Journal Pre-proof

Efficacy and Safety of Peppermint Oil in a Randomized Double-blind Trial of Patients With Irritable Bowel Syndrome

Z.Z.R.M. Weerts, A.A.M. Masclee, B.J.M. Witteman, C.H.M. Clemens, B. Winkens, J.R.B.J. Brouwers, H.W. Frijlink, J.W.M. Muris, N.J. De Wit, B.A.B. Essers, J. Tack, J.W.T. Snijkers, A.M.H. Bours, A.S. de Ruiter-van der Ploeg, D.M.A.E. Jonkers, D. Keszthelyi

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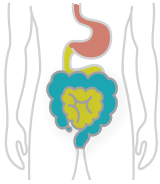
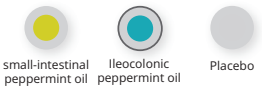
PERSUADE Peppermint oil in irritable bowel syndrome

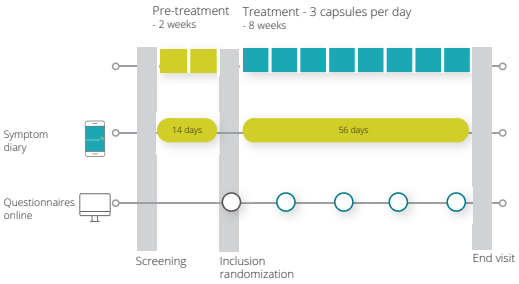
Concept





- 189 Patients with IBS (Rome IV)
- Mean age 34.0 years
- 77.8% female, 57.7% primary care

Design



- Primairy outcome: Abdominal Pain Response rate %
- Secondary outcomes, *e.g.*: IBS-symptom severity system (IBS-SSS)

Main results

Table 1:

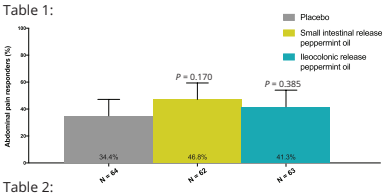
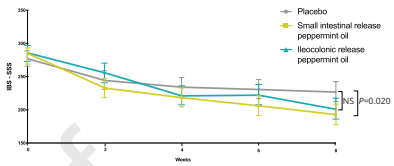


Table 2:



Gastroenterology

Efficacy and Safety of Peppermint Oil in a Randomized Double-blind Trial of Patients With Irritable Bowel Syndrome

SHORT TITLE

Effect of peppermint oil on IBS symptoms

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ABBREVIATIONS

AE: adverse event; BSFS: Bristol Stool Form Scale; CI: Confidence Interval; eCRF: electronic case report file; EMA: European Medicines Agency; FDA: Food and drug administration; GAD-7: Generalized Anxiety Disorder-7; GERD: Gastroesophageal reflux

disease; GI: Gastrointestinal; IBS-QoL: Irritable Bowel Syndrome Quality of Life; IBS-SSS: Irritable bowel syndrome symptom severity scoring system; IBS: irritable bowel syndrome; ITT: intention-to-treat; MUMC+: Maastricht University Medical Center; NNH: number needed to harm; NNT: number needed to treat; NRS: numerical rating scale; NSAID: Non-steroidal anti-inflammatory drug; OR: Odds Ratio; OTC: over-the-counter; PHQ-9: Patient Health Questionnaire-9; PP: per protocol; PPI: Proton pump inhibitor; TRP: Transient receptor potential

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DISCLOSURES / CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Z.Z.R.M. Weerts	Study concept and design, data collection, data analysis and interpretation, manuscript writing
A.A.M. Masclee	Study concept and design, obtained funding, data interpretation, constructive review of manuscript
B.J.M. Witteman	Study concept and design, constructive review of manuscript
C.H.M Clemens	Study concept and design, constructive review of manuscript
J.R.B.J. Brouwers	Study concept and design, constructive review of manuscript
H.W. Frijlink	Study concept and design, constructive review of manuscript
B. Winkens	Data analysis, constructive review of manuscript
J.W.M. Muris	Study concept and design, constructive review of manuscript
N.J. De Wit	Study concept and design, constructive review of manuscript
B.A.B. Essers	Study concept and design, constructive review of manuscript

J. Tack	Study concept and design, constructive review of manuscript
J.W.T. Snijkers	Data analysis
A.M.H. Bours	Data collection
A.S. de Ruiter	Data collection
D.M.A.E. Jonkers	Data interpretation, constructive review of manuscript
D. Keszthelyi	Study concept and design, obtained funding, data interpretation, constructive review of manuscript. All authors approved the final manuscript.

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ABSTRACT

Background & Aims: 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 (according to 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); 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 Food and Drug Administration: at least a 30% decrease in the weekly average of worst daily abdominal pain compared to baseline in at least 4 weeks. The co-primary endpoint was overall relief of IBS symptoms, as defined by the European Medicines Agency. Secondary endpoints included abdominal pain, discomfort, symptom severity, and adverse events.

Results: Abdominal pain response did not differ significantly between peppermint oil and placebo groups: 29/62 patients in the small intestinal-release peppermint oil group had a response (46.8%, *P*=.170 vs placebo), 26/63 patients in the ileocolonic-release peppermint oil group had a response (41.3%, *P*=.385 vs placebo), and 22/64 patients in the placebo group had a response (34.4%). We did not find differences among groups in overall relief (9.7%, *P*=.317 and 1.6%, *P*=.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*=.016), discomfort (*P*=.020), and IBS severity (*P*=.020). Adverse events, although mild, were more common in both peppermint oil groups (*P*<.005).

Conclusions: In a randomized trial of patients with IBS, 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. Clinicaltrials.gov no: NCT02716285

KEY WORDS: functional gastrointestinal disorder; PERSUADE study; RCT; treatment

INTRODUCTION

Irritable bowel syndrome (IBS) is a disorder of the gut-brain axis characterized by recurrent chronic abdominal pain and altered bowel habits[2]. IBS is highly prevalent with an estimated prevalence in the general population of 5-6% according to Rome IV criteria[3, 4]. IBS has a profound negative impact on quality of life and carries a substantial socioeconomic burden[5]. Although the number of therapeutic options has grown recently[6], 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[7], modulation of transient receptor potential (TRP) channel mediated visceral nociception[8-10], 5-hydroxytryptamine antagonism[11], antimicrobial and antifungal effects[12-14], and κ-opioid receptor agonism[15]. Enteric-coated capsules that release peppermint oil in the small intestine are currently available as an over-the-counter (OTC) drug in Europe[16] and as a medical food labeled product in the USA and Canada[17].

Guideline recommendations[18] 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[17, 19-23]. 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)[24] and the European Medicines Agency (EMA)[25] 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[1]. 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[8]. 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[26] 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 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 182mg of small-intestinal release peppermint oil (Tempocol®, WillPharma S.A.), 182mg of ileocolonic release peppermint oil (Tempocol®, core-capsules, coated with a Colopulse coating layer[1, 27]), or placebo (microcrystalline cellulose) intake orally. Randomization was done with ALEA Screening and Enrolment Application Software using the minimization method, accounted for inclusion center, IBS subtypes (diarrhea, mixed, constipation, undefined), gender, 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[17, 21]. This treatment duration was also selected to mitigate potential hazardous effects of long-term peppermint administration related to certain constituents[26]. Nevertheless, safety issues were later refuted by the EMA[28]. 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 for 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 H2-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)[29]; the Irritable Bowel Syndrome Quality of Life (IBS-QoL)[30], the EuroQoL-5D (EQ-5D-5L)[31, 32], the Generalized Anxiety Disorder-7 (GAD-7)[33], and the Patient Health Questionnaire-9 (PHQ-9)[34]. 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 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.

Efficacy assessment

Primary endpoints

The primary endpoint was the percentage of abdominal pain responders, according to FDA definition[24], 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[25], 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 does not influence bowel habit substantially. Therefore, improvements in bowel movements and stool consistency were not included into a combined primary efficacy endpoint[24], 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[35] 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 α-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[36]. 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 gender, 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 ≤ 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 α<0.025, is given in the *Supplementary Material*. Statistical analyses were carried out using IBM SPSS statistics 25.0 (Armonk, NY, USA) 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 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 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 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 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.

Primary efficacy outcomes

The proportion of abdominal pain responders did not differ significantly between groups: 46.8% in small-intestinal release peppermint oil (OR1.68; 95% CI0.80, 3.51; *P*=0.170; number needed to treat (NNT) 8.1) and 41.3% in ileocolonic release peppermint oil (OR1.39; 95%CI 0.66, 2.90; *P*=0.385; NNT 14.5), compared with 34.4% in placebo (*Table 2, Supplementary Table 2, Figure 1*).

The proportion of global relief responders did also not differ significantly between groups: 9.7% in small-intestinal release peppermint oil (OR2.12; 95%CI 0.49, 9.17; *P*=0.317), and 1.6% in ileocolonic release peppermint oil (OR0.33; 95%CI 0.03, 3.35; *P*=0.351), compared with 4.7% in placebo (*Table 2, Figure 1*).

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

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

Secondary efficacy outcomes

Results of exploratory secondary outcomes are presented in *Table 2* and *Supplementary Table 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 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 α=0.025) at eight weeks (*Figure 2, Supplementary Table 4)*.

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 3, Supplementary Table 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* 2*, Supplementary Figure* 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 8*). However, this did not reach the pre-specified level of significance (α=0.025).

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 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* 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 4*). All treatment groups showed improvements in quality of life that persisted over time, without a significant difference between groups (*Supplementary Table 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 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 6-7*). Efficacy outcomes did not differ significantly between primary and secondary/tertiary care patients (*Supplementary Table 9, Supplementary Material section 8*). 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 4*).

Adverse Events/Safety results

Table 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 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%)).

DISCUSSION

In this first randomized, double blind, placebo-controlled, clinical trial of peppermint oil in Rome IV-defined IBS patients, 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 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[35, 37], which indicated a difference in dichotomous overall abdominal pain improvement of 30% between placebo and peppermint oil[35]. 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 (182mg) 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[17]. 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[38-40]. 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[38], and plecanatide[39] and placebo. Of note is that the recent ACG Monograph[18] 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[35], 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[2], 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[37, 41]. 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[42, 43]. 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[8]. 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[38 40]. 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)[44]. 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[45]. 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[46]. 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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FIGURES

Figure 1. Percentage of patients who were abdominal pain responders (a) and global relief responders (b) in the ITT-population.

Figure 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.

Figure 2. Abdominal pain and discomfort scores in the ITT-population.

Figure 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.

Figure 3. IBS-SSS in the ITT-population.

Figure 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.

TABLES

Table 1. Summary of patient demographic and baseline characteristics (ITT-population)

	Placebo	Small-intestinal release Peppermint oil	Ileocolonic release Peppermint oil
	***N* = 64**	***N* = 62**	***N* = 63**
Demographic data			
Age, years			
Mean (SD)	35.5 (15.2)	32.0 (11.1)	34.4 (13.1)
Range	19-70	18-66	18-64
Gender, n (%)			
Female	49 (76.6)	51 (82.3)	47 (74.6)
Male	15 (23.4)	11 (17.7)	16 (25.4)
Race, n (%)			
Caucasian	63 (98.4)	60 (96.8)	58 (92.1)
Mixed[¶]	1 (1.6)	2 (3.2)	5 (7.9)
BMI, mean (SD)	24.6 (5.2)	25.6 (5.7)	26.5 (5.1)
Educational level, n (%)			
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)
Employment status, n (%)			
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
Setting, n (%)			
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)
IBS-Subtype, n (%)[‡]			
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)
Abdominal symptoms, mean (SD)			
Abdominal pain[§]	5.3 (1.3)	5.5 (1.2)	5.4 (1.4)
Abdominal discomfort[±]	6.3 (1.4)	6.4 (1.3)	6.5 (1.2)
Abdominal bloating[±]	6.4 (1.8)	6.4 (2.0)	6.7 (1.9)
Abdominal cramping[±]	6.2 (1.8)	6.0 (2.1)	6.3 (1.6)
Belching[±]	3.3 (2.5)	3.5 (2.4)	3.3 (2.7)
Nausea[±]	3.0 (2.4)	3.5 (2.7)	3.7 (2.5)
Bowel symptoms, mean (SD)			
Urgency[±]	6.2 (1.7)	6.3 (1.7)	6.6 (1.6)
IBS severity[†]			
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)

IBS Quality of Life, mean score (SD)[μ]	74.0 (14.2)	72.2 (14.7)	72.8 (16.6)
EQ-5D-5L, mean utility score (SD)[♮]	0.72 (0.2)	0.74 (0.2)	0.74 (0.2)
Psychological comorbidities[#]			
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/m2; *IBS* Irritable Bowel Syndrome.

¶ Self-reported race; placebo, *n*=1 mixed race is 1/4th Asian; small-intestinal release peppermint oil, *n*=1 mixed race is 1/4th Asian, and *n*=1 mixed race is 1/2nd unknown; ileocolonic release peppermint oil, *n*=4 mixed race is 1/4th Asian, and *n*=1 mixed race was 1/2nd 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=worse quality of life.

♮ The EQ-5D-5L measures 5-dimensions of QoL. Raw scores are transformed to utility scores[31], 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.

Table 2. Responder endpoints (ITT-population)

	Placebo ***N* = 64**	**Small-intestinal release Peppermint oil *N* = 62**			**Ileocolonic release Peppermint oil *N* = 63**		
			***P*-value**	**Odds Ratio (95% CI)**		***P*-value**	**Odds Ratio (95% CI)**
	No. responders (%)	*No. responders (%)*			*No. responders (%)*		
Primary endpoints							
Abdominal pain, 30%[¶]	22 (34.4)	29 (46.8)	0.170	1.68 (0.80 – 3.51)	26 (41.3)	0.385	1.39 (0.66 – 2.90)
Global relief[‡]	3 (4.7)	6 (9.7)	0.317	2.12 (0.49 – 9.17)	1 (1.6)	0.351	0.33 (0.03 – 3.35)
Secondary endpoints							
Moderate relief[§]	13 (20.3)	24 (38.7)	0.030	2.47 (1.09 – 5.56)	13 (20.6)	0.980	0.99 (0.41 - 2.38)
Abdominal pain, 50%[#]	8 (12.5)	16 (25.8)	0.062	2.51 (0.96 – 6.59)	13 (20.6)	0.220	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.

Table 3. Summary of treatment emerging adverse events (ITT-population)

	Placebo	**Small-intestinal release Peppermint oil**	**Ileocolonic release Peppermint oil**
	***N* = 64**	***N* = 62**	***N* = 63**
Total different AEs, mean (SE)	2.78 (0.34)	4.26 (0.37)*	4.45 (0.45)[#]
AEs[±], mean frequency (SE) / N (%)			
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)
AEs leading to discontinuation, total n	1	3	5
Headache, n	0	1	0
Palpitations, n	1	0	0
Diarrhea and abdominal cramps, n	0	0	1
Combination[¶], n	0	1	2

Combination‡, n	0	1	0
Combination§, n	0	0	2

AE adverse event; *SE* standard error; *GERD* Gastroesophageal Reflux Disease.

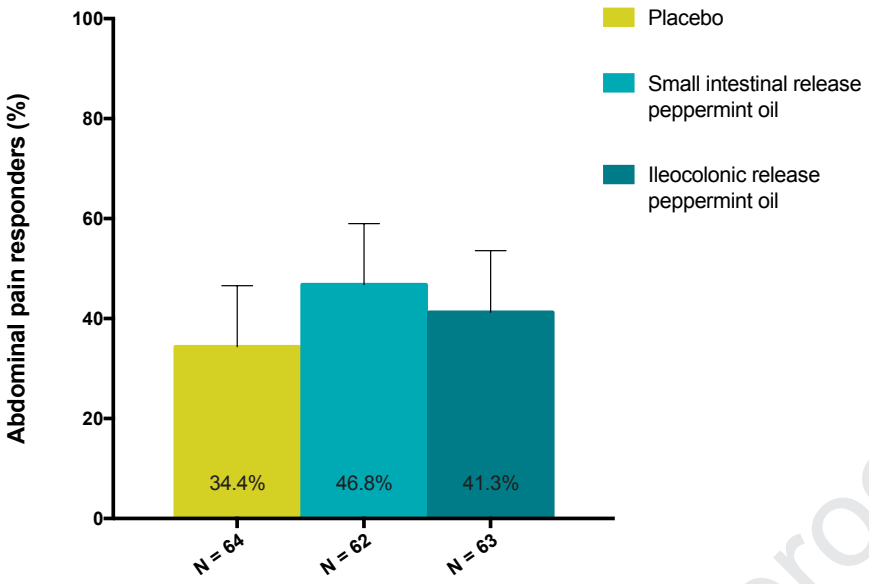
± Occurrence of AEs was self-reported in the daily symptom diary.

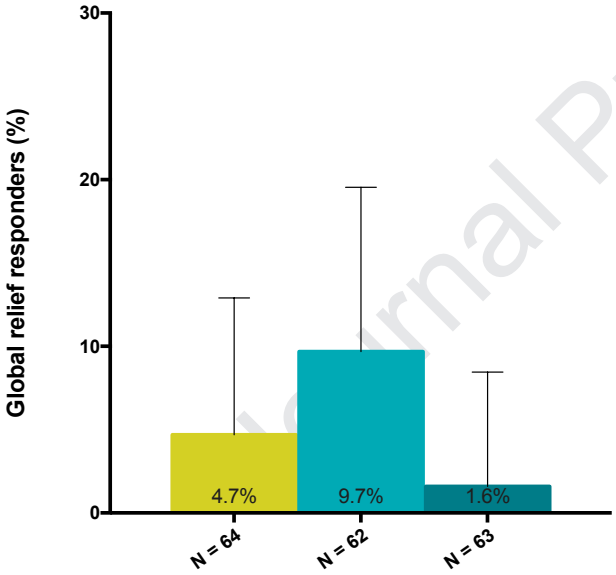
The total number of different AEs for small-intestinal release compared with placebo was significantly higher **P*=0.012, as well as for ileocolonic release peppermint oil compared with placebo #*P*=0.001.

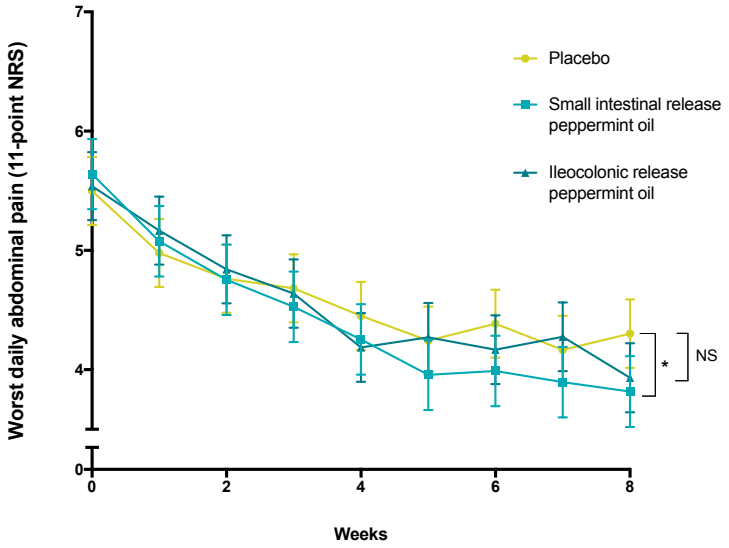
¶ Combination of *i.e.* flatulence, bloating, abdominal pain.

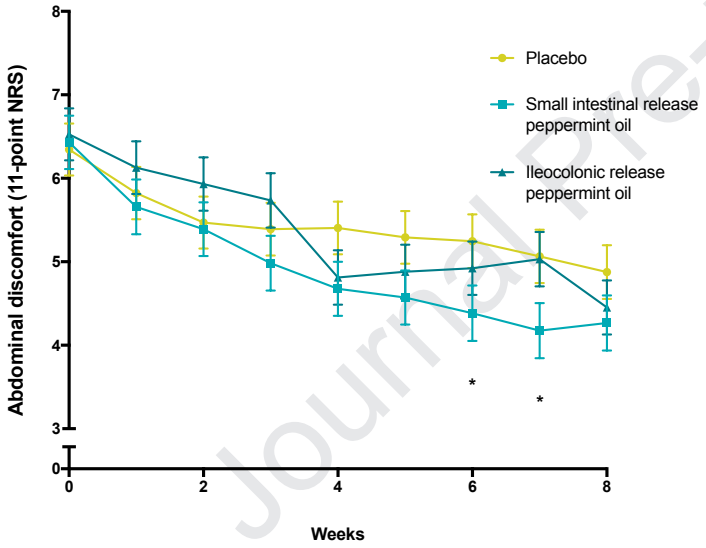
‡ Combination of *i.e.* headache, tightness of the chest, belching, bloating, muscle cramp.

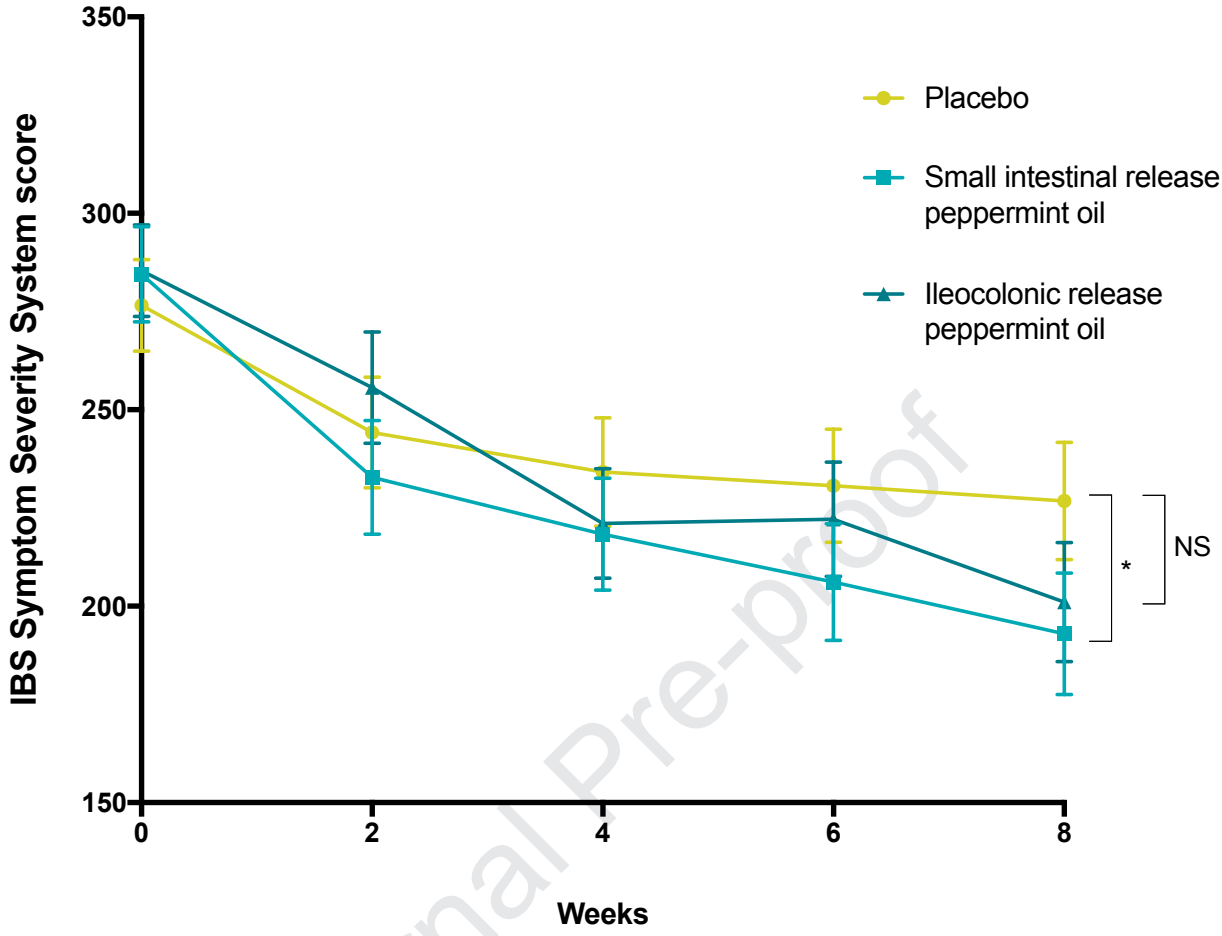
§ Combination of *i.e.* diarrhea, abdominal cramps, altered anal sensation, belching, altered taste.











What you need to know:

BACKGROUND AND CONTEXT: Peppermint oil is frequently used to treat irritable bowel syndrome (IBS), despite a lack of high quality evidence for efficacy. We studied the efficacy and safety of small intestinal-release peppermint oil in patients with IBS (Rome-IV) and explored the effects of targeted ileocolonic-release peppermint oil according to guidelines from regulatory authorities.

NEW FINDINGS: In a randomized trial of patients with IBS, 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. The small intestinal-release peppermint oil did, however, significantly reduce abdominal pain, discomfort, and IBS symptom severity.

LIMITATIONS: The primary outcome of this trial was a negative result. Improvements in secondary explorative endpoints should be interpreted with appropriate caution.

IMPACT: Peppermint oil can be considered a treatment option with moderate efficacy for patients with IBS.

Lay Summary: Peppermint oil does not significantly reduce abdominal pain or overall symptoms of relief in patients with IBS, according to the strict endpoints recommended by regulatory authorities. Small-intestinal release peppermint oil does, however, reduce abdominal pain, discomfort, and IBS symptom severity.

SUPPLEMENTARY MATERIAL

Efficacy and safety of peppermint oil in Irritable Bowel Syndrome: the PERSUADE randomized double-blind controlled clinical trial.

Original Research Article

SHORT TITLE

Effect of peppermint oil on IBS symptoms

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1. 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.

2. 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.

3. 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 1 error for a single comparison, an two-sided *P*-value ≤ 0.025 was considered statistically significant for secondary outcome analyses. Secondary outcomes were exploratory in nature.

4. DATA AVAILABILITY STATEMENT

The datasets analyzed during the current study are available for scientific researchers upon reasonable request through the first or last author.

5. SUPPLEMENTARY TABLES

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

	Placebo	**Small-intestinal release Peppermint oil**	**Ileocolonic release Peppermint oil**
	***N* = 64**	***N* = 62**	***N* = 63**
Adherent to study medication[¶]			
Number of patients (%)	62 (96.9)	56 (90.3)	58 (92.1)
Compliance rate to the digital symptom diary[±]			
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.

± 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.

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

	Small-intestinal release Peppermint oil	Ileocolonic release Peppermint oil
	***N* = 62**	***N* = 63**
Number needed to treat		
Based on primary abdominal response outcome[¶]	8.1	14.5
Based on moderate global relief outcome[§]	5.4	N.A.
Number needed to harm		
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.

Supplementary Table 3. Responder endpoints (PP-population)

	Placebo *N* = 59	Small-intestinal release Peppermint oil *N* = 55			Ileocolonic release Peppermint oil *N* = 56		
			***P*-value**	**Odds Ratio (95% CI)**		***P*-value**	**Odds Ratio (95% CI)**
	No. responders (%)	*No. responders (%)*			*No. responders (%)*		
Primary endpoints							
Abdominal Pain[¶]	21 (35.6)	25 (45.5)	0.335	1.46 (0.68 - 3.15)	26 (46.4)	0.204	1.64 (0.76 - 3.53)
Global Relief[‡]	3 (5.1)	6 (10.9)	0.243	2.44 (0.55 – 10.89)	1 (1.8)	0.359	0.34 (0.03 – 3.42)

The per-protocol-population included all randomly assigned patients who had at least 80% adherence to treatment and had completed the treatment period.

P-values, ORs and corresponding two-sided 95% CIs were calculated using multiple logistic regression adjusted for minimization variables.

¶ 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).

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

	Placebo	**Small-intestinal release Peppermint oil**			**Ileocolonic release Peppermint oil**		
	***N* = 64**	***N* = 62**			***N* = 63**		
Measurement	*Estimated means (SE)*	*Estimated means (SE)*	*Treatment effect (95% CI)*	*P*-value	*Estimated means (SE)*	*Treatment effect (95% CI)*	*P*-value
			Mean worst abdominal pain [§]				
Baseline	5.50 (0.29)	5.64 (0.29)	-	-	5.54 (0.29)	-	-
Week 1	4.98 (0.29)	5.08 (0.29)	-0.04 (-0.77; 0.68)	0.905	5.17 (0.29)	0.15 (-0.58; 0.87)	0.691
Week 2	4.76 (0.29)	4.75 (0.29)	-0.15 (-0.85; 0.55)	0.674	4.84 (0.29)	0.04 (-0.66; 0.74)	0.912
Week 3	4.68 (0.29)	4.53 (0.30)	-0.30 (-0.98; 0.39)	0.394	4.64 (0.29)	-0.08 (-0.76; 0.59)	0.808
Week 4	4.45 (0.29)	4.25 (0.30)	-0.34 (-0.99; 0.32)	0.310	4.19 (0.29)	-0.31 (-0.96; 0.35)	0.361
Week 5	4.24 (0.29)	3.96 (0.30)	-0.42 (-1.05; 0.19)	0.180	4.27 (0.29)	-0.01 (-0.64; 0.61)	0.970
Week 6	4.39 (0.29)	3.99 (0.30)	-0.54 (-1.13; 0.05)	0.074	4.17 (0.29)	-0.25 (-0.85; 0.33)	0.391
Week 7	4.17 (0.29)	3.89 (0.30)	-0.41 (-0.97; 0.14)	0.144	4.28 (0.29)	0.07 (-0.49; 0.63)	0.806
Week 8	4.30 (0.29)	3.82 (0.30)	-0.63 (-1.14; 0.12)	**0.016**	3.93 (0.29)	-0.41 (-0.92; 0.10)	0.117
			Abdominal discomfort [±]				
Baseline	6.35 (0.31)	6.43 (0.32)	-	-	6.53 (0.31)	-	-
Week 1	5.82 (0.31)	5.66 (0.33)	-0.25 (-1.19; 0.69)	0.601	6.13 (0.32)	0.12 (-0.81; 1.06)	0.795
Week 2	5.47 (0.31)	5.39 (0.32)	-0,16 (-1.07; 0.74)	0.722	5.93 (0.32)	0.28 (-0.63; 1.19)	0.546
Week 3	5.39 (0.32)	4.98 (0.33)	-0.49 (-1.39; 0.40)	0.282	5.74 (0.33)	0.16 (-0.74; 1.07)	0.722
Week 4	5.41 (0.32)	4.68 (0.33)	-0.81 (-1.68; 0.05)	0.064	4.81 (0.33)	-0.77 (-1.65; 0.10)	0.083

Week 5	5.29 (0.32)	4.57 (0.32)	-0.80 (-1.63; 0.02)	0.056	4.88 (0.32)	-0.59 (-1.42; 0.24)	0.164
Week 6	5.25 (0.32)	4.38 (0.33)	-0.95 (-1.74; -0.15)	**0.020**	4.92 (0.32)	-0.50 (-1.30; 0.29)	0.212
Week 7	5.07 (0.32)	4.18 (0.33)	-0.97 (-1.71; -0.24)	**0.009**	5.03 (0.33)	-0.21 (-0.95; 0.53)	0.572
Week 8	4.88 (0.32)	4.27 (0.33)	-0.69 (-1.36; -0.03)	0.041	4.45 (0.33)	-0.60 (1.27; 0.06)	0.076
			Abdominal bloating [±]				
Baseline	6.53 (0.38)	6.44 (0.39)	-	-	6.68 (0.38)	-	-
Week 1	5.51 (0.38)	5.15 (0.40)	-0.27 (-1.28; 0.73)	0.594	5.52 (0.38)	-0.15 (-1.15; 0.86)	0.772
Week 2	5.06 (0.38)	4.97 (0.39)	-0.00 (-0.98; 0.97)	0.994	5.45 (0.39)	0.23 (-0.75; 1.21)	0.646
Week 3	5.21 (0.38)	4.63 (0.40)	-0.49 (-1.45; 0.47)	0.316	5.11 (0.39)	-0.25 (-1.22; 0.72)	0.607
Week 4	5.03 (0.38)	4.19 (0.40)	-0.75 (-1.67; 0.17)	0.109	4.58 (0.39)	-0.61 (-1.54; 0.33)	0.203
Week 5	4.74 (0.38)	4.22 (0.40)	-0.44 (-1.32; 0.44)	0.328	4.77 (0.39)	-0.13 (-1.02; 0.77)	0.778
Week 6	5.04 (0.39)	4.10 (0.40)	-0.86 (-1.72; 0.00)	0.051	4.51 (0.39)	-0.69 (-1.55; 0.17)	0.114
Week 7	4.96 (0.39)	3.81 (0.40)	-1.07 (-1.87; -0.26)	**0.009**	4.06 (0.40)	-1.06 (-1.87; -0.24)	**0.011**
Week 8	4.52 (0.39)	4.03 (0.40)	-0.43 (-1.19; 0.33)	0.269	4.18 (0.39)	-0.52 (-1.28; 0.24)	0.180
			Abdominal cramping [±]				
Baseline	6.10 (0.33)	5.91 (0.34)	-	-	6.15 (0.33)	-	-
Week 1	4.87 (0.35)	5.07 (0.36)	0.38 (-0.38; 1.15)	0.322	5.93 (0.35)	1.01 (0.25; 1.77)	**0.010**
Week 2	4.87 (0.37)	4.73 (0.39)	0.04 (-0.85; 0.93)	0.929	5.56 (0.38)	0.64 (-0.26; 1.54)	0.163
Week 3	4.76 (0.39)	4.57 (0.40)	-0.01 (-0.96; 0.94)	0.984	5.12 (0.40)	0.31 (-0.66; 1.27)	0.533
Week 4	4.86 (0.37)	4.46 (0.38)	-0.21 (-1.10; 0.69)	0.648	4.86 (0.39)	-0.05 (-0.97; 0.86)	0.910
Week 5	4.97 (0.38)	4.36 (0.39)	-0.42 (-1.29; 0.45)	0.345	4.83 (0.39)	-0.19 (-1.08; 0.69)	0.668
Week 6	5.03 (0.39)	4.18 (0.40)	-0.66 (-1.54; 0.22)	0.140	4.85 (0.39)	-0.24 (-1.11; 0.64)	0.597
Week 7	4.40 (0.39)	3.69 (0.40)	-0.52 (-1.36; 0.31)	0.218	4.88 (0.40)	0.43 (-0.42; 1.27)	0.323

Week 8	4.36 (0.42)	3.83 (0.43)	-0.34 (-1.17; 0.49)	0.419	4.23 (0.42)	-0.18 (-1.02; 0.66)	0.673
			Belching [±]				
Baseline	3.41 (0.35)	3.65 (0.36)	-	-	3.41 (0.36)	-	-
Week 1	2.92 (0.35)	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)	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)	0.52 (-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)	0.24 ((-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)	-0.27 (-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)	0.29 (-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)	0.20 (-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)	0.17 (-0.58; 0.92)	0.655	1.74 (0.28)	-0.53 (-1.28; 0.23)	0.169
			Nausea [±]				
Baseline	2.89 (0.39)	3.29 (0.40)	-	-	3.56 (0.39)	-	-
Week 1	3.82 (0.40)	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)	-0.52 (-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)	-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)	-1.08 (-2.02; -1.44)	**0.024**	2.00 (0.41)	-1.42 (-2.38; -0.46)	**0.004**
Week 5	2.63 (0.40)	2.12 (0.41)	-0.91 (-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)	-0.66 (-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)	-0.71 (-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)	-0.67 (-1.50; 0.16)	0.115	1.81 (0.41)	-1.05 (-1.89; -0.21)	**0.015**

			Urgency [±]				
Baseline	6.41 (0.32)	6.55 (0.33)	-	-	6.73 (0.32)	-	-
Week 1	5.56 (0.33)	5.74 (0.34)	0.04 (-0.87; 0,96)	0.926	5.88 (0.33)	0.01 (-0.91; 0.92)	0.988
Week 2	5.34 (0.33)	5.73 (0.34)	0.25 (-0.64; 1.14)	0.577	5.93 (0.33)	0.28 (-0.62; 1.18)	0.541
Week 3	5.56 (0.33)	5.25 (0.34)	-0.45 (-1.33; 0.43)	0.316	5.70 (0.34)	-0.18 (-1.07; 0.71)	0.695
Week 4	5.42 (0.33)	5.15 (0.34)	-0.41 (-1.26; 0.43)	0.342	5.36 (0.34)	-0.38 (-1.23; 0.49)	0.395
Week 5	5.12 (0.33)	5.06 (0.34)	-0.20 (-1.02; 0.62)	0.634	5.41 (0.34)	-0.03 (-0.86; 0.81)	0.949
Week 6	4.90 (0.33)	4.68 (0.35)	-0.36 (-1.17; 0.45)	0.386	5.24 (0.33)	0.04 (-0.77; 0.84)	0.930
Week 7	5.09 (0.33)	4.61 (0.34)	-0.61 (-1.37; 0.15)	0.116	5.22 (0.34)	-0.18 (-0,96; 0.59)	0.645
Week 8	5.06 (0.33)	4.57 (0.35)	-0.63 (-1.35; 0.10)	0.091	4.97 (0.34)	-0.40 (-1.13; 0.33)	0.282
			IBS symptom severity [†]				
Baseline	276.53 (11.68)	284.52 (12.10)	-	-	285.42 (11.65)	-	-
Week 2	244.21 (14.10)	232.82 (14.50)	-19.38 (-48.01; 9.25)	0.183	255.65 (14.15)	2.56 (-26.06; 31.18)	0.860
Week 4	234.16 (13.77)	218.33 (14.21)	-23.81 (-54.61; 6.98)	0.129	221.10 (13.94)	-21.95 (-52.85; 8.95)	0.163
Week 6	230.68 (14.42)	206.13 (14.90)	-32.52 (-66.07; 1.03)	0.057	222.10 (14.58)	-17.45 (-15.01; 16.11)	0.306
Week 8	226.80 (14.97)	192.99 (15.45)	-41.79 (-76.88; -6.70)	**0.020**	201.05 (15.14)	-34.64 (-69.75; 0.48)	0.053
Month 6*	215.66 (13.97)	213.31 (14.42)	-8.86 (-42.03; 24.31)	0.600	224.28 (14.11)	-0.02 (-33.27; 33.23)	0.999
			IBS quality of life score [µ]				
Baseline	70.47 (2.47)	68.75 (2.56)	-	-	69.77 (2.47)	-	-
Week 4	73.66 (2.47)	72.87 (2.56)	0.93 (-1.75; 3.60)	0.495	74.01 (2.47)	1.05 (-1.65; 3.75)	0.443
Week 8	75.01 (2.50)	75.85 (2.59)	2.56 (-0.17; 5.29)	0.066	75.54 (2.50)	1.24 (-1.50; 3.97)	0.374

Month 6*	74.70 (2.52)	72.79 (2.61)	-0.31 (-3.34; 2.71)	0.838	73.92 (2.52)	-0.13 (-3.17; 2.90)	0.931
			EQ-5D-5L [ϟ]				
Baseline	0.70 (0.03)	0.71 (0.03)	-	-	0.72 (0.03)	-	-
Week 4	0.71 (0.03)	0.76 (0.03)	0.04 (-0.01; 0.10)	0.110	0.73 (0.03)	0.00 (-0.05; 0.06)	0.943
Week 8	0.72 (0.03	0.77 (0.03)	0.04 (-0.01; 0.10)	0.131	0.74 (0.03)	0.00 (-0.05; 0.06)	0.914
Month 6*	0.79 (0.03)	0.78 (0.03)	-0.02 (-0.75; 0.04)	0.509	0.77 (0.03)	-0.04 (-0.09; 0.02)	0.174
			Anxiety [#]				
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
			Depression [#]				
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.

ϟ 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[37], 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.

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

	Placebo	**Small-intestinal release Peppermint oil**			**Ileocolonic release Peppermint oil**		
	***N* = 64**	***N* = 62**			***N* = 63**		
Measurement	*Estimated means (SE)*	*Estimated means (SE)*	*Treatment effect (95% CI)*	*P*-value	*Estimated means (SE)*	*Treatment effect (95% CI)*	*P*-value
			Frequency scores [§]				
Baseline	9.10 (0.75)	9.38 (0.76)	-	-	8.55 (0.75)	-	-
Week 1	9.23 (0.67)	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)	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)	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)	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)	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)	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)	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)	4.66 (0.42)	0.32 (-1.97; 2.62)	0.783	4.00 (0.45)	0.49 (-1.82; 2.80)	0.678
			Stool consistency [±]				
Baseline	4.10 (0.16)	3.97 (0.16)	-	-	4.30 (0.16)	-	-
Week 1	4.06 (0.16)	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)	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)	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)	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)	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)	4.53 (0.16)	0.52 (0.09; 0.95)	**0.018**	4.61 (0.17)	0.27 (-0.16; 0.71)	0.219
Week 7	4.22 (0.16)	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)	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.

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

	Placebo	**Small-intestinal release Peppermint oil**			**Ileocolonic release Peppermint oil**		
	***N* = 29**	***N* = 25**			***N* = 29**		
Measurement	*Estimated means (SE)*	*Estimated means (SE)*	*Treatment effect (95% CI)*	*P*-value	*Estimated means (SE)*	*Treatment effect (95% CI)*	*P*-value
			Frequency scores in patients with IBS subtype diarrhea[§]				
Baseline	11.31 (1.20)	11.80 (1.29)	-	-	9.79 (1.20)	-	-
Week 1	11.90 (1.17)	11.16 (1.26)	-1.23 (-3.49; 1.03)	0.285	11.07 (1.17)	0.69 (-1.48; 2.86)	0.532
Week 2	9.46 (1.24)	10.96 (1.32)	1.01 (-2.12; 4.13)	0.525	8.65 (1.24)	0.71 (-2.32; 3.74)	0.644
Week 3	7.96 (1.05)	9.87 (1.15)	1.42 (-1.99; 4.82)	0.414	7.92 (1.05)	1.47 (-1.78; 4.73)	0.374
Week 4	8.01 (1.28)	9.91 (1.39)	1.41 (-2.67; 5.49)	0.498	8.46 (1.29)	1.97 (-1.95; 5.89)	0.323
Week 5	7.43 (1.06)	7.16 (1.17)	-0.76 (-4.70; 3.18)	0.704	6.75 (1.11)	0.83 (-2.98; 4.65)	0.667
Week 6	6.46 (1.23)	6.67 (1.30)	-0.28 (-4.63; 4.08)	0.901	7.05 (1.26)	2.11 (-2.15; 6.37)	0.330
Week 7	6.17 (0.86)	5.16 (0.96)	-1.50 (-5.43; 2.43)	0.452	5.11 (0.91)	0.46 (-3.34; 4.26)	0.813
Week 8	4.53 (0.63)	4.62 (0.71)	-0.40 (-4.13; 3.33)	0.832	4.72 (0.68)	1.71 (-1.89; 5.31)	0.350
			Stool consistency in patients with IBS subtype diarrhea[±]				
Baseline	4.64 (0.19)	4.81 (0.21)	-	-	5.10 (0.19)	-	-
Week 1	4.47 (0.19)	4.78 (0.21)	0.14 (-0.28; 0.56)	0.518	5.24 (0.19)	0.31 (-0.10; 0.72)	0.141
Week 2	4.49 (0.20)	4.87 (0.21)	0.20 (-0.26; 0.67)	0.385	5.10 (0.20)	0.15 (-0.30; 0.60)	0.521

Week 3	4.25 (0.20)	4.98 (0.22)	0.56 (0.06; 1.06)	0.028	5.26 (0.20)	0.55 (0.07; 1.02)	**0.023**
Week 4	4.27 (0.20)	4.87 (0.22)	0.42 (-0.11; 0.95)	0.118	5.28 (0.20)	0.54 (0.04; 1.05)	0.036
Week 5	4.75 (0.20)	5.19 (0.22)	0.26 (-0.27; 0.80)	0.333	5.39 (0.20)	0.18 (-0.34; 0.70)	0.496
Week 6	4.51 (0.20)	5.37 (0.21)	0.69 (0.14; 1.24)	**0.014**	5.40 (0.20)	0.43 (-0.11; 0.97)	0.119
Week 7	4.54 (0.20)	5.05 (0.23)	0.34 (-0.24; 0.92)	0.255	5.62 (0.21)	0.62 (0.06; 1.18)	0.030
Week 8	4.62 (0.21)	5.07 (0.23)	0.28 (-0.33; 0.89)	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.

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

	Placebo	**Small-intestinal release Peppermint oil**			**Ileocolonic release Peppermint oil**		
	***N* = 14**	***N* = 12**			***N* = 16**		
Measurement	*Estimated means (SE)*	*Estimated means (SE)*	*Treatment effect (95% CI)*	*P*-value	*Estimated means (SE)*	*Treatment effect (95% CI)*	*P*-value
			Frequency scores in patients with IBS subtype constipation [§]				
Baseline	6.79 (0.82)	5.96 (0.88)	-	-	5.56 (0.76)	-	-
Week 1	7.14 (0.83)	6.58 (0.90)	0.27 (-1.79; 2.32)	0.796	5.56 (0.78)	-0.36 (-2.27; 1.56)	0.711
Week 2	5.86 (0.70)	5.42 (0.76)	0.39 (-2.05; 2.82)	0.753	3.81 (0.65)	-0.82 (-3.08; 1.44)	0.473
Week 3	5.57 (0.72)	4.80 (0.80)	0.05 (-2.71; 2.82)	0.969	4.57 (0.70)	0.22 (-2.36; 2.81)	0.864
Week 4	5.86 (0.79)	3.67 (0.82)	-1.36 (-4.36; 1.64)	0.370	4.58 (0.74)	-0.05 (-2.88; 2.77)	0.972
Week 5	6.18 (1.01)	4.47 (1.10)	-0.88 (-4.49; 2.73)	0.630	4.59 (0.96)	-0.37 (-3.74; 3.00)	0.830
Week 6	5.19 (0.80)	3.90 (0.86)	-0.46 (-3.68; 2.76)	0.778	4.24 (0.76)	0.27 (-2.73; 3.28)	0.857
Week 7	5.86 (0.89)	3.72 (0.96)	-1.31 (-4.77; 2.16)	0.454	4.12 (0.89)	-0.52 (-3.80; 2.77)	0.755
Week 8	3.81 (0.78)	4.15 (0.83)	1.17 (-2.09; 4.42)	0.478	3.69 (0.78)	1.10 (-1.99; 4.19)	0.479
			Stool consistency in patients with IBS subtype constipation [±]				
Baseline	3.23 (0.32)	2.99 (0.34)	-	-	3.08 (0.30)	-	-
Week 1	3.07 (0.32)	3.47 (0.34)	0.64 (-0.35; 1.63)	0.202	3.57 (0.30)	0.65 (-0.27; 1.57)	0.167
Week 2	2.82 (0.32)	2.87 (0.34)	0.29 (-0.74; 1.31)	0.579	3.53 (0.30)	0.85 (-0.10; 1.80)	0.080
Week 3	3.73 (0.32)	3.43 (0.35)	-0.07 (-1.13; 1.00)	0.903	3.66 (0.31)	0.07 (-0.93; 1.07)	0.895
Week 4	3.35 (0.33)	3.15 (0.34)	0.04 (-1.06; 1.14)	0.945	3.68 (0.31)	0.48 (-0.57; 1.52)	0.372

Week 5	3.01 (0.33)	2.88 (0.35)	0.10 (-1.02; 1.23)	0.855	3.54 (0.31)	0.68 (-0.38; 1.74)	0.207
Week 6	3.10 (0.33)	3.42 (0.35)	0.56 (-0.59; 1.71)	0.337	3.55 (0.31)	0.59 (0.49; 1.67)	0.279
Week 7	3.17 (0.33)	3.81 (0.35)	0.87 (-0.30; 2.05)	0.142	3.92 (0.33)	0.90 (-0.22; 2.02)	0.115
Week 8	3.55 (0.35)	3.42 (0.37)	0.11 (-1.12; 1.34)	0.860	3.83 (0.35)	0.42 (-0.75; 1.60)	0.477

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.

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

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

¶ Mean frequency of use per patient during the eight-week treatment period.

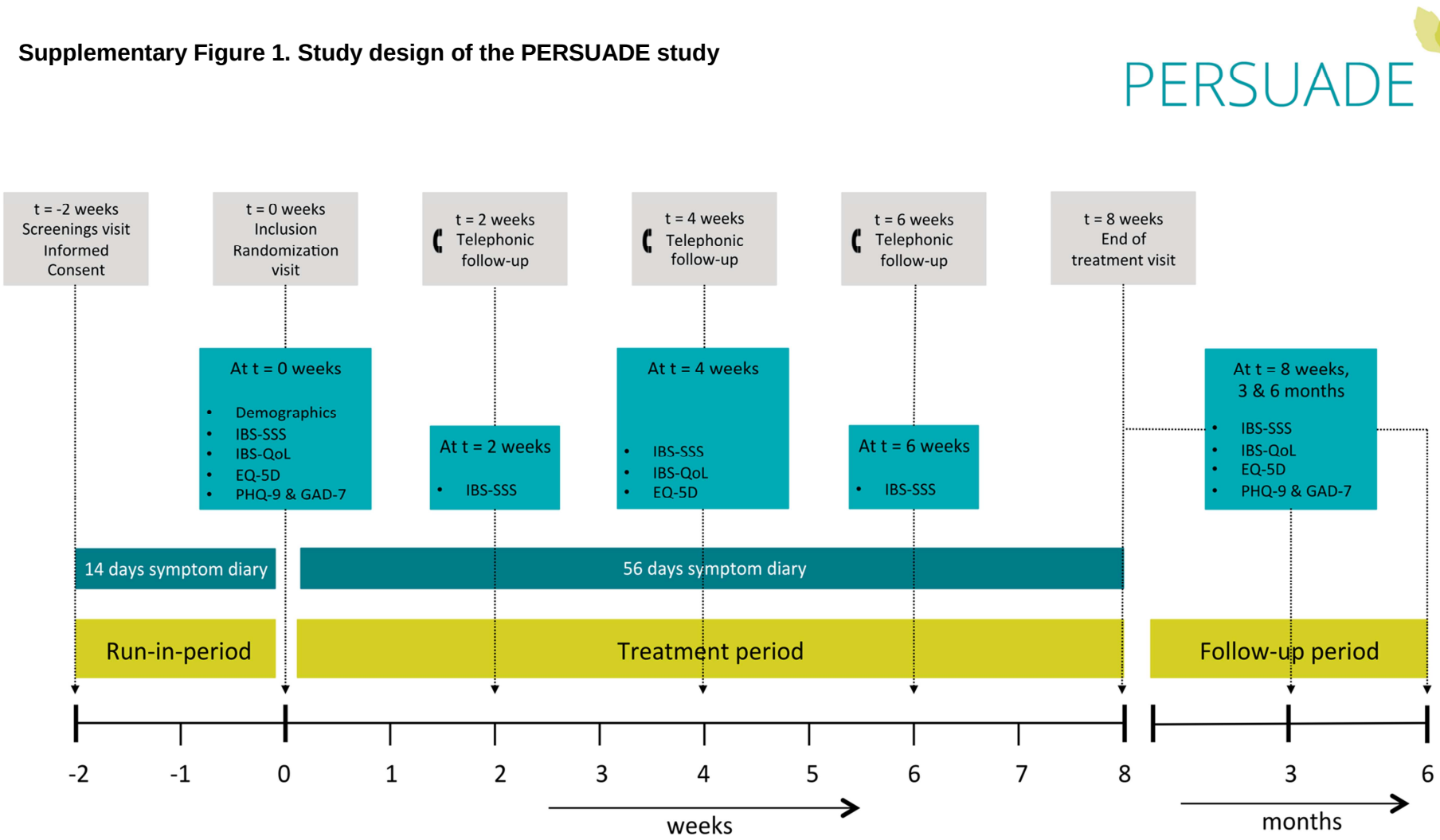
± GI-medication comprises of *i.e.* antacids, laxatives, and anti-diarrheal drugs.

‡ Number of patients and percentage of patients that used the medication at least once during the eight-week treatment period.

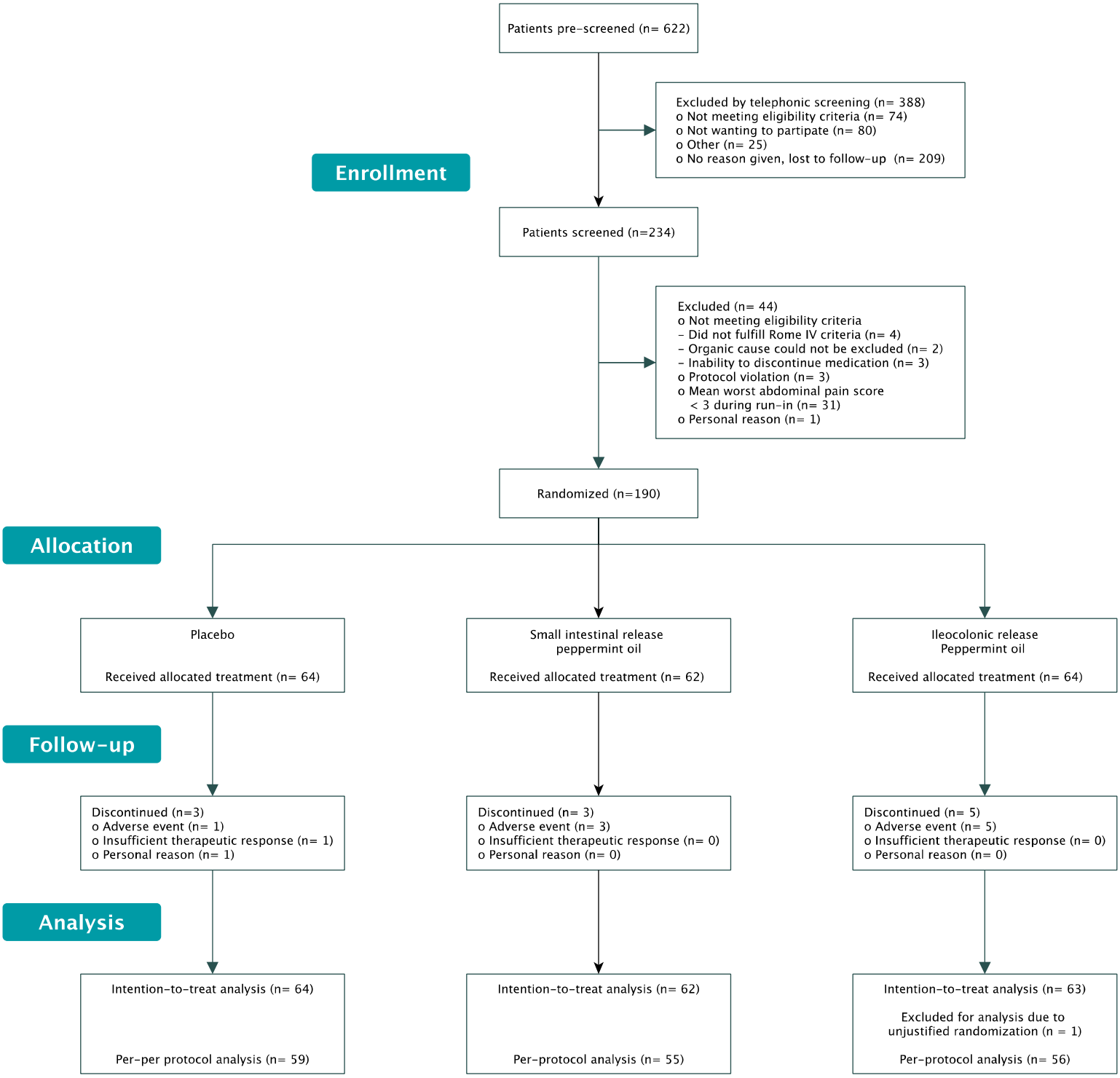
The differences in mean frequency between small-intestinal release peppermint oil and placebo (P=0.087 for pain medication, P=0.457 for GI-medication) and ileocolonic release peppermint oil and placebo (P=0.039 for pain medication, P=0.044 for GI medication) did not reach statistical significance (α=0.025).

6. SUPPLEMENTARY FIGURES

Supplementary Figure 1. Study design of the PERSUADE study



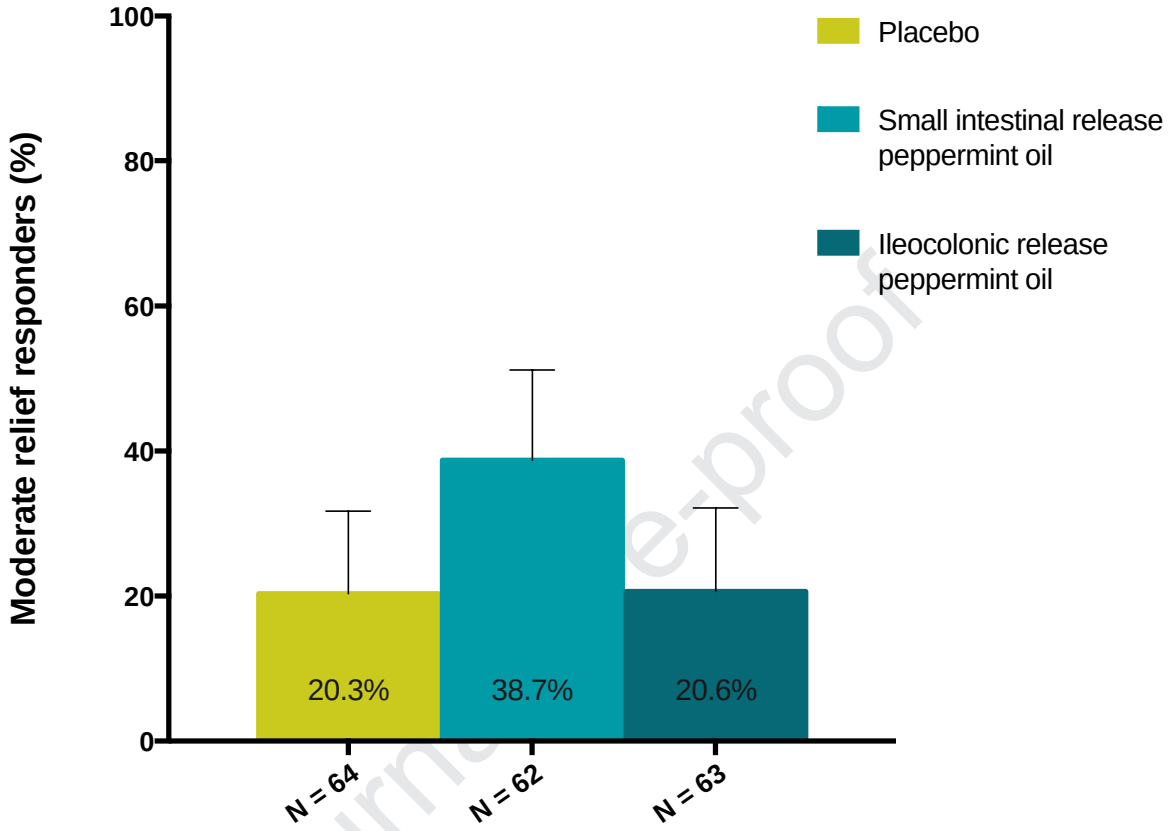
Supplementary Figure 2. CONSORT flowchart of patient flow throughout the study



Supplementary Figure 2. Flowchart of patients included in the PERSUADE study.

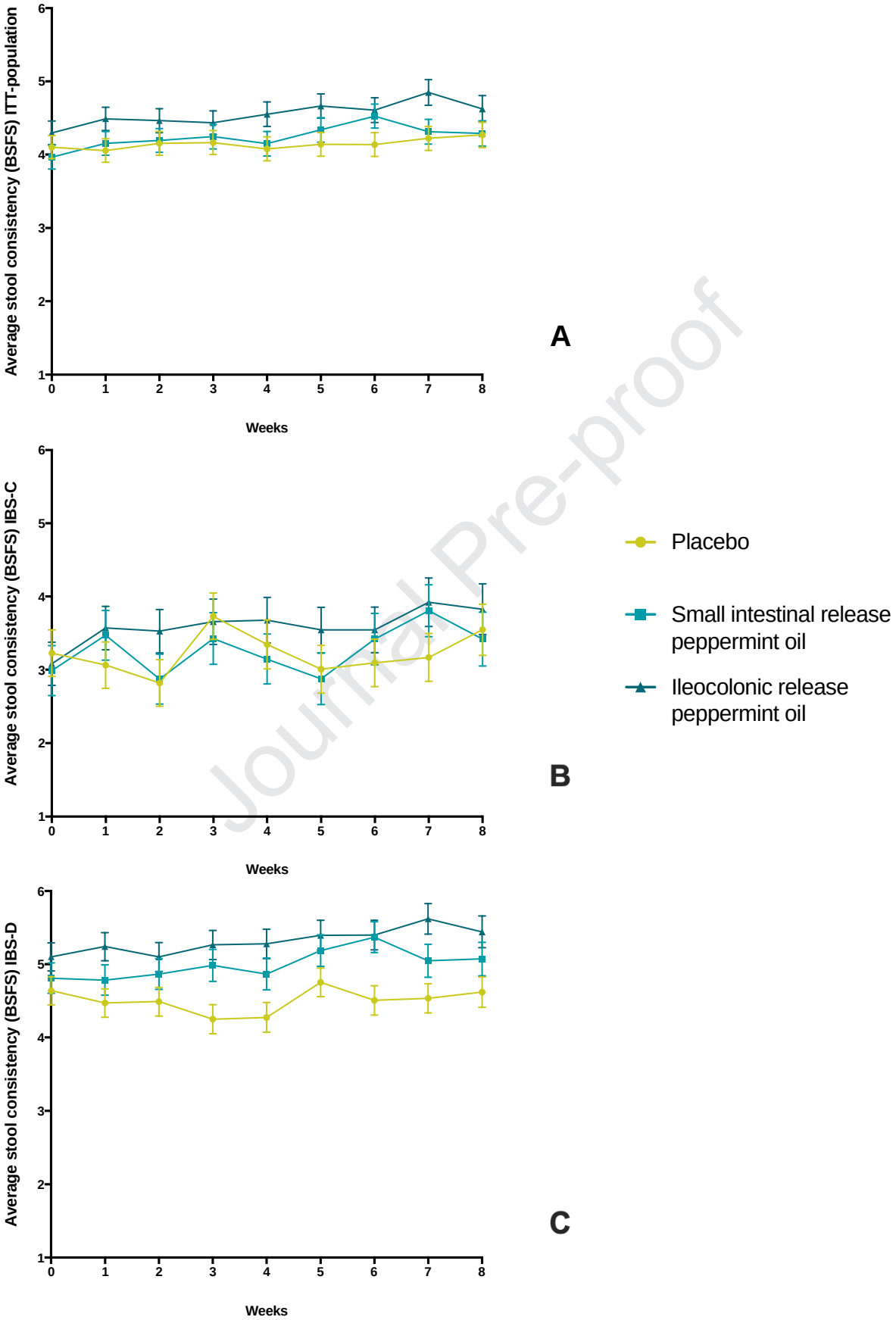
IBS; Irritable Bowel Syndrome.

Supplementary Figure 3. Percentage of moderate relief responders



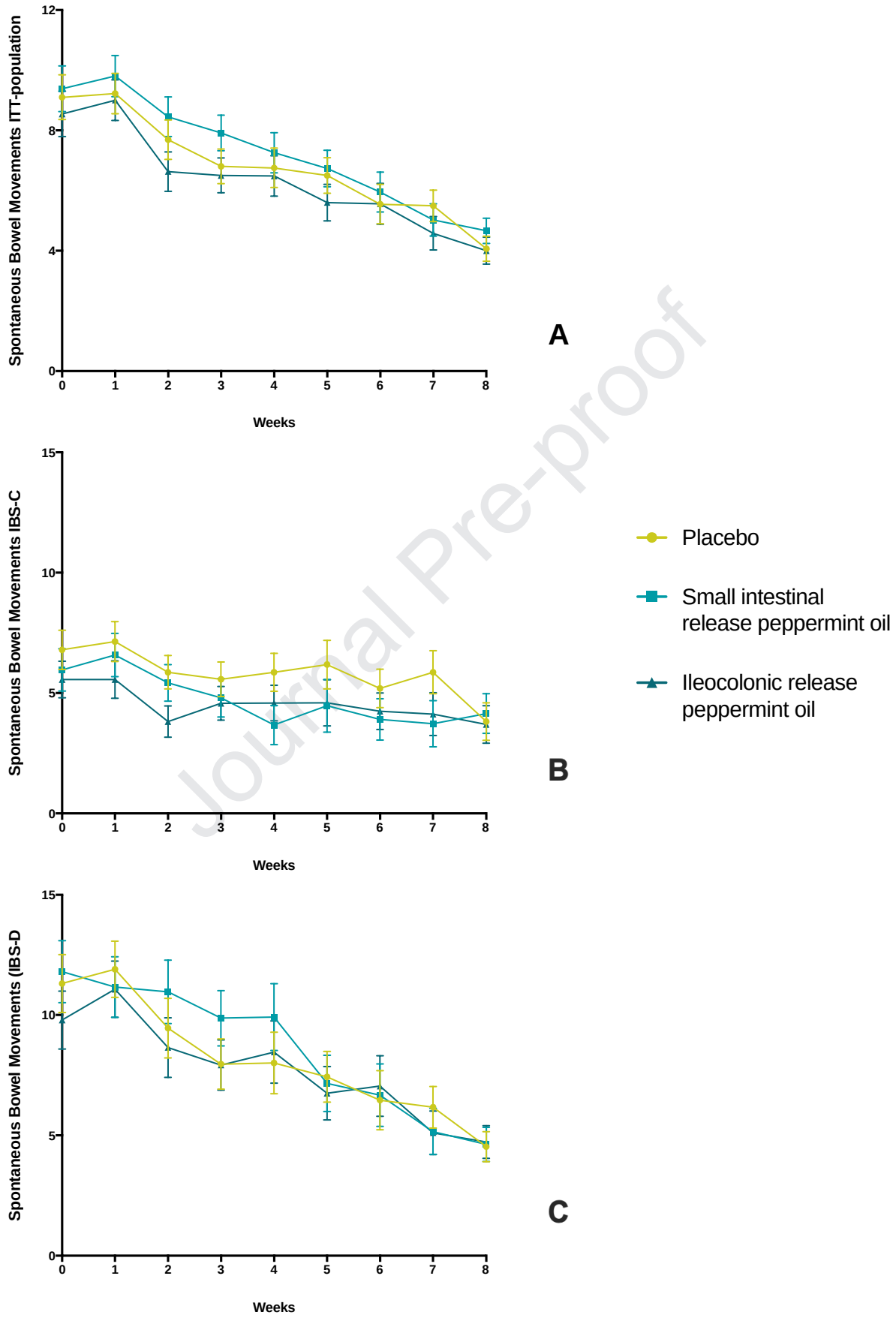
Supplementary Figure 3. Percentage of patients who were moderate relief responders in the ITT-population. A responder was a patient with at least a relief score of 5, 6 or 7 (on a 7-point NRS) in at least 4 weeks out of 8 weeks. Values are percentages, bars represent standard errors. *P*=0.030 for small-intestinal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

Supplementary Figure 4. Stool consistency



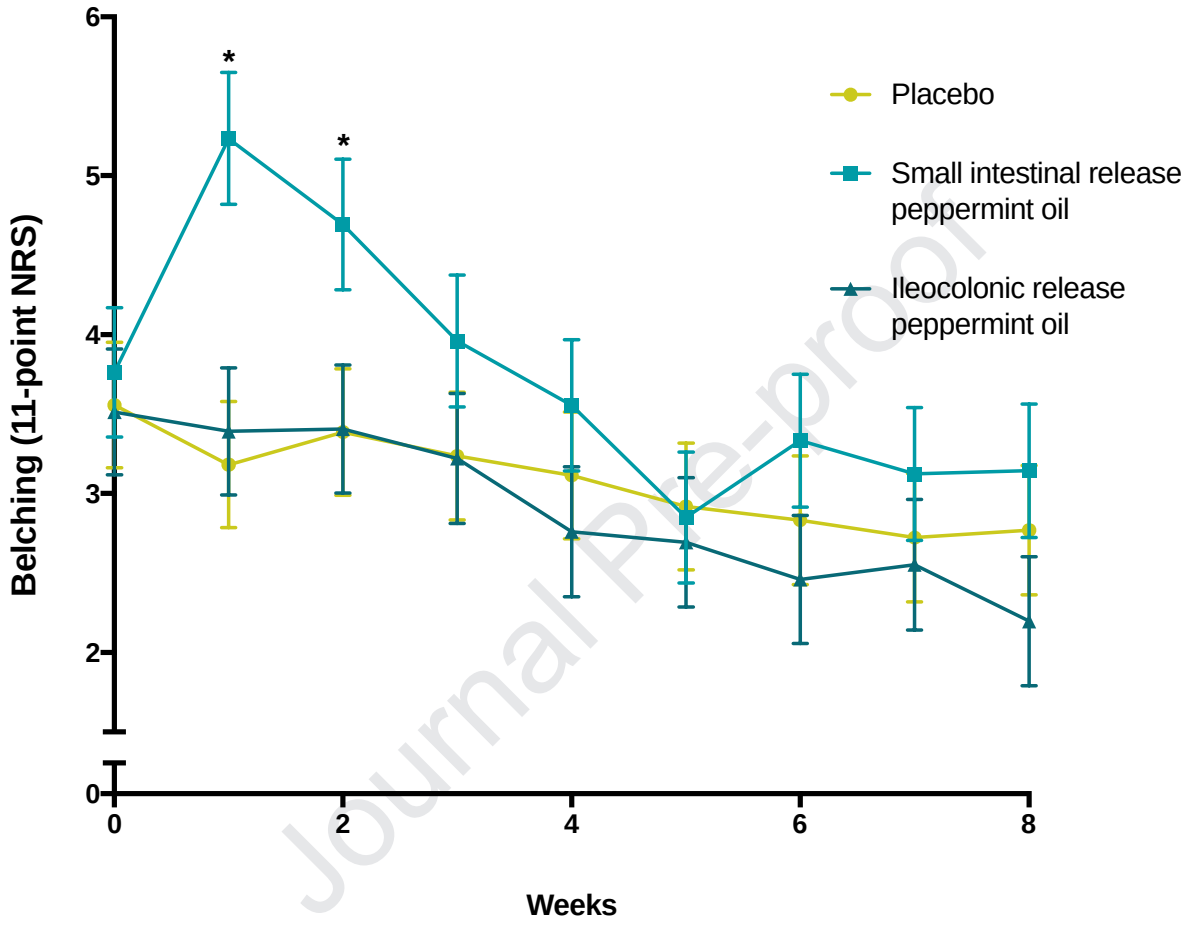
Supplementary Figure 4. **A**. Stool consistency in the ITT-population (*N*=189), **B**. Stool consistency in the IBS-C population (*N*=42), **C.** Stool consistency in the IBS-D population (*N*=83). Values are adjusted estimated marginal means derived from the linear mixed model, bars represent standard 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.

Supplementary Figure 5. Stool frequency



Supplementary Figure 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.

Supplementary Figure 6. Belching scores



Supplementary Figure 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.

7. Exploratory Supplementary Analyses of primary endpoints

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

	Placebo	**Small-intestinal release Peppermint oil**			**Ileocolonic release Peppermint oil**		
	***N* = 64**	***N* = 62**			***N* = 63**		
			***P*-value**	**Odds Ratio (95% CI)**		***P*-value**	**Odds Ratio (95% CI)**
	No. responders (%)	*No. responders (%)*			*No. responders (%)*		
Primary endpoints							
Abdominal pain[¶]							
IBS-D	11/29 (37.9)	10/25 (40.0)	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	0.90 (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	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	8.06 (0.84 – 77.12)	3/5 (60.0)	0.135	7.28 (0.54 – 98.64)
Global relief[‡]							

IBS-D	1/19 (3.4)	1/25 (4.0)	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	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	1.44 (0.09 – 23.43)	0/13 (0)	0.999	N.A.
IBS-U	0/9 (0)	2/10 (20.0)	0.999	N.A.	0/5 (0)	1.000	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).

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

	Placebo	**Small-intestinal release Peppermint oil**			**Ileocolonic release Peppermint oil**		
	***N* = 64**	***N* = 62**			***N* = 63**		
			***P*-value**	**Odds Ratio (95% CI)**		***P*-value**	**Odds Ratio (95% CI)**
	No. responders (%)	*No. responders (%)*			*No. responders (%)*		
Primary endpoints							
Abdominal pain[¶]							
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[‡]							
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).

8. 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 (OR9.14, 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% CI0.53; 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).