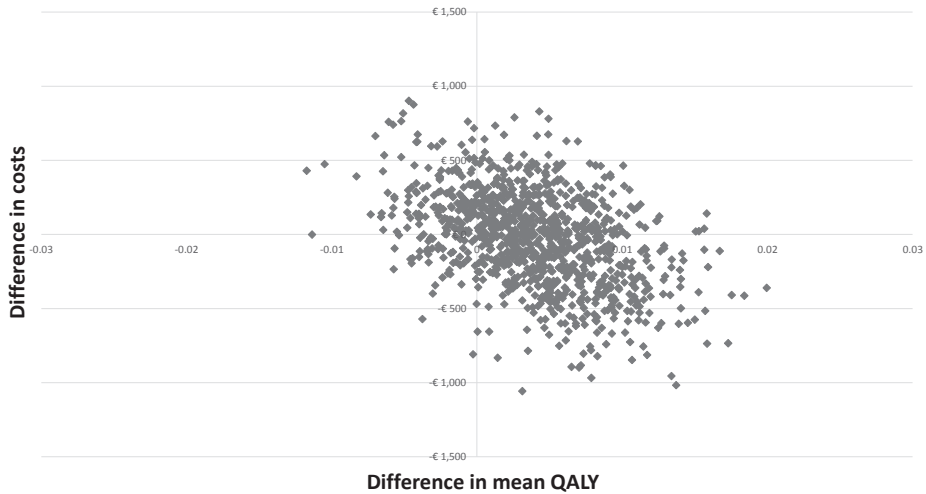
**Table 8.3** Total costs per category (ITT-population).

	Placebo N=64	Small-intestinal release Peppermint oil N=62	Difference in means <sup>‡</sup> (€) (95% CI)
<b>Costs, mean (SD) (€)</b>			
<b>Total direct costs</b>	355 (90)	161 (11)	-194 (-392;-35)*
Mental healthcare	287 (90)	69 (8)	-218 (-411;-57)*
General practice	29 (2)	19 (2)	-11 (-17;-5)*
Rehabilitation	0 (0)	0 (0)	0 (0)
Outpatient consultation	3 (1)	4 (1)	0 (-2; 3)
Company doctor	7 (2)	8 (2)	0 (-5; 6)
Homeopathy	7 (2)	8 (3)	0 (-6; 7)
Medication	2 (0)	1 (0)	-1 (-2;0)
Dietician	1 (0)	5 (1)	4 (2;5)*
Travelling-expenses	2 (0)	1 (0)	-1 (-2;0)
Treatment or diagnostics	17 (6)	6 (2)	-11 (-24;0)
Hospitalization	-	-	-
Study treatment costs	N.A.	42 (0)	-
<b>Total indirect costs</b>	818 (73)	975 (78)	157 (-55;370)
Absenteeism	386 (59)	453 (71)	71 (-103;256)
Presenteeism	364 (20)	371 (21)	7 (-50;65)
Productivity loss unpaid work	68 (10)	145 (19)	77 (37;120)*
<b>Total costs, mean (SD)</b>	1.175 (113)	1.132 (82)	-40 (-226;322)

<sup>‡</sup> Bootstrapped differences (means and confidence intervals) between small-intestinal release peppermint oil and placebo. CI Confidence Interval. \* significant (no zero in confidence interval).

## Cost-effectiveness analysis

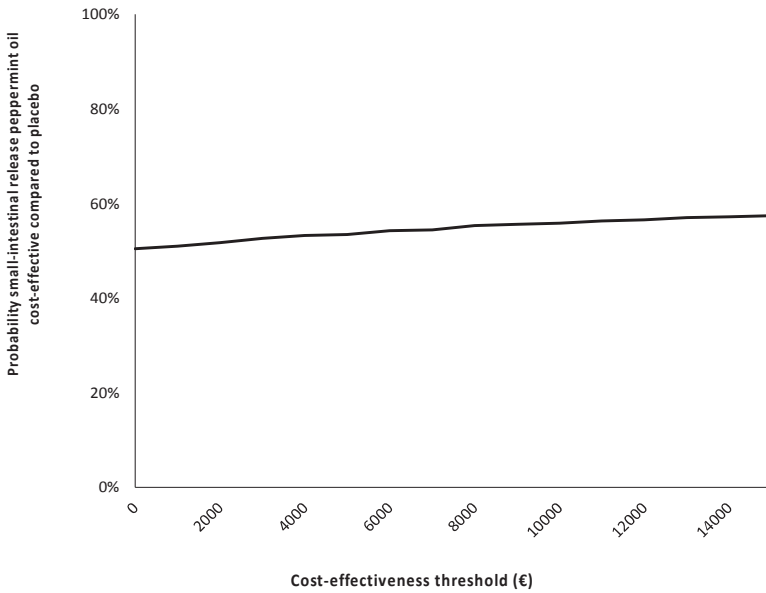
The incremental cost-savings of small-intestinal peppermint oil were €40.00, with an incremental corrected QALY gain over eight-weeks of 0.004 compared to placebo. The cost-effectiveness plane is presented in Figure 8.1 and shows that small-intestinal release peppermint oil is a dominant treatment compared to placebo in 46% of simulations (southeast quadrant, greater effectiveness at lower costs). Peppermint oil is more effective, but at a higher cost (northeast quadrant) in 31% of the simulated ratios, while it is inferior in 18% of simulations (northwest quadrant, less effective and higher costs). The net benefit acceptability curve showed that the probability of peppermint oil being cost-effective was 50% at a WTP-threshold of €1.000 and increased to 56% at a WTP threshold of €10.000 (Figure 8.2).



**Figure 8.1** Cost-effectiveness plane of small-intestinal release compared with placebo. Each data-point represents 1 bootstrapped estimate of incremental costs and baseline corrected QALYs. The bootstrapped ICERs cover all four quadrants in both planes, indicating some uncertainty of the data. 46% of simulations lie in the south-east quadrant, the quadrant indicating dominance of peppermint oil. 31% of simulations lie in the north-east quadrant, indicating higher efficacy but at higher cost. The cost-effectiveness acceptability curve (Figure 8.2) shows the probability peppermint oil is cost-effective at different WTP-thresholds.

### Sensitivity analyses

The results from the sensitivity analysis are presented in Table 8.4. When using the main clinical outcome instead of QALY, the proportion of abdominal pain responders did not differ significantly between both groups: 22/64, 29/62 in the placebo and small-intestinal peppermint oil group, respectively.<sup>9</sup> The cost-effectiveness plane showed that small-intestinal release peppermint oil is dominant in 51% of ICER simulations and more effective at higher cost in 41% of simulations. At a WTP-threshold of €5.000 per additional responder, the probability of small-intestinal release peppermint oil being cost-effective is 89% (Figure 8.3).

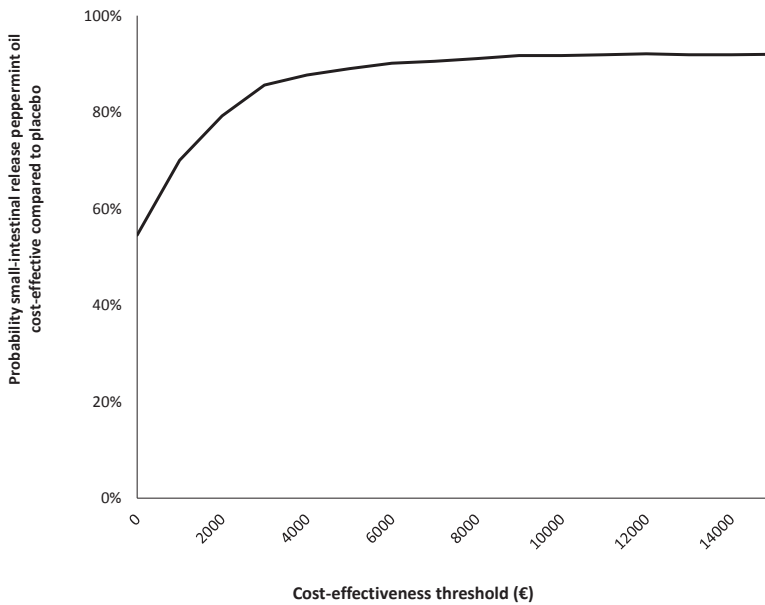


**Figure 8.2** Cost-effectiveness acceptability curve. The line indicates the probability (y-axis) of a treatment being cost-effective, *i.e.*, the proportion of replications small-intestinal release peppermint oil has the highest net monetary benefit, given various levels of willingness to pay (cost-effectiveness thresholds (x-axis)).

**Table 8.4** Results of primary and sensitivity analyses (ITT-population).

	Effect	Costs (€)	Quadrant (%)#				Probability of cost effectiveness at WTP (%)	
			NE	NW	SE	SW	€5.000	€10.000
<b>Cost utility, primary analysis (corrected QALY)</b>	0.004	-40	31	18	46	5	53	56
<b>Sensitivity analysis</b>								
Cost-effectiveness, responder ratio <sup>§</sup>	12.4	-40	41	5	51	3	89	92
Cost utility, uncorrected QALY	0.006	-40	40	6	51	3	56	58
Cost utility, health-care perspective	0.004	-195	15	5	65	15	83	85

WTP willingness to pay; NE North-east; NW North-west; SE south-east; SW south-west; QALY quality adjusted life years. # The four quadrants represent four different situations of cost-effectiveness compared to placebo. If the majority of the bootstrapped ICERs (incremental cost-effectiveness ratio) appear in the south-east quadrant of the figure, this indicates that treatment is dominant. If the majority of the bootstrapped ICERs appear in the north-west quadrant of the figure, this indicates that treatment is inferior; § The primary clinical endpoint was the percentage (%) of abdominal pain responders, according to FDA definition, with a responder being a patient with at least 30% decrease in the weekly average of worst daily abdominal pain (scored on an 11-point NRS) compared to baseline, in at least 50% of the treatment period, *i.e.* in this study four weeks.



**Figure 8.3** Cost-effectiveness acceptability curve of costs and abdominal pain responder (FDA definition). The line indicates the probability (y-axis) of small-intestinal release peppermint oil being cost-effective. At a WTP-threshold of 5.000, small-intestinal release has a probability of 89% of being cost effective when using the main clinical parameter, abdominal pain responder, as effect outcome.

When using uncorrected (for baseline differences) QALYs, peppermint oil is dominant in 51% of simulations and more effective at higher cost in 40% of simulations. When comparing uncorrected (for baseline differences) QALYs to corrected ones, the probability of peppermint oil being a cost-effective treatment at a WTP-threshold of €10.000 increases slightly from 56% to 58%.

When assessing cost-effectiveness from a healthcare perspective, small-intestinal peppermint oil is dominant compared to placebo in 65% of ICER simulations. Peppermint oil then has a 85% probability of being cost-effective at a WTP-threshold of €10.000.

## Discussion

Here, we report the results of the first trial-based economic evaluation of peppermint oil for IBS conducted in a multicenter, placebo-controlled RCT. The results show that



small-intestinal release peppermint oil may be considered cost-effective compared to placebo during an eight-week treatment, from a societal perspective at a conservative WTP-threshold of €10.000 per QALY. However, there is uncertainty surrounding the incremental cost-effectiveness ratios (ICERs). At a lower and highly conservative WTP-threshold of €1.000 per QALY, peppermint oil and placebo have an equal chance of being cost-effective. Sensitivity analyses showed similar results indicating uncertainty surrounding the cost-effectiveness of peppermint oil with the exception of the analyses using abdominal pain responder according to FDA-definition, and costs from a healthcare perspective. In these cases, peppermint oil has a much higher probability of being cost-effective compared to placebo.

IBS is highly prevalent and one of the most expensive conditions in gastroenterology.<sup>27</sup> We recently demonstrated that peppermint oil is a moderately effective treatment in patients with IBS, decreasing abdominal pain, discomfort, and IBS-symptom severity.<sup>9</sup> Although non-significant, small-intestinal release peppermint showed an abdominal pain response rate (FDA-defined) of 12.4%, which is numerically comparable to studies reporting statistically significant differences between linaclotide,<sup>28</sup> plecanatide<sup>29</sup> and tenapanor<sup>30</sup> versus placebo. These findings further warrant a trial-based economic evaluation such as the current study.

Peppermint oil is available as an OTC drug without reimbursement from healthcare insurance in many countries. Peppermint oil capsules are relatively inexpensive and this study indicates that they are likely cost-effective, showing that its use can be justified by the (albeit modest) gains in health-related QoL and cost-savings. Moreover, in light of its favorable adverse event profile, and the fact that no pharmacological treatment thus far has been able to cure IBS or improve stringent outcomes in more than half of the patients investigated, small-intestinal release peppermint oil can be a worthwhile treatment option. Peppermint oil seems particularly suited for primary care or as an initial step in therapy since more than half of patients were recruited in this setting. Our findings are further supported by preliminary model-based study suggesting cost-effectiveness of peppermint oil.<sup>31</sup> Other treatments with a high probability of being cost-effective as a treatment for IBS are anti-depressants, the low FODMAP diet, and cognitive behavioral therapy.<sup>27</sup> No direct comparisons can be made at this point due to different study designs and patient populations.

The ICER of eight weeks of peppermint oil treatment was dominant, indicating cost savings with a small health related QoL gain with the bootstrap analysis showing uncertainty surrounding the ratio. This short-term evaluation might underestimate cost-

effectiveness, since long-term savings and QALY gains are not taken into account. However, as guidelines do not currently recommend peppermint oil usage for longer than three months<sup>32</sup>, we did not perform any long-term analysis and did not extrapolate the data. Future studies should investigate the safety, effect and QALY gains of longer treatment periods.

This economic evaluation additionally investigated cost-effectiveness based on a clinical parameter instead of traditional QALYs. We used the stringent abdominal pain response outcome (FDA-defined) at a willingness-to-pay-threshold of €5000 and showed that while using this outcome, peppermint oil has an 89% probability of being cost-effective. Currently, healthcare policymakers have not defined willingness-to-pay threshold values when clinical effect measures are used instead of QALYs.<sup>24</sup> Nevertheless, given that the FDA-endpoint is recommended by drug regulatory authorities<sup>33,34</sup> and widely accepted as a primary outcome in IBS trials, we anticipate that more economic evaluations will present ICERs based on this endpoint in addition to ICERs based on more traditional QALY outcomes. This would enhance comparisons between treatments further.

The results of the current study should be considered in light of potential limitations. First, for the estimation of costs, we relied on self-reported healthcare usage and productivity losses, which may lead to recall- and social desirability bias. However, studies in the UK and the Netherlands have shown good agreement between health registry and self-reported data.<sup>35,36</sup> In addition, the bias would have been present in both groups and is therefore unlikely to have a noticeable effect. Second, a substantial part of the cost-savings within healthcare perspective was driven by differences in mental healthcare costs. This difference results in a higher probability of peppermint oil being cost-effective from a healthcare perspective as shown in the sensitivity analysis. It is questionable however, whether the difference in mental healthcare costs is a mere result of the treatment with peppermint oil in the relatively short period of 8 weeks. Baseline depression and anxiety scores were slightly higher in the placebo compared to the peppermint oil group. Therefore, despite using a valid randomization method stratified for potential effect modifiers, we cannot exclude this difference to be caused by chance and not treatment effect. Third, missing data regarding presenteeism (*Supplementary Table S8.1*), limits the validity of the results. Fourth, it is not always clear whether patients can make a distinction between IBS-related productivity loss and other comorbidities. Although we used expert opinion to make such distinction for the medical consumption questionnaire, this is not possible for the productivity questionnaire because of the generic questions. Fifth, since this was a trial-based cost-

effectiveness study we only compared to peppermint oil to placebo. A valid comparison to other treatments such as the low-FODMAP diet or cognitive behavioral therapy would require a model-based study. Sixth, patients were relatively young, female, and predominantly white. In addition, half of the population was recruited from primary care. Results may therefore not reflect cost-effectiveness in other populations. Nevertheless, the patient inclusion led to a population highly representative for IBS patients seeking treatment in daily routine clinical practice. Thereby our results are applicable to everyday practice and informative for both healthcare policy makers and providers.

In summary, treatment of IBS with small-intestinal release peppermint oil appears to be cost-effective, both from a societal and healthcare perspective, although there is uncertainty surrounding the ICER. When using abdominal pain responder instead of QALY as an outcome measure, peppermint oil has a very high probability of being cost-effective. The use of peppermint oil, which is a low-cost treatment, can be justified by the modest QALY gains and the slightly higher proportion of abdominal pain responders. More research and long-term data are necessary to confirm the cost-effectiveness of peppermint oil.

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# Supplementary Material

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## Supplementary Methods

### Patient inclusion

Patients had to be between 18 and 75 years of age and needed to fulfill the Rome IV diagnostic criteria for IBS. If alarm symptoms were present (e.g. unexplained rectal blood loss or weight loss), a colonoscopy or other relevant tests were performed to exclude organic disease. Exclusion criteria were inability to read or understand Dutch, history of GI disorders such as inflammatory bowel disease, celiac disease, or thyroid dysfunction (if not well-regulated), history of major abdominal surgery or radiotherapy interfering with GI function. An uncomplicated appendectomy, cholecystectomy, or hysterectomy were allowed unless within six months prior to screening. Other exclusion criteria were use of peppermint oil capsules in the three months prior to screening, a known allergic reaction to peppermint oil, current drug abuse, and a history of liver or gallbladder/biliary disease. Women had to use contraceptives and have a negative urine pregnancy test, or be postmenopausal for at least two years. The use of one antidepressant or one PPI was allowed, if a patient had been and would stay on a stable dose. Prohibited concomitant medications included opioids, prokinetics, stimulant laxatives (i.e. bisacodyl), linaclotide, prucalopride, and anti-spasmodic drugs. Regular use of NSAIDs, antibiotics, osmotic laxatives, and antidiarrheal drugs was prohibited.

### Determination of indirect costs

For the determination of absenteeism costs, self-reported dates of sick leave were used to determine the period for which absenteeism needed to be valued. Costs were calculated by multiplying the hours absent by average wage rates per hour (reference average wage rates used in this study can be found in *Table 8.1*).

For the determination of presenteeism costs, productivity losses were valued by multiplying hours in which work was impaired with average wage rates per hour. The number of hours in which work productivity was impaired, was determined using a self-reported inefficiency score. Productivity losses in unpaid work were valued by multiplying the hours lost in productivity with the average wage for domestic help (*Table 8.1, main manuscript*), as recommended by the Dutch costing manual.<sup>13</sup> For all other indirect costs, we used general average wage rates per hour instead of job-specific wages, as recommended by the Dutch costing manual, as this leads to less bias in favor of high-income earners. Because of general disparities in wages as a result of gender, different averages were used for women versus men (*Table 8.1, main manuscript*).

## Supplementary Results

### Compliance and missing data

Compliance to the web-based questionnaires was high: only a single patient did not complete the questionnaires at the end of the treatment period. All other patients completed all questionnaires until the end of the study or until discontinuation. However, due to a routing error, a single question regarding presenteeism was not presented to all patients at all time points. This led to missingness of this particular item in up to 50% of patients. Missingness on this topic was well balanced between treatment groups and the missing values were MCAR. Missing values regarding costs and utilities from the patients that discontinued the study ( $N=6$ ) were MAR. No other missing values occurred. An overview of the proportion of patients with missing data per cost component is given in *Supplementary Table S8.1*.



## Supplementary Tables

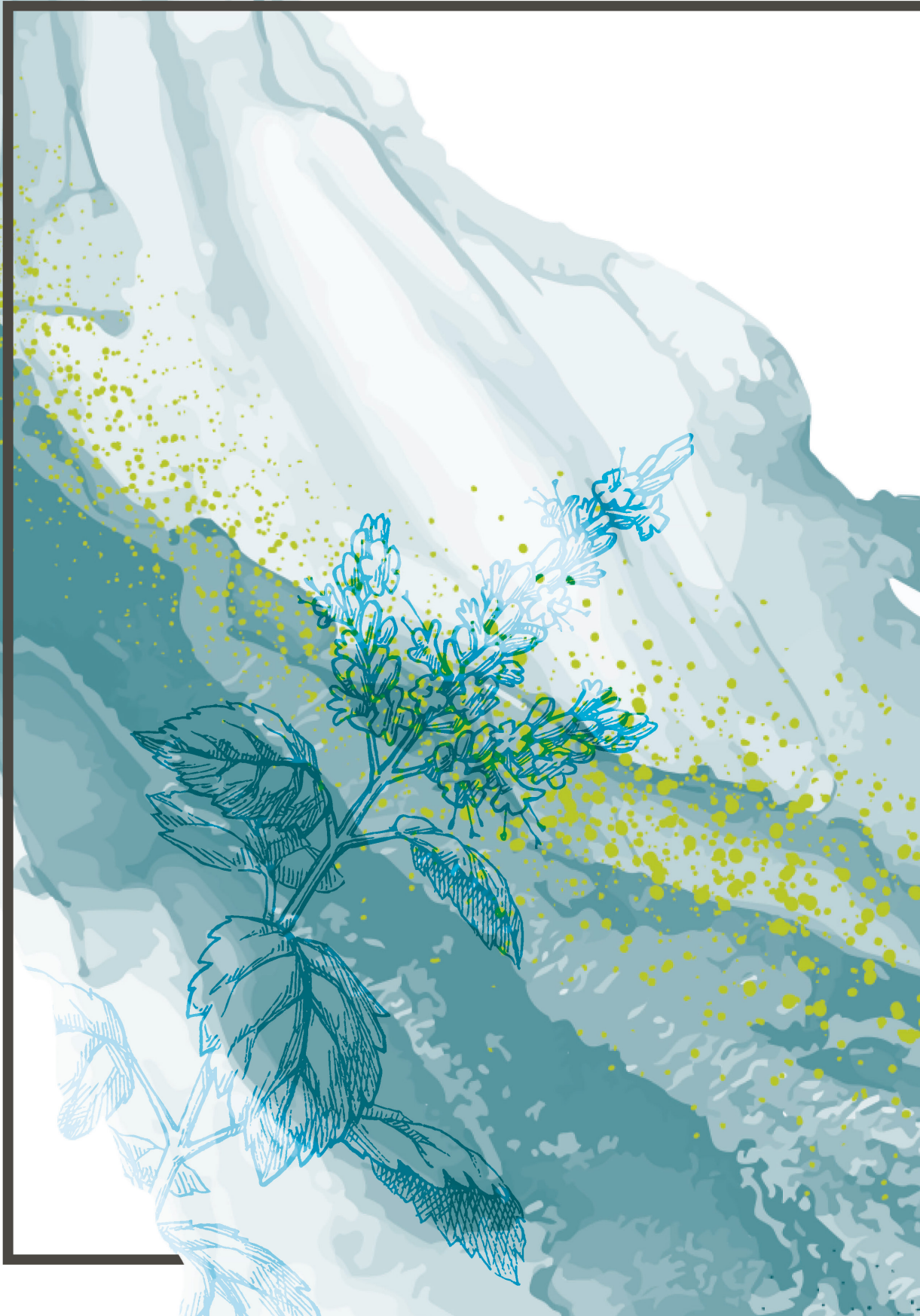
**Table S8.1** Proportion of patients with incomplete data by treatment allocation (ITT population).

	Placebo N=64	Small-intestinal release Peppermint oil N=62
<b>Utility, N (%)</b>		
Baseline	0	0
T = 4 weeks	1 (1.6)	1 (1.6)
T = 8 weeks	3 (4.7)	4 (6.5)
<b>Absenteeism, N (%)</b>		
Baseline	0	0
T = 4 weeks	1 (1.6)	1 (1.6)
T = 8 weeks	3 (4.7)	4 (6.5)
<b>Presenteeism, N (%)</b>		
Baseline	34 (53.1)	34 (54.8)
T = 4 weeks	32 (50.0)	33 (53.2)
T = 8 weeks	30 (48.4)	27 (43.5)
T = 6 months	15 (23.4)	16 (25.8)
<b>Resource use, N (%)</b>		
Baseline	0	0
T = 8 weeks	3 (4.7)	4 (6.5)

**Table S8.2** Utilities and IBS-QoL scores per measurement moment (ITT-population).

	Placebo	Small-intestinal release Peppermint oil	Treatment effect (95% CI)	P-value
	N=64 Estimated means (SE)	N=62 Estimated means (SE)		
<b>IBS quality of life score<sup>a</sup></b>				
Baseline	70.47 (2.47)	68.75 (2.56)	-	-
T = 4 weeks	73.66 (2.47)	72.87 (2.56)	0.93 (-1.75; 3.60)	0.495
T = 8 weeks	75.01 (2.50)	75.85 (2.59)	2.56 (-0.17; 5.29)	0.066
<b>EQ-5D-5L<sup>b</sup></b>				
Baseline	0.70 (0.03)	0.71 (0.03)	-	-
T = 4 weeks	0.71 (0.03)	0.76 (0.03)	0.04 (-0.01; 0.10)	0.110
T = 8 weeks	0.72 (0.03)	0.77 (0.03)	0.04 (-0.01; 0.10)	0.131

Treatment effect is the corrected difference in change from baseline between small-intestinal release peppermint oil and placebo, obtained from linear mixed modelling. P-value is level of significance of comparison between small-intestinal release peppermint oil and placebo. The treatment period consisted of eight weeks. <sup>a</sup> Assessed using the IBS-QoL questionnaire. <sup>b</sup> Assessed using the EuroQoL-5D (EQ-5D-5L) questionnaire.



# Chapter 9

Smart data collection for the assessment of  
treatment effects in irritable bowel syndrome:  
observational study

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# Abstract

## Introduction

End-of-day symptom diaries are recommended by drug regulatory authorities to assess treatment response in patients with irritable bowel syndrome (IBS). We developed a smartphone application to measure treatment response. Because the employment of an application to measure treatment response in IBS is relatively new, we aimed to explore patients' adherence to the diary and characteristics associated with adherence.

## Methods

A smartphone application was developed to serve as a symptom diary. IBS patients (based on Rome IV criteria) were instructed to fill out end-of-day diary questionnaires during the eight-week treatment. Additional online questionnaires assessed demographics, IBS symptom severity, and psychosocial comorbidities. Adherence rate to the diary was defined as the percentage of days completed out of total days. Adherence to the additional web-based questionnaires was also assessed.

## Results

Overall, 189 patients were analyzed (mean age  $34.0 \pm 13.3$  years, 77.8% female). The mean adherence rate to the diary was  $87.9 \pm 9.4\%$ . However, adherence to the diary decreased over time ( $P < 0.001$ ). No significant association was found between adherence and age, gender, or educational level, while higher anxiety scores were associated with lower adherence ( $P = 0.03$ ). Adherence to the online questionnaires was also high ( $>99\%$ ). Missing data due to technical issues was limited.

## Conclusions

The use of a smartphone application as a symptom diary to assess treatment response resulted in high patient adherence. The data-collection framework described led to standardized data-collection with excellent completeness and can be used for future RCTs. Due to the slight decrease in adherence to the diary use throughout the study, this method might be less suitable for longer trials.

## Introduction

Irritable Bowel Syndrome (IBS) is a highly prevalent chronic disorder of brain-gut-interaction characterized by recurrent abdominal pain and altered bowel habits.<sup>1</sup> Since well-defined organic causes and validated biomarkers for IBS are lacking, patient reported outcome measures (PROMs) are crucial to assess treatment response. In accordance, drug regulatory authorities currently recommend using end-of-day symptom scores in IBS trials to measure drug efficacy.<sup>2,3</sup> Diaries are generally considered suitable to measure these end-of-day gastro-intestinal (GI) symptom scores and have the ability to capture symptom variability over time.<sup>4</sup> The validity and reliability of paper diaries, however, may be impeded by fake adherence<sup>5</sup>, *i.e.* falsifying or backfilling written answers outside of the proposed time-window to forge good adherence.<sup>6</sup> The gap between reported and actual adherence to paper diaries has shown to be as large as 80% in some studies.<sup>7</sup> Because backfilling introduces considerable recall and ecological bias<sup>8</sup>, using paper diaries can distort trial results, which can ultimately lead to incorrect conclusions about treatments. Efficacy endpoints in clinical trials should therefore, preferably not be assessed by paper diaries.

Recent technological advancements and the widespread availability of smartphones have given rise to numerous health-related applications and electronic diaries in the last decade<sup>9-12</sup>, both in clinical and research settings. A digitalized data-collection provides several advantages over a paper-based data-collection as it results in higher data entry quality and more efficient data handling.<sup>13</sup> For example, responses can be verified automatically by built-in response requirements, routing, and data validation, and manual data transcription can be omitted. More importantly, data entry of previous days can be prevented and all entries can be given a date- and time-stamp, generating more valid (momentary) results and allowing assessment of actual adherence to the diary. Studies in (non-IBS) patients that have implemented electronic diaries have reported excellent adherence, ranging from 76%-100%.<sup>5,14,15</sup>

These advantages encouraged our group to implement a digital data-collection framework and develop a smartphone application that can be used as a digital symptom diary. This diary was used to collect Food and Drug Administration (FDA) recommended efficacy outcomes in our randomized placebo-controlled clinical trial (RCT) on the efficacy of peppermint oil in IBS, the PERSUADE study.<sup>16</sup> The current study describes the development and evaluates the performance of the overall digital framework used for data-collection in that clinical trial. Within the realms of IBS trials, the use of a digital symptom diary is relatively new; most previous studies did not report

the adherence to the assessment method used, and data on adherence in other populations can not necessarily be extrapolated to IBS. Therefore, our primary aim was to evaluate the performance of a custom-made digital symptom diary in patients with IBS, in particular by assessing patients' adherence. Since patient characteristics can impact adherence<sup>17,18</sup>, our secondary aim was to identify sociodemographic and clinical patient characteristics associated with adherence rate.

## Materials and methods

The present study was based on data from the PERSUADE study.<sup>16</sup> This was a randomized, double-blind, placebo-controlled trial conducted in four hospitals located throughout the Netherlands (*Supplementary Figure S9.1*). The study protocol was approved by the Maastricht University Medical Center+ (MUMC+) Ethics Committee and was registered in the US National Library of Medicine (Clinicaltrials.gov; NCT02716285). All study procedures were performed in compliance with Good Clinical Practice Guidelines and according to the revised Declaration of Helsinki.<sup>19</sup> All participants gave written informed consent prior to participation.

The study design of the PERSUADE study has been described in detail elsewhere.<sup>16</sup> In brief, the primary aim was to investigate the efficacy of peppermint oil, a conventional small-intestinal and a novel ileocolonic release formulation, in patients with IBS. To this end, patients between 18-75 years of age, who fulfilled the Rome IV-criteria for IBS and had a mean worst abdominal pain score at least 3 on an 11-point numerical rating scale (NRS, 0=no pain, 10=worst possible pain) during a 14-days pre-treatment period were included. Participants were randomized to placebo, small-intestinal release peppermint oil, or ileocolonic release peppermint oil for an eight-week treatment period.

Data-collection was performed using a customized framework for digital data-collection, specifically designed and developed for the trial, consisting of 1) a digital symptom diary (smartphone application); 2) an electronic Case Report File (eCRF, Castor EDC); 3) web-based patient questionnaires (Castor EDC); and 4) a planning tool (Ldot). During the 14-days pre-treatment and the eight-week treatment period, patients were instructed to register symptoms daily in the digital symptom diary. Study visits and telephone follow-up telephone interviews were documented in the eCRF. Patients were requested to complete several web-based questionnaires at different time-points within the study duration. The complete list of inclusion criteria and study overview with timing



of the questionnaires is given in the *Supplementary Material*. Primary efficacy results of the PERSUADE study have been described elsewhere.<sup>16</sup>

### **Digital symptom diary: smartphone application**

For the digital symptom diary, an electronic smartphone application was developed by the center for data and information management at Maastricht University (MEMIC), in close collaboration with the investigators. The app was programmed using Xamarin, a framework to develop cross-platform applications by using programming language C sharp (C#). The PERSUADE app supports Android and iOS devices. A Maastricht University industrial designer designed the visual content. A MEMIC team of data-managers and researchers of the MUMC+ Neurogastroenterology group tested the application and provided feedback throughout several phases of development. Additionally, a patient was asked to use the diary and provide feedback regarding its user-friendliness. Patient inclusion could commence once a version was reached that all agreed on.

The application's home screen consisted of three main elements, the daily end-of-day symptom questionnaire, a medication list, and the Bristol stool chart questionnaire (*Figure 9.1*). The end-of-day symptom questionnaire included one main question to assess the primary outcome (in accordance with FDA guidelines): "How would you rate your abdominal pain today? Think about the worst abdominal pain today" (0=no pain, 10=worst possible pain) (*Figure 9.2*). The daily symptom questionnaire was accessible between 6 and 12pm and was unavailable outside this time window, to avoid premature completion. Other daily questions were related to 'need of rescue medication', and 'adverse events experienced'. If a patient had not completed the daily entry before 10 pm of that particular day, one push notification was given. At the end of each week, the end-of-day questionnaire consisted of additional questions regarding abdominal discomfort, abdominal bloating, abdominal cramping, belching, nausea, and urgency during the last week (on an 11-point NRS, 0=no symptoms, 10=worst possible symptoms). There was no possibility to enter data from previous days and participants could not review prior entries. Automated routing, response requirements, and real-time data verification were built in to increase data-quality and completeness.

The medication list was used once to register all regular medication use. Patients were asked to keep their concomitant medication use as stable as possible. However, if alterations were needed, they were able to delete, add, or change dosage of (non GI-) drugs.

The Bristol stool form scale was used to register all bowel movements (Figure 9.3). There was no minimum or maximum number of registrations per day.

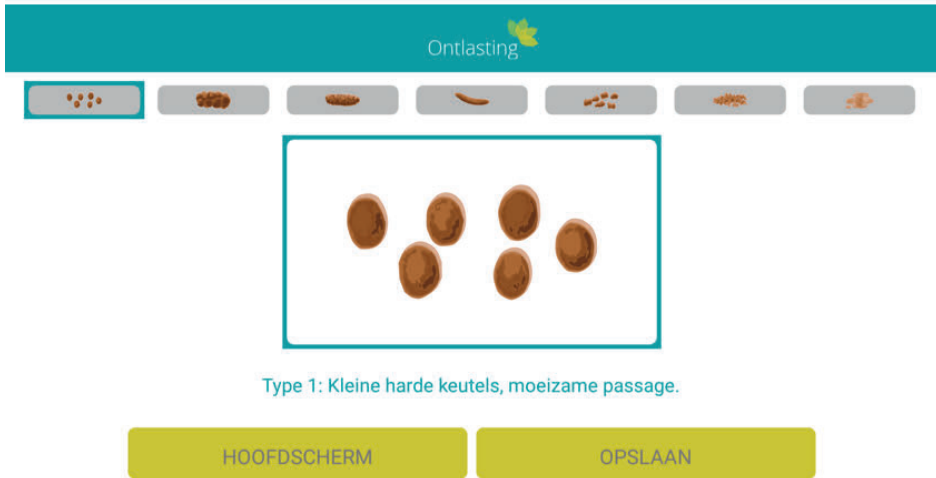


**Figure 9.1** Home screen, i.e. main menu, of the PERSUADE smartphone application. In Dutch, Medicijnenlijst means Medication list, Ontlasting rapportage means registration of Bowel habit, and Vragenlijst means questionnaire. Note that the questionnaire icon is grey, denoting that the end-of-day daily symptom questionnaire is disabled. The questionnaire was accessible and could be filled in between 6 and 12 pm each day, the icon would then become blue.



**Figure 9.2** Question in the PERSUADE smartphone application assessing the primary endpoint. Patients were requested to answer this question each night during the pre-treatment and treatment period. In Dutch, Hoe erg was vandaag de pijn in uw buik? means How would you rate your abdominal pain today? Denk hierbij aan de ergste pijn vandaag means Think about the worst pain today. Annuleren means cancel, and volgende means next.





**Figure 9.3** The Bristol Stool Chart questionnaire. Patients were requested to register each bowel movement by selecting the stool type most resembling theirs. In Dutch, ontlasting means defecation, kleine harde keutels, moeizame passage means small hard lumps, difficult to pass. Hoofdscherm means home screen, opslaan means save.

All patients received extensive verbal and written instructions during the screenings visit on how to use the application and were encouraged to contact the researchers if the application crashed or otherwise did not function properly. A personalized username and password were provided for access to the application. Patients were instructed to enable automatic updates of the application to ensure the most recent version was used. If a patient did not own a smartphone or tablet, a device was provided. During the complete pre-treatment and treatment periods, an alert system in the planning tool notified the investigators when a patient had failed to submit three or more daily entries. In addition, the development (IT) team received automated notifications of application crashes.

### Web-based patient questionnaires

At randomization, two, four, six, and eight weeks of treatment, and at three and six months of follow-up after treatment had ended, patients were requested to fill out web-based questionnaires. We chose not to implement these into the digital diary because of the large number of questions to be answered. Included were a questionnaire regarding demographics and lifestyle, and validated questionnaires regarding symptom severity (IBS Symptom Severity System), quality of life (IBS Quality of Life, EuroQoL EQ-5D-5L), comorbid symptoms of anxiety and depression (General Anxiety Disorder 7, Patient

Health Questionnaire 9), and healthcare utilization and productivity loss (Medical Consumption Questionnaire, Productivity Cost Questionnaire). Patients received invitations via email containing a HTML-link to the electronic environment (Castor EDC). If a patient had not completed the questionnaire within two days, two automatic reminders were sent via email. Automated routing, response requirements, and real-time data verification were built in to increase data-quality and completeness.

### **Electronic Case Report File (eCRF)**

During the study visits and telephone follow-up calls, investigators documented all findings in a cloud-based eCRF (Castor EDC). The eCRF forms were built by the first author with input from the other authors and contained items regarding e.g. demographics, Rome IV diagnostic criteria for IBS, history and physical examination, adverse events, and general wellbeing. Investigators were given unique usernames and passwords to view and add data for their respective inclusion centers. To achieve registration uniformity, the investigators were trained on how to enter data and additional step-by-step instructions were given in standard operating procedure documents. Real time automated data verification and corresponding pop-up notifications were built in to prevent typing errors or other erroneous entries. Automated routing of questions and response requirements ensured that correct items were displayed and filled in. An audit trail enabled tracking of all data changes.

Information on the eCRF, the web-based tool used by investigators to monitor study logistics, and privacy and data storage are given in the *Supplementary Material*.

### **Planning tool – Ldot**

Ldot is a web-based tool developed by MEMIC and was used to monitor study logistics. All personal patient data were entered into Ldot and the application supported the study workflow by indicating when each study event, e.g. randomization, follow-up call, etc., needed to take place per patient. Ldot was able to communicate with the digital diary and the web-based questionnaires (Castor EDC). For example, all email invitations for the questionnaires were sent automatically via Ldot. Patients' adherence to the diary and web-based questionnaires could be monitored within Ldot and investigators were notified if patients failed to complete three consecutive days in the diary. Investigators were also notified if patients failed to complete a web-based questionnaire after a reminder was given. To guarantee the anonymity and quality of research data, no research data could be entered into Ldot. Investigators could view and add personal data for their respective inclusion centers. There was no possibility of viewing data from

other inclusion centers, except for the coordinating investigator (first author) who had access to all data. An audit layer of the application tracked and stored information of all changes.

### **Storage, servers, and privacy**

All software and data storage complied with international ISO27001, ISO9001, and Good Clinical Practice guidelines, and Dutch NEN7510 guidelines. Electronic diary data, web-based-questionnaire and eCRF data, and privacy sensitive personal data (Ldot) were all stored on different (non-connected) servers. Several back-ups were made per day. Access to the servers was and will be restricted, with 24-hour on-site surveillance. Data will be stored for 15 years after study completion.

### **Outcome measures**

The primary outcome of the current study was patients' adherence to the digital symptom diary, defined as the mean percentage of entries and calculated by dividing the number of actually completed entries by the number of minimal requested entries (total number of days in study). Patients were instructed to complete a diary entry on all consecutive days during the 14-day pre-treatment and 56-day treatment period, or all days until discontinuation with the study.

Secondary outcomes were change in mean adherence per week over time, sociodemographic and clinical patient characteristics associated with adherence rate, mean time of diary completion, and difference in adherence between patients who were defined as responders to treatment versus non-responders. Potential data-loss and critical evaluation points were considered to explore the overall feasibility of a smartphone application as a primary data-collection tool in a RCT. Other secondary outcomes were patients' adherence to and completeness of the additional web-based questionnaires, and investigators' adherence to and completeness of the electronic case report file (eCRF).

### **Statistical analysis**

Statistical analyses were carried out using IBM SPSS statistics 25.0 for Macintosh (Armonk, NY, USA). Data are expressed as mean and standard deviation or as number plus percentage of total. Multivariable linear regression analysis was used to investigate the association between baseline patient characteristics and adherence to the digital diary, adjusting for minimization variables (age, gender, IBS-subtype, inclusion center and

treatment group). A repeated measures ANOVA was performed to assess the influence of time (weeks) on adherence. If the Mauchly's test indicated that the sphericity assumption was not met, the Greenhouse-Geisser corrected results were reported. A two-sided  $P < 0.050$  (2-sided) was considered statistically significant.

## Results

Overall, 190 patients were randomized. One patient was randomized erroneously, *i.e.* without fulfilling all inclusion criteria. Therefore, 189 patients were analyzed ( $N=64$  in the placebo group,  $N=62$  in the small intestinal release peppermint group,  $N=63$  in the ileocolonic release peppermint oil group: mean (SD) age 34.0 (13.3) years, 77.8% female, 95.8% Caucasian, 57.7% primary care). Eleven patients withdrew from the study during the treatment period (data until discontinuation were included in the analyses). Baseline characteristics are presented in *Table 9.1*. During recruitment, only a single patient stated the digital data-collection as a reason not to participate.

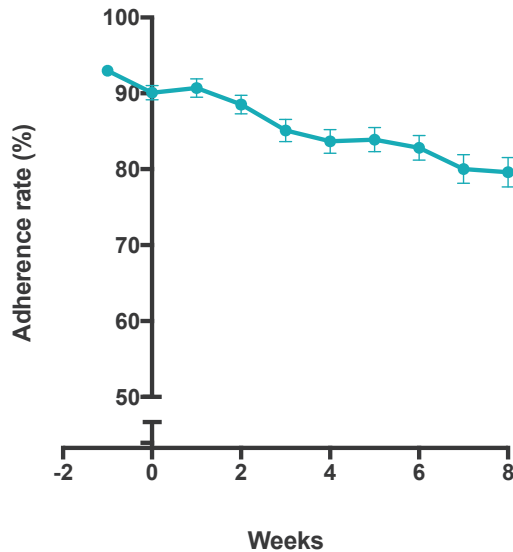
### Patients' adherence to the digital symptom diary

Most patients used their own smartphones, but four out of 189 patients needed a device provided by the investigators. Patient's adherence to the daily digital symptom diary was excellent during the entire study period, reflected by a mean (SD) completion rate of 87.9% (9.4), 91.5% (9.2) and 86.9% (10.8), during all 70 days of study duration, the 14-day pre-treatment period, and the eight-week treatment period, respectively. Adherence during the treatment period did not differ significantly between treatment groups being 87.2% for placebo, 88.3% for the small-intestinal release ( $P=0.67$  versus placebo), and 87.2% for the ileocolonic release peppermint oil ( $P=0.33$  versus placebo). Adherence did not differ between patients that were clinical responders to treatment versus patients that were non-responders, *i.e.* 88.0% and 86.2%, respectively. Over the complete study period of 70 days, a significant decrease in mean weekly patient' adherence to the end-of-day questionnaire was found, *i.e.*  $F(5.9, 1114.9)=15.5$ ,  $P < 0.001$  (*Figure 9.4*). Nevertheless, mean (SD) adherence was still good at the end of the study, *i.e.* 79.6% (26.61) (*Figure 9.4*).

**Table 9.1** Summary of patient demographic and baseline characteristics.

	N=189
<b>Demographic data</b>	
<b>Age, years</b>	
Mean (SD)	34.0 (13.3)
Range	18-70
<b>Gender, N (%)</b>	
Female	147 (77.8)
<b>Educational level, N (%)</b>	
No education	1 (0.5)
Low	15 (7.9)
Moderate	80 (42/3)
High	93 (49.2)
<b>Setting, N (%)</b>	
Primary care	109 (57.7)
Secondary care	41 (21.7)
Combined secondary & tertiary care	39 (20.6)
<b>IBS-subtype, N (%)<sup>a</sup></b>	
Diarrhea	83 (43.9)
Constipation	42 (22.2)
Mixed	40 (21.2)
Undefined	24 (12.7)
<b>IBS severity<sup>b</sup></b>	
Mean score (SD)	276.5 (71.9)
Mild, N (%)	15 (7.9)
Moderate, N (%)	100 (52.9)
Severe, N (%)	74 (39.2)
<b>IBS Quality of Life, mean score (SD)<sup>c</sup></b>	73.0 (15.1)
<b>EQ-5D-5L, mean utility score (SD)<sup>d</sup></b>	0.7 (0.2)
<b>Psychological comorbidities<sup>e</sup></b>	
Anxiety, mean (SD)	5.4 (4.3)
Depression, mean (SD)	6.8 (4.5)

<sup>a</sup> Determined in a face-to-face interview (according to Rome IV criteria); <sup>b</sup> The IBS Symptom Severity System consists of 5-items with a maximum score of 100, higher scores indicate more severe symptoms; <sup>c</sup> The IBS-Quality of Life questionnaire consists of 34-items with a 5-point Likert scale: 1=good, 5=worse quality of life; <sup>d</sup> The EuroQol-5D-5L measures 5-dimensions of QoL. Raw scores are transformed to utility scores<sup>20</sup>, which vary from 1 (perfect health) to 0 (death); <sup>e</sup> Anxiety; the GAD-7 consists of 7-items with a 4-point response scale: 0=not at all, 3=almost every day. Depression; the PHQ-9 consists of 9-items with a 4-point response scale: 0=not at all, 3=almost every day.



**Figure 9.4** Adherence rate per week to the digital symptom diary in the PERSUADE study, which was defined as the mean percentage of entries and calculated by dividing the number of actually completed entries over the number of minimal requested entries. Dots represent mean adherence rate (%) plus standard deviations.  $N=189$ . The decrease in adherence throughout the pre-treatment and treatment period is statistically significant,  $F(5.9, 1114.9)=15.5, P<0.001$ .

When exploring independent baseline predictors for adherence, the combined regression model that included all minimization variables (age, gender, IBS-subtype, inclusion center), treatment group, baseline IBS symptom severity, anxiety and depression scores, and educational level, showed that one of the four inclusion centers, *i.e.* center C (*Supplementary Figure S9.2*), (regression coefficient  $B -10.04$ , 95% CI  $-19.51$ ;  $-0.56$ ,  $P=0.04$ ) and anxiety scores at baseline ( $B -0.59$ , 95% CI  $-1.12$ ;  $-0.06$ ,  $P=0.03$ ) were negatively associated with adherence throughout the study. Indeed, when comparing adherence between the different inclusion centers, mean (SD) adherence in center C was lowest, *i.e.* 82.3% (12.5), compared with 88.3% (9.2), 84.7 (14.0), and 91.4% (7.7), in centers A, B, and D, respectively. Mean time of completing the end-of-day symptom diary was 9:46 pm, *i.e.* 14 minutes before receiving the push notification.

### Feasibility of a smartphone application as primary data-collection method

Several technical issues were noted by the investigators or reported by patients. In most cases, the cause was found and the issue was resolved by the app development team

without data-loss. Encountered hurdles included difficulties installing the application during the screening visit due to connectivity failure, not receiving reminder notifications, inaccurate visual scaling of questionnaires on smaller smartphone screens, updates of Android or iOS operating systems that interfered with prior processes, and connectivity failure due to server maintenance. All (documented) technical issues and their (short-term) consequences are presented in *Table 9.2*.

### **Web-based questionnaires: patients' adherence and completeness**

Adherence to the web-based questionnaires was also excellent. One patient did not complete the questionnaires at the end of the treatment period; all others completed all questionnaires until the end of the study or until discontinuation ( $N=11$  discontinued with the study). Halfway through the study duration, however, a routing error in one questionnaire became apparent. Although this mistake was corrected immediately, the error had already led to missing data for that (one) particular question in 23.3%-54.0% of all patients, depending on measurement moment (*Supplementary Table S9.1*). No missing items were found in other questionnaire items.

### **Investigators' adherence to the eCRF**

Adherence of the investigators to the eCRF was excellent with a completion rate of more than 99%. In total, there were 27 patients with at least one missing variable in the case report file, 11 of whom discontinued with the study during the treatment period (the missing values comprehend follow-up calls that were not conducted). The remaining 17 cases with missing data were because of missed follow-up calls (in 11 cases, one out of three follow-ups was missed), not registering if additional information about the six-months follow-up period was given, not registering the date of the last menstruation, not registering if the GP was informed about participation in the study, or not registering the number of capsules that were reported not to be taken during one of the follow-up calls.

**Table 9.2** Technical difficulties and consequences with regard to the digital symptom diary.

Description of technical issue	N patients affected	Consequence and if applicable, solution.
Low internet connectivity hindered installation of the application during the screening visit	15	In most cases, the problem was solved by moving to a location with better internet connectivity, or by postponing the installation to a later time.
Not receiving push-notifications as a reminder to complete the end-of-day questionnaires	12	In many cases, patients would complete the questionnaire regardless of receiving the notification. However, the exact effect is unknown and it may have negatively impacted adherence during days no notification was received. In most cases, the problem could be resolved by changing the telephone settings, e.g. by ignoring battery optimizations. In two cases in which the issue could not be resolved, reminders were given during the study period by setting the alarm of the device at 10pm. In the short period during which it was unknown how many devices were affected, additional text-messages were sent as a reminder.
Incomplete views of the questions due to a too large scaling on smaller smartphone screens.	8	The issue was resolved by adjusting the scaling in the application during updates. Because only a few letters were not depicted correctly and because all participants had received a manual that included the actual questions asked, the negative effect of short-term scaling issues is estimated to be negligible.
iOS or Android updates that interfered with prior settings of the applications	-	The issue did not lead to missing data because the small bugs did not shut down the application. The development team would provide updates that resolved the issues as soon as possible.
Maintenance of the hosting server	21	The issue led to missing data of one complete day, i.e. the day at which the maintenance took place, in all but 2 patients that were included at the time of the maintenance.

## Discussion

The results of the current study demonstrate that patients' adherence to the end-of-day questionnaire in the digital symptom diary was excellent, with a mean completion rate of 87.9% over 70 days of study duration. The total proportion of missing data and data-loss due to technical issues of the application was small, indicating that it is safe and realistic to use the application as a primary data-collection method. Furthermore, patients' adherence to the web-based questionnaires and investigators' adherence to the eCRF were also outstanding with completion rates of more than 99%.



In terms of electronic diary usage in clinical trials, the adherence rate found in this IBS study is at least comparable to or even higher than rates previously reported rates.<sup>5,14,15</sup> Most people in the Netherlands own a mobile phone, i.e. 90.3% (Statistics Netherlands).<sup>21</sup> Only few patients ( $N=4$ ) needed a device from the investigator team to participate in the study and only a single patient stated the digital data-collection as a reason not to participate. Mean adherence to the digital symptom diary decreased with  $\pm 11\%$  from the first week of the pre-treatment period, to the last week of the eight-week treatment period (Figure 9.4). A slight decrease in adherence to the diary during a study period, i.e. logging fatigability, is not uncommon and has also been observed in other studies investigating digital diaries.<sup>5,22</sup> Regarding the usage of digital diaries in RCTs to assess treatment response (according to FDA-recommended definitions) in IBS patients specifically, we are aware of one recent IBS study that applied an electronic diary to assess treatment effect. However, a direct comparison with this study was not possible, as details on the type of device, application, or adherence to the diary were not provided.<sup>23</sup>

With regard to sociodemographic and clinical patient characteristics associated with completing the daily entries in the diary, we found no evidence for a statistically significant effect of gender, age, or educational level on adherence. This differs from the results of some prior studies and meta-analysis, which observed for example a statistically significant positive effect of age on adherence.<sup>15,17</sup> Our interpretation is that this may be caused by the relatively young patient population in the current study. We observed that patients with higher anxiety scores had lower adherence to the digital symptom diary. These data are in line with those of Aaron *et al.*, showing that participants with higher stress levels may have lower completion rates.<sup>24</sup> Interestingly, a negative association was found between one inclusion center and adherence. All four inclusion centers were located in urban areas, but with a wide geographical spread throughout the Netherlands as shown in *Supplementary Figure S9.2*. The center with the negative association (C) was the center in the most urban and populated area, i.e. the Amsterdam-The Hague-Rotterdam-Utrecht urban agglomeration. No obvious demographical or baseline differences were observed between study populations in different inclusion centers. No significant association was found between the lower adherence and the investigator by whom the instructions were given. Although the reason for a lower adherence of patients included in this center is unclear, religious and cultural backgrounds of inhabitants of this agglomeration may have differed from those of the inhabitants of other geographical areas.<sup>25-27</sup> Nevertheless, overall mean (SD) adherence during the treatment period in this inclusion center was still good, i.e. 82.3% (12.5).

In terms of technical issues arising during the study, minor bugs occurring as a consequence of ever evolving smartphones and operating systems are practically inevitable. It is our experience therefore that continuous maintenance and software updating by a development (IT) team is crucial to avoid data-loss and potential agitation of the study participant due to application malfunctioning. Consequently, the feasibility of using a smartphone application as a primary data-collection method depends to a large extent on skills and availability of development team staff and research groups should check if appropriate support is available before opting for such methods.

Many high-quality IBS trials have used Interactive Voice Response Systems (IVRS) as the primary data-collection method.<sup>28-31</sup> In spite of this frequent use, the IVRS used in IBS trials have not been described in detail, thereby hampering replication and implementation of the used methodology in other trials. For comparison to our methodology, we therefore depended on what is known about IVRS in general. Akin to a digital symptom diary, the IVRS method allows control of time-windows in which surveys should be completed, provides automated time-stamps to answers, performs data verification and validation, follows a predefined routing schema, enables automatic reminders, collects and stores data “real-time”, and leads to an overall consistent survey administration. In addition, both methods equally depend on telephone- or internet-service, and require staff to program and maintain the software. A potential advantage of the IVRS over the digital diary is that it does not depend on literacy skills of the participant. An IVRS may also need fewer software updates than required by smartphone applications due to the high paced updates of operating systems. Potential disadvantages of the IVRS compared to a digital symptom diary are e.g. 1) the inability to get clarification during the survey, whereas a digital symptom diary can have built-in optional clarification of questions; 2) not all IVRS are equipped with speech-recognition, open-ended questions then require transcription by a data-manager; 3) the quality of open-ended question recordings is dependable on enunciation, background noise, and connection; and 4) usage of the IVRS requires extensive participant training and could be less user-friendly.<sup>32</sup> As for patient adherence to the IVRS, this was reported by only one recent IBS trial. They reported a mean adherence rate of 71% and 73% in the two groups examined, when adherence was defined as completing at least 80% of the scheduled calls to the IVRS.<sup>30</sup> The adherence to the IVRS in that study was thus notably lower than the adherence to the digital symptom diary found in the current study.

The current study described the overall framework for digitalized data-collection used in the PERSUADE study. In addition to the digital symptom diary, the electronic framework used in this drug trial consisted of web-based patient questionnaires and an

electronic CRF to collect additional secondary outcomes. A troublesome issue that occurred was a routing error in one of the questionnaires that was discovered too late and had already led to a high proportion of missing data (*Supplementary Table S9.1*). This applied to only a single question, but routing errors can have potentially disastrous consequences. As such, investigators and data-managers should take appropriate care and time when testing questionnaires. Data-exports should furthermore be examined in an early testing phase and preferably by more than one investigator and data-manager. Similar to the diary, the web-based questionnaires and the eCRF featured built-in routing of questions, data validation, and response requirements to stimulate data-quality and completeness. Overall, these steps allowed for guaranteed standardized data-collection with a completeness of more than 99% for the web-based questionnaire and eCRF items.

Additional advantages of the combined framework for digitalized data-collection are: 1) the ability to monitor patients and their adherence; 2) a reduction in paperwork and physical archiving, e.g. in this study the paperwork was reduced to one single informed consent file; 3) manual data transcription can be omitted as research data enter the database immediately; 4) the possibility to adjust and individualize the smartphone application, eCRF, and web-based questionnaires according to the needs of each particular study and 5) more accurate and standardized data reporting as no error-prone re-entry necessary as compared to paper diaries. The described framework for digitalized data-collection can therefore be employed across different disease entities.

Our findings should be interpreted in light of some potential limitations. First, the study was not primarily designed for the analysis of adherence to the digital symptom diary, but for measuring the main clinical outcome.<sup>16</sup> However, since almost 200 patients were included, the sample size is sufficiently large to estimate adherence with enough precision. Second, adherence rate was not assessed within a controlled trial with a more traditional method of data collection (i.e. paper-and-pencil diaries, IVRS) as a comparison. However, the rapid diffusion toward digitalized approaches in healthcare and clinical research renders such comparison less meaningful from a practical point of view as the use of such techniques become inevitably ubiquitous. In addition, it is unlikely that these traditional approaches to data collection would result in even higher adherence than those observed here. Another limitation was that patient satisfaction with the digital diary or web-based questionnaires was not quantified by means of a questionnaire. In the current study, the feasibility of the used framework was evaluated primarily on the basis of patients' and investigators' adherence and the proportion of complete data, whereas quantified patient satisfaction was not taken into account.

However, a low patient satisfaction would have likely led to a lower adherence and thereby a higher proportion of missing data. Therefore, it is unlikely that applying such a questionnaire would have altered our main findings.

In conclusion, in this IBS drug trial, the use of a smartphone application as a digital symptom diary to assess treatment response was found to be highly feasible and resulted in high quality data-collection with an excellent patient adherence of more than 86% during the complete study period. The combination of the digital diary with the eCRF, planning tool and web-based-questionnaires in this study led to an overall standardized state-of-the art data-collection with excellent completeness and can be used as a framework for future RCTs. Due to the slight decrease in patient adherence to the digital diary throughout the study, caution is needed when using such methods in long-term studies. Although this framework was designed for IBS clinical trials, the results reported here are of added value for a far broader range of disorders for which the collection of PROMs is required. Future studies should preferably include a control group, for example a group using the IVRS or a group using the app without receiving reminding notifications, to compare adherence and to ascertain specific factors driving high adherence.

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# Supplementary Material

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## Supplementary Methods

### In- and exclusion criteria

#### Eligibility criteria for pre-treatment period

In order to be eligible to participate in the run-in period of this study, subjects must meet all the following criteria:

1. Age between 18 and 75 years;
2. Diagnosed with Irritable Bowel Syndrome according to the Rome-IV criteria<sup>26</sup>:
  - Recurrent abdominal pain, at least 1 day/week for the last 3 months;
  - Symptom onset at least 6 months prior to diagnosis
  - Associated with two or more of the following:
    1. Pain related to defecation;
    2. Pain associated with a change in frequency of stool;
    3. Pain associated with a change in form (appearance/consistency) of stool;
3. Based on the medical history and previous examination, no other causes for the abdominal complaints can be defined. Especially no history of:
  - a. Inflammatory Bowel Disease;
  - b. Celiac Disease;
  - c. Thyroid dysfunction (if not well-regulated);If alarm symptoms (including unexplained rectal blood loss or weight loss) are present, a colonoscopy has been performed and was negative for other causes.
4. Women in fertile age (<55 years old) must use contraception or be postmenopausal for at least two years.

#### Exclusion criteria for pre-treatment period

A potential subject who meets any of the following criteria will be excluded from participation in this study:

1. Insufficient fluency of the Dutch language;
2. Any previous use (also incidental use) of peppermint oil capsules in the last 3 months prior to inclusion (the use of peppermint tea, menthol candy etc. is allowed);
3. The inability to stop regular use of medication affecting the gastro-intestinal system (such as Non Steroidal Anti Inflammatory Drugs (NSAID), laxatives, prokinetics, opioids, spasmodolytics and anti-diarrhoeal drugs). This use should be halted at least 1 week before enrollment into the run-in period;
  - a. The use of 1 antidepressant drug is allowed, providing dosing has been stable for  $\geq 6$  weeks before enrollment;
  - b. The use of 1 proton pump inhibitor (PPI) is allowed, providing dosing has been stable  $\geq 6$  weeks before enrollment;
4. Previous major abdominal surgery or radiotherapy interfering with gastrointestinal function:
  - a. Uncomplicated appendectomy, cholecystectomy and hysterectomy allowed unless within the past 6 months;



- b. Other surgery upon judgment of the principle investigator;
- 5. History of liver disease, cholangitis, achlorhydria, gallstones or other diseases of the gallbladder/biliary system;
- 6. Pregnancy, lactation;
- 7. Using drugs of abuse;
- 8. Known allergic reaction to peppermint.

### Inclusion criteria for the actual treatment period

In order to be eligible to participate in this study, subjects must meet all of the following criteria:

- 1. No changes in in- and exclusion criteria for the run-in period have occurred;
- 2. Average worst abdominal pain score (on 11-point NRS) of > 3, during the 14-days pre-treatment period.

## Supplementary Table

**Table S9.1** Proportion of patients with missing data in web-based questionnaires.

	N=189
<b>Missingness of all questionnaires due to discontinuation or non-response, N (%)</b>	
Baseline	0
T = 4 weeks	6 (3.2)
T = 8 weeks	12 (6.3)
T = 3 months	11 (5.8)
T = 6 months	11 (5.8)
<b>Missingness in question regarding presenteeism due to a routing error, N (%)</b>	
Baseline	102 (54.0)
T = 4 weeks	95 (50.3)
T = 8 weeks	86 (45.5)
T = 3 months	69 (36.5)
T = 6 months	44 (23.3)

# Supplementary Figures

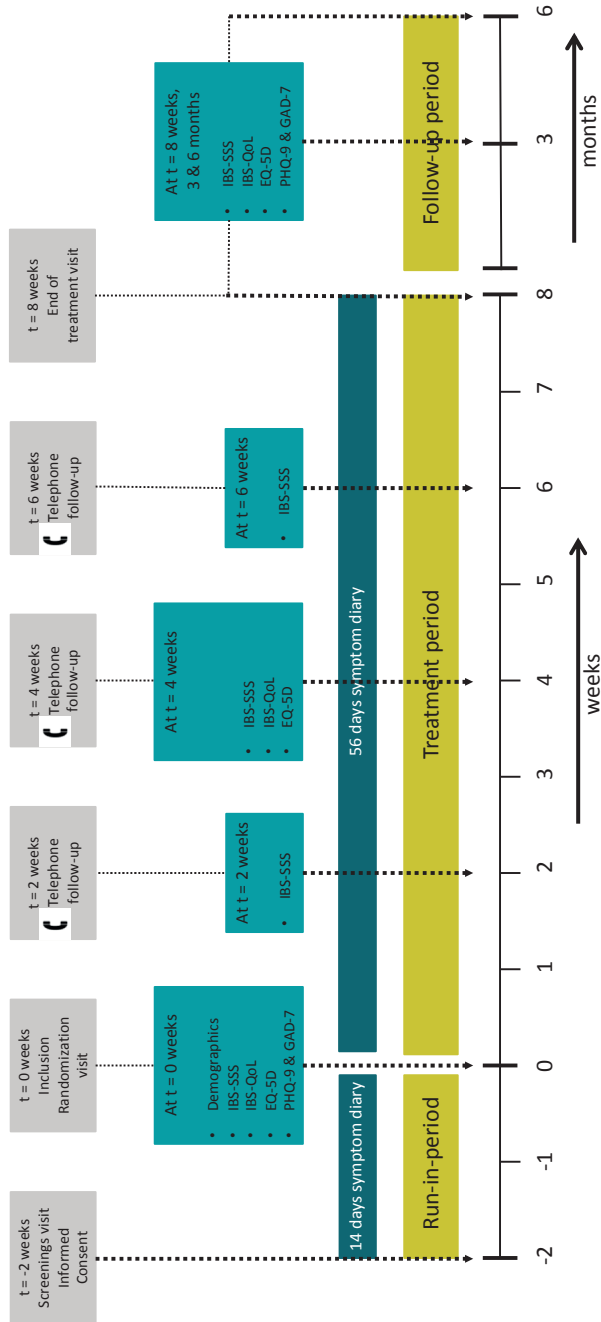
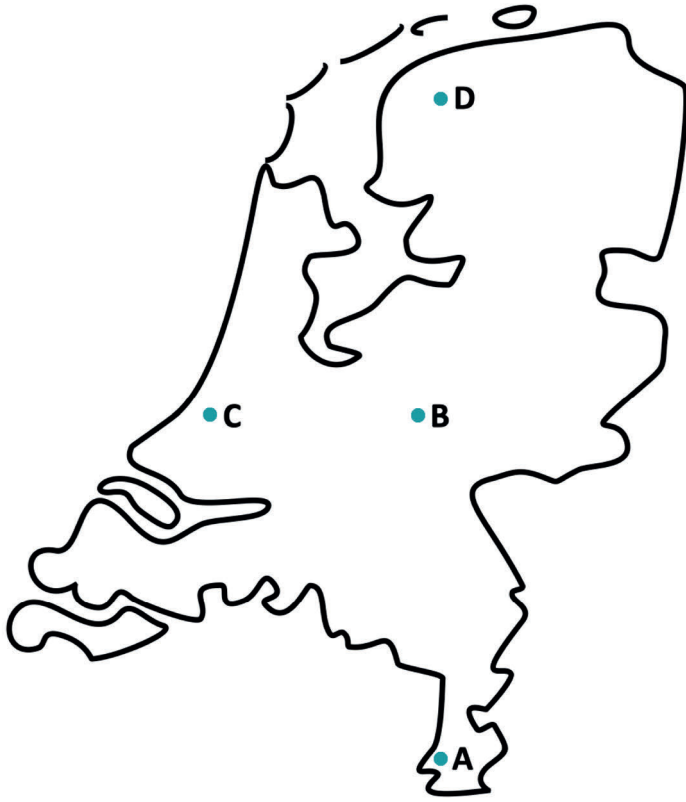
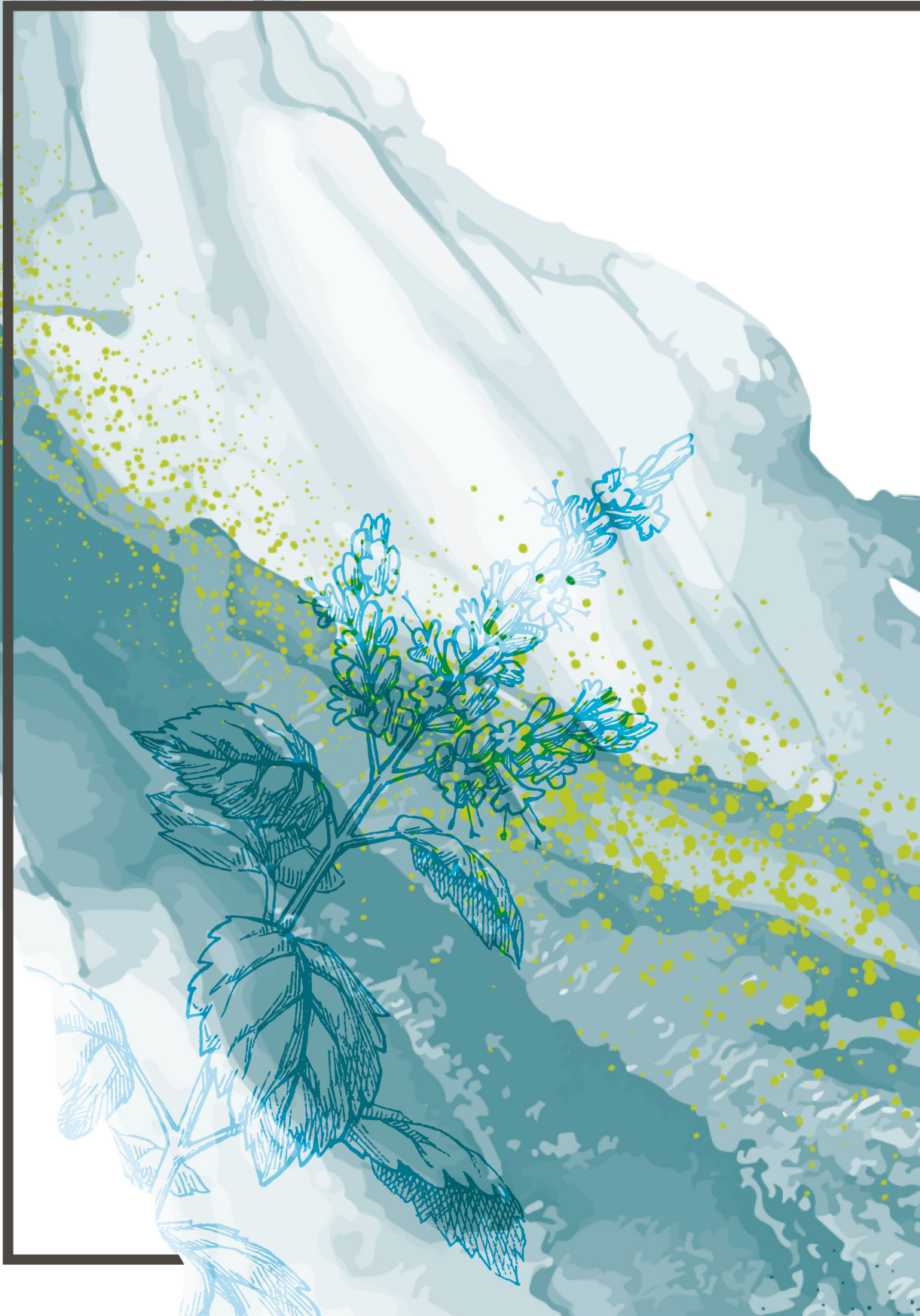


Figure S9.1 Study design of the PERSUADE study.

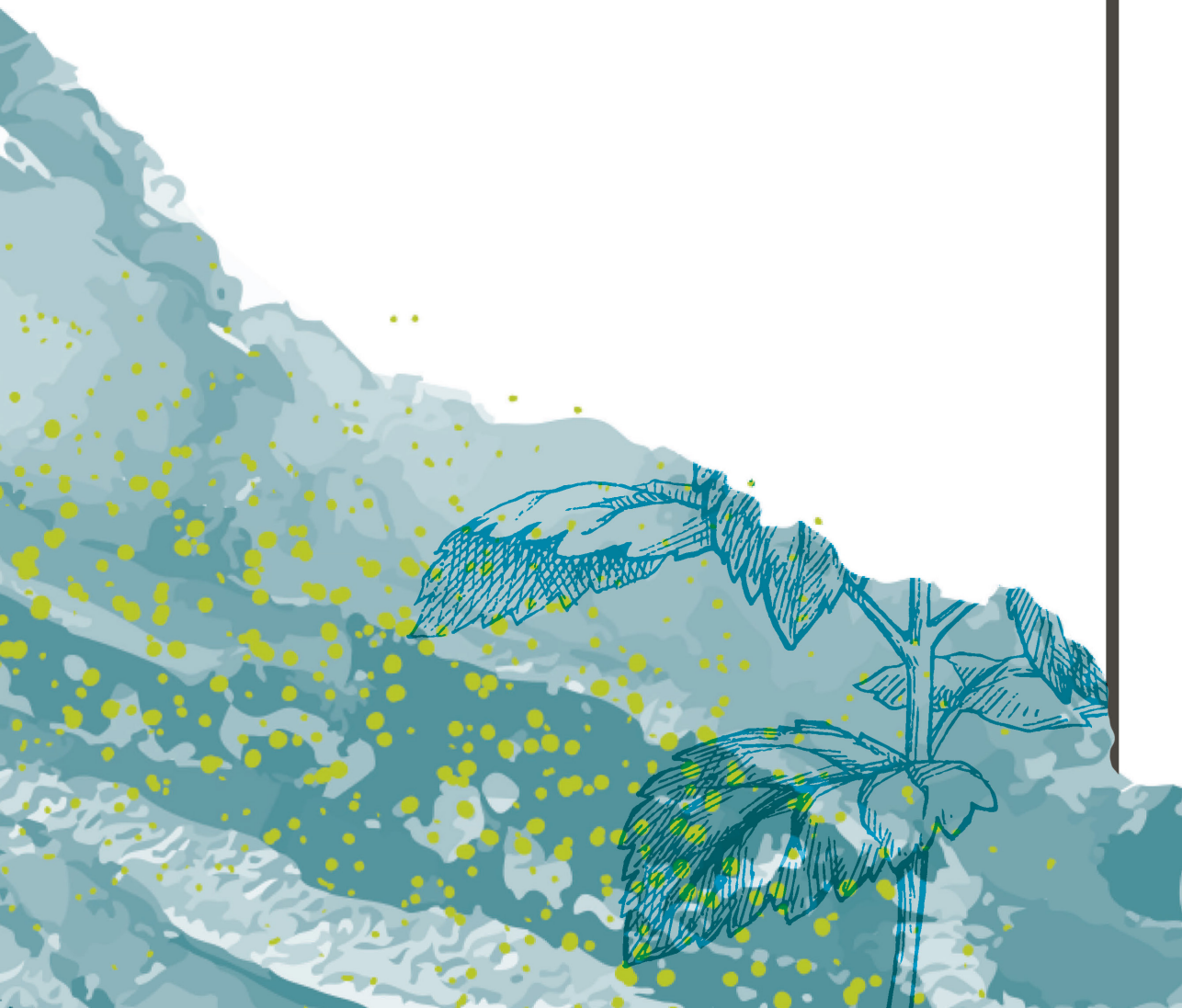


**Figure S9.2** Inclusion centers.



# Chapter 10

General discussion





## General discussion

In this thesis, we have focused on the diagnostic criteria, targeted treatment, and socioeconomic burden of irritable bowel syndrome (IBS). We showed that patients who fulfill the updated and more restrictive Rome IV criteria are more likely to be younger women, with more severe gastrointestinal (GI) symptomatology, more severe psychological comorbidities, and lower quality of life, compared to patients with similar symptoms who do not fulfill the Rome IV criteria (**chapter 2**). In **chapter 3**, we found that 30% of patients did no longer fulfill the Rome III criteria for IBS after a follow-up period of five years. However, this reduction in GI symptoms was not paralleled by improvement in quality of life or life satisfaction. Our data suggest that quality of life and general well-being are more related to concurrent anxiety and depression scores than to GI symptom severity. To further improve patient outcome and quality in life in IBS, better and possibly more targeted treatments are needed.

In **chapter 4**, we provided a comprehensive overview of transient receptor potential channels as novel treatment strategies in patients with IBS. Transient receptor potential melastatin 8 (TRPM8) is of particular interest because menthol, the primary constituent of peppermint oil, is an agonist for the TRPM8 receptor. Peppermint oil itself is a commonly used therapeutic agent in IBS. In **chapter 5**, we therefore focused more specifically on the TRPM8 channel and explored neuro-immune interactions possibly underlying symptom generation in IBS. By using various experimental techniques, we shed light on the potential anti-inflammatory function of TRPM8 in IBS. Because of a relative lack in high-quality evidence for efficacy of peppermint oil, we went on to further evaluate the clinical performance of peppermint oil in IBS. First, in **chapter 6**, we demonstrated that a newly developed ileocolonic release peppermint oil formulation had a significantly later mean peak menthol concentration in the blood, in comparison with the conventional small-intestinal release peppermint oil. This finding points to a more distal, presumably ileocolonic, release of peppermint oil in the intestine when given in the ileocolonic release peppermint oil formulation. Thereafter, in **chapter 7**, we investigated the efficacy and safety of small-intestinal and ileocolonic release peppermint oil in patients with IBS in a large multicenter randomized controlled trial, the PERSUADE study. We found that peppermint oil, in both formulations, was not superior compared to placebo when using the FDA recommended primary outcome of abdominal pain response. Small-intestinal release peppermint oil, however, showed significant improvements in secondary outcomes of IBS symptoms. We also showed that small-intestinal peppermint oil appears to be cost-effective in patients with IBS during an eight-week treatment period (**chapter 8**). In the final chapter, **chapter 9**,

we described the custom-made framework for digitalized data collection in which we used a smartphone application as a digital end-of-day symptom diary. We found that our method led to high patient and investigator compliance and an overall state-of-the-art standardized data collection with excellent completeness.

## Diagnostic criteria

As IBS is a disorder of gut-brain interaction without a clear organic cause, the diagnosis is currently based on the presence of typical symptoms. The Manning criteria, published in 1978, were the first criteria described to aid the diagnosis of IBS<sup>1</sup>, followed by the Rome diagnostic criteria for FGIDs.<sup>2</sup> Generally, the symptom-based Rome diagnostic criteria have benefited IBS research as they have provided a clear definition of criteria patients should fulfill in IBS studies. This increases the reproducibility of research findings. Nevertheless, applicability of the criteria in daily clinical practice is limited<sup>3</sup>, as was also shown by a study of our group. Using a survey across 11 European countries, our colleagues found that only about one third of general practitioners (GP) use the Rome diagnostic criteria to diagnose IBS in daily practice.<sup>4</sup> This may reflect that the diagnostic process in IBS is challenging, despite the fact that diagnostic criteria have been defined and generally accepted. Reasons for this include: 1) IBS symptoms can resemble those of other organic disorders, e.g. inflammatory bowel disease or microscopic colitis, and the criteria do not provide clear leads to differentiate between these and IBS<sup>5</sup>; 2) IBS symptoms vary considerably both between and within patients; 3) underlying pathophysiological mechanisms may differ between subgroups of IBS patients<sup>6</sup>; 4) precise, objective and non-invasive biomarkers are currently not available, hence, there is no simple test that can accurately diagnose the disorder<sup>7</sup>; and 5) clinicians are not aware of the symptom-based diagnostic criteria or may not be confident enough to employ them. A new problem arose when in 2016 the previous Rome III criteria were updated to the more restrictive Rome IV criteria: what to do with patients who do no longer fulfill the diagnostic criteria for IBS? These patients remain to have the same symptoms and unfulfilled needs. Using end-of-day symptom diary based surrogate markers, we investigated how the change to more stringent criteria would impact IBS prevalence (**chapter 2**). We found that 12.6% to 38.4% of Rome III-positive patients would no longer fulfill the updated Rome IV criteria for IBS, depending on definitions used. Our results were corroborated by another study, in which the investigators found that 15% did no longer fulfill the updated IBS criteria.<sup>8</sup> More recent population-based studies have also confirmed the decrease in IBS prevalence when using the Rome IV criteria: Van Houte *et al.* and Jossan *et al.* found prevalence rates of 5.5% in the general Belgian population and 5.1% in a Canada-UK-US population, respectively.<sup>9,10</sup> This is a



decrease of approximately 6% compared to population prevalence rates on the basis of the Rome III criteria.<sup>11</sup> Consequently, clinicians need to be aware of the fact that a significant percentage of patients presenting with IBS symptoms, does not fulfill the more restrictive Rome IV criteria, though they are in need for therapy, similar to patients fulfilling the criteria. Under the new Rome IV criteria, the majority of patients would rather be categorized as having functional constipation, functional diarrhea, functional abdominal bloating/distention or unspecified bowel disorder. The diagnostic confusion may lead to delay or lack of appropriate treatment. Furthermore, patients who are Rome IV negative are excluded from participation in clinical trials on novel therapeutic strategies that may otherwise benefit them. Although this is directly related to the update in criteria, we showed that even patients who did no longer fulfill the preceding Rome III criteria for IBS after a 5-year follow-up period still presented with moderate GI symptoms and had comparable scores of impaired quality of life and general well-being as those who still fulfilled the criteria (**chapter 3**). Failing to treat symptoms that hamper quality of life and affect patients' everyday functioning may eventually result in increased health-associated costs. We therefore pragmatically advise that patients who present with functional GI symptoms, but do not fulfill the diagnostic Rome IV criteria, should still be managed as having a functional GI disorder.

Diagnosing IBS in daily practice is complicated by it often being a diagnosis by exclusion.<sup>12</sup> As a consequence, a large proportion of patients undergo numerous diagnostic investigations to exclude organic disorders, even though studies have shown that the yield of these is rather low and not substantiated or evidence-based.<sup>12,13</sup> The diagnostic work-up of IBS often is redundant, with significant costs and challenges to the financially restrictive healthcare system. The high prevalence of IBS, the associated frequent usage of healthcare resources and loss in work productivity (*i.e.* absenteeism and presenteeism) result in a considerable global impact on society and associated economic burden.<sup>14</sup>

### **The challenging (re)search for better therapies**

Another major contributing factor to the substantial socioeconomic burden is the limited efficacy of most therapeutic modalities for IBS. Neither low-cost traditional, nor new and more costly pharmacological agents for IBS have been able to provide more than 8-20% therapeutic gain over placebo.<sup>15</sup> Novel therapeutic strategies that not only provide more global relief, but are also cost-effective are therefore urgently needed.

Peppermint oil is a promising low-cost drug for IBS, but evidence from high quality randomized controlled trials was still limited. We investigated its efficacy in a well-designed trial according to FDA and European Medicines Agency (EMA) guidelines (**chapter 7**). In addition to the conventional enteric-coated peppermint oil with small-intestinal release, a newly developed ileocolonic release peppermint oil (**chapter 6**) was studied. We hypothesized that both formulations would be superior compared to placebo, but no statistically significant differences were found when using the pre-specified strict FDA and EMA recommended endpoints. In this regard, the first clinical trial with peppermint oil in a Rome IV-defined IBS population of both primary and secondary care patients showed a negative primary outcome when using these robust stringent endpoints. However, the difference in primary abdominal pain response rate (FDA recommended endpoint) between placebo and small-intestinal release peppermint oil in our study, *i.e.* 12.4%, is comparable to differences in response rate of other IBS therapeutic trials; *e.g.* using linaclotide<sup>16</sup>, or plecanatide.<sup>17</sup> It should be noted that in these studies much larger patient populations were studied compared to our study. Although the PERSUADE study is the largest peppermint oil RCT in patients with IBS up to date, we cannot exclude that our study was underpowered to detect statistically significant effects when using these pre-specified stringent endpoints. A type-II error may have occurred since we powered the study for an expected 30% difference that had previously been reported by a meta-analysis.<sup>18</sup>

#### *The importance of appropriate outcome measures*

In general, clinical trials investigating treatment for IBS are hindered by high placebo response rates estimated at around 40%<sup>19</sup>, the chronic relapse-remitting nature, and potentially elusive outcome measures. With regard to the latter, there has been ongoing debate about whether current instruments are able to fully evaluate the complex and heterogeneous IBS condition and represent all relevant dimensions of health for patients with IBS.<sup>20</sup> Both the FDA and EMA recommended endpoints for IBS trials treating more than one IBS-subtype, are dichotomous endpoints of being an abdominal pain- or global relief responder or non-responder, respectively. It has been argued that these dichotomous endpoints provide an incomplete clinical understanding and fail to represent smaller benefits that are clinically important.<sup>21</sup> Therefore, trial results in IBS may reflect a lack of efficacy of treatments given, but could also reflect a lack of appropriate outcome measures. In case of the PERSUADE study, conflicting findings were found across different outcome measures. On the one hand, no statistically significant differences were found between groups in primary outcomes recommended by the FDA and EMA, nor in health-related quality of life scores. This may seem at odds with previous meta-analyses that reported higher efficacy rates.<sup>22</sup> On the other hand,

small-intestinal release peppermint oil did show statistically significant improvements in secondary outcomes of IBS symptoms that indicate clinically meaningful differences, such as IBS symptom severity, abdominal discomfort, and more relief of IBS symptoms (**chapter 7**). The difference in efficacy rates might therefore not be at odds with prior work, but merely a consequence of reporting the rigorous and stringent outcome measures predefined by the FDA/EMA for the first time in peppermint oil research. Inclusion of our study data and thus a broader array of outcome measures in the meta-analysis subsequently led to widening of the uncertainty around the estimate of efficacy.<sup>23,24</sup> Future research is needed to clarify the role of these different outcome measures on efficacy results.

#### *The importance of an appropriate target population*

Another reason for the general low level of efficacy in IBS trials may be related to the definition of the target population. The Rome criteria recommend to include only the Rome IV-defined IBS population in clinical trials to increase standardization and increase reproducibility of study findings. We did not find any association between baseline abdominal pain and being an abdominal pain responder in our study (**chapter 7**). Neither do we know whether inclusion of additional Rome-III positive Rome IV-negative patients with 'milder' IBS symptoms might have resulted in better efficacy of peppermint oil compared to inclusion of Rome IV-positive patients only. It should be noted that we included patients from both primary and secondary/tertiary care and this may have led to a higher applicability of trial results to the general population. Nevertheless, one could speculate on the outcome of efficacy of peppermint oil when a pragmatic trial in real-world setting would have been conducted. In such a trial, all patients in routine care with IBS-like symptoms and without an organic cause that have IBS according to their doctor's opinion (without Rome IV criteria confirmation necessarily). To date, only very few pragmatic trials have been performed in IBS, e.g. one investigating the efficacy of acupuncture<sup>25</sup> and one investigating on-demand usage of antispasmodics.<sup>26</sup> Further insight into pathophysiological mechanisms underlying IBS can also aid selection of the best treatment for a specific patient. Subsequently, this may lead to more cost-effective treatment of patients with IBS. Our group previously performed a trial with probiotics selected to affect visceroperception in a subgroup of patients with visceral hypersensitivity. Unfortunately, preselection of patients did not lead to a better outcome.<sup>27</sup> IBS is generally considered a complex multifactorial disorder. For tailored treatment strategies, better insight in single or combined factors contributing to predominant symptoms in subgroups of patients is needed.

*The importance of appropriate measurement tools*

Use of inappropriate symptom measurement tools is another factor that may impede clinical trial design, the measurement of treatment response, and therefore the development of novel (cost-effective) therapies. In the PERSUADE study, we used a custom-made smartphone application as a digital symptom diary, web-based patient questionnaires, and an electronic case report file, collectively resulting in standardized data collection with high patient and investigator compliance (**chapter 9**). Compared to traditional paper diaries and questionnaires, this digital approach has led to a reduction in recall- and ecological bias, shows a higher adherence and provides more real time data, contributing to the methodological soundness of our RCT. Optimal symptom measurement tools may diminish biased trial results and enable clinicians and policy makers to draw more valid conclusions about treatment efficacy. Another example of a more real time registration of IBS symptoms, is the Experience Sampling Method (ESM). Akin to the digital end-of-day symptom diary used in the PERSUADE trial, the ESM is an electronic questioning method. In contrast to end-of-day symptom questionnaires, the ESM applies random and repeated measurements in the subject's natural environment for several consecutive days. Our research group is in the process of validating an ESM patient reported outcome measure (PROM) specifically for IBS and this will help to gain further insight into fluctuations in IBS symptoms and potential triggers.<sup>28</sup> This more detailed insight will further enable a more personalized medicine approach (that may also be more cost-effective). In addition, validated multi-item ESM-PROMs developed according to FDA guidelines can also be used in clinical trials to evaluate treatment response. Future studies should assess whether this would alter findings of efficacy endpoints, and if yes – to what extent, in comparison to digital end-of-day questionnaires or Interactive Voice Response (IVR) systems.

**Targeted treatment with peppermint oil**

Regardless of whether we had selected the appropriate outcome measures in our RCT, assessed them with the most optimal measurement tool, or included the correct target population, we had anticipated greater treatment efficacy of peppermint oil in patients with IBS. In contrast to the highly favorable number needed to treat of 2-3 that had been found in prior (less well designed) studies, we showed a number needed to treat of 8.

Advantages of small-intestinal release peppermint oil as an initial treatment compared to other pharmacological options for IBS are its availability as a low-cost over the counter (OTC) drug and the general mild and transient adverse event profile without the risk of

serious adverse events. As treatment effects emerged towards the end of the eight-week treatment period, future studies should evaluate the efficacy and safety of longer treatment with small-intestinal release peppermint oil. It would also be of value to assess if longer treatment periods will be cost-effective, since we found that small-intestinal release peppermint oil is likely cost-effective on the short term. In addition, it may be interesting to explore the presence of pharmacogenetic effects on treatment response, as part of the mechanism of action of peppermint oil is via TRPM8 stimulation through its main constituent menthol.<sup>29</sup> Mutations in transient receptor potential channels are known from genetics analyses in pain research<sup>30</sup> and may affect treatment response.

In **chapter 5**, we investigated TRPM8 as the potential mechanism of action of peppermint oil, and postulated that activation of this receptor results in diminished release of inflammatory mediators which contribute to pain symptom generation. As this mechanistical study was small and preliminary, future research will need to elucidate these mechanisms further to optimize targeted treatments such as peppermint oil for IBS.

We had hypothesized that the ileocolonic release would further increase efficacy, but this was not shown by our results. While reducing upper GI side effects, the ileocolonic release peppermint oil unexpectedly showed an increase in abdominal cramping. As ileocolonic-release peppermint oil also showed lower efficacy than small-intestinal release peppermint oil, we do not recommend its further development in the current form. Further insight into the differential intestinal expression of the receptor, *i.e.* expressional differences between duodenum, proximal colon and sigmoid, may lead to better understanding of the small-intestinal versus the ileocolonic release peppermint oil performance.

### **Integrating mental health care**

In **chapter 3**, we showed that a reduction in IBS symptom severity was not paralleled by improvement in quality of life and general life satisfaction in patients with IBS. Our data suggested that quality of life and general well-being were related to concurrent anxiety and depression risk scores, rather than GI symptom severity. Moreover, in the cost-effectiveness analysis of the PERSUADE study, we found that a large proportion of the total associated socioeconomic costs was driven by mental healthcare usage (**chapter 8**). In patients with IBS, the prevalence of psychological co-morbidities is high and there is long-standing appreciation for a connection of anxiety and depression and altered stress responses and IBS. In view of the generally unsatisfactory results of

pharmacological treatment and our findings of **chapter 3 and 8**, it can be argued that future models of care and novel treatment strategies should preferably integrate the management of psychological comorbidities. A multidisciplinary and multidimensional approach likely leads to better treatment outcomes than standard care given by GPs and gastroenterologists. Treatment guidelines are indeed increasingly incorporating the promising biopsychosocial model of care, which simultaneously addresses both mood disorders and GI symptoms in patients with IBS.<sup>31</sup> The efficacy of psychological therapies alone, e.g. cognitive behavioral therapy<sup>32,33</sup> and hypnotherapy<sup>34</sup> has also been demonstrated in high quality studies. Nevertheless, therapist-led interventions are generally time consuming, relatively costly and the availability may be limited in some geographical areas due to a limited number of trained therapists. Psychological treatments that require less resources may increase cost-effectiveness, patient-satisfaction, and availability. Such treatments include 1) group hypnotherapy that has been shown to be non-inferior to individual hypnotherapy<sup>35</sup>; 2) internet-delivered exposure-based cognitive behavioral therapy (CBT) that has been shown to reduce IBS symptoms<sup>36</sup>; and 3) a primarily home-based CBT that has been shown to be at least as effective as standard CBT.<sup>32</sup> In that light, our group recently started a study to explore whether online hypnotherapy is non-inferior compared to individual face-to-face hypnotherapy in treatment of IBS symptoms (NCT03899779). It is hypothesized that such approaches can decrease treatment-associated costs, while simultaneously increasing general well-being and quality of life in patients with IBS.

## General conclusions

This thesis has assessed the redefinition of diagnostic criteria and how this impacts the clinical diagnostic process in IBS. The potential effect on study populations in research has been described. Furthermore, this thesis has given an overview of current knowledge on TRP channels in IBS and provided the first *in vivo* data on the TRPM8 channel in IBS. Study findings described in this thesis are key in paving the way to develop novel TRP targeted therapies for IBS.

At the center of this thesis is the PERSUADE study, the largest RCT with peppermint oil in IBS to date. This trial not only contributed to knowledge on peppermint oil, but also assessed efficacy using both strict endpoints according to regulatory guidelines and other clinically relevant but less robust endpoints. Findings in this thesis showed that peppermint oil is not only moderately effective, but also appears to be cost-effective. Trial data were collected using a digital data-collection method which is detailed further in this thesis and led to excellent adherence.

This chapter has addressed some of the major challenges of IBS research, such as selecting the appropriate study outcomes and patient population, and employing a more digital and daily-life approach to data-collection.

Although this thesis provides new insights in the broader sense of IBS research and treatment, the high prevalence, substantial socioeconomic burden, and impact on quality of life of IBS will continue to necessitate extensive future research into the field of disorders of gut-brain interaction and particularly IBS.

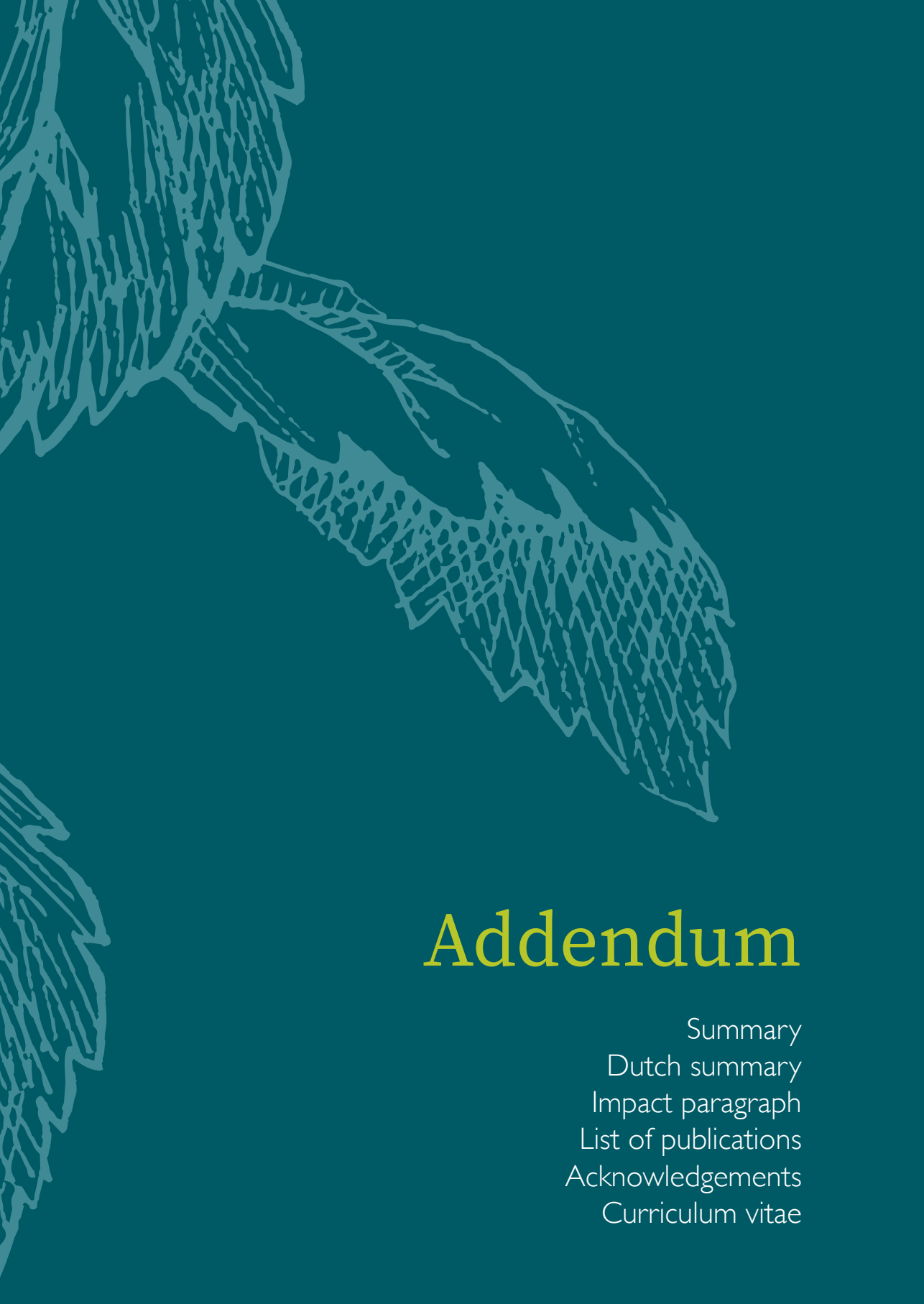
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# Addendum

Summary  
Dutch summary  
Impact paragraph  
List of publications  
Acknowledgements  
Curriculum vitae



## Summary

Irritable bowel syndrome (IBS) is disorder of gut-brain interaction, previously known as a functional gastrointestinal disorder and is characterized by chronic recurrent abdominal pain and altered bowel habits. It is highly prevalent affecting 5-15% of the population depending on the criteria used and is associated with a pronounced impact on quality of life. The underlying pathophysiology of IBS is multifactorial, complex and incompletely understood and as a consequence, reliable biomarkers to diagnose IBS are currently lacking. This makes IBS a diagnosis which is, according to current consensus, merely based on symptoms. Symptoms vary widely both within and between patients, reflecting the complex and heterogeneous phenotype of IBS. Despite its prevalence and substantial negative impact on patients, treatment options are currently limited and are moderately effective at best. The relative lack of effective therapies adds further to the large associated socioeconomic burden.

This thesis covers several aspects concerning IBS, from the diagnostic Rome criteria and symptom evolution over time (**part I**), to the investigation of therapeutic targets, with a special focus on treatment with peppermint oil, its potential mechanisms of action and its cost-effectiveness (**part II**).

### **Part I - Epidemiological aspects and clinical manifestations of Irritable Bowel Syndrome**

Currently, diagnosis of IBS is made based on the presence of typical clinical symptoms which are incorporated in the Rome criteria and include abdominal pain and aberrant defecation such as constipation, diarrhea or a combination of the two. The Rome criteria have been modified every few years, which has led to some variation in IBS patient populations over time, depending on which criteria were used to diagnose IBS. The most recent version of the criteria is the Rome IV, published in 2016, which was expected to be more stringent than the prior Rome III due to the requirement of abdominal pain at least once a week instead of three days per month. In addition, the presence of pain instead of discomfort alone is a necessity. In **chapter 2**, we compared the prevalence of IBS according to the Rome III and IV criteria in the well characterized Maastricht IBS Cohort. Of 404 patients that were diagnosed with IBS using the Rome III criteria, between 87% and 62% was likely to also fulfill the Rome IV criteria. When comparing clinical characteristics between Rome III IBS patients and those also fulfilling the Rome IV criteria, we found that the Rome IV IBS population reflected a subgroup of Rome III IBS patients that was more often female, with more severe GI

symptomatology, psychological comorbidities and lower quality of life. This implies that results from Rome III IBS studies may not be directly transferable to Rome IV IBS populations. In clinic however, both groups should be considered as having IBS.

Besides variations in population characteristics due to different diagnostic criteria used, symptoms within patients are also known to vary over time. Given that insight in factors affecting the natural disease course can help in the search for new patient-tailored targeted treatment strategies, we evaluated symptom evolution and characteristics that could predict the disease course over a five-year follow up period in **chapter 3**. Of 161 Rome III IBS patients of the Maastricht IBS cohort, 30% did not fulfill the Rome III criteria any longer and had significantly lower levels of GI symptoms and GI-specific anxiety after five years follow-up. Nevertheless, comorbid anxiety and depression and quality of life were comparable between patients that did and did no longer fulfill the diagnostic criteria at follow-up. No baseline predictors for being Rome-IV positive at follow-up were found. These results suggest that long-term quality of life and general wellbeing might depend on concurrent psychological symptoms, rather than GI symptom improvement over time. Therefore, therapeutic efforts should also be aimed at addressing mental health issues.

## **Part II - Transient receptor potential channels as therapeutic target**

Although the number of therapeutic options for IBS has grown in the last decades, treatment of abdominal pain in particular remains challenging and is often unsatisfactory. In the search for cost-effective treatments, Transient Receptor Potential (TRP) channels are promising targets for therapeutic interventions. TRP channels are known to have temperature-sensing properties, among many other functions. Growing evidence indicates that TRP channels in the GI tract are involved in the propagation and processing of abdominal pain signals in IBS.

To gain an overview of current knowledge on the role of various TRP channels in visceral nociceptive processes in IBS, we first performed an extensive literature search and summarized the findings in **chapter 4**. An extensive overview of knowledge on TRPV1, TRPV4, TRPA1, TRPM8 and their potential implications for treatment in IBS was given. Of these, TRPV1 is most widely-studied and its stimulation is associated with increased visceral nociception, hypersensitivity and increased neurogenic inflammation. Although direct antagonism of TRPV1 led to excellent analgesia, safety issues impair its clinical use. Knowledge regarding another important channel, the TRPM8 channel, is mostly derived from animal studies. These have shown that TRPM8 appears to have an

anti-nociceptive and possibly anti-inflammatory effect. Peppermint oil, which has been used for decades to treat abdominal pain in IBS, has menthol as its main constituent. Interestingly, menthol is a TRPM8 agonist suggesting a possible effect through this TRP channel.

In **chapter 5**, we then investigated molecular mechanisms underlying the potential beneficial effect of TRPM8 agonism in IBS. As human data on intestinal TRPM8 was limited to a single study in Crohn's disease and a brief report on polymorphisms in IBS, this was the first study investigating TRPM8 in intestinal tissue of IBS patients. In biopsies from both a London and Maastricht IBS cohort, we showed that TRPM8-immunoreactivity was colocalized with immune cells being predominantly of the dendritic cell lineage and in close approximation to nerve endings. In addition, we demonstrated that TRPM8 immunoreactivity and mRNA expression was increased in IBS patients as compared to controls. Ex vivo treatment of IBS patient biopsies with TRPM8 agonist icilin reduced the release of pro-inflammatory cytokines. These data indicate that TRPM8 may have important anti-inflammatory properties and by this virtue can impact neuro-immune disease mechanisms in IBS.

Peppermint oil is one of the pharmacotherapeutic entities currently used for IBS and is also an agonist for TRPM8. Enteric-coated capsules that release peppermint oil in the small intestine are currently available as an over-the-counter drug in Europe. However, studies investigating the clinical efficacy of peppermint oil in IBS are hampered by methodological shortcomings that impede the ability to draw firm conclusions. In addition, the Rome diagnostic criteria have been updated and the European Medicines Agency (EMA) and Food and Drug Administration (FDA) have defined robust endpoints for clinical trials in IBS since then. Together, a methodologically well-designed trial to assess efficacy of peppermint oil in Rome IV diagnosed IBS patients was warranted.

Although the mechanism of action of peppermint oil is multifactorial (e.g. intestinal smooth muscle relaxation, inhibition of serotonin receptors, anti-microbial and anti-fungal), part of its effect is likely effectuated through TRPM8 stimulation via its main constituent, menthol. We hypothesized that an increased local colonic peppermint oil concentration would perhaps increase therapeutic efficacy and decrease burdensome upper GI adverse events from small-intestinal release peppermint oil, such as heartburn and belching, due to the more distal release. Therefore, an ileo-colonic release peppermint oil capsule was developed and its pharmacokinetic performance was assessed and compared to the already existing small-intestinal peppermint oil capsule in a crossover study, described in **chapter 6**. We showed in eight healthy volunteers, that

the time to reach the maximum concentration of the menthol metabolite, menthol-glucuronide, was significantly longer for ileocolonic release compared to small-intestinal release peppermint oil, *i.e.* 360 versus 180 min. The lag time – time to reach a systemic concentration - was also significantly longer, *i.e.* 225 compared to 37 min, respectively. These results point to the likely release of peppermint oil in the more distal part of the intestine, the ileocolonic region.

We then investigated both formulations of peppermint oil in the PERSUADE trial, described in **chapter 7**. The primary objective of this multicenter, randomized, placebo-controlled study was to determine the efficacy of small-intestinal release peppermint oil according to FDA and EMA guidelines. Moreover, we aimed to explore efficacy of the novel ileocolonic release peppermint oil. In 189 Rome IV diagnosed IBS patients, both formulations of peppermint oil did not result in statistically significant abdominal pain reduction or global symptom relief, when using stringent FDA and EMA recommended endpoints. The small-intestinal peppermint oil did, however, show greater improvements versus placebo in secondary outcomes, *i.e.* abdominal discomfort, IBS severity, and moderate relief. Adverse events were mild but more common in both peppermint oil groups as compared to placebo. As our results did not show benefits of ileocolonic release peppermint oil, *i.e.* upper GI adverse events were indeed reduced but abdominal cramping was increased in some patients, findings did not support the further development of ileocolonic release peppermint oil for IBS. Small-intestinal release peppermint oil, however, can be considered a moderately effective treatment based on the results of our study being the largest RCT with peppermint oil to date.

Ideally, treatments should not only be effective, but also cost-effective. It is known that IBS not only has a large negative impact on quality of life, but is also associated with increased direct healthcare costs (e.g. consultations, emergency room visits) and indirect healthcare related costs (e.g. sick leave, decreased productivity at work). In **chapter 8**, we described a cost-effectiveness study of the small-intestinal release peppermint oil compared to placebo that was performed alongside the clinical RCT (PERSUADE study). The peppermint oil group had a slightly higher increase in utility scores (quality adjusted life years) than placebo during treatment. In addition, the peppermint oil group appeared to incur fewer total costs during treatment. Results of the cost-effectiveness analysis showed that peppermint oil is likely cost-effective with a probability varying between 56% and 89%, depending on the outcome measure and willingness to pay threshold used. Data analyses showed some uncertainty surrounding these results.



Summarizing, we demonstrated a moderately positive effect of peppermint oil on IBS symptoms (**chapter 7**). Peppermint oil is inexpensive, widely available and has few adverse events. Taken together with the modest gains in quality of life adjusted years and the results of **chapter 8** where we show that peppermint is likely cost-effective, we conclude that peppermint oil seems an appropriate first-line treatment for patients with IBS. More research and long-term data are necessary to confirm the effectiveness and cost-effectiveness of peppermint oil.

Since there are no reliable biomarkers in IBS, end-of-day symptom diaries are considered the gold standard to assess treatment response. For the PERSUADE study, we used a custom-made digital framework for data-collection consisting of a daily digital symptom diary (custom smartphone application), web-based patient questionnaires (regarding e.g. demographics and life style, IBS symptom severity, and anxiety and depression), and an electronic case report file (eCRF) for investigators to report in. This digital framework and the results of the study investigating the feasibility of this digital data collection method is described in **chapter 9**. We showed that the mean overall compliance rate to the symptom diary was 88%. Patients' compliance to the additional web-based questionnaires and investigators' compliance to the eCRF were also very high, *i.e.* both more than 99%. Missingness due to technical issues was limited to one question. The data collection framework thereby was shown to lead to a standardized state-of-the art data collection with excellent completeness and can be used for future RCTs. Due to a slight decrease in patient compliance to the digital diary throughout the study, this method might be less suitable for trials of longer duration.

Finally, in **chapter 10**, we provide an overview of the major findings presented in this thesis, integrated the different topics that were discussed in the different chapters, tried to place results in the broader scope of IBS research and discussed new insights and future perspectives.

Although this thesis provides new insights in different aspects of IBS research and treatment, its high prevalence, associated substantial socioeconomic burden, and impact on quality of life on patients affected will continue to necessitate extensive future research into the field of disorders of gut-brain interaction and particularly IBS.



## Dutch summary

Het Prikkelbare Darm Syndroom (PDS), oftewel Irritable Bowel Syndrome (IBS) in het Engels, is een aandoening die gekenmerkt wordt door een verstoorde functie van de hersen-darm as, voorheen een functionele maagdarmaandoening genoemd. PDS wordt gekarakteriseerd door chronisch terugkerende buikpijn en een veranderd ontlastingspatroon. Het is een veelvoorkomende aandoening die bij 5-15% van de bevolking voorkomt, afhankelijk van de gebruikte criteria voor diagnose. De onderliggende pathofysiologie van PDS is nog niet volledig opgehelderd, maar is complex en multifactorieel bepaald. Als gevolg daarvan ontbreken momenteel betrouwbare biologische markers om PDS te diagnosticeren. Dit maakt PDS een diagnose die, volgens de huidige consensus, louter is gebaseerd op klachten die door patiënten gerapporteerd worden. Deze klachten, oftewel symptomen, kunnen sterk verschillen per patiënt en over de tijd waardoor PDS gekenmerkt wordt door een heterogene patiëntenpopulatie. Ondanks de hoge prevalentie en de aanzienlijke vermindering in kwaliteit van leven waarmee PDS gepaard gaat, zijn behandelopties op dit moment beperkt en op zijn best matig effectief. Het ontbreken van effectieve therapieën draagt bij aan de grote sociaaleconomische last van PDS.

Dit proefschrift richt zich op meerdere aspecten van PDS. **Deel I** richt zich op de Rome criteria om PDS te diagnosticeren en verandering van symptomen over de tijd. **Deel II** richt zich op onderzoek naar gerichte aangrijpingspunten voor therapie, met een bijzondere nadruk op de behandeling van PDS met pepermuntolie, potentiële werkingsmechanismen, en de kosteneffectiviteit van deze behandeling.

### **Deel I - Epidemiologische aspecten en klinische manifestaties van het Prikkelbare Darm Syndroom**

Conform de huidige consensus wordt de diagnose van PDS gesteld op basis van de aanwezigheid van typische klinische symptomen die zijn opgenomen in de Rome criteria, namelijk buikpijn en een afwijkend ontlastingspatroon zoals obstipatie, diarree of een combinatie van beide. De Rome criteria zijn over de jaren steeds enigszins gewijzigd op basis van wetenschappelijk onderzoek, waardoor afhankelijk van de gehanteerde versie van de Rome criteria de PDS-patiënten populaties van elkaar kunnen verschillen. De meest recente versie van de criteria is de Rome IV, gepubliceerd in 2016, die naar verwachting strenger zou zijn dan de voorgaande Rome III criteria, vanwege het criterium dat er ten minste sprake dient te zijn van één keer per week buikpijn in plaats van drie dagen per maand. Bovendien dient er sprake te zijn van pijn en is enkel de

aanwezigheid van ongemak onvoldoende. In **hoofdstuk 2** vergeleken we de prevalentie van PDS afhankelijk van het gebruik van de Rome III of IV criteria in een goed gekarakteriseerd cohort van PDS-patiënten, het Maastricht IBS Cohort. Van de 404 patiënten bij wie PDS werd gediagnosticeerd met behulp van de Rome III criteria, voldeed tussen 87% en 62% waarschijnlijk ook aan de strengere Rome IV criteria. Bij het vergelijken van klinische kenmerken tussen Rome III PDS-patiënten en patiënten die ook voldeden aan de Rome IV criteria, ontdekten we dat de Rome IV PDS-populatie een subgroep weerspiegelde die vaker vrouwelijk was, met ernstigere maag-darm klachten, meer psychologische co-morbiditeit en een lagere kwaliteit van het leven. Dit betekent dat de resultaten van wetenschappelijk onderzoek binnen de Rome III PDS-populatie mogelijk niet direct vergelijkbaar zijn met die van een Rome IV PDS-populatie. In de klinische praktijk is het echter belangrijk om beide groepen als PDS te beschouwen en zodanig te behandelen.

Naast variaties in populatiekenmerken als gevolg van verschillen in de gebruikte diagnostische criteria over de tijd, is het ook bekend dat symptomen binnen patiënten kunnen variëren over de tijd. Aangezien kennis over het natuurlijke ziekteverloop kan helpen bij het zoeken naar nieuwe, op de patiënt toegesneden, gerichte behandelstrategieën, evalueerden we in **hoofdstuk 3** de variatie van symptomen over een periode van vijf jaar en zochten we naar voorspellers van ziektebeloop. Na vijf jaar follow-up rapporteerde 30% van 161 Rome III PDS-patiënten van het Maastricht IBS cohort gemiddeld minder maag-darmklachten en hieraan gerelateerde angst en voldeed deze 30% niet langer aan de Rome III criteria. Psychologische klachten, zoals angst en depressie, en de kwaliteit van leven waren echter vergelijkbaar tussen patiënten die nog wel en zij die niet meer aan de diagnostische criteria voldeden op het moment van follow-up. Er konden geen duidelijke voorspellers voor het ziekteverloop worden gevonden. Deze resultaten suggereren dat het algemeen welbevinden en de kwaliteit van leven op de lange termijn mogelijk meer afhangen van psychologische symptomen op dat moment, dan van verbetering van maag-darm klachten in de loop van de tijd. Deze resultaten pleiten tevens voor therapeutische interventies voor PDS die gericht zijn of mede gericht zijn op mentale gezondheid.

## **Deel II - Transient Receptor Potential kanalen als therapeutisch doelwit**

Hoewel het aantal behandelopties voor PDS de afgelopen decennia is toegenomen, blijft de behandeling van met name buikpijn een uitdaging en is deze vaak onbevredigend. In de zoektocht naar kosteneffectieve therapieën zijn Transient Receptor Potential (TRP) -

receptoren veelbelovende aangrijpingspunten voor therapeutische interventies. Van TRP-receptoren is bekend dat ze temperatuurgevoelige eigenschappen hebben, naast vele andere functies. Zo is de TRPM8 receptor verantwoordelijk voor het gevoel van koude en wordt dit aangrijpingspunt ook geactiveerd door menthol, wat het frisse gevoel van menthol verklaart. De TRPV1 receptor is verantwoordelijk voor een gevoel van warmte en wordt ook geactiveerd door capsaicine, een stofje in chilipepers. Activatie van de TRPV1 receptor door capsaicine verklaart het hete pittige gevoel van rode peper. Toenemend bewijs toont dat TRP-receptoren die zich op cellen in het maagdarmlkanaal bevinden (in het slijmvlies), betrokken zijn bij de signalering, verspreiding en verwerking van buikpijnsignalen. De verhoogde prikkelgevoeligheid en gevoelsgevaarwording vanuit de darm wordt viscerale hypersensitiviteit genoemd.

Om een overzicht te krijgen van de huidige kennis over de rol van verschillende TRP-receptoren in pijnverwerking en prikkelgevoeligheid bij PDS, hebben we eerst een literatuuronderzoek uitgevoerd en de bevindingen samengevat in **hoofdstuk 4**. Een uitgebreid overzicht van kennis over TRPV1, TRPV4, TRPA1, TRPM8 en hun mogelijke implicaties voor de behandeling van mensen met PDS werd gegeven. Van deze receptoren is TRPV1 het meest bestudeerd en wordt de stimulatie ervan geassocieerd met verhoogde prikkelgevoeligheid, viscerale hypersensitiviteit en ontstekingsreactie oftewel inflammatie. Hoewel bleek dat een directe blokkering oftewel antagonisme van TRPV1 tot uitstekende pijnstilling leidde, belemmerden veiligheidsproblemen het klinische gebruik ervan. Kennis over een andere belangrijke receptor, de TRPM8-receptor, is grotendeels afkomstig uit dierstudies. Deze hebben ons laten zien dat TRPM8 een pijnstillende en mogelijk ontstekingsremmende werking lijkt te hebben. Interessant is dat pepermuntolie, dat al tientallen jaren wordt gebruikt om buikpijn bij PDS te behandelen, menthol als hoofdbestanddeel heeft en menthol de TRPM8 receptor direct kan stimuleren. Dit suggereert mogelijk een gunstig effect van pepermuntolie via deze TRP-receptor.

In **hoofdstuk 5** hebben we vervolgens moleculaire mechanismen onderzocht die ten grondslag liggen aan het potentieel gunstige effect van TRPM8 stimulatie in PDS. Aangezien de wetenschappelijke kennis over TRPM8 bij mensen in de darm zich beperkt tot één enkele studie bij de ziekte van Crohn en een kleine studie naar polymorfismen oftewel genetische variaties bij PDS, was dit de eerste studie die TRPM8 expressie en activiteit in darmweefsel van PDS-patiënten onderzocht. In darm biopten van PDS-patiënten uit Londen en Maastricht toonden we aan dat TRPM8 reactieve cellen zich vlakbij cellen van het immuunsysteem bevonden, en vlakbij zenuwuiteinden. Bovendien bleek dat TRPM8 activiteit en de genetische (mRNA) expressie verhoogd was bij PDS-

patiënten in vergelijking met mensen zonder PDS. Behandeling van darmbiopten van PDS-patiënten met een middel dat de TRPM8 receptor kan stimuleren, iciline, verminderde de afgifte van stoffen die voor meer ontsteking kunnen zorgen oftewel pro-inflammatoire cytokines. Deze resultaten tonen dat TRPM8 belangrijke ontstekingsremmende eigenschappen kan hebben en daardoor mogelijk de neuro-immun gerelateerde ziektemechanismen in PDS kan beïnvloeden.

Een van de farmacotherapeutische middelen die momenteel voor PDS gebruikt wordt en die ook TRPM8 kan stimuleren, is pepermuntolie. Speciaal gecoate maagsap-resistente capsules zorgen ervoor dat de pepermuntolie wordt afgegeven in de dunne darm en zijn verkrijgbaar in Europa zonder recept. Wetenschappelijke studies uit het verleden naar de klinische werkzaamheid van pepermuntolie bij PDS werden echter belemmerd door methodologische beperkingen die het moeilijk maken om eenduidige conclusies te trekken over de daadwerkelijke effectiviteit van pepermuntolie. Bovendien zijn de diagnostische Rome criteria herzien naar versie IV en hebben de European Medicines Agency (EMA) en de Food and Drug Administration (FDA) inmiddels nieuwe robuuste eindpunten gedefinieerd voor geneesmiddelenstudies binnen PDS. Derhalve was een methodologisch goed opgezette studie nodig om de werkzaamheid van pepermuntolie bij Rome IV PDS-patiënten te beoordelen.

Hoewel het werkingsmechanisme van pepermuntolie multifactorieel is (bijv. relaxatie van de gladde spieren van de darmen, remming van serotoninereceptoren, antimicrobiële en antischimmel activiteit), wordt een deel van het effect waarschijnlijk bewerkstelligd door TRPM8-stimulatie via het hoofdbestanddeel, menthol. Onze hypothese was dat een toename van de lokale pepermuntolie concentratie in het colon oftewel de dikke darm wellicht de therapeutische werkzaamheid zou verhogen. Tevens zou een meer distale afgifte van de pepermuntolie kunnen leiden tot vermindering van bovenste gastro-intestinale bijwerkingen zoals zuurbranden en oprispingen. Daarom werd er een nieuwe pepermuntolie capsule ontwikkeld die de olie vanaf het ileocecale gedeelte van de darm in het colon afgeeft in plaats van in de dunne darm. De farmacokinetische eigenschappen van deze nieuwe capsule werden onderzocht en vergeleken met de reeds bestaande dunne darm pepermuntolie capsule in een cross-over studie, beschreven in **hoofdstuk 6**. Bij acht gezonde vrijwilligers werd aangetoond dat de tijd om de maximale concentratie van de menthol metaboliet, mentholglucuronide, te bereiken significant langer was voor de capsule met dikke darm afgifte in vergelijking met capsules met dunne darm pepermuntolie afgifte, namelijk 360 versus 180 minuten. De tijd waarin een systemische concentratie van mentholglucuronide bereikt werd was ook significant langer, namelijk 225 versus 37 min. Deze

resultaten wijzen op de afgifte van pepermuntolie in het meer distale deel van de darm ofwel de dikke darm, het colon.

Vervolgens zijn beide formuleringen van pepermuntolie onderzocht in de PERSUADE-studie, beschreven in **hoofdstuk 7**. Het primaire doel van dit multicenter, gerandomiseerde, placebo-gecontroleerde onderzoek was het bepalen van de werkzaamheid van dunne darm afgifte pepermuntolie volgens de vernieuwde FDA- en EMA-richtlijnen. Bovendien wilden we de werkzaamheid van de nieuwe pepermuntolie met dikke darm afgifte onderzoeken. In 189 Rome IV PDS-patiënten, resulteerde pepermuntolie (beide formuleringen) echter niet in een statistisch significante vermindering in buikpijn of globale symptoomverlichting wanneer de strenge FDA en EMA aanbevolen eindpunten als primaire uitkomstmaat gebruikt werden. De dunne darm pepermuntolie gaf wel significante verbeteringen ten opzichte van placebo in secundaire uitkomstmaten, namelijk buikpijn, ernst van de PDS, en een algehele redelijke verlichting in symptomen. Bijwerkingen waren mild maar kwamen vaker voor in beide pepermuntoliegroepen ten opzichte van placebo. Onze resultaten toonden geen voordelen van pepermuntolie met dikke darm afgifte. Hoewel het voorkomen van enkele bovenste gastro-intestinale bijwerkingen inderdaad minder was, nam de gemiddelde mate van buikkrampen bij sommige patiënten toe. Onze bevindingen ondersteunen daarom de verdere ontwikkeling van pepermuntolie met dikke darm afgifte voor PDS niet. Op basis van de resultaten van de PERSUADE-studie, de grootste RCT met pepermuntolie tot nu toe, kan pepermuntolie met dunne darm afgifte echter worden beschouwd als een matig effectieve behandeling voor PDS.

Idealiter is een behandeling niet alleen effectief, maar ook kosteneffectief. Het is namelijk bekend dat PDS niet alleen een grote negatieve invloed op de kwaliteit van leven heeft, maar ook geassocieerd is met verhoogde directe kosten van de gezondheidszorg (bijvoorbeeld kosten van een consult, spoedeisende hulp bezoeken) en indirecte kosten (bijvoorbeeld ziekteverzuim, verminderde productiviteit op het werk). In **hoofdstuk 8** hebben we de kosteneffectiviteit van dunne darm afgifte pepermuntolie vergeleken met placebo in een studie die werd uitgevoerd binnen het kader van het klinische gerandomiseerd onderzoek (PERSUADE-studie). De pepermuntolie groep toonde tijdens de behandeling een iets grotere stijging in kwaliteit van leven (uitgedrukt als 'quality adjusted life years') dan de met placebo behandelde groep. Daarnaast bleek de pepermuntoliegroep tijdens de behandeling iets minder totale (gezondheid gerelateerde directe en indirecte) kosten te hebben. Resultaten van de kosteneffectiviteitsanalyse toonden dat pepermuntolie waarschijnlijk kosteneffectief is met een waarschijnlijkheid variërend tussen 56% en 89%, afhankelijk van de uitkomstmaat en de drempel van

betalingsbereidheid (willingness to pay) die gebruikt worden. Sensitiviteitsanalyses toonden enige onzekerheid rondom deze resultaten.

Samenvattend vonden we dat pepermuntolie een overwegend positief effect heeft op PDS symptomen (**hoofdstuk 7**). Pepermuntolie is daarnaast goedkoop, overal verkrijgbaar en heeft weinig bijwerkingen. Samengenomen met de bescheiden winst in kwaliteit van leven en de resultaten van **hoofdstuk 8**, waar we tonen dat pepermuntolie capsules waarschijnlijk kosteneffectief zijn, kunnen we concluderen dat pepermuntolie een passende eerstelijnsbehandeling is voor patiënten met PDS. Meer onderzoek en data over effecten op langere termijn zijn nodig om de effectiviteit en kosteneffectiviteit van pepermuntolie verder te bevestigen.

Aangezien er op dit moment nog geen betrouwbare biologische markers zijn voor PDS, worden dagelijkse symptoomdagboeken beschouwd als de gouden standaard om de respons op een behandeling te beoordelen. Voor de PERSUADE-studie gebruikten we een op maat gemaakt digitaal raamwerk voor het verzamelen van gegevens, bestaande uit een dagelijks digitaal symptoomdagboek (op basis van een smartphone-applicatie), elektronische vragenlijsten voor patiënten (met betrekking tot bijvoorbeeld demografie en leefstijl, ernst van PDS-symptomen en angst en depressie), en een elektronische case report file (eCRF) voor onderzoekers om verslag in te doen. Dit digitale raamwerk en de resultaten van ons onderzoek naar de haalbaarheid van deze methode van digitale gegevensverzameling worden beschreven in **hoofdstuk 9**. We toonden aan dat de gemiddelde compliance oftewel het nalevingspercentage van het symptoom dagboek 88% was, wat inhoudt dat van alle dagen binnen het onderzoek het dagboek gemiddeld 88% trouw werd ingevuld door de deelnemers. Compliance van patiënten betreffende de aanvullende elektronische vragenlijsten en compliance van onderzoekers betreffende het eCRF waren ook hoog, d.w.z. beide meer dan 99%. Dataverlies door technische problemen bleef beperkt tot één vraag. Kortom, het digitaal raamwerk resulteerde in een gestandaardiseerde dataverzameling van hoge kwaliteit met hoge mate van compleetheid en kan worden gebruikt voor toekomstige onderzoeken waarbij therapie-effect gemeten dient te worden. Vanwege de lichte afname in patiënt compliance betreffende het digitale dagboek over de tijd, is deze methode mogelijk minder geschikt voor onderzoeken van langere duur.

Ten slotte wordt in **hoofdstuk 10** een overzicht gegeven van de belangrijkste bevindingen van dit proefschrift, integreerden we de onderwerpen die in de verschillende hoofdstukken werden besproken, plaatsten we de resultaten in breder perspectief en bespraken we nieuwe inzichten en toekomstmogelijkheden.



Hoewel dit proefschrift nieuwe inzichten biedt op verschillende vlakken binnen PDS-onderzoek en behandeling, is verder wetenschappelijk onderzoek noodzakelijk vanwege de hoge prevalentie, de bijbehorende substantiële sociaaleconomische last en de impact op de kwaliteit van leven van patiënten met een aandoening van hersen-darm interactie waaronder PDS.



## Impact paragraph

Irritable bowel syndrome is a highly prevalent disorder affecting 5-15% of the population and is associated with a substantial financial and societal burden. The studies described in this thesis contribute to the long-term goal to further optimize IBS treatment.

### Impact on healthcare providers

The results from this thesis are relevant for people involved in the care of patients with IBS such as general practitioners, gastroenterologists, psychologists, and dieticians.

Treatment of cardinal IBS symptoms such as abdominal pain is exceptionally challenging. The large randomized controlled trial on the efficacy of peppermint oil in IBS, the PERSUADE study, showed that peppermint oil is a moderately, though most-likely cost-effective treatment. This allows for appropriate positioning of peppermint oil in the therapeutic arsenal and for more informed decision making. Upper GI symptoms may occur as side effects during treatment with peppermint oil. We demonstrated for the first time that belching severity decreases after three weeks of continues peppermint oil treatment. This particular finding can help healthcare providers who prescribed peppermint oil treatment to better inform patients when this highly burdensome but harmless side-effect occurs.

Although part of this thesis focused on (GI-)targeted treatment with peppermint oil, we also showed that improvement in GI symptom severity does not necessarily result in an improvement in quality of life in patients with IBS. Furthermore, we showed that a large part of the total healthcare costs in patients with IBS was spent on mental healthcare. Findings like these can inform healthcare providers about the importance of a multidimensional and integrated treatment and will eventually help them in treating IBS patients successfully.

Furthermore, results from the different studies described in this thesis are largely applicable to primary care where general practitioners diagnose and see the majority of patients with IBS. The Maastricht IBS Cohort studies included 28-33% of patients recruited from primary care. The PERSUADE study included 57.7% of patients recruited from primary care. This in itself represents the largest of such population examined with regards to peppermint oil efficacy and led to a high applicability of the results to every-day clinical practice.

## **Impact on research**

This thesis is relevant for researchers in the field of functional gastrointestinal disorders. After redefinition of the diagnostic criteria our results were one of the first to show how this affected prevalence and characteristics of IBS patient populations.

In addition, the studies included in this thesis provide a solid basis to optimize treatment response measurement in functional GI disorders by designing and describing a framework for digitalized data-collection in RCTs. This data-collection method showed excellent adherence from both patients and researchers in our PERSUADE study. In addition, future Dutch studies benefit from the national multicenter network established at the start of our peppermint oil RCT. Currently, the (slightly adapted) framework for data-collection is being used for symptom measurement in the multicenter RCT of our research group on the efficacy of face-to-face versus online hypnotherapy for patients with IBS.

This thesis has contributed to science by giving an overview of current knowledge on transient receptor potential channels and by investigating the role of the TRPM8 receptor in the human (IBS) colon. The results in this thesis showed for first time that colonic mRNA expression levels are significantly higher in patients with IBS compared to healthy volunteers and that intestinal TRPM8 activation results in a decrease of pro-inflammatory cytokines. Shedding light onto this pathophysiological mechanism may lead to the proposal of new mechanistic studies of which the outcomes can eventually lead to the development of more targeted treatment for patients with IBS.

## **Impact on patients with IBS and society**

Importantly, the research topics described in this thesis provide benefit to patients with IBS in a broad sense. By increasing knowledge on key factors involved in pathophysiology of IBS and in quality of life, more targeted treatment can be developed and investigated. This will then hopefully lead to improvements in healthcare with better health outcomes on the long-term for patients with IBS.

Although there is long-standing appreciation for the magnitude of the societal and economic burden imposed by IBS, Dutch data are still sparse. Data included in this thesis indicated that a large part of the substantial healthcare costs incurred by Dutch patients with IBS was actually driven by mental and not GI-related healthcare. In addition, it was shown that patients who improve in GI symptom severity did not necessarily have a better quality of life. These findings point to a large impact of

psychological comorbidities on total associated costs and call for a change in quality of care models for IBS. Implementing the biopsychosocial model in healthcare systems could lead to an early recognition of psychological comorbidity in IBS patients, which may further lead to significant economic benefit for the healthcare system and society in general.

In addition, this thesis provided the first trial-based data suggesting that treatment with small-intestinal release peppermint oil is cost-effective. This finding can impact clinical decision making, implementing the cost-effective peppermint oil in routine practice and hence lowering total IBS associated costs while high quality care is maintained.

### **Knowledge translation**

A prerequisite for the implementation of novel scientific knowledge is the dissemination of research findings to the scientific community, healthcare providers, policy makers, and patients. Therefore, the chapters in this thesis were or will be published in international peer-reviewed journals. Moreover, results were presented at various national and international research meetings such as the Dutch Digestive Disease Days, the European NeuroGASTRO meeting, the United European Gastroenterology (UEG) congress, and the Federation of Neurogastroenterology and Motility Meeting.

At this moment, the joint multidisciplinary clinical guideline of the Dutch College of General Practitioners (Nederlands Huisartsen Genootschap) and the Dutch society of gastroenterologists (Nederlandse Vereniging van Maag-Darm-Leverartsen) on IBS is under revision. As a result of the studies in this thesis, the novel guideline can incorporate data on prevalence after the change from Rome III to Rome IV diagnostic criteria. Furthermore, based on a meta-analysis that included data from our study<sup>1</sup>, it is expected that peppermint oil will be included as a moderately effective and low-cost first-line treatment option for IBS. Taken together with the publications on the PERSUADE study in *Medisch Contact*<sup>2</sup>, *Nederlands Tijdschrift voor Geneeskunde*<sup>3</sup> and *Huisarts en Wetenschap*<sup>4</sup>, and the publication of trial results by the Maastricht University Medical Center and Zuyderland Medical Center websites and social media accounts, the national dissemination was successful and will likely lead to further implementation throughout the Netherlands.

Results of this RCT have been picked up by the international gastroenterologist community as well. Features included an editorial about our study design and results in *Gastroenterology*<sup>5</sup>, a citation as one of the major highlights of the past decade by the

editors of *The American Journal of Gastroenterology*<sup>6</sup>, a laudatory summary in *The New England Journal of Medicine (NEJM) Journal watch*<sup>7</sup>, and a discussion and summary in the *BMJ Evidence Based Medicine*.<sup>8</sup> Most recently, our study findings have been incorporated in the *American College of Gastroenterology* clinical guideline on the management of IBS.<sup>9</sup>

With regards to knowledge distribution to patients with IBS, findings of the Maastricht IBS Cohort and PERSUADE study have been summarized and sent to patients who participated in the studies. Members of the Dutch IBS patient organization were informed about results in layman's articles published in their members magazine *Prikkels*. In addition, Maastricht University Medical Center has organized various patient information evenings to keep patients up to date with current knowledge.

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## Curriculum vitae

Zsa Zsa Weerts was born on October 13<sup>th</sup> 1989 in Geleen, the Netherlands. After graduating from Graaf Huyn College, Geleen, cum laude in 2008, she started medical school at Maastricht University. During her bachelor, she followed a course Internal Health Care Policy at the University of Birmingham, UK. During her master, she did several internships abroad, including at Kusturba Medical College in Manipal India, Ailsa hospital in Ayr, Scotland, and Universidad de San Francisco de Quito, in Quito, Ecuador. In the last year of her master, she starting working on a research project regarding Irritable Bowel Syndrome under direct supervision of dr. Z. Mujagic. After obtaining her Medical Doctor's degree, she became a PhD candidate at the department of Gastroenterology-Hepatology at the Maastricht University Medical Center, under the supervision of Prof. dr. A. Masclee, Prof. dr. Jonkers, and dr. D. Keszthelyi. During her time as a PhD candidate, she conducted a large multicenter clinical trial and had the opportunity to present her work at several international conferences. In June 2019, she started working as a resident Internal Medicine (ANIOS) at the Zuyderland Medical Center in Sittard-Geleen and Heerlen under supervision of dr. J. Buijs. This clinical experience convinced her to become a gastroenterologist. In August 2020, she started her training to become a gastroenterologist (AIOS) under the supervision of Prof. dr. A. Masclee and dr. J. Kruiemel. Zsa Zsa currently lives in Maastricht, the beautiful capital of the southern part of the Netherlands.







