TOWARDS AN INTEGRATED PSYCHONEUROPHYSIOLOGICAL APPROACH OF IRRITABLE BOWEL SYNDROME

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INTRODUCTION

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EPIDEMIOLOGY

Irritable bowel syndrome (IBS) is among the most frequently occurring functional bowel disorders and is characterized by recurrent abdominal pain or discomfort accompanied by altered bowel habits[1]. Its prevalence ranges from 6% in the Netherlands[2] to 22% in other Western countries[3]. Approximately two-third of patients is female and symptom onset generally occurs below the age of 35. IBS has considerable economic impact, accounting for total annual direct costs of £ 45.6 million on average in the United Kingdom[4]. In the Netherlands, health care utilization and absence from work in IBS patients is approximately twice that of the general population[5].

DIAGNOSIS

In 1978, Manning was the first to introduce diagnostic criteria for IBS after an era in which diagnosis was made by exclusion of organic disease[6]. The Manning criteria required onset of abdominal pain associated with more frequent and looser bowel movements, pain relieved with defecation, visible abdominal bloating, and subjective sensation of incomplete evacuation and mucous stools more than 25% of the time. In 1992, an international committee of specialists known as the Rome Working Team refined the Manning criteria and formulated the Rome I criteria for IBS. These were re-evaluated in 1998 (Rome II criteria, applied in this thesis; Table 1)[1] and recently in 2006 (Rome III criteria)[7,8]. According to Rome III criteria, irritable bowel syndrome is defined as recurrent abdominal pain or discomfort at least 3 days per month in the last 3 months, associated with 2 or more of the following: 1) improvement with defecation and/or 2) onset associated with a change in frequency of stool and/or 3) onset associated with a change in form (appearance) of stool[8]. Additional symptoms that support the diagnosis but are not part of these criteria include abnormal stool frequency (≤ 3 times per week or ≥ 3 times per day), abnormal stool form (hard/lumpy stool or loose/watery stool), defecation straining, urgency, sensation of incomplete bowel movement, passage of mucus, and bloating. In daily practice, subgroups are recognized according to predominant bowel habit, i.e. IBS with diarrhoea (IBS-D), IBS with constipation (IBS-C), alternating or mixed IBS (IBS-A, both hard/lumpy and loose stools) and unsubtyped IBS (insufficient abnormality of stool consistency to meet criteria for IBS-D, IBS-C or IBS-A). From a clinical point of view, the Rome criteria help physicians to make a more firm diagnosis of IBS. In research, they allow standardization of patient recruitment and comparison of patient groups between studies.

Table 1. Rome II criteria for irritable bowel syndrome

Diagnostic criteria
At least 12 weeks, which need to be consecutive, in the preceding 12 months of abdominal discomfort or pain that has two of three features:
1. Relieved with defecation; and/or
2. Onset associated with a change in frequency of stool; and/or
3. Onset associated with a change in form (appearance) of stool
Supportive symptoms of the irritable bowel syndrome
1. Fewer than three bowel movements a week
2. More than three bowel movements a week
3. Hard or lumpy stools
4. Loose (mushy) or watery stools
5. Straining during a bowel movement
6. Urgency (having to rush to have a bowel movement)
7. Feeling of incomplete bowel movement
8. Passing mucus (white material) during a bowel movement
9. Abdominal fullness, bloating or swelling
Diarrhoea-predominant
1 or more of 2, 4, or 6 and none of 1, 3, or 5
Constipation-predominant
1 or more of 1, 3, or 5 and none of 2, 4, or 6

PATHOPHYSIOLOGY

Despite the growing body of literature, the pathophysiology of IBS remains poorly understood. Currently, IBS is viewed as a multifactorial condition in which clinical expression results from interplay between physiological and neuropsychological factors[9,10]. These factors are integrated in the brain-gut axis, a conceptual framework which has recently emerged in an attempt to improve our understanding of the etiology, pathogenesis and clinical expression of IBS. They include autonomic dysfunction[11,12], altered processing of afferent sensory information[13,14], disturbed intestinal motility[15,16], enhanced visceral sensitivity[17,18], inflammatory processes[19,20], altered immune activity[21,22], and psychological disturbances[23,24]. Dysfunction at different levels of the brain-gut axis may be responsible for these alterations.

Autonomic dysfunction

Several studies have demonstrated some form of autonomic dysregulation in IBS[11,12,25,26], but the nature of autonomic dysfunction remains elusive and results have been far from congruent. For instance, spectral analysis of heart rate variability has suggested increased sympathetic activity in IBS patients[25], both during waking and sleep[26]. These data are supported by findings showing hypertensive episodes during sigmoidal balloon distension in both IBS and health, pointing to upregulated

sympathetic tone[27]. In contrast, it has also been shown that rectal balloon distension depresses blood pressure in IBS patients (but not in controls)[11], suggesting down-regulated sympathetic activity during visceral stimulation.

Autonomic control of gastrointestinal motor and sensory functioning is complex. In short, it is governed by the dorsal vagal complex[28], an integrated central structure comprising the motor nucleus of the vagus from which autonomic outflow to the colon arises, and the nucleus tracti solitarii (NTS) which integrates viscerosensory input from the gut and other organs[29]. Physiological information from the gut *proximal* to the splenic flexure is carried by cranial nerve afferents that terminate in the NTS, while noxious viscerosensory information is transmitted by sympathetic spinal fibers. From the NTS, interneurons project to the ventrolateral medulla (VLM), which controls sympathetic outflow, and to higher centers. Sensory information originating *distal* from the splenic flexure (descending colon and rectum) is exclusively conveyed by spinal afferent fibers that terminate in the thalamus, but collaterals also reach the NTS and VLM[30,31]. This key role of the NTS suggests that the altered autonomic outflow observed in IBS may result from either a normal or abnormal reflex response to disturbed afferent viscerosensory information from the gut.

Altered intestinal motility

Both small intestinal and colonic motility are altered in IBS[32,33]. Intraluminal small intestinal pressure recordings have revealed shorter intervals between fasting migrating myoelectric complexes, more clusters of jejunal pressure activity and more ileal propulsive waves in IBS-D compared to controls, implying increased small bowel motility. The latter abnormality was associated with cramping abdominal pain[32]. Manometry of the left hemicolon in IBS patients has demonstrated increased colonic frequency patterns, a higher motility index, and an increase in mean number and peak amplitude of high amplitude propagating contractions (HAPCs), which coincided with the occurrence of abdominal pain in more than 90%[33]. Other studies, however, have not been able to demonstrate significant differences in colonic motility between IBS patients and healthy controls[34]. Autonomic dysfunction may be seen as circumstantial evidence for altered intestinal motility in IBS. However, it remains elusive which intestinal motor abnormalities contribute to symptom generation.

Visceral hypersensitivity

Visceral hypersensitivity is considered a hallmark in IBS[35,36] and has even been proposed as a biological marker[17]. Typical findings in IBS patients are increased visceral sensitivity to nocious stimuli, such as rapid rectal balloon distension, while physiological stimuli elicit similar responses as in controls[17]. The pathophysiology of this visceral hyperalgesia is poorly understood, but it may result from disturbances at

different levels of the brain-gut axis. First, sensitization of peripheral nerve endings at the intestinal level may occur during or after acute inflammation[37,38], leading to higher excitability and/or increased firing of these neurons. Second, alterations in the spinal dorsal horn neurons and upregulation of spinal nerve endings may play a role in the extended viscerosomatic referral pattern that is often seen in IBS[17,37]. Third, altered processing of afferent visceral information in the brain, particularly in the prefrontal cortex, anterior cingulated cortex, and thalamus, has repeatedly been demonstrated in IBS patients[14,39,40]. These regions are not only involved in pain processing but are also part of the emotional limbic system and are therefore involved in numerous psychological and cognitive events[41,42]. Although the prevalence of visceral hypersensitivity in IBS patients differs between studies and its role in the pathophysiology is not clear, it is one of the few reproducible phenomena in IBS.

Inflammation and immune system alterations

The role of low-grade inflammation and (mucosal) immune system activation in the pathogenesis of IBS has received much attention over the last decade. The risk to develop IBS after dysenteric illness is increased[19,20,43]. Histological studies found increased numbers of immunocompetent cells in colonic and small bowel mucosa of patients with post-infectious IBS (PI-IBS)[21,44,45]. Even more interestingly, large bowel mucosal samples in subgroups of IBS patients show activated mast cells with signs of degranulation and inflammatory mediator release in the proximity of mucosal nerve endings, especially in patients who are hypersensitive to balloon distension[21,46]. This implies that mucosal inflammation may contribute to symptom generation. In addition, increased or decreased secretion of several pro- and anti-inflammatory cytokines that are known to modulate the (intestinal) immune response[47] may play a role in this mucosal inflammation. For instance, a number of single nucleotide polymorphisms (SNPs) in the promoter region of the gene coding for the anti-inflammatory cytokine interleukin-10 (IL-10), leading to increased production of IL-10, appear to be less prevalent in IBS patients[22]. Very recent data involving microarray gene expression profiling of sigmoid colon mucosa even suggest stable alterations in colonic mucosal immunity in IBS[48]. These data strongly suggest that inflammation of the gut mucosa plays a role in the clinical expression of IBS in at least a subset of patients.

Psychopathology

Symptoms in IBS are associated with psychological factors, which may affect clinical outcome[23]. Whether psychological disturbances contribute to the pathophysiology of IBS as such or only occur as comorbidity is not yet clear. Although an increased prevalence of several psychiatric conditions such as anxiety, depression and somatization has been demonstrated in IBS[49-51], these disorders may particularly be

related to health care seeking[51]. There is also evidence to suggest that psychological disorders do not play a significant role in the pathophysiology of IBS when levels of visceral hypersensitivity are accounted for[52]. Alternatively, altered processing of afferent visceral information in the prefrontal cortex, anterior cingulated cortex, and thalamus has been demonstrated in IBS[39,40]. Nociception (becoming aware of a painful stimulus) and emotional pain management both occur in these brain regions, which are also part of the emotional limbic system[41,42], suggesting that psychological disturbances may be related to visceral hypersensitivity and IBS.

AIMS AND OUTLINES

The concept of the brain-gut axis as a model to improve our understanding of the pathophysiology of IBS has been the basis of research in IBS over the last decades and the framework for this thesis. The primary objective was to gain further insight in the many parameters and variables that are involved in this model, and their relationship. The second goal was to study the efficacy of a brief psychological group intervention for the treatment of IBS symptoms. Third, we aimed to test the validity of a previously published comprehensive working model of IBS, based on the brain-gut axis.

Evidence for abnormal activity of the autonomic nervous system, reflected in the cardiovascular system by altered heart rate variability (HRV)[25,26] and in the digestive system by disturbed motility[32,33], suggests disturbed viscerosensory-autonomic reflexes in IBS. In rats, electrical stimulation of abdominal vagal afferents increases sympathetic outflow and also decreases baroreflex sensitivity (BRS), pointing to the possible involvement of the arterial baroreflex in IBS[53]. Altered baroreflex functioning during gastrointestinal stress (i.e., abdominal pain) may constitute a pathophysiological key in IBS, as the arterial baroreflex not only modulates sympathetic and parasympathetic autonomic outflow, but also affects cortical arousal[54] and somatic[54,55] and visceral[53] pain perception. Since this topic has not been studied in humans, we evaluated systolic blood pressure, heart rate and BRS involvement in IBS patients and healthy controls under baseline conditions and during a gastrointestinal stressor (rectal balloon distension). The results of this study are presented in **Chapter 2.**

Several gut peptides are known to be involved in the regulation of gastrointestinal motor and sensory function. For instance, cholecystokinin (CCK) stimulates colonic motility and increases rectal sensitivity to balloon distension in healthy individuals[56,57]. Motilin is involved in the regulation of interdigestive motility of the stomach and small intestine[58], but also affects colorectal motor function[59]. Peptide YY (PYY)

delays proximal gastrointestinal motility[60] and the number of PYY-containing colonic enteroendocrine cells is increased in symptomatic IBS patients after an acute infectious gastroenteritis[44]. **Chapter 3** investigates plasma levels of gut peptides released from the upper (CCK and motilin) and lower (PYY) small intestine under fasting and postprandial conditions in IBS patients, as well as the influence of age, gender, IBS subtype and visceral hypersensitivity on gut hormone secretion.

With an increased risk of developing IBS after acute gastroenteritis[19,20,43], it has become increasingly clear that inflammation and mucosal immune system activation may be important in IBS symptom generation[61]. Larger numbers of immunocompetent cells are found in rectal mucosa of patients with post-infectious IBS up to 1 year after infection[44]. Since pro- and anti-inflammatory cytokines are important modulators of the (intestinal) immune response, imbalances in cytokine secretion may play a role in the ongoing mucosal inflammation. A recent study showed that the high producer IL-10 genotype (anti-inflammatory cytokine; -1082 G/G Single Nucleotide Polymorphism, SNP) is less prevalent in IBS patients compared to healthy controls[22]. The study described in **Chapter 4** was conducted to investigate the prevalence of gene promoter SNPs of IL-10 and TNF-α (pro-inflammatory cytokine) that are known to be associated with low IL-10 or high TNF-α secretion, in IBS patients and in healthy controls.

Chapter 5 studies reflex rectocolonic motor inhibition in IBS patients and healthy controls under both fasting and postprandial conditions. This inhibitory reflex has previously been demonstrated in healthy individuals[62,63]. Our study was undertaken to characterize this inhibitory reflex in IBS in an attempt to better understand the motor disturbances that occur in these patients, and in particular postprandial symptom deterioration[64].

Visceral hypersensitivity appears to play an important role in the pathophysiology of IBS[35,36] and has even been proposed as a biological marker[17]. Although processing of afferent visceral information and emotional pain management both occur in the same brain regions[41,42], little is known about the relationship between psychological variables and visceral hypersensitivity. Such information is relevant because it may provide a better understanding of the pathogenesis of IBS and its treatment. In **Chapter 6**, we explore the prevalence of rectal hypersensitivity, levels of psychological distress and symptom severity in IBS patients, and we attempt to address which demographical, clinical and psychological variables predict the occurrence of visceral hypersensitivity in IBS.

Curative treatment for IBS is not available[65] and therefore therapeutic interventions are directed towards reducing predominating symptoms. These include medication such as antispasmodics, laxatives or antidiarrhoeals in addition to patient education, reassurance, and dietary advice[9]. Novel therapies focus on serotonergic and psycho-

tropic agents, but therapeutic gain is at best restricted to subgroups of patients[66-69]. The efficacy of psychological interventions such as cognitive behavioural therapy, dynamic psychotherapy and hypnotherapy has been demonstrated in a number of studies[70-74]. As most forms of psychotherapy incorporate a relaxation technique, we conducted a randomized controlled trial to determine short and long-term efficacy of relaxation training, a brief psychological group intervention, when added to standard medical care, on symptom severity and psychological wellbeing in IBS patients. The results of this study are described in **Chapter 7.**

With disturbances at different levels of the brain-gut axis as the central, conceptual framework for understanding the pathogenesis underlying IBS, a biobehavioral model would be of great assistance to verify different pathophysiological hypotheses. One of few attempts to construct such a model came from Naliboff and colleagues in 1998, who proposed an initial but comprehensive working model of IBS, incorporating the central nervous system, visceral sensory and motor functioning, and cognitive-behavioral systems[75]. In **Chapter 8**, we evaluate a modified version of this model by using Structural Equation Modeling (SEM) in order to calculate reciprocal and chronological relationships between the model variables and thereby test its validity.

Finally, **Chapter 9** summarizes the various studies presented in this thesis and discusses the new insights that have been obtained in the light of the current knowledge on the pathopysiology and clinic aspects of IBS.

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VISCEROSENSORY-CARDIOVASCULAR REFLEXES: ALTERED BAROREFLEX SENSITIVITY IN IRRITABLE BOWEL SYNDROME

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ABSTRACT

Background: Animal studies have demonstrated that visceral afferent stimulation alters autonomic cardiovascular reflexes. This mechanism might play an important role in the pathophysiology of conditions associated with visceral hypersensitivity, such as irritable bowel syndrome (IBS). As such studies in humans are lacking, we measured viscerosensory-cardiovascular reflex interactions in IBS patients and healthy controls.

Methods: Blood pressure (SBP), heart rate (HR) and arterial baroreflex sensitivity (BRS) were studied in 87 IBS patients and 36 healthy controls under baseline conditions and during mild (15 mmHg) and intense (35 mmHg) visceral stimulation by rectal balloon distension. BRS was computed from continuous ECG and arterial blood pressure signals (Finapres-method) during 5 min periods of 15/min metronome respiration.

Results: Baseline SBP and HR were not different between patients and controls. In both groups, SBP increased similarly during rectal stimulation, whereas HR decreased during mild and increased during intense stimulation. BRS was significantly higher in patients compared to controls at baseline (7.9±5.4 *vs.* 5.7±3.7 ms/mmHg, P=0.03) and increased significantly in both groups during mild stimulation. This increase persisted in controls during intense stimulation, but BRS returned to baseline in patients. BRS was not significantly different between groups during rectal distension.

Conclusion: This study demonstrates the presence of a viscerosensory-cardiovascular reflex in healthy individuals and in IBS patients. The increased BRS in IBS patients at baseline may either be a training-effect (frequent challenging of the reflex) or reflects altered viscerosensory processing at the nucleus tracti solitarii.

INTRODUCTION

Irritable bowel syndrome (IBS) is a frequently occurring functional disorder with a prevalence ranging from approximately 6 to 22%[1,2]. It is characterized by recurrent abdominal pain and disturbed bowel habits. In the absence of an established biological substrate, the diagnosis is symptom-based and made according to the Rome II criteria[3].

IBS is a multifactorial condition in which disturbances in the brain-gut axis have been identified. In particular, visceral hypersensitivity, which may be induced by a number of factors such as post-inflammatory tissue injury[4] or persistent mucosal immune activation[5,6], is thought to play a central role in the pathophysiology[7,8]. In addition, abnormal activity of the autonomic nervous system, reflected in the cardiovascular system by altered heart rate variability (HRV)[9,10] and in the gastrointestinal tract by disturbed motility[11,12], has been reported. These observations suggest disturbed viscerosensory-autonomic reflexes in IBS.

Gastrointestinal functioning is controlled by the dorsal vagal complex (DVC)[13]. This is an integrated structure comprising the motor nucleus of the vagus (DMV) from which autonomic outflow to the colon arises; the nucleus ambiguus (NA), where parasympathetic outflow to the cardiovascular system is generated; and the nucleus tracti solitarii (NTS), which integrates viscerosensory input from the gut, cardiovascular system (e.g. carotid and aortic baroreceptors) and other organs[14,15]. Interneurons from the NTS also reach the NA.

Noxious viscerosensory information from the gut down to the splenic flexure is transmitted by sympathetic spinal fibers, while physiological information is carried by cranial nerve afferents that terminate in the NTS. From here, interneurons project to the ventrolateral medulla (VLM), which governs sympathetic outflow, and to higher centers. Sensory information from the descending colon and rectum is exclusively conveyed by spinal afferent fibers that terminate in the thalamus, but collaterals also reach the NTS and VLM[16,17]. The key role of the NTS suggests that the altered autonomic outflow observed in IBS may result from an abnormal reflex response to disturbed afferent viscerosensory information from the gut.

Results of a study by Saleh et al. point to the possible involvement of the arterial baroreflex in IBS. They demonstrated that, in rats, electrical stimulation of abdominal vagal afferents increased sympathetic outflow and also decreased baroreflex sensitivity (BRS)[18]. Altered baroreflex functioning during gastrointestinal stress may constitute a pathophysiological key in IBS, as the arterial baroreflex not only modulates sympathetic and parasympathetic autonomic outflow, but also affects cortical arousal[19,20] and somatic[19,21] and visceral[18] pain perception.

Thus far, no human studies have addressed BRS involvement in IBS. As, in general, BRS is reduced in disease[22-24], we expected that baseline BRS is depressed in IBS patients. Furthermore, we anticipated an exaggerated BRS reduction during gastrointestinal stress in IBS patients compared to healthy controls[25]. Both assumptions would explain at least part of the previously observed abnormal activity of the autonomic nervous system (*i.e.*, increased sympethetic predominance) and the increased visceral pain perception in IBS patients. The following study was done to corroborate this hypothesis.

METHODS

The local ethics committee approved the study protocol.

Participants

Between March 2001 and July 2002, IBS patients were recruited through the outpatient department of Gastroenterology and Hepatology of the Leiden University Medical Center and through local advertisement. Eligible patients were seen by one of the investigators (PvdV). Exclusion criteria were the presence of organic disease, previous major abdominal surgery apart from cholecystectomy and appendectomy, dependence on analgesics and pregnancy. Patients who were taking cardio-active or antihypertensive drugs were excluded. Other medication such as antispasmodics, laxatives, bulking agents and occasional use of analgesics was permitted. All included patients met the Rome II criteria for IBS[3]. Age and sex matched healthy volunteers were recruited by advertisement. Each participant provided informed consent before entering the study.

Visceral stimulator

An electronic visceral stimulator, *i.e.* barostat (Synectics Visceral Stimulator, Synectics Medical, Stockholm, Sweden), was used to study the effect of a visceral stressor on blood pressure, heart rate and BRS. Using electronic feedback regulation, this device is able to apply isobaric distensions. Constant pressure is maintained within a highly compliant, polyethylene bag (maximum capacity 1000 mL) tied to the end of a multilumen tube (19 Fr) by injecting air when the rectal wall relaxes and aspirating air during rectal contraction[26]. Intrabag pressure is directly measured via a separate lumen.

BRS instrumentation

The finger cuff of a noninvasive blood pressure measurement device (Finapres, TNO, Amsterdam, NL) was attached to the middle finger of the subjects' right hand to continuously record arterial blood pressure and heart rate. When this did not yield a good signal, the cuff was attached to another finger on the same hand. The cuff of an automatic sphygmomanometer (Accutorr, Datascope Corp, Montvale, NJ, USA) was attached to the subject's left upper arm. A surface ECG was obtained with a Marquette Case-12 electrocardiograph (Marquette Electronics Inc., Milwaukee, USA). Thoracic impedance was measured by two electrodes attached to the lateral sides of the lower part of the thorax to monitor subject's compliance with the metronome respiration protocol described below. An indicator for metronome respiration was visualized on a computer screen. The ECG, finger blood pressure and thoracic impedance signals were digitally stored (sampling rate 500 Hz, sample size 16 bits).

Study design

Recordings were performed in a quiet, air-conditioned room with a constant temperature of 20 °C. No individuals except the investigator were allowed to enter the room during measurements. Subjects were allowed a standardized small, fat-free breakfast at 8:00 am. Upon arrival at our department at 11:00 am, a tap water enema was given to empty the rectosigmoid area. Next, subjects were placed in a bed, which was in a 6° head-down position to abolish gravitational effects of the abdominal contents on the rectal balloon. The bag was inserted into the rectum and the catheter was connected to the barostat. Subsequently, ECG, Finapres and Accutorr devices were connected during a 30 min adaptation period. In this period, aortic and carotid baroreceptors could adjust to the supine blood pressure that was maintained throughout the entire recording period.

The experimental procedure is outlined in Figure 1. Each BRS measurement sequence consisted of a 5-min 15/min metronome respiration episode, preceded by three Accutorr blood pressure measurements to determine systolic blood pressure (SBP). Metronome respiration at 0.25 Hz prevents the direct mechanical component of respiration and the respiratory gating effect to enter the low-frequency band (0.04-0.15 Hz) in which we compute baroreflex sensitivity[27,28]. Subjects were asked not to speak during metronome respiration, but to report any discomfort. Free chosen tidal volume was permitted to assure comfortable breathing.

After a baseline BRS measurement procedure at 0 mmHg rectal pressure, a slow ramp distension (5-30 mmHg, 1 mmHg/min) was performed to measure rectal pain perception. This was done using a 10 cm Visual Analog Scale (VAS) anchored 'none' to 'unbearable' that was administered at every even pressure. Pain perception scores > 1 cm were considered significant. Perception measurements during the BRS mea-

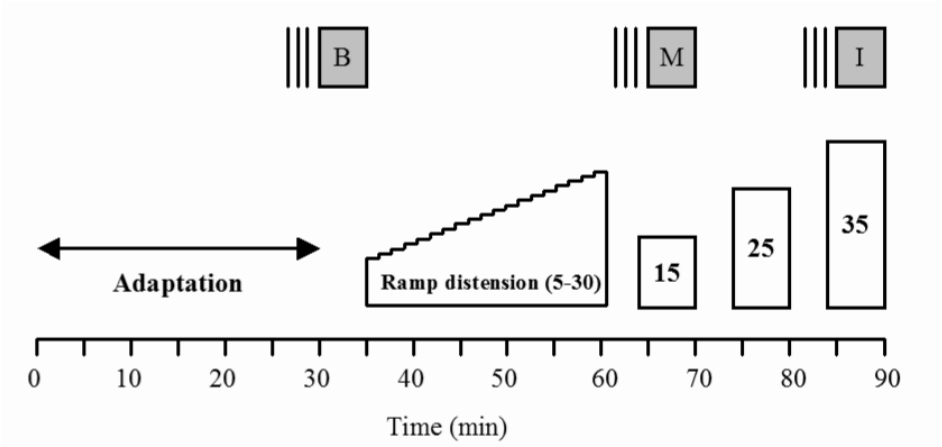

Figure 1. Study design. The three vertical lines next to shaded boxes denote the Accutorr systolic blood pressure measurements. Shaded boxes denote metronome respiration period for baroreflex sensitivity (BRS) assessment. B, baseline, M, mild rectal stimulation, I, intense rectal stimulation. Open boxes denote ramp distension (5-30 mmHg) or phasic rectal distensions of 15, 25 and 35 mmHg.

surement sequence were not feasible because of interference with metronome respiration. After balloon deflation, BRS measurement sequences were carried out during isobaric phasic distensions of 15 mmHg (mild, non-painful stimulus) and 35 mmHg (intense, mostly painful stimulus)[29]. Each distension lasted 6 min and was preceded by a 4-min period at 5 mmHg. Metronome respiration commenced one minute after each rectal distension onset. A 25 mmHg isobaric distension was performed in between the mild and strong stimuli to provide a gradual transition.

BRS signal analysis

To characterize arterial baroreflex function we computed baroreflex sensitivity (BRS), the reflex-induced increase/decrease of the interval between heart beats in milliseconds when arterial blood pressure rises/falls by 1 mm Hg. First, the longest arrhythmia free and stationary period in each metronome respiration episode was selected (sinus rhythm and a stationary signal are prerequisites for a reliable BRS value). Then, BRS was computed in the selected episode using the POLYAN software[30]. This algorithm calculates the transfer function between the systolic blood pressure variability (baroreflex input) and the interbeat interval variability (output), averaged over the 0.04-0.15 Hz band. BRS assessment was deemed impossible if this period was less than 90 seconds. Data selection and BRS computations were performed by two independent analysts.

The Accutorr arm cuff was not inflated during the BRS measurement procedures to avoid any possible interaction with the rectal distension stimulus. Instead, we calculated blood pressure during this period by computing the difference between the Finapres BP in the 3 min prior to the BRS measurement procedure and the Finapres

BP during the subsequent BRS measurement procedure. This difference was added to the Accutorr BP measured prior to the BRS assessment.

Statistical analysis

Linear mixed model analysis was used to detect overall differences in BRS, SBP and HR between IBS patients and controls (SPSS for Windows 11.0, Chicago IL, USA). Condition (baseline or rectal distension), group (IBS patients or controls), and condition by group interaction were analyzed as separate contributors. Subjects with missing data were not excluded from the analysis. Within-group changes from baseline in BRS, SBP, HR, and pain perception scores were analyzed using t statistics or Wilcoxon Signed Ranks Tests, and between-group differences were compared by t statistics or Mann-Whitney tests where appropriate. Data are expressed as mean ± SD in text and tables and, for clarity purposes, as mean ± SE in figures. The level of significance was set at $P \leq 0.05$.

RESULTS

Subject characteristics

We screened 130 patients, 26 of whom did not meet Rome II criteria, and 40 healthy volunteers. All 40 volunteers and 104 patients provided informed consent. From these, 17 patients and 4 control subjects were excluded from the analysis: 10 patients and 1 control subject used cardio-active or antihypertensive medication, 4 patients and 3 controls had cardiac arrhythmias and 1 patient had a pacemaker. Two more patients were excluded due to technical difficulties during the BRS measurements. Thus, 87 patients and 36 controls were included in the final analysis. Mean age and gender distribution were comparable in patients and controls (Table 1). Pain perception was significantly increased in patients from 8 mmHg onward, but in controls from 22 mmHg onward, indicating hypersensitivity to balloon distension in patients (Fig 2).

Baseline assessment

Opposite to what we expected, baseline BRS was higher in IBS patients compared to controls (7.9 ± 5.4 versus 5.7 ± 3.7 ms/mmHg, $P=0.03$) (Fig 3). Baseline SBP (Table 2) and HR (Table 3) were not significantly different between patients and controls.

Table 1. Baseline characteristics of IBS patients and healthy controls

	IBS (n=87)	Controls (n=36)
Age (yr)	40.0 ± 13	39.5 ± 15
Females	60 (69)	21 (58)
Bowel habit		
diarrhea	31 (36)	0
constipation	27 (31)	0
alternating	22 (25)	0
currently unknown	7 (8)	-
normal	-	36 (100)

Numbers within parentheses show percentages. IBS, irritable bowel syndrome; n, number of patients or controls.

BRS, blood pressure and heart rate during phasic rectal distension

BRS

Figure 3 shows mean BRS in patients and controls during baseline and 15 and 35 mmHg rectal distensions. The condition by group interaction was significant (P=0.01). BRS was not different between patients and controls during 15 mmHg (9.0 ± 5.7 versus 9.2 ± 6.4 ms/mmHg, respectively, P=0.68) and 35 mmHg distensions (7.3 ± 4.3 versus 7.9 ± 4.3 ms/mmHg, respectively, P=0.40). BRS was significantly increased in controls (P<0.0001) and in patients (P<0.05) during 15 mmHg, but only in controls (P=0.002) and not in patients (P=0.25) during 35 mmHg distensions.

Systolic blood pressure

Mixed model analysis showed that neither condition by group interaction nor the group factor was significant for systolic blood pressure (P=0.37 and P=0.41, respectively), indicating that the SBP response to rectal distensions was similar in patients and control subjects. In contrast, condition was significant (P<0.0001), indicating that blood pressure changed similarly in both groups. SBP was significantly increased in controls (P=0.002) with a similar trend in patients (P=0.08) during 15 mmHg distension, and in both groups during 35 mmHg distension (P<0.001) (Table 2).

Heart rate

HR condition by group interaction was not statistically significant (P=0.13), nor was group (P=0.07), but condition was significant (P<0.0001). Compared to baseline, HR decreased significantly in patients (P<0.0001) and controls (P=0.003) during 15 mmHg and increased significantly in patients (P<0.0001) and controls (P=0.05) during 35 mmHg distension (Table 3).

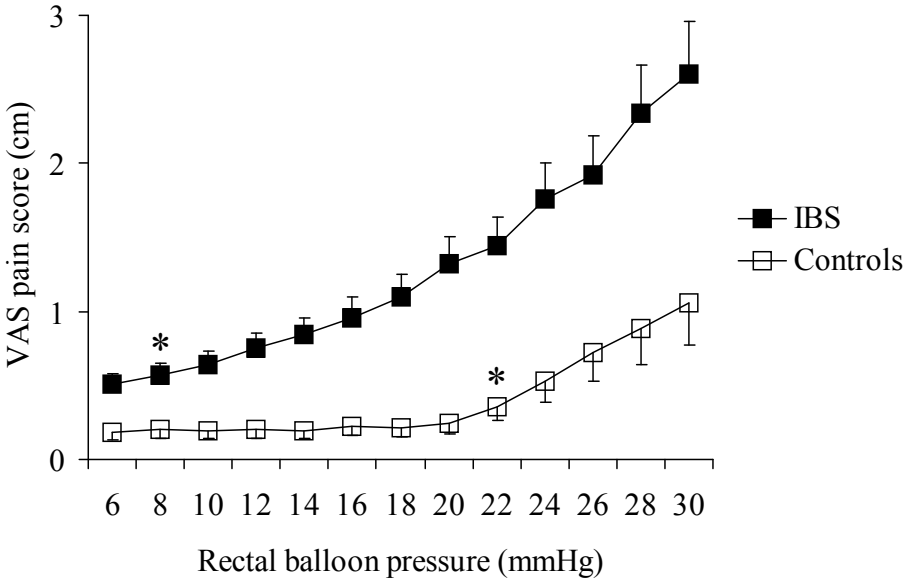

Figure 2. Pain perception during ramp distension. Visual Analog Scale (VAS, range 0-10) scores for rectal pain perception (mean ± SE) during the ramp distension procedure in IBS patients (closed squares) and healthy controls (open squares). Asterisks denote the first pressure at which the perception score was significantly increased compared to 6 mmHg ($P<0.05$), which was at 8 mmHg for IBS patients and at 22 mmHg for controls.

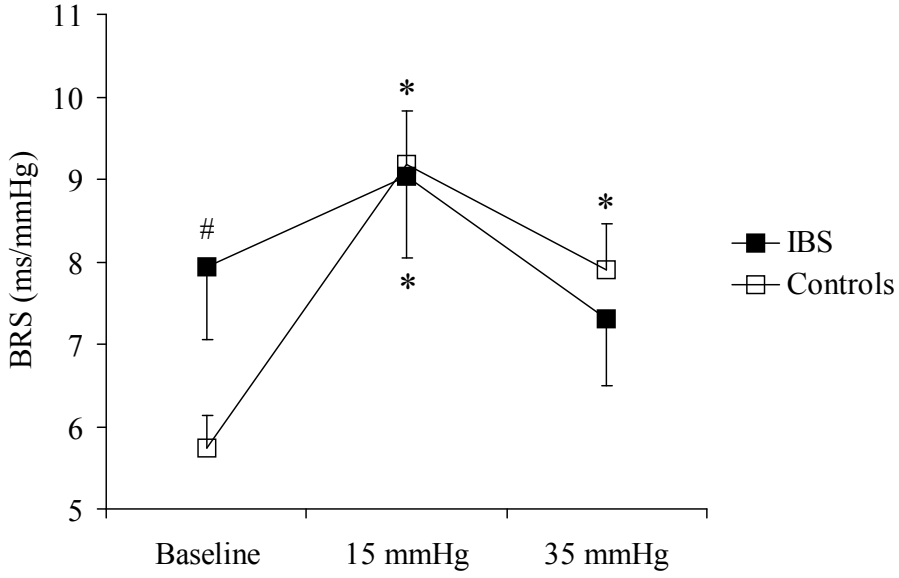

Figure 3. BRS (mean ± SE) at baseline and during mild (15 mmHg) and intense (35 mmHg) rectal stimulation in IBS patients (closed squares) and healthy controls (open squares). Baseline BRS was significantly larger in patients compared to controls (#, $P=0.025$). * significant increase from baseline ($P<0.05$).

Table 2. Mean systolic blood pressure at baseline and during mild and intense rectal stimulation in IBS patients and healthy controls

	baseline	15 mmHg	P-value*	35 mmHg	P-value†
IBS (n=87)	120.7± 14.8	122.5 ± 17.7	0.08	130.6 ± 13.6	<0.001
Controls (n=36)	116.4 ± 12.7	121.6 ± 12.8	0.002	129.5 ± 14.5	<0.001
P-value‡	0.23	0.91		0.90	

Data are expressed as mean ± SD. * 15 mmHg versus baseline; † 35 mmHg versus baseline; ‡ IBS patients versus control subjects.

DISCUSSION

Our study demonstrates that stimulation of visceral afferents by a standardized stimulus, *i.e.,* pressure-driven rectal balloon distension, produces significant changes in systolic blood pressure and heart rate in healthy subjects and in patients with IBS. Moreover, this stimulus increases baroreflex sensitivity in healthy individuals and in IBS patients. In addition, resting BRS is significantly larger in IBS patients compared to healthy subjects.

Physiologic mechanisms underlying the cardiovascular response to rectal distension

Heart rate and blood pressure

Several studies have reported that stimulation of visceral afferents produces cardiovascular responses, notably in blood pressure and heart rate. Yet, the results are contradictory, which may be caused by widely varying experimental designs. For instance, abdominal vagal nerve stimulation in anesthetized rats did not alter blood pressure and heart rate[18]. Azpiroz and colleagues reported that neither jejunal balloon distension below the perception threshold, nor distension at the discomfort threshold or above affected heart rate in healthy volunteers (blood pressure data were not reported)[31]. Cardiovascular responses to colorectal distension were measured in rats[32] and in humans[33]. In awake rats, blood pressure and heart rate increased during colorectal distension in a dose-dependent manner[32]. In healthy volunteers, a similar graded response was observed in blood pressure (heart rate was not reported)[33].

Table 3. Mean heart rate at baseline and during mild and intense rectal stimulation in IBS patients and healthy controls

	baseline	15 mmHg	P-value*	35 mmHg	P-value†
IBS (n=87)	67.1 ± 10.1	64.0 ± 9.6	<0.001	72.0 ± 14.7	<0.001
Controls (n=36)	64.2 ± 9.3	61.4 ± 8.9	0.003	66.5 ± 12.0	0.05
P-value‡	0.14	0.33		0.07	

Data are expressed as mean ± SD. * 15 mmHg versus baseline; † 35 mmHg versus baseline; ‡ IBS patients versus control subjects.

Our findings are consistent with a graded hypertensive response in healthy individuals and in IBS patients. The response in heart rate was, however, biphasic in both groups: heart rate decreased during mild rectal distension (15 mmHg) but increased during more intense stimulation (35 mmHg).

Most likely, the primary autonomic response to the stimulus we applied is sympathetic activation. This hypothesis is supported by the consistent blood pressure increases as demonstrated in this study and by others[32,33]. The hypertension-associated baroreceptor loading reflexly reduces the increase in sympathetic outflow (thereby reducing the original blood pressure rise and tachycardic response) while enhancing vagal outflow (which lowers heart rate, but not peripheral vascular resistance and thereby blood pressure). Thus, a mild hypertensive stressor may leave heart rate unaffected or even cause a slight decrease. Thus far, heart rate decreases have been reported during mental stress[34,35]. To our knowledge, we are the first to demonstrate this phenomenon during viscerosensory stimulation.

In contrast, a high blood pressure increase (e.g. during 35 mmHg distension) will be counteracted by the baroreflex to a lesser degree as the baroreceptor firing characteristic is S-shaped[36]. Consequently, the significant baroreceptor loading during high pressure rectal distension will lead to less reduction of the increase in sympathetic tone and less stimulation of parasympathetic outflow. This may explain our finding that during high rectal distension pressure, not only blood pressure but also heart rate increased.

Individual heart rate responses differed in sign and magnitude. Approximately 80% of our study population (IBS patients plus control group) exhibited a heart rate decrease during mild stimulation. Six percent (5/87 patients and 2/36 controls) had a heart rate decrease of more than 10 bpm and in one subject in the IBS group, heart rate lowered by 12 bpm from 62 to 50 bpm. On intake, this patient had reported defecation syncope on several occasions. It has been long hypothesized that straining during defecation (Valsalva maneuver) plays a dominant role in this form of fainting. However, recently, syncope was recorded during colonic air insufflation in a patient with recurrent defecation syncope that was not specifically associated with straining. A cardiac pacemaker resolved these symptoms completely[37]. It is hence conceivable that the colorectal-cardiovascular reflex response to mild distension as measured in our study provides an alternative clue to the mechanism that underlies this form of syncope.

Baroreflex sensitivity

We measured an increase in baroreflex sensitivity under mild rectal distension in healthy subjects and in IBS patients. During intense stimulation, the BRS increase compared to baseline persisted in healthy controls, albeit to a lesser extent, whereas

BRS returned to baseline in patients. These findings are opposed to our original hypothesis that BRS would lower under stress. This expectation was based on a study in rats, showing that sympathetic output increased and baroreflex sensitivity decreased following stimulation of general gastric afferents[18]. Several incompatibilities may account for this difference. First, anesthetized rats were used[18], while our study subjects were not sedated. Thus, cortical perception (stimulus awareness) may have played a role in the BRS increase we observed. In addition, it has been shown that anesthetic agents as used in the rat study considerably depress the arterial baroreflex[38]. Second, the insertion of catheters into the femoral artery and vein may additionally have influenced the autonomic conditions[39] in the rat experiment. Third, it cannot be ruled out that the spinal afferent viscerosensory input caused by the rectal distensions in our study is processed differently at the level of the brainstem from the cranial nerve (vagal) afferent input in the rat study.

The mechanism responsible for the BRS increase can only be speculated upon. Possibly, projections of the viscerosensory afferents ending at the NTS produce a neurotransmitter that directly enhances the baroreflex gain. Substance P, which is known to enhance the baroreflex by modulating the transmission from the baroreceptive afferents to the NTS neurons, would be a candidate neurotransmitter to achieve this effect[10,40]. Substance P production at the level of the NTS has been demonstrated for somatosensory afferents[20], while a high density of substance-P-containing fibers originating from the gastrointestinal tract have also been found in the pigeon NTS[41]. Alternatively, enhanced parasympathetic tone as a reflex response to rectal stimulation may have enhanced BRS by facilitating deeper modulation of the parasympathetic outflow, *i.e.* allowing increased heart rate fluctuation, rather than by increasing baroreflex gain.

Differences between IBS patients and healthy control subjects

Baseline supine heart rate and blood pressure were not significantly different between IBS patients and controls, although patients tended to have slightly higher values (Tables 2 and 3). The non-significant trend (P=0.14) to higher supine baseline HR values in IBS patients we observed was also reported by several other groups[9,42-46]. HR was similar during mild distension in patients and controls (P=0.33), but again tended to be higher in IBS patients during intense rectal distension (P=0.07). Few published numerical data are available regarding baseline blood pressure differences between IBS patients and healthy controls. Levine et al. found that baseline systolic blood pressure was significantly higher in patients[45].

The most striking difference between IBS patients and healthy control subjects was the 39% elevated BRS-value in patients (7.9 ± 5.4 versus 5.7 ± 3.7 ms/mmHg, P=0.03). This difference no longer existed during mild and intense rectal distension.

The marked elevated baseline BRS in IBS patients may provide an explanation for autonomic alterations reported in patients[10,47,48]. The baroreflex plays a key role in the generation of heart rate variability as it transfers respiration induced blood pressure variability into fluctuations in sympathetic and parasympathetic outflow, eventually leading to modulation of the discharge rate of the cardiac pacemaker[28]. Differences in heart rate variability (HRV) and HRV-derived assessments of the sympathovagal balance[49,50] as reported by several research groups[10,47,48] might therefore at least partly be explained by differences in baroreflex function.

Our study does not provide information on the basis of which the elevated baseline BRS value in IBS patients and its functional role in IBS can be explained. We speculate that the frequently experienced viscerosensory stimuli, *e.g.*, abdominal pain, entail a training-effect, possibly materialized in chronic elevated substance P concentrations at the NTS level[20,40,41,51]. Such a training-mechanism can only be further investigated in animal models of visceral afferent stimulation. Alternatively, the elevated baseline BRS value may reflect an intrinsic autonomic characteristic in which IBS patients differ from healthy individuals. Altered baroreflex function could witness altered information processing at the NTS level. For the esophagus, a vago-vagal reflex from/to the gastrointestinal tract (GI-GI reflex pathway) has been demonstrated involving the NTS as well as the NA[52]. In analogy, spino-spinal GI-GI sensorimotor reflex pathways, although not identified yet, may be involved in reflexes regarding the distal gut.

It is tempting to interpret the enhanced baseline baroreflex vigor as an anticipatory phenomenon and to expect benefits from that anticipation in the form of inhibition of cortical arousal[19,25] and visceral pain perception[18] during irritating stimuli such as abdominal pain. However, our finding that no differences in BRS values exist between IBS patients and control subjects during rectal distension renders such a hypothesis unlikely.

A limitation of our study was that we did not measure rectal perception during the applied rectal stimuli (phasic distensions), as this was not feasible due to the imposed metronome respiration. It may, however, be inferred from the pain scores during ramp distension (Fig 2) that pain perception was increased in IBS patients compared to controls. Furthermore, the lack of baseline values in the patient group prior to disease onset should be appreciated when interpreting our results. Finally, although we controlled for age and gender in this study, which have been shown to be strong determinants of spontaneous baroreflex sensitivity, there are other variables that may also affect baseline BRS[53].

Conclusions

In summary, our study provides evidence for the existence for a colorectal-cardiovascular reflex, characterized by a blood pressure increase, slight heart rate decrease, and an increase of baroreflex sensitivity during mild stimuli. Intense stimuli increase heart rate and blood pressure, while baroreflex sensitivity seems to be impaired compared to mild stimulation. This reflex, that was evident in normals as well as in IBS patients, might well be involved in defecation syncope.

Our study also provides evidence for baroreflex involvement in irritable bowel syndrome, as IBS patients have a higher baseline BRS-value than healthy controls. This finding renders the hypothesis unlikely that IBS patients are hypersensitive due to diminished baroflex function. We provide two possible explanations for the higher baseline BRS in IBS: 1) a "training-effect" (frequent challenging of the reflex by IBS-associated abdominal discomfort); 2) altered information processing at the NTS that causes BRS increases and, in parallel, abnormal GI-GI sensorimotor reflexes. While the first explanation considers the autonomic changes as a consequence of IBS, the second one recognizes a role for the autonomic nervous system in the pathophysiology of IBS and explains both altered HRV and changes in gastrointestinal motility as observed in this condition[54]. The latter hypothesis requires further corroboration.

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3

PROXIMAL AND DISTAL GUT HORMONE SECRETION IN IRRITABLE BOWEL SYNDROME

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ABSTRACT

Background: Sensory and motor dysfunction of the gut are both important characteristics of irritable bowel syndrome (IBS). Several gut peptides contribute to the regulation of gastrointestinal function but little is known on gut hormone secretion in IBS.

Methods: We evaluated perceptual thresholds and fasting and postprandial plasma levels of proximal (cholecystokinin (CCK), motilin) and distal (peptide YY) gut peptides up to 1 hour after ingestion of a high caloric meal in 99 IBS patients and 40 age and sex matched healthy controls.

Results: Fasting plasma CCK levels were significantly elevated in patients (1.2 ± 0.8 pM) compared to controls (0.8 ± 0.7 pM, P=0.006), as was the incremental postprandial CCK response (72 ± 73 *versus* 40 ± 42 pM·60 min, respectively; P=0.003). No differences in fasting and postprandial motilin or PYY levels were found. The postprandial PYY response was significantly increased in hypersensitive compared to normosensitive patients (215 ± 135 *versus* 162 ± 169 pM, P=0.048). Patients with a diarrhoea predominant bowel habit had higher fasting motilin levels compared to constipated patients or alternating type IBS patients (82.1 ± 36.5 *versus* 60.8 ± 25.1 *versus* 57.5 ± 23.9 pM, one-way ANOVA P=0.003).

Conclusion: IBS patients have increased fasting and postprandial plasma levels of CCK. Changes in plasma levels of motilin and PYY may contribute to the clinical expression of IBS, such as the presence of visceral hypersensitivity or predominant bowel habit.

INTRODUCTION

Irritable Bowel Syndrome (IBS) is a frequently occurring disorder that has received much attention over the last decades. However, its pathophysiology remains poorly understood. Disturbances at different levels of the brain-gut-axis have been proposed in symptom generation, including low-grade chronic intestinal inflammation[1], immune system alterations[2], autonomic dysfunction[3], and altered central processing of afferent sensory input[4]. In particular, enhanced visceral perception is considered to be important as it has been reported that up to 94% of patients are hypersensitive to rectal balloon distension[5]. There is also evidence pointing to altered gut motility in IBS[6]. IBS patients exhibit abnormal postprandial colonic motor activity[7] and reduced perception thresholds for gas, discomfort and pain[8] after a meal. Symptoms often deteriorate postprandially[9].

Several gut peptides are known to be involved in the regulation of gastrointestinal motor and sensory function. For instance, cholecystokinin (CCK) is a proximal gut hormone, released upon fat and protein ingestion, that delays gastric emptying[10] and stimulates contraction of the gallbladder[11] and exocrine pancreatic secretion[12]. Studies in healthy individuals have shown that infusion of CCK stimulates colonic motility and increases rectal sensitivity to balloon distension[13,14]. Motilin is also released from the proximal intestine and is involved in the regulation of interdigestive motility of the stomach and small intestine[15], but also affects colorectal motor function[16]. Peptide YY (PYY) is a distal gut peptide that has been shown to delay proximal gastrointestinal motility[17]. Spiller et al. recently showed that the number of PYY-containing colonic enteroendocrine cells is increased in IBS patients who develop symptoms after an acute infectious gastroenteritis[18].

Little is known about gut hormone secretion in patients with IBS. We hypothesize that changes in gut hormone secretion may contribute to the observed alterations in gut motor and sensory function in IBS. Therefore, we studied plasma levels of gut peptides released from the upper (CCK and motilin) and lower (PYY) small intestine under fasting and postprandial conditions in a large cohort of IBS patients. In addition, the influence of age, gender, IBS subtype and visceral hypersensitivity on gut hormone secretion was evaluated.

METHODS

Participants

Between March 2001 and July 2002, IBS patients between 18 and 65 years of age were invited to participate in a large clinical trial on psychological therapy, which in-

cluded assessment of psychological function, autonomic nerve function, postprandial gut hormone secretion, rectal barostat measurements, and evaluation of the efficacy of relaxation training for the treatment of IBS. This study reports on postprandial gut peptide response tests.

Patients were recruited through a tertiary referral centre (the outpatient department of Gastroenterology of the Leiden University Medical Centre (LUMC)) and through local advertisement. Healthy volunteers were recruited through advertisement. Eligible participants were screened by one of the investigators (PvdV). All patients met Rome II criteria for IBS[19]. Exclusion criteria were organic disease, previous abdominal surgery (except cholecystectomy and appendectomy), and pregnancy. Use of antispasmodics, bulking agents, laxatives, and occasional use of analgesics was permitted. Informed consent was obtained from each participant. The LUMC ethics committee had approved the study protocol.

Hypersensitivity testing

An electronic barostat (Synectics Visceral Stimulator, Synectics Medical, Stockholm, Sweden) was used to assess visceral hypersensitivity. This device is able to maintain constant pressure within a highly compliant, polyethylene bag tied to the end of a multilumen tube, as described elsewhere[20]. Perception of rectal pain was quantified on a 100-mm Visual Analog Scale (VAS) at every even pressure, with end points ranging from 'none' to 'unbearable'. Pain thresholds were defined as the first pressure level at which perception scores exceeded 10 mm. Hypersensitivity to rectal balloon distension was defined as a pain threshold that was 2 SD or more below the mean threshold in healthy controls.

Subjects were permitted a small standardized breakfast at 8.00 AM and arrived at our department at 10.00 AM. A tap water enema was used to evacuate the rectum and the barostat bag was positioned as described previously[21]. Barostat recordings commenced after 30 min. The experimental protocol consisted of a slow ramp distension to assess rectal compliance. Intrabag pressure was increased at a rate of 1 mmHg/min, from 5 to 30 mmHg. At all even pressures (6, 8…30 mmHg), patients rated the urge to defecate and pain using the 100-mm VAS scale. After the experiment had ended, the rectal balloon was removed.

Meal

Fifteen minutes after the barostat experiment, an intravenous canula was inserted in the antecubital vein of one arm and a fasting blood sample was obtained (t=0). At 13.00 AM, patients were offered an 800 kcal solid meal, consisting of 2 slices of brown bread, 10 g of margarine, 1 slice of fat cheese, 1 slice of cooked ham, 350 ml of semi-skimmed milk, 1 boiled egg, 300 ml of yoghurt, and 10 g of honey (44

g of protein, 46 g of fat and 69 g of carbohydrates). Additional blood samples were obtained at t = 15, 30, 45 and 60 min.

Plasma peptide assays

Blood samples were collected in ice-chilled tubes containing 2 g/L EDTA. All samples were centrifuged at rate of 3000 rpm for 15 min at a constant temperature of 4 °C and stored at -20 °C until peptide levels were determined. Plasma CCK was measured by a sensitive and specific RIA as described previously[22]. Levels of PYY were determined using antiserum generated in rabbits by intracutaneous injections of synthetic human PYY (BACHEM AG, Bubendorf, Switzerland). PYY was labelled with ^{125}I using chloramine T. There is no cross-reactivity with pancreatic polypeptide or vasoactive intestinal peptide. The detection limit is 10 pM and both PYY (1-36) and PYY (3-36) bind to the antibody in dilutions up to 1:250.000. Plasma motilin concentrations were determined using a sensitive and specific radioimmunoassay as described previously[23].

Statistical analysis

All statistical analyses were carried out using SPSS for Windows, version 11.0.1 (SPSS Inc., Chicago IL, USA). An incremental postprandial response was computed for each peptide by calculating the incremental area under the curve. Linear mixed model analysis was used to detect overall differences in plasma peptide levels between groups over time. Plasma peptide level, subject group and the interaction were analysed as separate contributors to the model. Patient numbers were used to indicate repeated measurements. Demographical characteristics were compared between groups by Student-t or Mann-Whitney analysis and chi square analysis where appropriate. Between-group differences in plasma peptide concentrations were compared by Mann-Whitney or ANOVA with post-hoc Tukey's correction for multiple group-wise comparisons. Within-group changes relative to fasting were analysed using Wilcoxon Signed Ranks Tests. Data are expressed as mean ± SD. The level of statistical significance was set at $P<0.05$.

RESULTS

Subject characteristics

We screened 130 patients and 40 healthy volunteers. Twenty-six patients did not meet Rome II criteria for IBS[19]. Blood sampling was unsuccessful in 5 patients, so that 99 patients and 40 healthy controls were included in the final analysis. All provided informed consent. Thirty-one patients (31%) were recruited through the

Table 1. Baseline characteristics of IBS patients and healthy controls

	IBS patients (n=99)	Controls (n=40)
Age (yr)	41.9 ± 14.0	39.7 ± 15.0
Females n (%)	71 (72)	25 (62)
Bowel habit		
diarrhoea	34	0
constipation	34	0
alternating	22	0
not specified (IBS)/normal (controls)	9	40

IBS, irritable bowel syndrome; n, number of patients or controls.

outpatient department, and 68 patients (69%) and all healthy controls were recruited through advertisement. Demographical and clinical characteristics are listed in Table 1. Mean age and gender distribution was comparable between groups.

Plasma CCK

Fasting and postprandial plasma CCK levels are shown in Figure 1. Fasting plasma CCK concentrations were significantly higher in patients compared to controls (Table 2). The postprandial plasma CCK response was significantly different between patients and controls (CCK concentration by group interaction, $P<0.001$). Plasma CCK concentrations increased significantly in patients and controls from 15 min onward ($P<0.001$ for all time points in both groups), reaching a peak at t=30 min in both

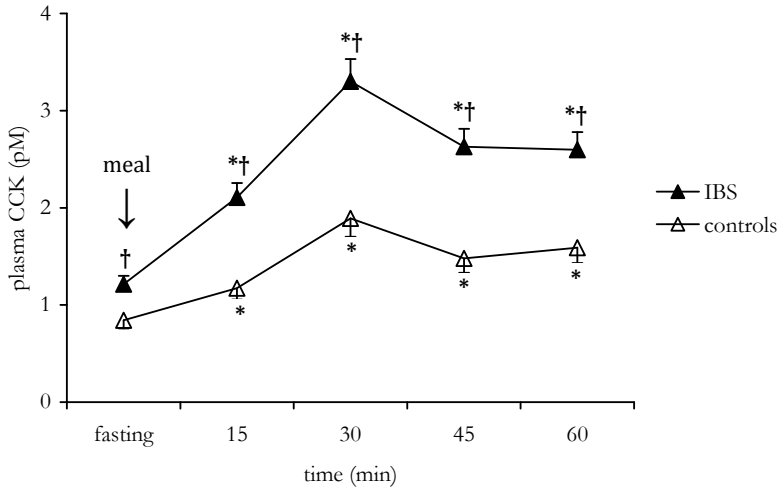

Figure 1. Fasting and postprandial plasma CCK concentrations in IBS patients (closed triangles) and controls (open triangles).
* P<0.001 compared to fasting, † P<0.001 compared to controls.

Table 2. Fasting plasma concentrations and incremental postprandial responses of CCK, PYY and motilin in IBS patients and healthy controls

	IBS patients			Controls		
	all (n=99)	male (n=28)	female (n=71)	all (n=40)	male (n=15)	female (n=25)
Age (yr)	41.9±14.0	41.1±12.7	42.2±14.6	39.7±15.0	43.2±17.2	37.6±13.5
CCK						
fasting	1.2±0.8*	0.8±0.6	1.4±0.8†	0.8±0.7	0.9±0.5	0.8±0.8
AUC meal	71.7±72.6#	59.3±52.9	76.6±78.9	40.5±42.1	35.2±27.7	43.6±49.0
Motilin						
fasting	69.2±31.3	81.9±40.1‡	64.2±25.8	65.3±29.5	70.2±32.0	62.4±28.1
AUC meal	-615±1039	-813±1145	-536±991	-427±825	-594±899	-328±778
PYY						
fasting	17.5±6.0	16.4±4.3	17.9±6.5	17.5±8.9	15.1±3.1	18.9±10.8
AUC meal	181±159	153±163	192±157	247±294	228±228	258±331

AUC, Area Under the Curve. Fasting concentrations are expressed as pM. AUC is expressed as pM•60 min. * P=0.006 versus controls; # P=0.003 versus controls; † P=0.012 versus male IBS patients and P=0.009 versus female controls; ‡ P=0.046 versus female IBS patients.

groups. The incremental postprandial CCK response was significantly increased in patients compared to controls (Table 2).

Plasma motilin

Fasting and postprandial motilin levels are shown in Figure 2. Fasting plasma motilin levels were not different between patients and controls. Plasma motilin concentra-

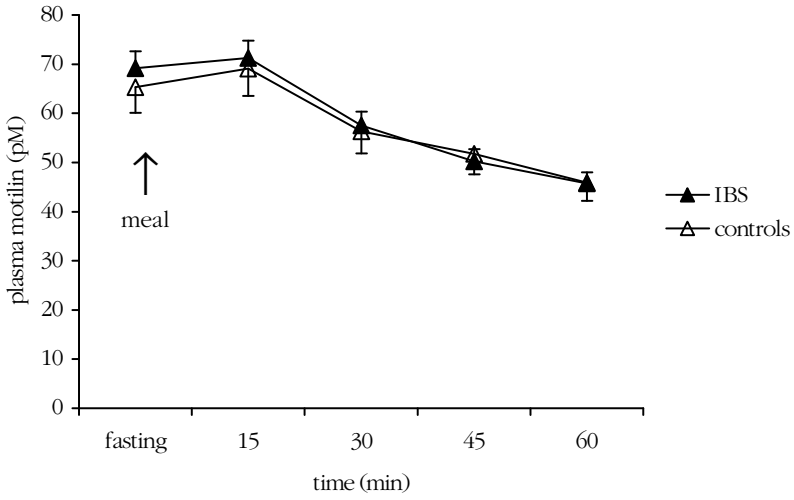

Figure 2. Fasting and postprandial plasma motilin concentrations in IBS patients (closed triangles) and controls (open triangles). * P<0.001 compared to fasting.

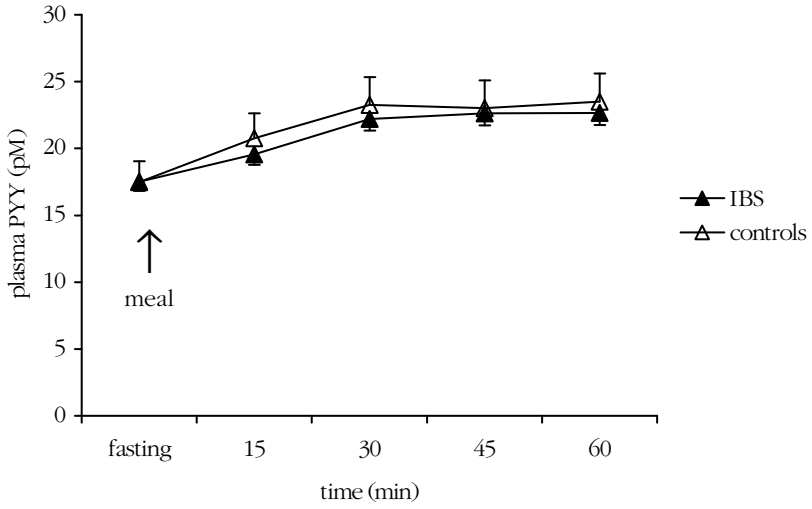

Figure 3. Fasting and postprandial plasma PYY concentrations in IBS patients (closed triangles) and controls (open triangles).
* P<0.001 compared to fasting.

tions decreased significantly after the meal in both groups. The postprandial motilin response was similar in both groups (plasma motilin concentration by group interaction *P*=0.49) (Table 2).

Plasma PYY

Figure 3 illustrates fasting and postprandial PYY levels in patients and controls. Fasting PYY concentrations were similar in patients and controls. The overall plasma PYY response was similar in both groups (PYY concentration by group interaction, *P*=0.80). Plasma PYY concentrations increased significantly in both groups from 15 min to 60 min (*P*<0.001). The incremental postprandial PYY response was not significantly different between patients and controls (Table 2).

Influence of age and gender

Fasting CCK levels were significantly correlated with age in the whole group (*r*=0.33, *P*<0.001), but the postprandial CCK response was not (*r*=0.05, *P*=0.58). A small but significant correlation was found between age and fasting levels of motilin for the whole group (*r*=0.19, *P*=0.03). Age was neither correlated with the postprandial motilin response, nor with baseline nor postprandial levels of PYY.

A significant gender effect was found for fasting plasma levels of CCK (one-way ANOVA, *P*=0.001), which were significantly elevated in female IBS patients compared to male IBS patients (Tukey's *P*=0.012) and female controls (Tukey's *P*=0.009) (Table 2 and Figure 4). This was not accounted for by age, as mean age was similar between groups. Linear regression analysis showed that both age and gender were

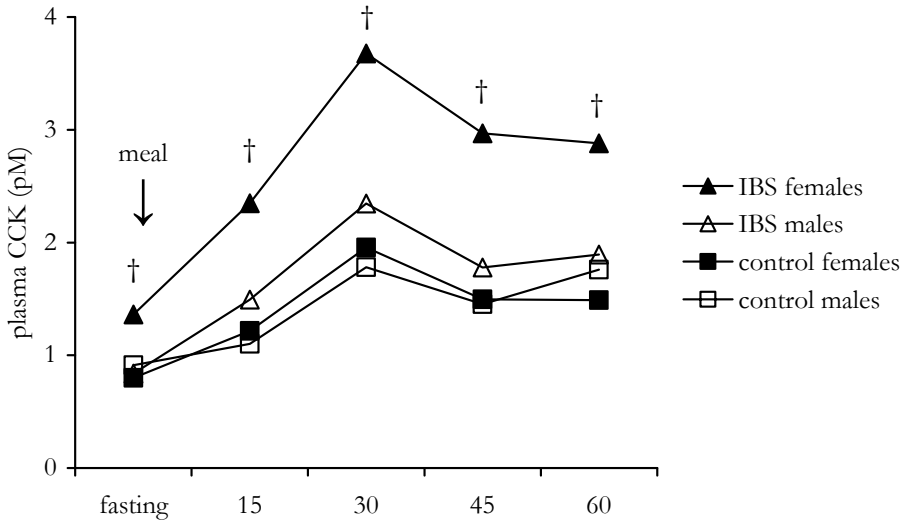

Figure 4. Fasting and postprandial plasma CCK concentrations in female IBS patients (closed triangles), male IBS patients (open triangles), female controls (closed squares) and male controls (open squares). † P<0.001 compared to male patients and male and female controls.

independently correlated with fasting plasma CCK concentration (*P*=0.007 for gender and *P*<0.001 for age).

Figure 4 shows that postprandial CCK levels were also significantly increased in female IBS patients compared to the other subgroups. While the ANOVA indicated an overall difference in the incremental CCK response between these subgroups (*P*=0.05), no significant differences were found between female patients compared to male patients and female controls after adjustment for multiple comparisons (Table 2).

Fasting levels of motilin were significantly higher in male IBS patients compared to female patients (Tukey *P*=0.046). No differences were found between female and male control subjects (Table 2). Neither postprandial motilin levels nor fasting and postprandial levels of PYY were different between males and females (Table 2).

IBS subgroups

Fasting plasma levels of CCK and PYY were not different between the three IBS subgroups, but basal plasma concentrations of motilin were significantly increased in IBS-D compared to IBS-C and IBS-A (Table 3). No differences were found with respect to the incremental postprandial responses of CCK, PYY or motilin.

Table 3. Fasting plasma concentrations and incremental postprandial responses of CCK, PYY and motilin in IBS subgroups according to predominant bowel habit

	IBS-D (n=34)	IBS-C (n=34)	IBS-A (n=22)	P-value*
Age (yr)	43.3 ± 13.0	39.0 ± 15.3	42.4 ± 14.1	
Females n (%)	20 (59)	27 (79)	19 (86)	
CCK				
fasting	1.1 ± 0.9	1.3 ± 0.7	1.4 ± 0.8	0.364
AUC meal	63 ± 66	80 ± 66	68 ± 67	0.577
Motilin				
fasting	82.1 ± 36.5†	60.8 ± 25.1	57.5 ± 23.9	0.003
AUC meal	-655 ± 1390	-547 ± 774	-495 ± 867	0.846
PYY				
fasting	17.6 ± 7.7	17.5 ± 3.7	18.3 ± 6.4	0.897
AUC meal	191 ± 168	185 ± 175	164 ± 123	0.829

AUC, Area Under the Curve. Fasting concentrations are expressed as pM. AUC is expressed as pM•60 min. * P-value for overall difference between subgroups (chi-square or one-way ANOVA) † P=0.011 versus IBS-C, P=0.009 versus IBS-A.

Visceral hypersensitivity and gut peptides

Two of 99 patients declined to participate in the barostat study. Thirty-two of the remaining 97 patients (33%) were classified as hypersensitive to rectal balloon distension. No differences between hypersensitive and normosensitive patients were found for fasting plasma levels of CCK, PYY and motilin or postprandial responses, apart from an increased plasma PYY response in hypersensitive patients (*P*=0.048) (Table 4).

Table 4. Fasting plasma concentrations and incremental postprandial responses of CCK, PYY and motilin in hypersensitive and normosensitive IBS patients

	hypersensitive (n=32)	normosensitive (n=65)	P-value
Age (yr)	41.3 ± 12.8	42.5 ± 14.8	
Females n (%)	23 (72)	48 (74)	
CCK			
fasting	1.2 ± 0.9	1.2 ± 0.8	0.908
AUC meal	73 ± 71	72 ± 75	0.214
Motilin			
fasting	73.2 ± 30.1	67.0 ± 31.6	0.155
AUC meal	-496 ± 1150	-678 ± 1000	0.710
PYY			
fasting	18.9 ± 7.4	16.9 ± 5.1	0.220
AUC meal	215 ± 135	162 ± 169	0.048

AUC, Area Under the Curve. Fasting concentrations are expressed as pM. AUC is expressed as pM•60 min.

DISCUSSION

Our study demonstrates that both fasting plasma CCK concentrations and the postprandial CCK response are significantly increased in IBS patients compared to healthy controls. In contrast, neither fasting plasma levels of peptide YY and motilin nor the postprandial responses of these peptides are different between patients and controls.

The effects of CCK on gastrointestinal function are well-known and include increased sensitivity and motor activity of the distal gut[10,11,13,14,24]. Previous studies in patients with IBS have pointed to disturbed CCK release and altered organ sensitivity to CCK. Infusion of CCK in IBS patients leads to excessive intestinal motor activity[25], reduced pain thresholds[26], and increased gallbladder smooth muscle sensitivity[27]. A study by Sjölund et al. indicated that the release of CCK after ingestion of emulgated maize oil was higher in IBS patients compared to healthy controls[28]. Our findings in a large cohort of IBS patients confirm that postprandial CCK secretion is exaggerated in IBS. Additionally, we found that fasting levels of CCK were elevated in IBS patients. This was not observed by Sjölund et al., possibly due to the smaller sample size in that study (n=18).

One could argue whether the relatively small difference in postprandial plasma CCK concentrations between patients and controls (i.e., approximately twofold increase in IBS) is sufficient to contribute to exaggerated sensorimotor responses in IBS. Niederau et al demonstrated that only infusion of pharmacological doses of cerulein, a CCK agonist, resulted in significantly increased colonic motor activity[13]. Similarly, Sabate and colleagues showed decreased rectal sensory thresholds to balloon distension during CCK infusion at pharmacological but not physiological levels[14]. Unfortunately, these experiments were carried out only in healthy individuals. We previously demonstrated decreased rectal sensory thresholds during CCK infusion in IBS patients[26]. It is possible that increased sensitivity to CCK together with twofold increased postprandial plasma levels are, in part, responsible for altered gastrointestinal sensory and motor function in IBS.

Infusion of CCK has been shown to increase rectal pain sensitivity in IBS[26]. One could hypothesize that elevated plasma levels of CCK may contribute to the pathophysiology of visceral hypersensitivity[5]. Yet, our finding that neither fasting nor postprandial CCK levels were different between hypersensitive and normosensitive patients renders a contribution of changes in CCK secretion to the pathogenesis of enhanced visceral perception unlikely. However, increased CCK release after a meal may well be involved in the exaggerated postprandial colonic motor response in IBS patients[6,7,13]. There is also evidence to suggest that CCK infusion aggravates symptom severity in patients with functional abdominal pain syndromes, including

IBS[29]. Therefore, CCK antagonists such as loxiglumide are considered to have clinical potential in IBS[30].

Plasma levels of CCK correlated significantly with age, which confirms previous findings[31]. Interestingly, the elevated fasting and postprandial plasma CCK levels in IBS patients were almost completely attributable to female patients. This was not accounted for by age, as mean age was similar between groups. Our finding is particularly interesting in view of the female predominance in IBS. Thus far, no studies on gender differences with respect to CCK secretion in humans have been published. One animal study, however, demonstrated gender differences in sphincter of Oddi sensitivity during CCK infusion, evidenced by a greater change in phasic wave amplitude in female compared to male dogs[32]. CCK probably does not play a role in IBS subtypes, as fasting and postprandial CCK levels were not different between patient subsets divided by bowel habit.

Fasting and postprandial plasma levels of motilin were comparable between IBS patients and controls. Similar results have been reported by others[28,33], although increased[8] and decreased[28] motilin secretion after a meal has also been observed in IBS. Remarkably, plasma motilin levels decreased after meal ingestion in both groups. One should realise that motilin contributes to motility in the interdigestive and not in the digestive state, and is involved in triggering phase III of the migrating motor complex (MMC). Motilin levels fluctuate in accordance with the various phases of the MMC. Fasting motilin levels may have been obtained during phase III in some individuals, yielding higher mean plasma motilin concentrations, while in the first hour after meal ingestion phase III is suppressed, which may explain the observed decrease in plasma motilin concentrations. Furthermore, fasting motilin levels were significantly elevated in patients with diarrhoea predominance compared to those with constipation and alternating bowel habits. These findings may be clinically important as motilin is known to stimulate human colonic motility in vitro[34] and in vivo[35] and may therefore play a role in the accelerated colonic transit that has been demonstrated in diarrhoea predominant IBS[36].

Fasting and postprandial plasma peptide YY levels did not differ between IBS patients and controls, a finding that is in line with a previous study[8]. Others have more specifically studied the density of PYY secretory cells in the distal gut mucosa of IBS patients. One study suggested that local tissue levels of PYY in the descending colon are reduced in IBS patients compared to controls[37]. In contrast, another study showed increased numbers of PYY-containing enteroendocrine cells in rectal biopsy specimens of patients who developed IBS symptoms after an episode of acute dysenteric illness[18]. The latter findings point to a role for PYY in the pathophysiology of post-infectious IBS and visceral hypersensitivity. Our observation that patients

who were hypersensitive to rectal balloon distension have a greater PYY response supports this hypothesis.

Finally, it should be recognized that plasma hormone levels do not necessarily represent efficacy at target organ level. Peptides may act via endocrine, but also through paracrine and neurocrine pathways.

It is concluded that 1) fasting plasma motilin levels are significantly increased in diarrhoea subtype IBS patients, 2) postprandial PYY secretion is significantly increased in patients with visceral hypersensitivity, and 3) fasting and postprandial CCK levels are significantly increased in (female) IBS patients. The observed changes in gut hormone secretion, especially of CCK, support a role for gut peptides in the pathophysiology of IBS.

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4

ROLE OF TUMOR NECROSIS FACTOR-α AND INTERLEUKIN-10 GENE POLYMORPHISMS IN IRRITABLE BOWEL SYNDROME

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ABSTRACT

Background: Imbalances in the genetically controlled pro- and anti-inflammatory cytokine production may promote ongoing low-grade inflammation after an acute gastroenteritis, and, subsequently, IBS (post-infectious IBS, PI-IBS). We studied gene promoter single nucleotide polymorphisms (SNPs) of tumor necrosis factor α (TNF-α, pro-inflammatory) and interleukin 10 (IL-10, anti-inflammatory) in IBS patients and controls.

Methods: DNA was extracted from peripheral blood leucocytes of 111 IBS patients and 162 healthy controls. Genotype and allele frequencies were assessed by analyzing SNPs at position –308 (TNF-α) and -1082 and -819 (IL-10).

Results: Homozygous high producers for TNF-α (A/A) were rare (overall prevalence 2.6%). The heterozygous TNF-α genotype (G/A, high producer) was significantly more prevalent in IBS compared to controls (41% versus 26%, *P*=0.02). More patients (41%) than controls (30%) were positive for the A allele (*P*=0.044; OR 1.68, 95% CI 1.01-2.79), with a similar trend for diarrhoea (54%) versus constipation and alternating subtypes (<33%, *P*=0.079), but not for subgroups according to a history of acute gastroenteritis. IL-10 genotypes were similarly distributed in patients and controls for both SNPs. Possession of a high producer TNF-α *and* a low producer IL-10 genotype was significantly more prevalent in IBS (9%) versus controls (3%, *P*=0.035; OR 3.11, 95% CI 1.03-9.36) and in diarrhoea (20%) compared to other IBS subtypes (<4%, *P*=0.026).

Conclusion: Our results support the emerging hypothesis that genetically determined immune activity plays a role in the pathophysiology of IBS. Future studies in larger, clinically relevant, IBS subgroups are warranted to establish definite associations with cytokine gene polymorphisms.

INTRODUCTION

Irritable Bowel Syndrome (IBS) is a common functional bowel disorder characterized by recurrent abdominal pain and altered bowel habits[1,2]. Several mechanisms have been proposed in the pathophysiology of IBS, including visceral hypersensitivity[3,4], altered gut motility[5,6] and psychosocial factors[7,8]. In addition, inflammation and mucosal immune system activation may be important[9]. Recent studies demonstrated an increased risk for developing IBS after dysenteric illness[10-12] and increased numbers of immunocompetent cells in rectal mucosa of patients with post-infectious IBS (PI-IBS) up to 1 year after infection[13], implying that low-grade inflammation may contribute to symptom generation.

Pro- and anti-inflammatory cytokines are important modulators of the immune response and play a role in intestinal inflammation[14]. Cytokine production is under genetic control and imbalances in cytokine secretion may affect disease susceptibility and clinical outcome of various conditions. For instance, secretion of tumor necrosis factor alpha (TNF-α), a pro-inflammatory cytokine[15], is associated with a single nucleotide polymorphism (SNP) in the promoter region of the TNF-α gene (G→A substitution at position –308)[16,17]. Possession of the A allele (A/A or G/A) is associated with increased TNF-α production[18]. Homozygotes for the A allele have worse outcome of cerebral malaria[19] and virus-induced renal failure[20]. Likewise, production of the counter-inflammatory cytokine interleukin 10 (IL-10)[21] is associated with SNPs at positions –1082 (G→A) and –819 (C→T)[22]. Genetic predisposition for low IL-10 production (A/A for the –1082 and T/T for the –819 SNP)[22] is associated with inflammatory bowel disease, particularly ulcerative colitis[23], and acute rejection after liver transplantation[24]. IL-10 knock-out mice spontaneously develop chronic enterocolitis[25]. A recent study by Gonsalkorale et al.[26] showed that the high producer IL-10 genotype (-1082 G/G) is less prevalent in IBS patients compared to healthy controls. However, persisting low-grade inflammation may result from decreased production of anti-inflammatory cytokines, e.g. IL-10, as well as from high levels of pro-inflammatory cytokines such as TNF-α[27] or IL-1beta[28], or from imbalance between these cytokines. Our primary aim was therefore to study gene promoter SNPs of IL-10 and TNF-α in IBS patients and in healthy controls. In addition, we aimed to explore the frequencies of these SNPs in IBS subgroups based on post-infectious symptom onset and predominant bowel habit.

METHODS

Subjects

Patients were recruited through the outpatient department of Gastroenterology and Hepatology of the Leiden University Medical Center (LUMC) and through advertisement in a local newspaper. Healthy control subjects were recruited among spouses of non-IBS patients who attended our department and through advertisement. All participants were screened by one of the investigators (PvdV) and all patients met Rome II criteria for IBS[1]. Exclusion criteria for both groups were: presence of organic disease, previous abdominal surgery (cholecystectomy and appendectomy excluded), pregnancy and dependence on analgesics. Although the presence of immunological (astma, celiac disease) or other disorders was not excluded by means of physical, radiological or laboratory investigations, patients were explicitly requested to report the presence of any disease, now or in the past, and to specify any GI disorder in particular. Informed consent was obtained from each participant. The LUMC ethics committee had approved the study protocol.

Study design

Each subject completed a questionnaire concerning medical history and current abdominal symptoms and bowel habits. In a separate item, we explored whether symptom onset was associated with an episode of acute diarrhoea, fever and vomiting. Subsequently, blood samples were obtained.

Genotype assessment

Blood samples were collected in ice-chilled tubes containing EDTA and transported to the laboratory on ice. All samples were centrifuged at 1000 g for 10 min at 4°C. DNA was extracted from peripheral blood leucocytes according to the salting out procedure[29]. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used to determine the TNF-α G-308A, IL-10 G-1082A and C-819T SNPs. Genotype assessment was done as previously described[30-32]. Briefly, gene specific primers were used to generate 147 bp (TNF-α) and 360 bp (IL-10) products. Restriction enzyme digestion yielded fragments, which were analyzed by electrophoresis on a 4% agarose gel and visualized under UV light (Fig 1A and 1B).

Statistical analysis

We aimed to enroll at least 100 subjects in both groups, based on 1) a 24% prevalence of the high producer IL-10 genotype (G/G) in the Dutch population[31], 2) a power of 0.80, and 3) 11% difference in genotype prevalence between IBS patients and controls[26]. Genotype frequencies were compared between groups by Pearson's

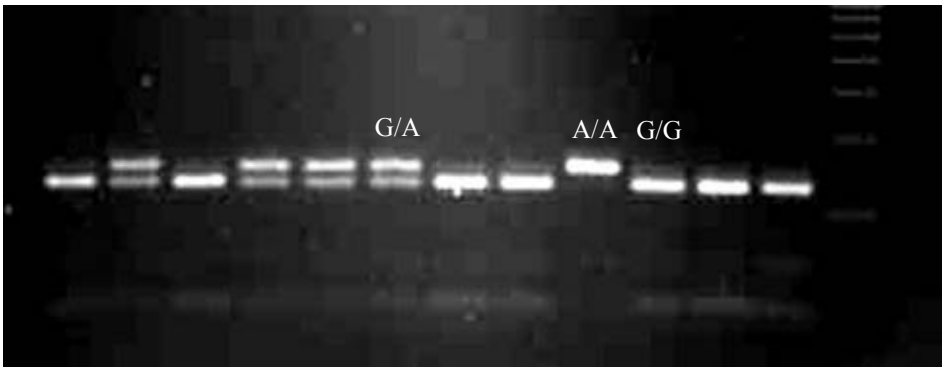

Figure 1A. Example of the TNF-α genotyping method using PCR-RFLP. A/A and G/A, high producer; G/G, low producer.

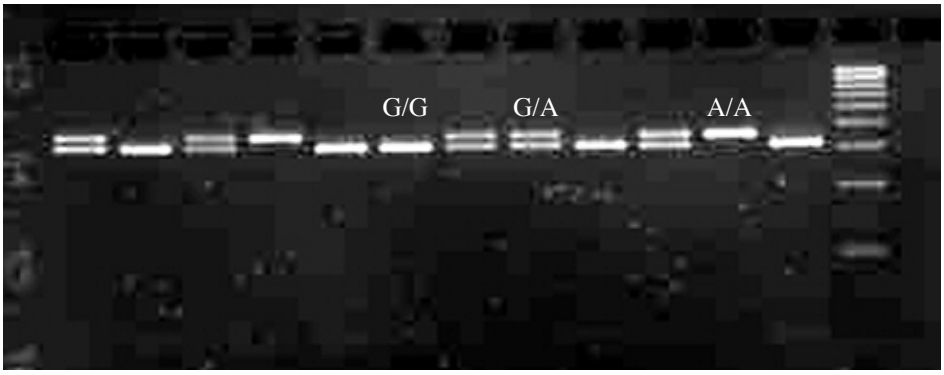

Figure 1B. Example of the IL-10 genotyping method using PCR-RFLP. G/G, high producer; G/A, intermediate producer; A/A, low producer.

chi-square analysis for each polymorphism. Allele and high/low producer genotype frequencies were compared by calculation of odds ratios. Data are expressed as mean (SD) or as number of cases (percentage) where appropriate. The level of significance is set at $P \leq 0.05$.

RESULTS

Subject characteristics

A total of 111 IBS patients and 162 healthy control subjects were eligible and included in the study. Table 1 displays patient and control group characteristics.

Twenty-three patients (21%) reported symptom onset after an episode of acute diarrhoea, vomiting and fever, and were marked as PI-IBS. Fifteen patients (13%) did not report their current bowel habit. Normal bowel habits were reported by 139 controls, and occasional occurrence of diarrhoea or constipation (less than 1 time

Table 1. Characteristics of study participants

Characteristic	IBS patients (n=111)	Controls (n=162)
Females	76 (84)	61 (98)
Age	48.6 (12.9)	37.6 (15.6)
Bowel habit		
diarrhoea	32 (35)	6 (10)
constipation	24 (27)	4 (6)
alternating	31 (34)	4 (7)
currently unknown	13 (15)	-
normal	-	86 (139)

Numbers without parentheses show percentages, numbers within parentheses show absolute numbers or SD (Age). IBS, irritable bowel syndrome; n, number of patients or controls.

per month) without abdominal pain was reported by 23 controls (Rome II negative). In both groups, more than 95% of participants were of Caucasian origin.

TNF-α and IL-10 genotype and allele frequencies

Genotype and allele frequencies for TNF-α are shown in Table 2. Homozygote high producers were rare (overall prevalence 2.6%). The heterozygous genotype (G/A) was significantly more prevalent in IBS patients compared to controls (41% versus 26%, *P*=0.02), with more patients than controls being positive for the A allele (A/A or G/A; 41% versus 30%, *P*=0.044; odds ratio (OR) 1.68, 95% confidence interval (CI)

Table 2. TNF-α G-308A genotype and allele distribution in IBS patients and controls

	IBS patients (n=111)		Controls (n=162)	
	n	%	n	%
Genotype				
A/A (high)	1	1	6	4
A/G (high)	45	41†	42	26
G/G (low)	65	59	114	70
Genotype				
A+ (A/A or A/G)	46	41‡	48	30
A- (G/G)	65	59	114	70
Allele frequency				
-308A (high)	47	21	54	17
-308G (low)	175	79	270	83

† $\chi 2=7.83$, $P=0.020$ versus controls; ‡ $\chi 2=4.07$, $P=0.044$ versus controls; odds ratio (OR) 1.68, 95% CI 1.01 - 2.79.

Table 3. IL-10 G-1082A genotype and allele distribution in IBS patients and controls

	IBS patients (n=111)		Controls (n=162)	
	n	%	n	%
Genotype				
G/G (high)	29	26	45	28
G/A (intermediate)	57	51	83	51
A/A (low)	25	23	34	21
Genotype				
G+ (G/G or G/A)	86	77	128	79
G- (A/A)	25	23	34	21
Allele frequency				
-1082G (high)	115	52	173	53
-1082A (low)	107	48	151	47

1.01 - 2.79). A allele frequencies were not different between patients and controls (21% versus 17%, *P*=0.18; OR 1.34, 95% CI 0.87 - 2.07).

Table 3 shows genotype and allele frequencies for the IL-10 G-1082A SNP. The low producer genotype (A/A) was similarly distributed in patients and controls (23% versus 21%, *P*=0.93). Likewise, frequencies of the A allele (low IL-10 production) were comparable between IBS patients and controls (48% versus 47%, *P*=0.71; OR 1.07, 95% CI 0.76 - 1.50). Similar results were obtained for the IL-10 C-819T SNP. Frequencies of the low-producer genotype (T/T) did not differ between patients and controls (6% versus 7%, *P*=0.73), nor did T allele frequencies (24% versus 27%, respectively, *P*=0.43; OR=0.85, 95% CI 0.58 – 1.27).

Combined high TNF-α and low IL-10 producer genotypes

Possession of both a low producer IL-10 genotype (–1082 A/A) and a high producer TNF-α genotype (-308 A/A or G/A) may make an individual particularly susceptible to an exaggerated inflammatory response or prolonged low-grade inflammation. Therefore we explored the frequencies of the presence of both genotypes in patients and controls. This combination was considerably more prevalent in IBS patients compared to controls (9% versus 3%, *P*=0.035; OR 3.11, 95% CI 1.03 - 9.36) (Table 4). The frequencies of the other genotype combinations were similar in patients and controls (Table 4). The combination of a high producer TNF-α genotype (A/A or G/A) and the other low producer IL-10 genotype (-819 T/T) was not significantly different between patients (3%) and controls (1%) (*P*=0.16; OR 4.47, 95% CI 0.46 – 43.56; other combinations not shown).

Table 4. Combined TNF-α G-308A and IL-10 G-1082A genotypes in IBS patients and controls

	IBS patients (n=111)		Controls (n=162)	
	n	%	n	%
Combination				
high TNF-α / low IL-10	10	9†	5	3
low TNF-α / high IL-10	50	45	85	53
high TNF-α / high IL-10	36	32	43	27
low TNF-α / low IL-10	15	14	29	18

† $\chi 2=4.45$, $P=0.035$ versus controls; OR 3.11, 95% CI 1.03 - 9.36.

IBS subgroups

Exact statistical comparisons between some subgroups according to reported post-infectious symptom onset or predominant bowel habit were not feasible due to small numbers in these groups. Yet, explorative analysis indicated a trend for the high producer TNF-α genotypes (A/A or G/A) to be more prevalent in IBS-D (54%) patients compared to IBS-C (33%) and IBS-A patients (29%) ($P=0.079$) (Table 5), but was found to be present in 48% of PI-IBS patients compared to 40% of non-PI-IBS patients ($P=0.49$) (Table 5). No differences were found regarding the IL-10 genotypes. Furthermore, the prevalence of a combined high producer TNF-α and low producer IL-10 genotype (-1082 A/A) appeared remarkably higher in IBS-D (20%) compared to IBS-C (4%) and IBS-A (3%) ($P=0.026$), but was similar in the PI-IBS and non-PI-IBS subgroups (9% versus 9%, $P=0.95$) (Table 5).

Table 5. TNF- and IL-10 genotype distributions and combinations in PI-IBS and non-PI-IBS patients, and in IBS subgroups according to predominant bowel habit

	PI-IBS (n=23)		non-PI-IBS (n=88)		diarrhea (n=35)		constipation (n=27)		alternating (n=34)	
	n	%	n	%	n	%	n	%	n	%
TNF-α G-308A										
high (A+)	11	48	35	40	19	54†	9	33	10	29
low (A-)	12	52	53	60	16	46	18	67	24	71
IL-10 G-1082A										
high (G+)	19	83	67	76	24	69	24	89	26	77
low (G-)	4	17	21	24	11	31	3	11	8	24
Combined										
high TNF-α / low IL-10	2	9	8	9	7	20‡	1	4	1	3
low TNF-α / high IL-10	10	44	40	46	12	34	16	59	17	50
high TNF-α / high IL-10	9	39	27	31	12	34	8	30	9	27
low TNF-α / low IL-10	2	9	13	15	4	11	2	7	7	21

† $\chi 2=5.08$, $P=0.079$ compared to IBS-C and IBS-A; ‡ $\chi 2=7.33$, $P=0.026$ compared to IBS-C and IBS-A.

DISCUSSION

This study demonstrates that the high producer TNF-α genotype is more prevalent in IBS patients compared to healthy controls. Although homozygous high producers were rare in both groups, the heterozygous genotype, which is also associated with a high TNF-α production phenotype[17], was present in 41% of patients versus only 26% of controls.

TNF-α is produced by monocyte-derived activated macrophages, which have a crucial role in chronic inflammatory states such as Inflammatory Bowel Disease[33] and rheumatoid arthritis[34]. It has been shown that patients with persisting symptoms after an acute infectious gastroenteritis have a fivefold increase in the number of these activated macrophages in the rectal lamina propria[13]. Macrophage TNF-α production can be stimulated by enteric pathogens such as Campylobacter jejuni, Salmonella and Shigella[35], which are important in the onset of PI-IBS[13,36,37]. Increased macrophage TNF-α production in patients carrying the A allele may contribute to the ongoing low-grade inflammation that is demonstrable in a subgroup of patients after an infectious enteritis[13,28]. The largest proportion of individuals positive for the A allele was indeed found in the PI-IBS group (48%) relative to the non-PI-IBS (40%), although this did not reach statistical significance. This does, however, not account for individuals carrying the A allele in the non-PI-IBS group. It is possible that low-grade inflammation can be provoked by unknown non-infectious stimuli, especially in patients who are genetically predisposed to an enhanced pro-inflammatory response. In addition, several other pro- and anti-inflammatory cytokines apart from TNF-α play a role in the regulation of the inflammatory process and may be involved in persistent low-grade inflammation. Finally, recall bias may have affected the composition of the PI-IBS and non-PI-IBS groups, as some patients had symptoms for more than 15 years.

Genotype frequencies for IL-10 at positions –1082 and –819 were not different between IBS patients and controls. We found that the high producer genotype (-1082 G/G) was present in 26% of patients and 28% of control subjects. These findings are in contrast with the recent preliminary observations by Gonsalkorale et al., showing a significant reduction in the high producer IL-10 genotype frequency in IBS patients compared to controls (21% versus 32%)[26]. When comparing these and our data, it is important to recognize that genotype frequencies vary according to ethnicity[31,38]. For instance, a recent study showed that the frequency of the high producer IL-10 genotype is much higher in the Irish population (34%) than in Africans (9.5%) or Singapore Chinese (0%)[39]. In our patient and control groups, more than 95% of individuals were of Caucasian origin, and the IL-10 –1082 high producer genotype frequencies that we found in controls (28%) are similar to those previously reported

in the Dutch population (24%)[31]. Although the study by Gonsalkorale et al.[26] provides no information on the ethnic origin of patients and controls, this may well explain the disparity between their study and ours.

The role of the C–819T SNP in IL-10 production is incompletely understood. This polymorphism is in linkage disequilibrium with C–592A, another SNP in the promoter region of the IL-10 gene[40]. Three haplotypes for the G-1082A, C-819T and C-592A SNPs are common in Caucasians, i.e. GCC, ACC, and ATA, respectively. Although a direct link between the C-819T SNP and levels of IL-10 production has not yet been established, the GCC/GCC genotype is more common in IL-10 high producers, whereas ATA/ATA is associated with low IL-10 production[22]. In our study, the –819 SNP was similarly distributed in patients and controls, supporting our observation that the genetic make-up for IL-10 production levels does not differ between these groups. However, other SNPs in the promoter region of the IL-10 gene may also be associated with increased or decreased IL-10 production. For instance, recent studies indicate that T-3575A, G-2849A, and C-2763A SNPs are associated with susceptibility to systemic lupus erythematosus[41] and leprosy[42] and disease severity in leprosy[42]. It may therefore be important to address these and other SNPs and haplotypes in IBS in future studies.

The combined presence of a high producer TNF-α and low producer IL-10 (-1082 A/A) genotype within one individual was 3 times more prevalent in IBS patients compared to controls. This finding is clinically relevant, since IL-10 is known to inhibit TNF-α synthesis as well as the initial inflammatory response[21]. Individuals with an inherited predisposition to produce high levels of TNF-α, which are not adequately counterbalanced due to a genetically determined low IL-10 secretion, may be particularly at risk to develop ongoing low-grade inflammation and IBS-like symptoms. However, only 1 in 10 patients had this genotype combination, implying that other mechanisms are also important in the pathogenesis of IBS.

Our study was not primarily designed to compare patient subgroups based on post-infectious symptom onset or predominant bowel habit. Patient numbers in these subgroups were small and therefore these results should be interpreted with caution. However, our data indicated that the proportion of individuals positive for the high producer TNF-α A-allele was relatively large in IBS patients with a diarrhoea predominant bowel habit (54%) compared to patients with constipation (33%) or alternating bowel habits (29%). Moreover, the combination of a high producer TNF-α genotype and a low producer IL-10 genotype appeared more prevalent in IBS-D compared to IBS-C and IBS-A (20% versus 4% and 3%, respectively). These are potentially interesting results, as several studies indicate that TNF-α is associated with the occurrence of diarrhoea. For instance, TNF-α is an important mediator of distal colonic secretion[43,44] and stool TNF-α concentrations are elevated in IBD[45] and

infectious HIV-related diarrhoea[46]. Decreased IL-10 mediated inhibition of TNF-α may further add to its biological actions in patients with this specific genotype combination. Our data indicate that IBS subgroups may exhibit different cytokine producer genotypes that might be involved in disease expression, and further studies in larger populations are warranted to confirm these preliminary results.

In conclusion, we have demonstrated that the high producer TNF-α genotype is more prevalent in IBS patients compared to healthy controls. Whereas the low producer IL-10 genotype is similarly distributed, the combination of a high producer TNF-α genotype and a low producer IL-10 genotype is also more prevalent in IBS. Our study contributes to the growing body of evidence that altered immune activation may be important in at least a subset of IBS patients. Future studies should further address the role of cytokine production in the pathophysiology of IBS and focus on clinically relevant subgroups.

ACKNOWLEDGEMENTS

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5

RECTO-COLONIC REFLEX IS IMPAIRED IN PATIENTS WITH IRRITABLE BOWEL SYNDROME

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ABSTRACT

Background: Motor and sensory dysfunction of the gut are present in a subset of patients with irritable bowel syndrome (IBS). Recent studies have demonstrated the presence of a recto-colonic inhibitory reflex in healthy humans. It is not known whether this reflex exists in IBS.

Methods: We studied rectal compliance, perception and the recto-colonic reflex by measuring volume responses of the descending colon to rectal distentions by barostat in 26 IBS patients and 13 healthy controls under both fasting and postprandial conditions.

Results: In the fasting state, rectal distention inhibited colonic tone and phasic motility to a similar extent in health and IBS. After a meal, rectal distention inhibited colonic tone and phasic motility to a lesser degree ($P<0.05$) in IBS than health. Under postprandial but not fasting conditions, rectal distentions of increasing intensity were associated with higher pain scores in IBS than in health.

Conclusion: Rectal distention inhibits tonic and phasic motility of the descending colon in healthy controls and in IBS patients. Postprandially this recto-colonic inhibitory reflex is impaired and attenuated in IBS patients compared to controls. These findings point to an altered reflex function in IBS and have implications for pathophysiology and therapy.

INTRODUCTION

Irritable bowel syndrome (IBS) is a functional bowel disorder that affects 5 to 20% of the general population and is characterized by recurrent abdominal pain and disturbed bowel habits[1,2]. The pathophysiology of IBS is poorly understood, but disturbances at various levels of the brain-gut-axis have been identified, including post-inflammatory changes[3], inappropriate mucosal immune activation[4,5], hyperexcitability of spinal dorsal horn neurons[6] and altered central processing of sensory afferent information[7]. These alterations may result in visceral hypersensitivity, which is considered a hallmark of IBS[8]. In addition, motor dysfunction may occur in IBS. However, disturbed gut motor and sensory functions are present only in a subset of IBS patients, emphasizing the need for alternative explanations for the pathophysiology of IBS.

Reflex inhibition of proximal gastrointestinal motor activity in response to stimulation of a distal segment of the small bowel has been demonstrated in healthy individuals[9,10]. Recent observations in humans[11-13] suggest the presence of recto-colonic and colorectal reflexes in the large bowel. These reflexes differ from the peristaltic reflex as they affect intestinal motility at much more distant segments. To date, the recto-colonic reflex has not been characterized in IBS.

Symptoms in IBS are typically provoked by a meal or, when already present, deteriorate postprandially. Simren et al. demonstrated that duodenal lipid perfusion reduces perception thresholds for first sensation, gas, discomfort and pain in IBS patients, but only for gas in healthy controls[14]. These data suggest an exaggerated sensory response to a meal in IBS. Our aim was to evaluate the recto-colonic reflex in IBS patients under both fasting and postprandial conditions and to compare the results with those obtained in healthy controls.

METHODS

Subjects

Twenty-six IBS patients between 18 and 65 years of age were recruited at the outpatient department of Gastroenterology and Hepatology of the Leiden University Medical Centre (LUMC). The diagnosis of IBS was based on Rome II criteria[2]. Medication for IBS was permitted but had to be stopped 4 days prior to the experiment. Thirteen healthy control subjects were recruited through advertisement. All participants provided informed consent and the LUMC ethics committee had approved the study protocol. Patient characteristics are shown in Table 1.

Table 1. Baseline characteristics of IBS patients and controls

	IBS patients (n=26)	Controls (n=13)
Age (yr)	40.5 ± 15.8	37.2 ± 11.3
Females n (%)	16 (62)	6 (46)
Bowel habit n (%)		
diarrhea	11 (42)	0
constipation	5 (19)	0
alternating	10 (39)	0
normal	0	13 (100)

Numbers within parentheses show percentages. IBS, irritable bowel syndrome; n, number of patients or controls.

Barostat

Two electronic barostats (Synectics Visceral Stimulator, Synectics Medical, Stockholm, Sweden) were used to study the recto-colonic reflex. One barostat was used to perform phasic rectal distentions, while the other measured changes in colonic tone. Pressure and volume were continuously monitored and recorded on a personal computer (Polygram for Windows SVS module, Synectics Medical, Stockholm, Sweden). The barostat assembly is shown in Figure 1.

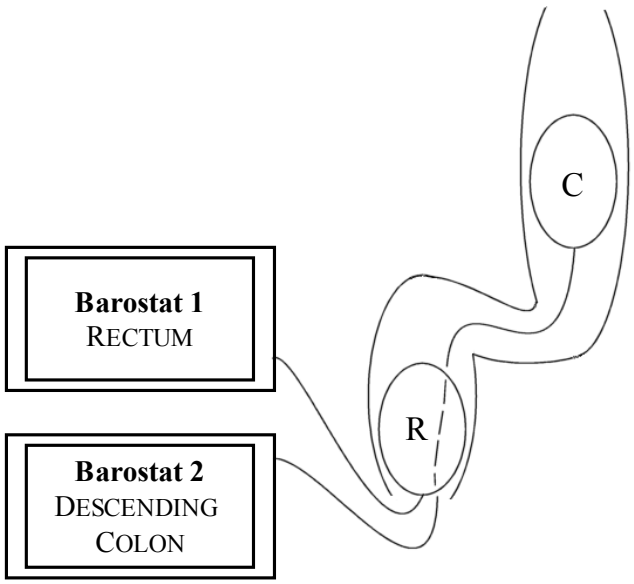

Figure 1. Dual barostat assembly with one bag in the rectum (R) and one bag in the descending colon (C). Both bags are connected to separate barostats.

Experimental design

All experiments were performed on one day to reduce subject discomfort. Therefore, we were unable to randomize the intervention (meal versus fasting), but all measurements were performed in the same order, i.e. first under fasting and thereafter under fed conditions.

The bowel was cleansed with 2 liters of polyethylene glycol (KleanPrep®) the day before the experiment. After an overnight fast, subjects reported at our department at 7.30 AM and received a tap water enema. A flexible guide wire was placed in the transverse colon by endoscopy. Then a barostat catheter with bag was positioned over the guide wire into the descending colon under fluoroscopic control. A second barostat catheter was placed in the rectum, approximately 5 cm from the anal verge. Experiments were performed with subjects in a 10° recumbent supine position (Trendelenburg), lying in a bed.

The experimental protocol is shown in Figure 2. After a 30-min resting period, colonic operating pressure (OP, defined as the pressure that provides a continuous intrabag volume of 80 ml) was determined during slow ramp distention (1 mmHg/min increments until 80 ml bag volume was reached). Next, colonic bag pressure was set at OP and kept constant throughout the experiment. After 30 min, a rectal distention protocol was started, consisting of 5 phasic bag distentions of 10, 15, 20, 25 and 30 mmHg of 5 min duration each. Each distention was followed by a 5 min rest period at 5 mmHg. The rectal distention protocol ended after 50 min and was followed by a 30-min rest period while maintaining colonic bag pressure. After 15

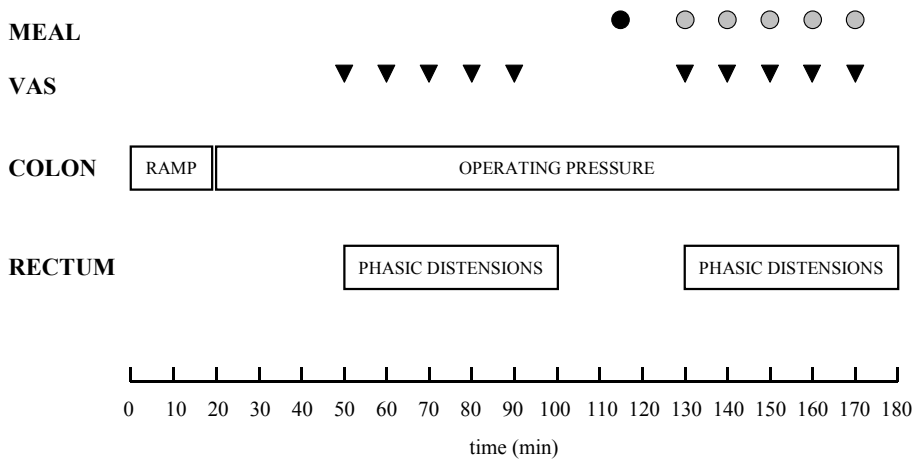

Figure 2. Experimental design. Two identical phasic rectal distention paradigms were performed during fasting and after meal ingestion, while colonic bag pressure was set at operating pressure. Meal ingestion consisted of 200 ml of Nutridrink™ (t=115 min, black circle), followed by 40 ml of Nutridrink™ at the beginning of each rectal distention (grey circles). Urge and pain perception was scored at 30 sec after rectal distention onset (triangles).

min, subjects ingested a 200 ml liquid test meal (Nutrison™, Nutricia, Zoetermeer, The Netherlands; 600 kCal; 13% proteins, 48% carbohydrates, 39% fat). The rectal distention protocol was repeated 15 min after the onset of meal ingestion. An additional 40 ml of Nutrison™ was administered at the beginning of each rectal distention to maintain a nutritional steady state during the experiment. At the end of the experiment, the position of both bags was checked using fluoroscopy, and the bags were removed.

The perception of urge to defecate and abdominal pain was quantified on a 100-mm Visual Analogue Scale (VAS) at 30 sec after the onset of rectal distention, with end points ranging from 'none' to 'unbearable'.

Data analysis

Rectal compliance was calculated by measuring the slope of the volume-pressure relationship from the onset of distention until the maximum pressure was reached. Mean colonic volumes during rectal distention were computed per minute. Subsequently, the relative change was calculated as the maximal volume per distention divided by the average volume in the 5-min pre-distention period (baseline volume). Phasic motility was defined as a 10% volume reduction below baseline, lasting for 10 - 60 seconds, and expressed as number of phasic volume events (PVEs)/5 min.

Statistical analysis

Linear mixed model analysis (SPSS for Windows 11.0.1, SPSS Inc., Chicago IL, USA) was performed to detect differences in colonic bag volume changes, perception scores and number of PVEs, over time, between patients and controls. Group, condition (rectal distention level) and group by condition interaction were analyzed as separate contributors to the model. Changes relative to the 10-mmHg distention and pre- and postprandial values within groups were analyzed using paired t statistics or Wilcoxon Signed Ranks Tests where appopriate. Between-group differences were compared by unpaired t statistics or Mann-Whitney tests. Correlations were calculated using Pearson's linear regression analysis. Data are expressed as mean ± SD. *P*-values less than 0.05 were considered significant.

RESULTS

Baseline barostat characteristics

Rectal compliance was reduced in IBS patients compared to controls, but the difference was only significant in the fed state (patients versus controls, fasting state: 101 ± 35 ml/5 mmHg versus 131 ± 86 ml/5 mmHg, *P*=0.13; Fig 3A; fed state 110

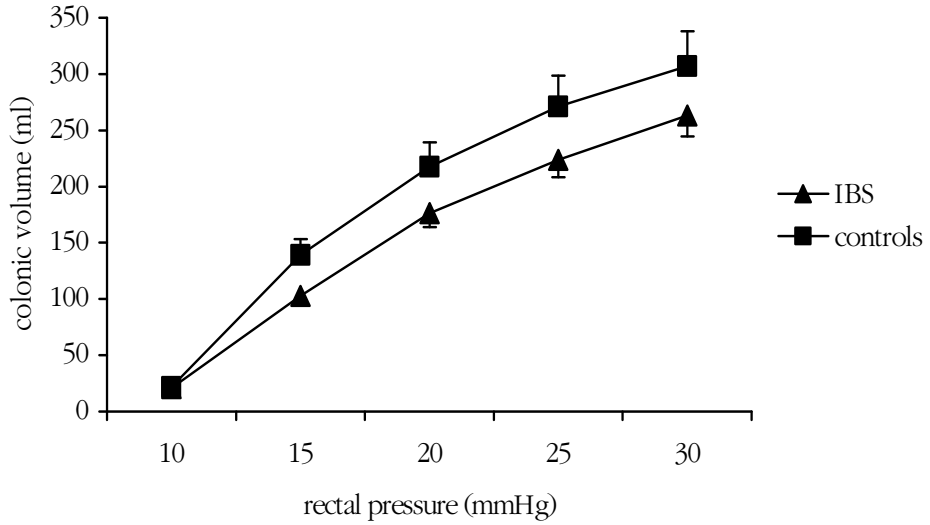

Figure 3A. Rectal compliance expressed as mean volumes (ml ± SEM) during successive distentions in healthy control subjects (squares) and IBS patients (triangles) under fasting conditions.

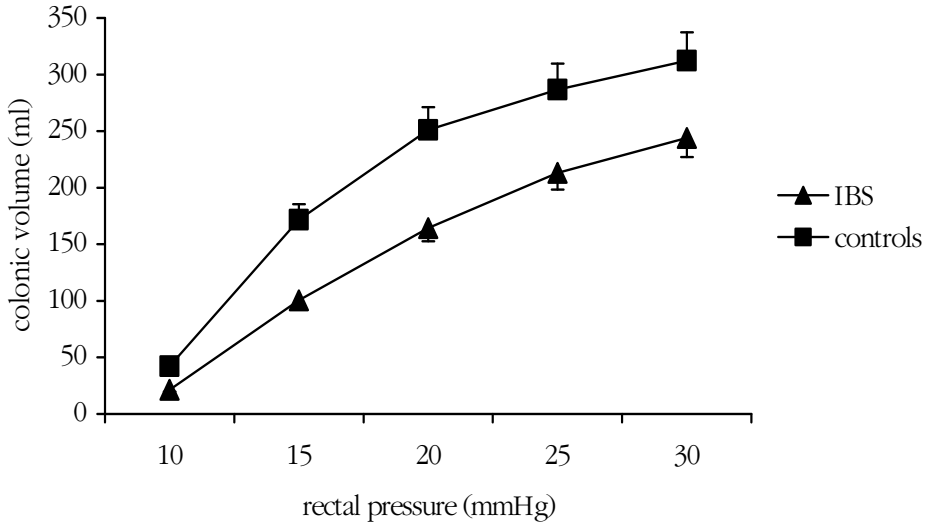

Figure 3B. Rectal compliance expressed as mean volumes (ml ± SEM) during successive distentions in healthy control subjects (squares) and IBS patients (triangles) under postprandial conditions.

± 37 ml/5 mmHg versus 140 ± 52 ml/5 mmHg, *P*=0.05; Fig 3B). However, analysis of covariance showed that postprandial compliance was not significantly different between health and IBS after adjusting for fasting compliance. No significant differences between patients and controls were found in baseline operating pressure,

Table 2. Baseline barostat characteristics of IBS patients and controls

	IBS patients (n=26)	Controls (n=13)	P-value
Fasting			
Operating pressure (mmHg)	14.5 ± 4.5	12.6 ± 4.0	0.21
Baseline colonic volume (ml)	137 ± 42	122 ± 33	0.26
PVEs (n/5 min in predistention episode)	2.9 ± 2.8	3.7 ± 2.8	0.42
Postprandial			
Baseline colonic volume (ml)	145 ± 42	125 ± 55	0.21
PVEs (n/5 min in predistention episode)	4.6 ± 2.3	4.3 ± 3.0	0.70

PVE, phasic volume event; IBS, irritable bowel syndrome.

colonic bag volumes, and number of PVEs in the fasting state and colonic volumes and number of PVEs in the postprandial state (Table 2).

Colonic volume during rectal distentions

Relative colonic bag volumes during rectal distentions in the fasting state are shown in Figure 4A. Mixed model analysis showed that colonic volumes differed across rectal distentions (condition, $P<0.001$). However, the magnitude of colonic relaxation was not different between IBS patients and healthy controls (interaction, $P=0.70$). Figure 5 represents an example of the colonic tracing during fasting in a healthy control subject.

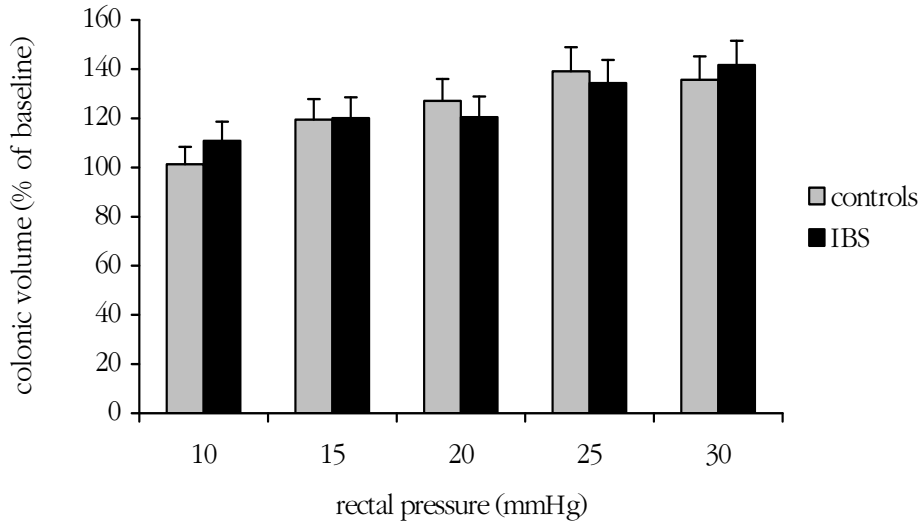

Figure 4A. Colonic bag volumes (% ± SEM) relative to baseline during rectal distentions in healthy control subjects (grey bars) and IBS patients (black bars) under fasting conditions.

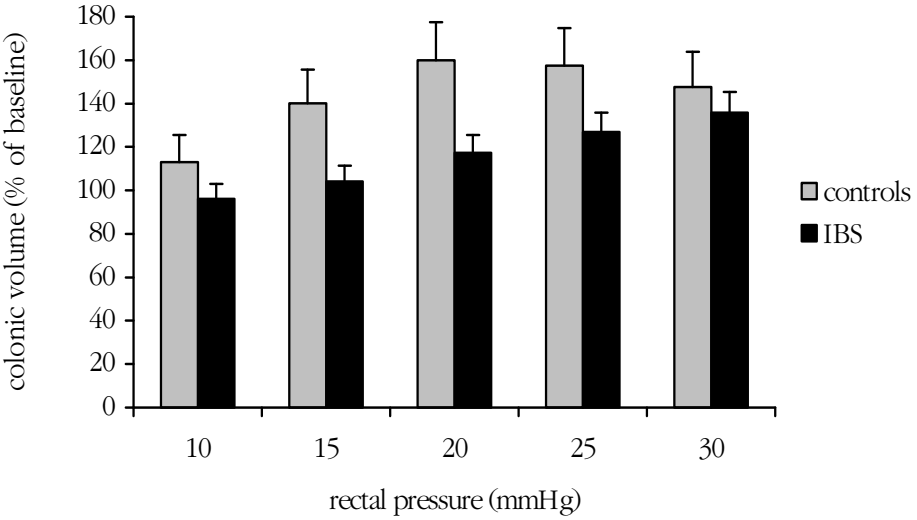

Figure 4B. Colonic bag volumes (% ± SEM) relative to baseline during rectal distentions in healthy control subjects (grey bars) and IBS patients (black bars) under postprandial conditions.

During the postprandial period, the interaction between condition and group was significant (*P*=0.01), suggesting that the effect of rectal distention on colonic volume differed between patients and controls. Figure 4B suggests that colonic relaxation was less pronounced in IBS than in health.

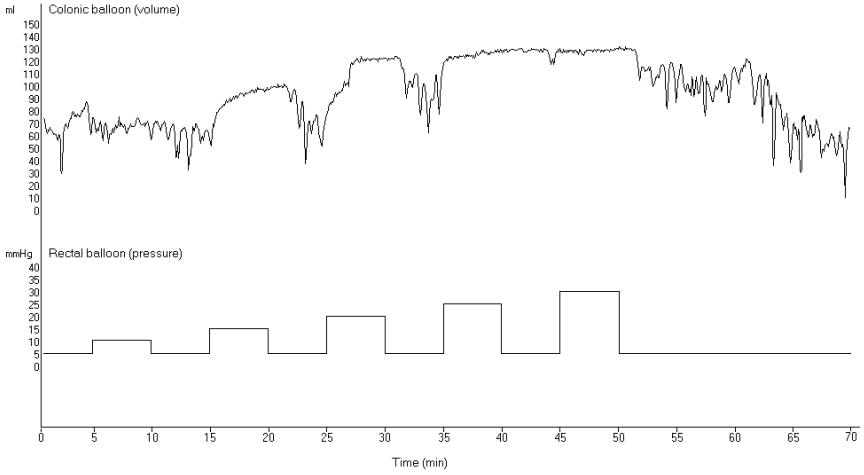

Figure 5. Example of a colonic volume tracing (upper curve) during increasing phasic rectal distentions (lower curve) in a healthy volunteer. From 15 mmHg onward, colonic volume increases while the number of PVEs is reduced. The colonic bag volume returns to baseline after the rectal distention protocol has ended.

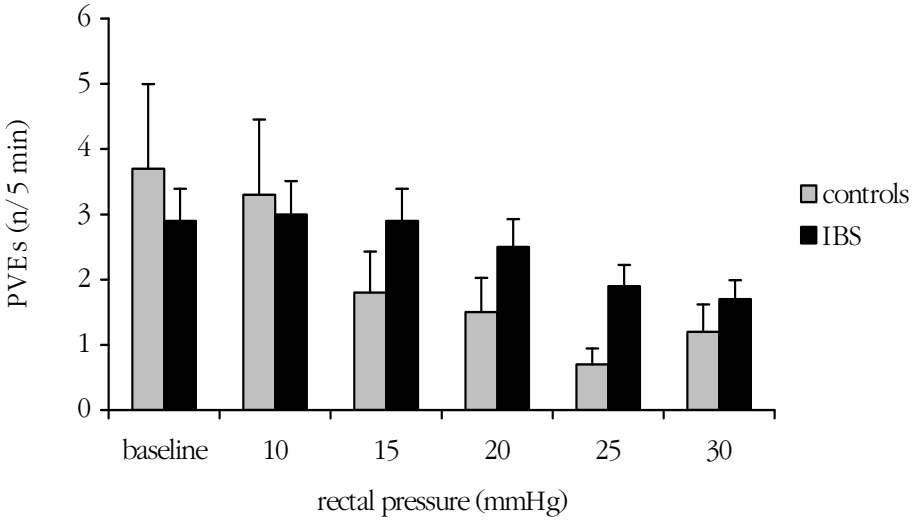

Figure 6A. Number of colonic Phasic Volume Events (PVEs) ± SEM at baseline and during rectal distentions under fasting conditions.

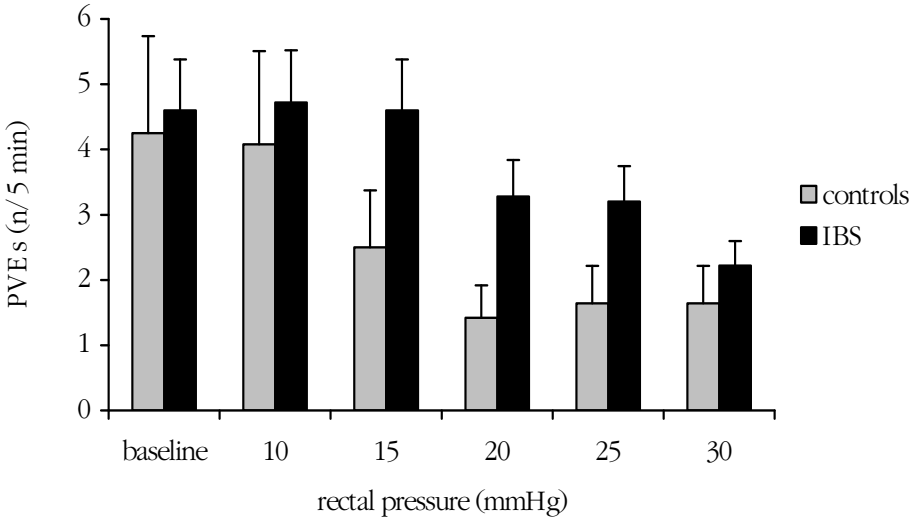

Figure 6B. Number of colonic Phasic Volume Events (PVEs) ± SEM at baseline and during rectal distentions under postprandial conditions.

Phasic motility

During fasting, rectal distentions inhibited colonic motility, reflected by reduced number of PVEs to a similar degree in both groups (condition, P<0.001; group by condition interaction, *P*=0.41) (Fig 6A).

In the absence of rectal distention, more PVEs were observed after compared to before a meal. The increase was significant in IBS patients from 3.4 ± 2.6 to 4.6 ±

2.3 PVE's/5 min (P=0.02), but not in controls (from 3.9 ± 3.4 to 4.3 ± 3.0 PVE's/5 min, P=0.52). During rectal distention after a meal, analysis of colonic PVEs revealed an interaction (P<0.05) between condition and group. Figure 6B suggests that more PVE's occurred in patients compared to controls.

Perception

Urge

During fasting, urge scores increased similarly in patients and controls at increasing bag pressures (condition, P<0.001; group by condition interaction, P=0.87) (Fig 7A). Similarly, urge increased significantly in both groups after the meal (condition, P<0.001), without significant between-group differences (P=0.95 for the interaction).

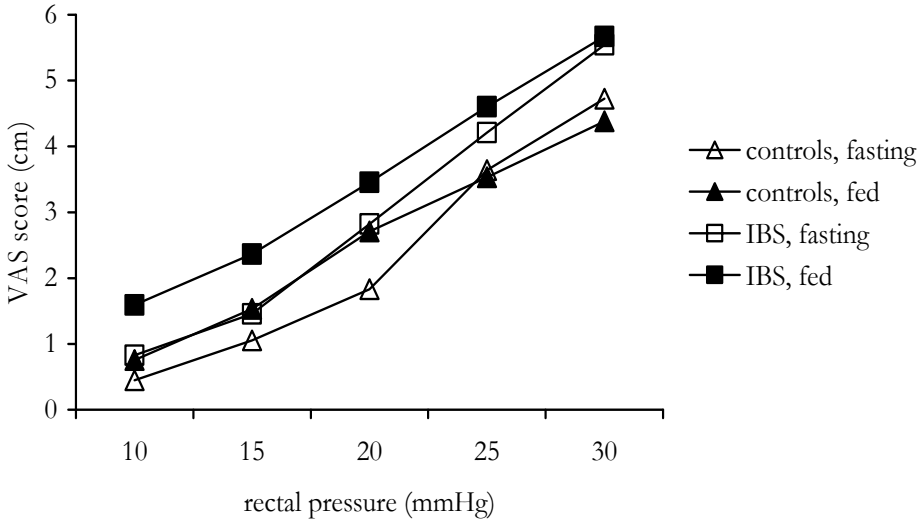

Figure 7A. Perception of urge to defecate during fasting in patients (open squares) and controls (open triangles) and after meal ingestion in patients (closed squares) and controls (closed triangles).

Pain

Under fasting conditions, pain scores in patients appeared higher compared to controls, but the interaction was not significant (P=0.08) (Fig 7B). Postprandially, the group by condition interaction for pain was significant (P=0.01). Figure 7B shows that pain after a meal was increased in IBS patients compared to controls.

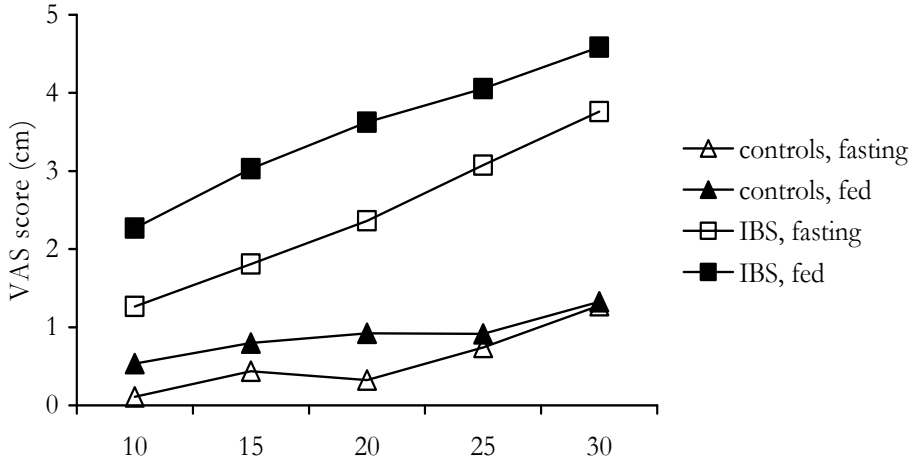

Figure 7B. Perception of pain during fasting in patients (open squares) and controls (open triangles) and after meal ingestion in patients (closed squares) and controls (closed triangles).

DISCUSSION

This is the first study to compare fasting and postprandial recto-colonic reflexes in health and IBS. Colonic motility was characterized by assessing tone and phasic volume events with a barostat. Our results show that 1) in controls, colonic tone and phasic volume events decline during rectal distention under fasting and postprandial conditions, 2) during fasting, colonic relaxation during rectal distention is comparable between IBS patients and healthy controls, and 3) after a standardized meal, colonic relaxation during rectal distention is impaired in IBS patients compared to controls. Under fasting conditions, rectal distention inhibited colonic tone and phasic volume events in an intensity-dependent manner in both health and IBS.

Reflex inhibition of colonic motility during rectal distention has previously been demonstrated in humans. Law et al. showed that colonic bag volumes increased during ramp and phasic rectal distentions in healthy volunteers[11]. In addition, our results also suggest for the first time that the magnitude of colonic relaxation was correlated to the intensity of rectal distention during fasting conditions. By contrast, Ng et al. reported that while 7 out of 14 subjects exhibited colonic dilatation during rectal distention, there was no significant overall group response[12]. Among our healthy subjects, colonic volumes increased by 10% or more in 9 of 13 subjects during rectal distention by 25 mmHg and in 10 of 13 subjects during 30 mmHg distention. Our results therefore support the observations by Law et al.[11] that a recto-colonic inhibitory reflex exists in humans. Differences in study design may explain the discrepancy between our study and a previous study[12]. For instance, Ng studied the colonic

volume response to only one rectal distention, while in our study and that of Law et al. several rectal distentions were employed and a dose response relationship could be established. Recently, Ng et al. studied the colorectal reflex by dual barostat assembly and found the reflex to be significantly attenuated in IBS patients compared to controls[13].

Under postprandial conditions, reflex inhibition of colonic motility, as measured by colonic volumes, was impaired in IBS patients compared to healthy controls. It is unlikely that the differences were attributable to differences in baseline colonic bag volumes, which were not significantly different. However, similar to previous studies, IBS patients had an exaggerated postprandial colonic contractile response[15,16]. Perhaps, exaggerated postprandial colonic motor activity impairs the ability of the colon to relax and thereby attenuates rectocolonic reflexes in IBS patients after a meal.

Consistent with previous studies, pain scores during rectal distentions were higher in IBS patients than in controls[8,17]. Furthermore, patients experienced more pain in the fed state compared to controls, while preprandial pain scores were not different between groups. Simren et al. showed that duodenal lipid infusion reduced perception thresholds for first sensation, gas, discomfort and pain in IBS patients, but only for gas in healthy controls[14], suggesting an exaggerated sensory response to a meal or nutrients in IBS patients. Recently, Caldarella and colleagues demonstrated that intraduodenal infusion of lipids reduced thresholds for discomfort during rectal distention in IBS patients, but not in healthy controls. However, thresholds for perception were significantly lower in IBS compared to controls, with no additional effect of lipid infusion[18]. Our findings confirm these findings, and clinical observations suggest that IBS symptoms deteriorate after a meal. However, the repeated distentions in our study may have also contributed to increased postprandial pain perception[19].

The role of postprandial recto-colonic inhibitory reflexes in the pathophysiology of IBS is not clear. Recent reports point to impaired reflexes at other locations in the gastrointestinal tract in patients with functional bowel disorders. For instance, impaired reflex fundic relaxation following intestinal administration of nutrients has been shown in patients with functional dyspepsia[20]. Our finding that colonic relaxation during rectal distention is impaired after a meal, taken together with the more pronounced effect of a meal on rectal sensation in IBS compared to controls, is consistent with the hypothesis of a generalized disturbance of postprandial colonic sensori-motor functions in IBS. This impairment should primarily be looked upon as a marker of disturbed gastrointestinal motor and sensory function, perhaps attributable to autonomic dysfunctions. In addition, disordered reflexes may also contribute to IBS symptoms, particularly postprandial exacerbation.

Finally, all measurements were performed in the same order, i.e. increasing rectal pressure distentions during fasting conditions followed by the same sequence after a meal. This was done to minimize discomfort to participating subjects. This is, however, a potential limitation of the study.

In conclusion, we have demonstrated the existence of a recto-colonic inhibitory reflex in healthy individuals and in IBS patients. The magnitude of this response is in the same range in both groups under fasting conditions, but is impaired in IBS patients after a meal. Since the role of disturbed colonic motor and sensory function in IBS has not been fully elucidated, future studies should focus on the involvement of retrograde reflexes in the pathophysiology of functional bowel disorders and characterize recto-colonic reflex dysfunction in IBS subgroups.

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6

SYMPTOM SEVERITY BUT NOT PSYCHOPATHOLOGY PREDICTS VISCERAL HYPERSENSITIVITY IN IRRITABLE BOWEL SYNDROME

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ABSTRACT

Background & Aims: Visceral hypersensitivity is a hallmark of irritable bowel syndrome (IBS), but the relationship with clinical symptoms and psychological factors has not been fully established. We aimed to 1) evaluate these variables in a large cohort of IBS patients, recruited from both hospital and general practice, and in healthy controls; 2) assess which of these factors predicts the occurrence of visceral hypersensitivity in IBS.

Methods: Rectal compliance and perception (intensity, perception thresholds; VAS 0-100 mm) were assessed by a rectal barostat study (ramp distension) in 101 IBS patients and 40 healthy volunteers. IBS symptom severity was scored using a 14-day 5-item diary. Anxiety, depression, somatization, vigilance, pain coping, dysfunctional cognitions, psychoneuroticism, and quality of life were assessed using psychometric questionnaires.

Results: Rectal compliance was significantly reduced in IBS patients compared to controls ($P<0.01$), as were thresholds for pain (27 ± 15 vs. 35 ± 8 mmHg; $P<0.01$) and urge ($P<0.05$). Levels of anxiety, depression, neuroticism, somatization and dysfunctional cognitions were significantly increased in IBS patients vs. controls while pain coping and quality of life were significantly worse. Hypersensitivity to rectal distension occurred in 33% of patients and was associated with increased symptom severity ($P=0.016$), but not with demographical characteristics or psychological disturbances.

Conclusion: Hypersensitivity to balloon distension occurs in 33% of IBS patients and is predicted by symptom severity but not by psychological or demographical characteristics.

INTRODUCTION

Irritable Bowel Syndrome (IBS) is characterized by recurrent abdominal discomfort or pain and disturbed bowel habits[1]. Several pathophysiological mechanisms have been suggested in symptom generation, including altered intestinal motility[2], autonomic dysfunction[3,4], inflammation[5,6], and immune system alterations[6-8]. Particularly, visceral hypersensitivity appears to play an important role[9,10] and has been proposed as a biological marker of IBS[11].

Visceral hypersensitivity may result from disturbances at different levels of the brain-gut axis, in which peripheral sensitization of intestinal nerve endings[12], hyperexcitability of spinal dorsal horn neurons[13] and altered central processing of visceral afferent information[14] are implicated. Abnormalities in regional brain activation, especially in areas involved in pain processing such as the anterior cingulated cortex and thalamus, have been reported in IBS patients in response to rectal balloon distension[15]. These regions belong to the emotional limbic system and are involved in psychological and cognitive events[16,17].

IBS symptomatology is associated with psychological factors and these may affect clinical outcome[18]. For instance, psychological distress is more prevalent among IBS patients who seek health care[19]. Little is known about the relationship between psychological variables and visceral hypersensitivity. Such information is relevant because it may provide a better understanding of the pathogenesis of IBS and its treatment. The few studies that explored this relationship have been criticized because of methodological shortcomings such as sample size and patient selection (tertiary referrals)[9,11,19].

The aims of the present study were to 1) explore in a large cohort of IBS patients the prevalence of rectal hypersensitivity, levels of psychological distress and IBS symptom severity, and 2) assess which demographical, clinical and psychological variables predict the occurrence of visceral hypersensitivity in IBS.

METHODS

Participants

This study was part of a large randomized controlled trial of psychological treatment in IBS, the results of which will be published elsewhere. IBS patients between 18 and 65 years of age were invited to participate. Baseline evaluation included detailed psychological assessment, rectal barostat measurements and IBS symptom severity scores.

To obtain a representative sample from the IBS population, patients were recruited from both the hospital IBS population (patients referred to the outpatient Department of Gastroenterology of the Leiden University Medical Center) and from the general population through local advertisement. Healthy volunteers were recruited through advertisement for comparison with the patient sample. All eligible participants were screened by one of the investigators (PvdV). Each patient met Rome II criteria for IBS[1]. Exclusion criteria were organic disease, previous abdominal surgery (except cholecystectomy and appendectomy), and pregnancy. Use of antispasmodics, laxatives, bulking agents and occasional use of analgesics was permitted. We used the Mini International Neuropsychiatric Interview (Dutch version 5.0.0)[20] to exclude patients with severe psychopathology (psychosis or risk of suicide). Informed consent was obtained from each participant. The Leiden University Medical Center ethics committee had approved the study protocol.

Barostat

An electronic barostat (Synectics Visceral Stimulator, Synectics Medical, Stockholm, Sweden) was used to assess rectal compliance and perception. This device measures rectal motor activity as volume changes in a rectal balloon, in which constant pressure is maintained by injecting air when the rectal wall relaxes and aspirating air during rectal contraction. Intrabag pressure is directly measured via a separate lumen. Maximal airflow is 38 mL/s. Pressure and volume are continuously monitored and recorded on a personal computer (Polygram for Windows SVS module, Synectics Medical, Stockholm, Sweden).

Visceroperception

Perception of urge to defecate and abdominal pain during rectal distension was quantified on a 100-mm Visual Analogue Scale (VAS). End points ranged from 'none' to 'intolerable'.

Demographical characteristics

The demographical group characteristics of interest were age, sex, and level of health care (general practice or referral).

Symptom severity

Patients and controls rated the severity of any abdominal discomfort, abdominal pain, constipation, diarrhea, and bloating, daily for 14 days, on a 5-point Likert scale (0 = no symptoms, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe symptoms) using a symptom diary card. A composite score was computed by summing up the 14-day mean scores for each symptom (range 0-20).

Psychological assessment

A battery of questionnaires was administered to both IBS patients and control subjects to determine the following psychological characteristics of each group.

Anxiety and depression. We the used the Symptom Checklist 90 (SCL-90) to measure levels of anxiety (10 items) and depression (16 items). The SCL-90 is a validated survey and consists of 90 items addressing a range of physical and psychological problems[21].

Psychoneuroticism. The level of psychoneuroticism was determined by summing up all 90 items of the SCL-90.

Somatization. We used the abridged Dutch version (NVM) of the Minnesota Multiphasic Personality Inventory (MMPI) to measure somatization, which is 1 of 5 subscales on this questionnaire[22].

The role of the abovementioned psychological factors in IBS has been studied previously[9,10,19]. In addition, we considered the following psychological variables relevant, as they may confound the abovementioned determinants:

Vigilance. We used the previously validated 10-item Somatosensory Amplification Scale (SAS)[23] to determine the extent to which an individual is likely to report enhanced perception of physical symptoms (i.e. lower cognitive perception thresholds).

Cognitions. The recently developed 31-item Cognitive Scale for Functional Bowel Disorders (CSFBD) was used to measure patients' levels of dysfunctional cognitions concerning their IBS[24].

Pain coping. Pain coping was measured by 1 of 4 subscales of the Pain Coping and Cognition List (PCCL). This inventory has been widely used in The Netherlands and awaits future validation. Patients were asked to rate the extent to which they agreed with 11 statements concerning pain coping on a 7-point scale, ranging from "I completely disagree" to "I completely agree".

Somatic symptoms. The SCL-90 was also used to record non-IBS-related somatic symptoms. There are 12 items concerning general complaints, including headache, vertigo, backache, myalgia, difficulties with breathing, intolerance for high or low temperatures, dysphagia, etc.

Quality of life. Quality of life was assessed using the validated SF-36 questionnaire[25]. This survey measures quality of life in 8 domains, i.e. physical functioning, social functioning, role limitations due to physical problems and emotional problems, mental health, vitality, bodily pain and general health.

Experimental design

A small standardized, low caloric breakfast was permitted at 8.00 AM on the day of the barostat recordings. After arrival at our department at 10.00 AM, subjects filled

out all questionnaires consecutively. Each participant was allowed the necessary time to complete the questionnaires, which took 80-90 min on average.

After completion, the rectum was evacuated using a tap water enema. Participants were then placed in a hospital bed and with the subject in the left lateral position, a lubricated and tightly folded highly compliant, polyethylene bag (maximum capacity 1000 mL) tied to the end of a multilumen tube (19 Fr) was inserted through the anus and positioned in the rectal ampulla. Bag position was checked by manual inflation of 150 mL of air and subsequent retraction of the catheter until prevented by the external anal sphincter. After balloon deflation, the catheter was introduced an additional 2 cm, secured to the subjects upper leg by a piece of tape, and connected to the barostat. The hospital bed was placed in a 15° recumbent supine position (Trendelenburg) to avoid interference of abdominal mass with barostat measurements. Barostat measurements commenced approximately 4 hours after the light breakfast.

The experimental protocol consisted of a slow ramp distension to assess rectal compliance. Intrabag pressure was increased at a rate of 1 mmHg/min, starting at 5 mmHg, until a maximum of 30 mmHg. Patients rated the urge to defecate and level of abdominal pain on the 100 mm VAS scale at all even pressures (6, 8,…, 30 mmHg). After the experiment had ended, the rectal balloon was deflated and removed and each participant was provided with a 14-day symptom diary card and a stamped envelope to return the diary. Subjects were instructed to start filling out their symptom diary on the day after the experiment.

Barostat analysis

Dynamic compliance was assessed by calculating volume increments for each individual pressure step in each study participant. Compliance was defined by the largest volume increment (i.e., the steepest slope of the pressure-volume curve) for each participant and averaged over groups. Perception scores are expressed as the mean score at each pressure step. Perception thresholds were defined as the first pressure level at which perception scores exceeded 10 mm.

Visceral hypersensitivity

Patients with a pain perception threshold ≥ 2 SD below the mean threshold in controls were considered to be hypersensitive to balloon distension.

Statistical analysis

We aimed to enroll at least forty subjects in each group to be able to detect a 5 mmHg difference in mean pain threshold, which we considered clinically relevant, with a power of 0.80 and SD of 8 mmHg based on previous studies by our group.

All statistical analyses were carried out using SPSS for Windows, version 11.0.1 (SPSS Inc., Chicago IL, USA). Demographical characteristics were compared between groups by Student-t, Mann-Whitney or chi square analysis as appropriate. Differences in rectal compliance and visceroperception were analyzed for statistical significance using mixed models, using patient numbers as indicator for repeated measurements. One model analyzed pressure, volume, and pressure by volume interaction as separate contributors to the model; a second model did the same for pressure, visceral perception, and pressure by perception interaction. Compliance, perception of urge and pain at maximum rectal pressure (30 mmHg) and perception thresholds for urge were compared by Mann-Whitney (patients *versus* controls) or Kruskal-Wallis analysis (IBS subgroups). Because the pain threshold during ramp distension was not reached in all participants (see results), the best estimates for the mean pain threshold and SD was obtained by Maximum Likelihood Estimation using software for parametric survival models. Normal distribution for the pain scores was assumed. These estimates were compared by log rank analysis.

Finally, binary logistic regression and backward stepwise analysis (method Likelihood Ratio; entry at 0.05 probability, removal at 0.10 probability) was performed to identify demographical, clinical (symptom severity) and psychological characteristics that predict the occurrence of visceral hypersensitivity. Age, gender, health care level, predominant bowel habit, post-infectious symptom onset, rectal compliance, symptom severity, anxiety, depression, somatic symptoms, psychoneuroticism, dysfunctional cognitions regarding functional bowel disorders, vigilance, pain coping, somatization, and quality of life (general health subscale) were entered in the analysis as separate predictors. Data are expressed as mean ± SD. The level of significance was set at $P<0.05$.

RESULTS

Subject characteristics

We screened 130 patients, 26 of whom did not meet Rome II criteria, and 40 healthy volunteers. Two patients declined to participate in the barostat study, and one patient was diagnosed with conversion disorder. All healthy volunteers and 101 patients provided informed consent and were included in the final analysis. Thirty-one patients (31%) were recruited through the outpatient department and 70 patients (69%) were recruited through advertisement. All patients in the latter group had previously consulted a physician and had been evaluated for their abdominal symptoms. Healthy controls were also recruited through advertisement.

Demographical, clinical and psychological characteristics of patients and controls are listed in Table 1. Mean age and male to female ratio were not different between groups. Symptom severity and levels of anxiety, depression, psychoneuroticism, somatization, other somatic symptoms, and dysfunctional cognitions were all slightly but significantly increased in IBS patients compared to healthy controls. Pain coping scores were significantly reduced in IBS. Compared to controls, patients had

Table 1. Baseline demographical, clinical, and psychological characteristics of IBS patients and healthy controls

	IBS patients (n=101)	Healthy controls (n=40)
Demographics		
Age (yr)	42.0 ± 13.9	39.7 ± 15.0
Female sex (%)	73	63
Bowel habit (%)		
Diarrhea	34	0
Constipation	35	0
Alternating	24	0
Not specified/normal (controls)	8	100
Symptoms		
IBS symptom score (0-20)	4.4 ± 2.5*	0.43 ± 0.57
Psychological profile		
Anxiety (10-50)	13.4 ± 4.6†	12.2 ± 3.7
Depression (16-80)	22.5 ± 6.9*	20.7 ± 8.3
Somatic symptoms (12-60)	18.3 ± 5.6*	15.0 ± 3.7
Psychoneuroticism (90-450)	123.8 ± 31.9*	113.3 ± 30.7
Dysfunctional cognitions (31-217)	110.3 ± 35.8*	85.7 ± 37.3
Vigilance (0-40)	9.7 ± 5.8	7.7 ± 4.7
Pain coping (6-1)	3.4 ± 1.0†	3.7 ± 0.8
Somatization (0-2)	0.6 ± 0.4*	0.3 ± 0.3
Quality of life (0-100)		
Physical functioning	82.0 ± 20.4*	94.1 ± 10.5
Role limitations-physical	60.0 ± 42.0*	87.2 ± 28.6
Bodily pain	62.1 ± 19.6*	90.3 ± 16.1
Mental health	75.2 ± 16.3	78.5 ± 13.4
Role limitations-emotional	80.8 ± 35.3	91.0 ± 26.8
Social functioning	73.2 ± 23.7*	90.9 ± 14.3
Vitality	58.5 ± 16.9*	70.8 ± 15.8
General health	61.2 ± 18.8*	75.1 ± 14.6

Score ranges from best to worst are indicated after each parameter. Data are expressed as mean ± SD. * $P<0.01$ versus healthy controls; † $P < 0.05$ versus healthy controls.

Table 2. Psychological profile of patients recruited from the tertiary referral center and from the general population

	Referral center (n=31)	General population (n=70)
Anxiety (10-50)	12.9 ± 3.4	13.7 ± 5.0
Depression (16-80)	22.2 ± 5.1	22.7 ± 7.6
Somatic symptoms (12-60)	18.6 ± 4.0	18.2 ± 6.3
Psychoneuroticism (90-450)	122.1 ± 22.5	124.6 ± 35.7
Dysfunctional cognitions (31-217)	109.6 ± 34.2	110.6 ± 36.7
Vigilance (0-40)	8.2 ± 4.2	10.4 ± 6.3
Pain coping (6-1)	3.5 ± 1.0	3.4 ± 1.0
Somatization (0-2)	0.7 ± 0.3	0.6 ± 0.4

Score ranges from best to worst are indicated following each parameter. Data are expressed as mean ± SD.

impaired quality of life on 6 out of 8 SF-36 subscales. Psychological measures were not different between patients from the tertiary referral center and those from the general population (Table 2).

Rectal compliance and perception

Rectal compliance was significantly reduced in the IBS group compared to healthy control subjects (29.7 ± 12.6 ml/mmHg versus 41.8 ± 18.3 ml/mmHg, $P<0.0001$) (Fig 1A). Subgroup analysis showed that rectal compliance was particularly reduced in patients with a diarrhea predominant bowel habit (IBS-D; $P=0.04$) and those with alternating bowel habit (IBS-A; $P=0.05$) compared to constipation predominant IBS (IBS-C) (Fig 1B).

Urge perception at high rectal pressure distension (30 mmHg) was not significantly different between IBS patients (6.6 ± 2.7 cm) and controls (6.1 ± 2.6 cm) ($P=0.30$). The pressure-urge curves were also not significantly different between patients and controls (pressure by group interaction $P=0.82$; Fig 2). In contrast, pain perception at high rectal pressure was significantly increased in IBS patients compared to controls (2.5 ± 2.7 cm versus 1.0 ± 1.4 cm, $P=0.003$) and the pressure-pain curves differed significantly between groups (pressure by group interaction $P<0.0001$; Fig 3). No differences between IBS subgroups were found (Table 3).

Perception thresholds

Urge thresholds were reached in all participants, but were somewhat reduced in IBS patients (15.6 ± 6.1 mmHg) compared to controls (18.1 ± 6.0 mmHg) ($P=0.042$). No differences were found between IBS subgroups (Table 3). In contrast, only 10 of 40 control subjects (25%) compared to 55 of 101 IBS patients (54%) reached the threshold for rectal pain during balloon distension ($\chi^2=10.01$, $P=0.002$) (Fig 4). Maximum Likelihood Estimation of the mean pain threshold and SD in each group and subse-

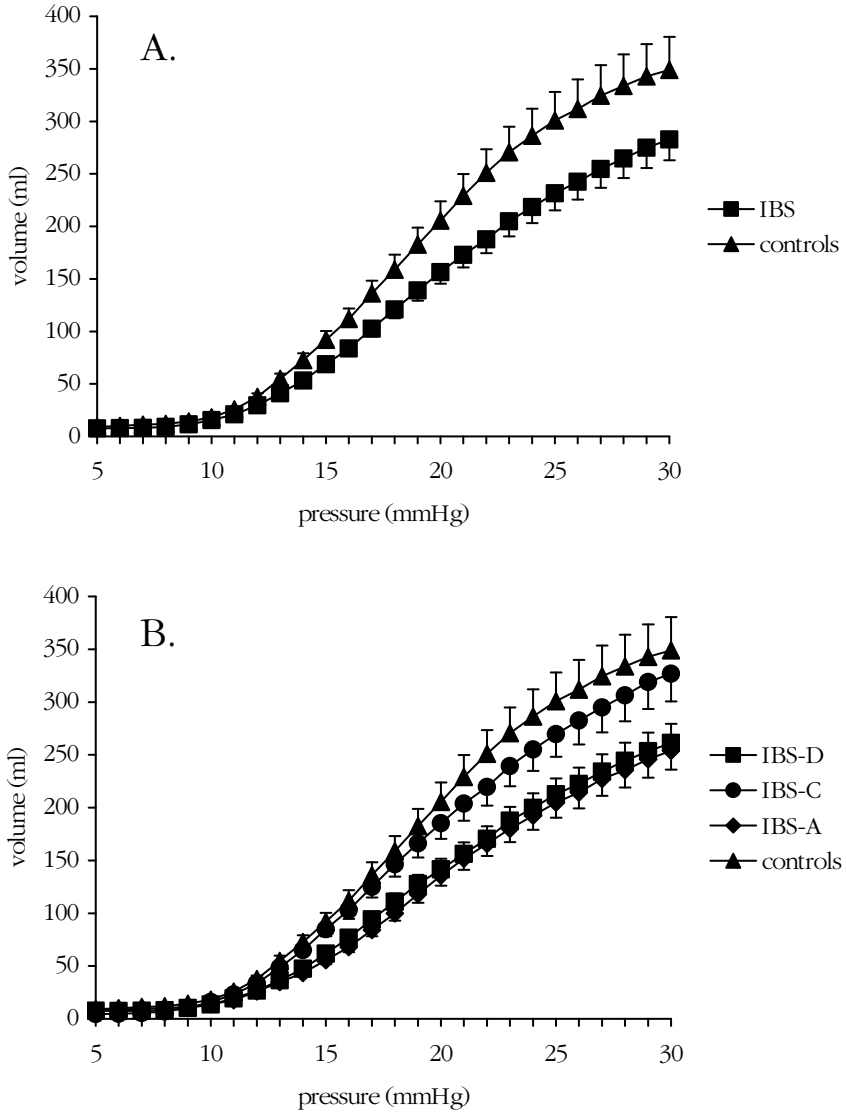

Figure 1. Dynamic rectal compliance (ml/mmHg) in IBS patients and controls (A.) and IBS-D, IBS-C and IBS-A patients and controls(B.). Compliance was significantly increased in all IBS patients compared to controls and in IBS-C compared to IBS-D and IBS-A. Data are expressed as mean ± SEM.

quent log rank analysis showed that the threshold was significantly reduced in IBS patients (27.5 ± 15.1 mmHg) compared to controls (35.3 ± 8.2 mmHg) (P=0.0009), but did not differ between IBS subgroups (Table 3).

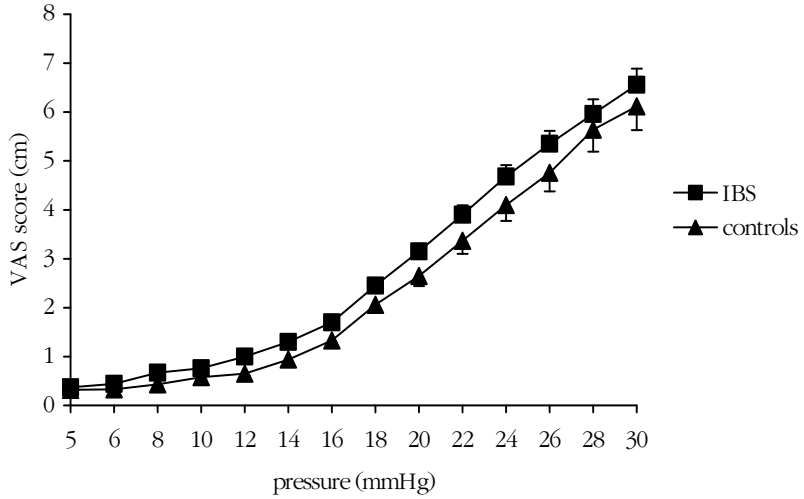

Figure 2. Intensity of urge perception in 101 IBS patients (squares) and 40 controls (triangles). Urge did not differ between patients and controls. Data are expressed as mean ± SEM.

Visceral hypersensitivity

The threshold for hypersensitivity to balloon distension was set at 18.9 mmHg (35.3 *minus* 16.4 mmHg). Thirty-three IBS patients (33%) compared to 0 controls were identified as hypersensitive to balloon distension (χ^2=17.06, P<0.0001) (Table 4). Thus, pain thresholds fell outside the range of control subjects in approximately 1 in 3 IBS patients.

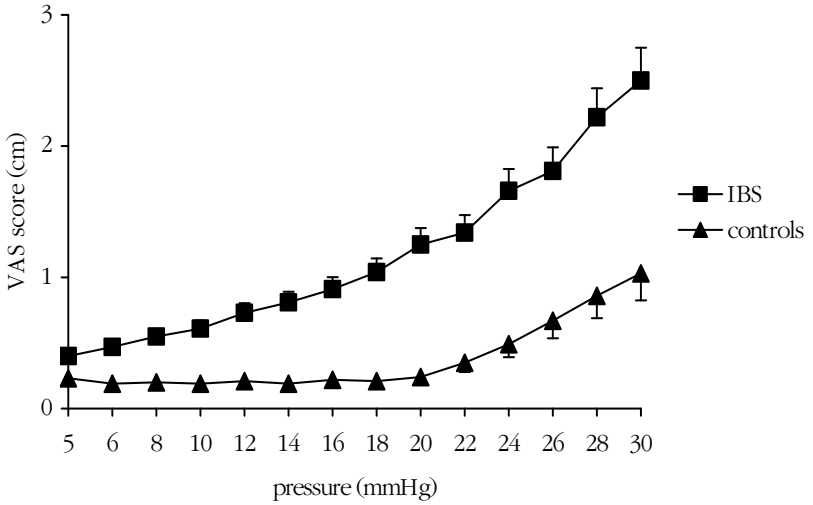

Figure 3. Intensity of pain perception in 101 IBS patients (squares) and 40 controls (triangles). Pain perception was significantly increased in patients compared to controls (pressure by group interaction, P<0.0001). Data are expressed as mean ± SEM.

Table 3. Rectal compliance and perception in IBS patients, IBS subgroups and healthy controls

	IBS patients				Controls
	IBS-D (n=34)	IBS-C (n=35)	IBS-A (n=24)	all patients (n=101)	(n=40)
Compliance (ml/mmHg)	27.2 ± 11	35.2 ± 14 †	26.6 ± 11	29.7 ± 13*	41.8± 18
Urge at 30 mmHg (cm)	6.5 ± 2.9	6.7 ± 2.6	6.7 ± 2.9	6.6 ± 2.7	6.1 ± 2.6
Pain at 30 mmHg (cm)	2.2 ± 2.6	2.9 ± 2.7	2.6 ± 3.0	2.5 ± 2.7*	1.0 ± 1.4
Threshold urge (mmHg)	16.9 ± 6.6	14.2 ± 5.6	15.6 ± 6.1	15.6 ± 6.1‡	18.0 ± 6.0
Threshold pain (mmHg)	31.3 ± 18	23.6 ± 13	29.6 ± 15	27.5 ± 15*	35.3 ± 8.2

* $P<0.01$ compared to controls; † $P<0.05$ compared to IBS-D and IBS-A; ‡ $P<0.05$ compared to controls. Data for the group with unknown bowel habit are not shown due to the small number of patients (N=8). Data are expressed as mean ± SD.

Predictors of visceral hypersensitivity

Of all tested variables, only IBS symptom severity remained as a predictor of visceral hypersensitivity in the logistic regression analysis (OR=1.25, 95% CI 1.04-1.50; P=0.016). Table 5 lists demographical, clinical and psychological characteristics in hypersensitive and normosensitive patients. IBS symptom scores were significantly higher in hypersensitive compared to normosensitive patients (5.4 ± 2.5 versus 4.0 ± 2.4, P=0.007). No other differences were found.

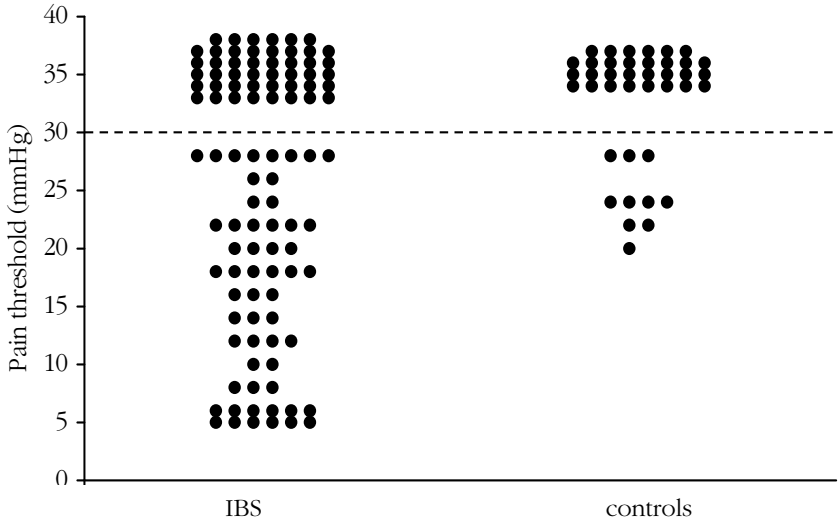

Figure 4. Individual pain thresholds in IBS patients and healthy controls. Significantly more patients (N=55, 54%) compared to controls (N=10, 25%) reached the pain threshold before the end of the ramp distension (dotted line, 30 mmHg).

Table 4. Visceral hypersensitivity in IBS patients and healthy controls

	Hypersensitive	Normosensitive
IBS (n=101)	33 (33%)*	68 (67%)
Controls (n=40)	0 (0%)	40 (100%)

* $P<0.001$ compared to controls.

Table 5. Demographical, clinical, and psychological characteristics of hypersensitive and normosensitive IBS patients

	Hypersensitive (n=33)	Normosensitive (n=68)
Age (yr)	40.7 ± 12.4	42.6 ± 14.5
Female sex (%)	73	74
Recruitment (%) advertisement	68	71
Bowel habit (%)		
Diarrhea	33	34
Constipation	46	29
Alternating	18	27
Not specified/normal	3	10
Post-infectious (%)	11	13
Dynamic compliance	31.8 ± 14.9	28.6 ± 11.3
IBS composite score	5.4 ± 2.5*	4.0 ± 2.4
Dyscomfort	1.38 ± 0.8‡	1.17± 0.62
Pain	1.34 ± 0.95†	0.98 ± 0.72
Constipation	0.73 ± 0.64†	0.37 ± 0.56
Diarrhea	0.45 ± 0.86	0.48 ± 0.69
Bloating	1.37 ± 0.79†	1.01 ± 0.75
General health	62.7 ± 16.4	60.5 ± 19.9
Anxiety	13.9 ± 5.0	13.2 ± 4.4
Depression	23.1 ± 6.5	22.3 ± 7.1
Somatic symptoms	19.0 ± 4.5	18.0 ± 6.1
Psychoneuroticism	126.5 ± 32.2	122.5 ± 32.0
Dysfunctional cognitions	106.8 ± 35.3	111.9 ± 36.1
Vigilance	9.2 ± 5.3	9.9 ± 6.1
Pain coping	3.5 ± 1.1	3.3 ± 0.9
Somatization	0.6 ± 0.4	0.6 ± 0.4
Antispasmodics (%)	15	12
Laxatives or bulking agents (%)	30	31

* $P=0.007$ *versus* normosensitive patients (range 0 (no symptoms) to 20 (worst imaginable)); † $P<0.02$ *versus* normosensitive patients; ‡ $P=0.072$ *versus* normosensitive patients.

DISCUSSION

The present study shows that 1) visceral hypersensitivity is an important feature of irritable bowel syndrome, but not present in all patients, and 2) hypersensitivity to rectal balloon distension is predicted by IBS symptom severity, but not by demographical or psychological characteristics.

Our data confirm previous findings that rectal compliance and pain thresholds are reduced and that the intensity of pain perception is increased in IBS patients when compared to healthy controls. Urge intensity at any given pressure was similar in patients and controls, with slightly lower thresholds for urge in IBS patients. Our observation that pain perception rather than urge is increased, is consistent with other reports demonstrating decreased perception thresholds in IBS only for noxious stimuli, and not for stool[11].

It is presumed that a phasic distension protocol (i.e. rapid balloon inflation to predefined pressure levels) is the preferred procedure to test visceral hypersensitivity, since this would elicit rectal sensations at lower volumes or pressures compared to slow ramp distension[26,27]. However, we chose to perform only ramp distensions because we considered rectal compliance to be an important factor in the model on predictors of visceral hypersensitivity, and compliance is best measured by means of slow ramp distension[27]. Phasic distensions were not performed, since assessment of sensory thresholds during phasic distensions after preceding ramp distension may introduce perceptual response bias, and phasic distensions prior to ramp distension may affect subsequent rectal compliance measurements. The pain thresholds we observed during ramp distension are similar to those reported by others using phasic distensions[10,28,29], which supports previous findings that the type of distension procedure (phasic, ramp, etc) does not affect perception[30].

One of our main findings is that hypersensitivity to balloon distension was less likely to occur in patients with milder symptoms. This challenges the view that visceral hyperalgesia is a biological marker of IBS[11], since hypersensitivity may be absent in Rome II positive patients with mild symptoms. The difference in the proportion of hypersensitive patients between that study (95%) and ours (33%) may in part be due to the use of different parameters to define visceral hypersensitivity. Mertz et al. used 3 parameters to score rectal perception simultaneously (i.e. perception thresholds, intensity of sensations and altered viscerosomatic referral), whereas we only identified patients having decreased pain thresholds and not those having decreased discomfort thresholds or altered pain referral patterns. It is, of course, essential to use equal definitions of visceral hypersensitivity when comparing its prevalence between studies. Since no accepted definition of visceral hypersensitivity is currently available, we decided to use a statistical point of view and consider patients with a

pain perception threshold ≥ 2 SD below the mean threshold in healthy controls as hypersensitive to rectal balloon distension. In general, this method is accepted to define 'outliers'. While this cut-off is arbitrary, our data suggest that hypersensitivity to rectal distension is not a suitable biological marker to identify patients with IBS.

The pathophysiology of visceral hyperalgesia in IBS remains poorly understood. Recent evidence suggests that disturbances may occur at different levels of the brain-gut axis. First, sensitization of peripheral nerve endings at the intestinal level may occur during or after acute inflammation[12,13], leading to higher excitability and/or increased firing of these neurons. Second, some studies suggest that alterations in the spinal dorsal horn neurons may provide an explanation for the extended viscerosomatic referral pattern that is often seen in IBS[11,12]. Third, altered processing of afferent visceral information in the brain, particularly in the prefrontal cortex, anterior cingulated cortex, and thalamus, has repeatedly been demonstrated in IBS patients[15,31]. These regions are not only involved in pain processing but are also part of the emotional limbic system and are therefore involved in numerous psychological and cognitive events[16,17]. Since nociception (becoming aware of a painful stimulus) and emotional pain management both occur in similar regions of the brain, we hypothesized that psychological disturbances are related to visceral hypersensitivity. However, our results do not support this hypothesis, as none of the psychological variables we studied predicted the occurrence of hypersensitivity to balloon distension. These findings substantiate previous observations that psychological characteristics as anxiety, somatization, and neuroticism do not correlate with sensory thresholds[9,11,19]. Similar results were obtained in recent study, in which multivariate analysis demonstrated that abdominal pain and bloating were significantly associated with altered rectal perception whereas psychological symptoms were not[32]. Our data also show that rectal hyperalgesia is not associated with other psychological factors (vigilance, dysfunctional cognitions, pain coping), demographical characteristics (age, gender), quality of life, or predominant bowel habit.

Previously Whitehead et al. proposed a model for psychological factors that influence pain perception in IBS[33]. It was suggested that low pain thresholds in IBS are influenced by two related cognitive traits, i.e. selective attention to gut sensations and a tendency to interpret these sensations as symptoms of disease. Our data show that neither vigilance (selective somatic attention) nor cognitions regarding functional bowel disorders (interpretation of normal sensations as symptoms of disease) were different between hypersensitive and normosensitive IBS patients. These findings suggest that hypersensitive patients do not perceive or manage their symptoms differently from normosensitive patients. Although vigilance and cognitions on functional bowel disorders differed significantly between patients and controls, these parameters were not associated with increased rectal sensitivity.

We aimed to obtain a representative sample from the IBS population by recruiting patients both from the outpatient clinic and by advertisement. Levels of psychological distress were low and did not differ significantly between groups. One may argue that low levels of psychopathology explain why we found no correlation between psychological variables and visceral hypersensitivity, since a certain degree of parameter variability is required for correlations to be detected. Although some studies found significantly more psychological disturbances in IBS patients recruited from tertiary care[18,19,34], one of these studies found no relation between psychological distress and visceral hypersensitivity in clinic patients with IBS[19], supporting our finding that visceral hypersensitivity is not affected by psychopathology, regardless of level of health care.

Allowing patients to take antispasmodics, laxatives and, occasionally, analgesics during barostat measurements is a limitation of this study as it may interfere with visceral sensitivity and affect sensory thresholds in general. While use of these medications was similar in hypersensitive and normosensitive patients (Table 5), prohibiting the use of these medications may have further increased the number of patients with hypersensitivity to balloon distension in both groups.

In conclusion, we found that patients with IBS have impaired rectal compliance and reduced sensory thresholds to rectal distension compared to controls. Visceral hypersensitivity is present in one third of our IBS population and is associated with increased symptom severity. Although psychological parameters do not predict the occurrence of visceral hypersensitivity, this does not exclude a common neuropsychological basis in the pathophysiology of IBS. Future studies should focus on the role of the brain-gut axis in the development of irritable bowel syndrome.

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7

SHORT AND LONG TERM BENEFIT OF RELAXATION TRAINING FOR IRRITABLE BOWEL SYNDROME

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ABSTRACT

Background: Psychotherapy is effective in treating Irritable Bowel Syndrome (IBS), but the effect of relaxation training (RT), a brief psychological group intervention, is not known.

Aim: To determine the efficacy of RT in a large cohort of IBS-patients.

Methods: Ninety-eight IBS-patients were included in this randomized controlled trial. Forty-six patients received standard medical care (CON) and 52 received four 90-minute sessions of RT in small groups in addition to standard medical care. IBS symptom severity, medical consumption and quality of life were assessed at baseline in patients and in 38 healthy controls and evaluated in patients at 3, 6 and 12 months after intervention.

Results: IBS symptom severity was significantly reduced in the RT group compared to CON at 3, 6 and 12 months after treatment (time by treatment interaction, P=0.002). The number needed to treat for long term improvement was 5. Quality of life was improved (General Health, P=0.017; Health Change, P=0.05). Frequency of doctor visits was reduced (P=0.039).

Conclusion: Relaxation training is a brief group intervention that significantly improves symptom severity, general health perception and medical consumption in IBS patients immediately after, as well as 6 and 12 months after intervention.

INTRODUCTION

Irritable Bowel Syndrome (IBS), a frequently occurring functional bowel disorder, is characterized by recurrent abdominal discomfort or pain accompanied by altered bowel habits[1]. IBS has considerable economic impact[2], accounting for total annual direct costs of £ 45.6 million on average in the United Kingdom[3]. In the Netherlands, health care utilization and absence from work in IBS patients is approximately twice that of the general population[4].

Since curative treatment is currently not available[5], therapeutic interventions are directed against predominating symptoms. These interventions include antispasmodics, laxatives or antidiarrhoeals in addition to patient education, reassurance, and dietary advice[6]. Novel therapies focus on serotonergic and psychotropic agents, but therapeutic gain is at best restricted to subgroups of patients[7-10]. In addition to pharmacotherapy, efficacy of psychological interventions such as cognitive behavioural therapy, dynamic psychotherapy and hypnotherapy has been demonstrated in a number of studies[11-15]. Most of these interventions, however, require multiple sessions in individual patients and are therefore time-consuming and expensive.

Relaxation training (RT) is a brief psychological intervention that can not only be provided to individuals, but also to groups of patients. Most forms of psychotherapy incorporate a relaxation technique, but sound data on the efficacy of RT as solitary treatment for IBS are lacking[16]. Two studies on the efficacy of RT in IBS provided promising results but had methodological limitations (small patient number, high drop-out rate)[17-18]. We conducted a randomized controlled trial to determine short and long-term efficacy of group RT, when added to standard medical care (CON), in a large cohort of IBS patients.

MATERIALS AND METHODS

Patients

Between March 2001 and July 2002, IBS patients between 18 and 65 years of age were invited to participate. To obtain a representative sample from the IBS population, patients were recruited both through the outpatient Department of Gastroenterology and Hepatology of the Leiden University Medical Centre (LUMC) and through advertisement in a local newspaper. All eligible patients were screened by one of the investigators (PvdV) to confirm that each participant met Rome II criteria for IBS[1]. Exclusion criteria were presence of any organic disease (particularly inflammatory bowel disease and thyroid disease), previous abdominal surgery (except cholecystectomy and appendectomy), pregnancy and dependence on analgesics. Use

of antispasmodics, laxatives, bulking agents and occasional use of analgesics was permitted. The Mini International Neuropsychiatric Interview (Dutch version 5.0.0)[19] was used to exclude patients with psychotic disorder, substance use disorder or risk of suicide. Thirty-eight age and sex-matched healthy volunteers were included for baseline comparisons. Informed consent was obtained from each participant. The study protocol was approved by the LUMC ethics committee.

Study design

Randomization

This study was designed and conducted as a randomized controlled trial. To guarantee participation of 5 patients per RT group, block wise randomization was carried out in 10 patients using sealed envelopes by a co-worker who was not involved in the study. The day and time of treatment was decided on the agenda of the trainers, not of the patients. Patients randomized to RT, who were unable to attend all scheduled sessions, were asked to participate in the subsequent RT group. If this was not possible, they were replaced by a patient from the control group (CON) who was not yet informed about the randomization results. This procedure was also performed by the same co-worker (Fig 1).

Patient characteristics

Baseline demographics, clinical characteristics and quality of life were assessed in patients and in healthy volunteers. To further characterize the patient group, levels of anxiety, depression, somatic symptoms and psychoneuroticism were measured in patients and healthy controls using the Symptom Checklist 90[20]. The presence of dysfunctional IBS related cognitions was assessed by the Cognitive Scale for Functional Bowel Disorders[21].

Intervention

During the screening visit, all patients received information on gut function in IBS. The physician provided a positive diagnosis for IBS with explanation and a rationale for the specific symptoms. In the control treatment arm patients were instructed to have, upon request and for the duration of the study, free access to specialized gastroenterological care including symptom-oriented pharmacotherapy. No attempt was made to control for contact time between therapist and patient in the control versus the RT arm. The primary aim was to make the control condition credible, plausible and acceptable for the patient. Patients in the RT group were also allowed free access to specialized gastroenterological care and pharmacotherapy.

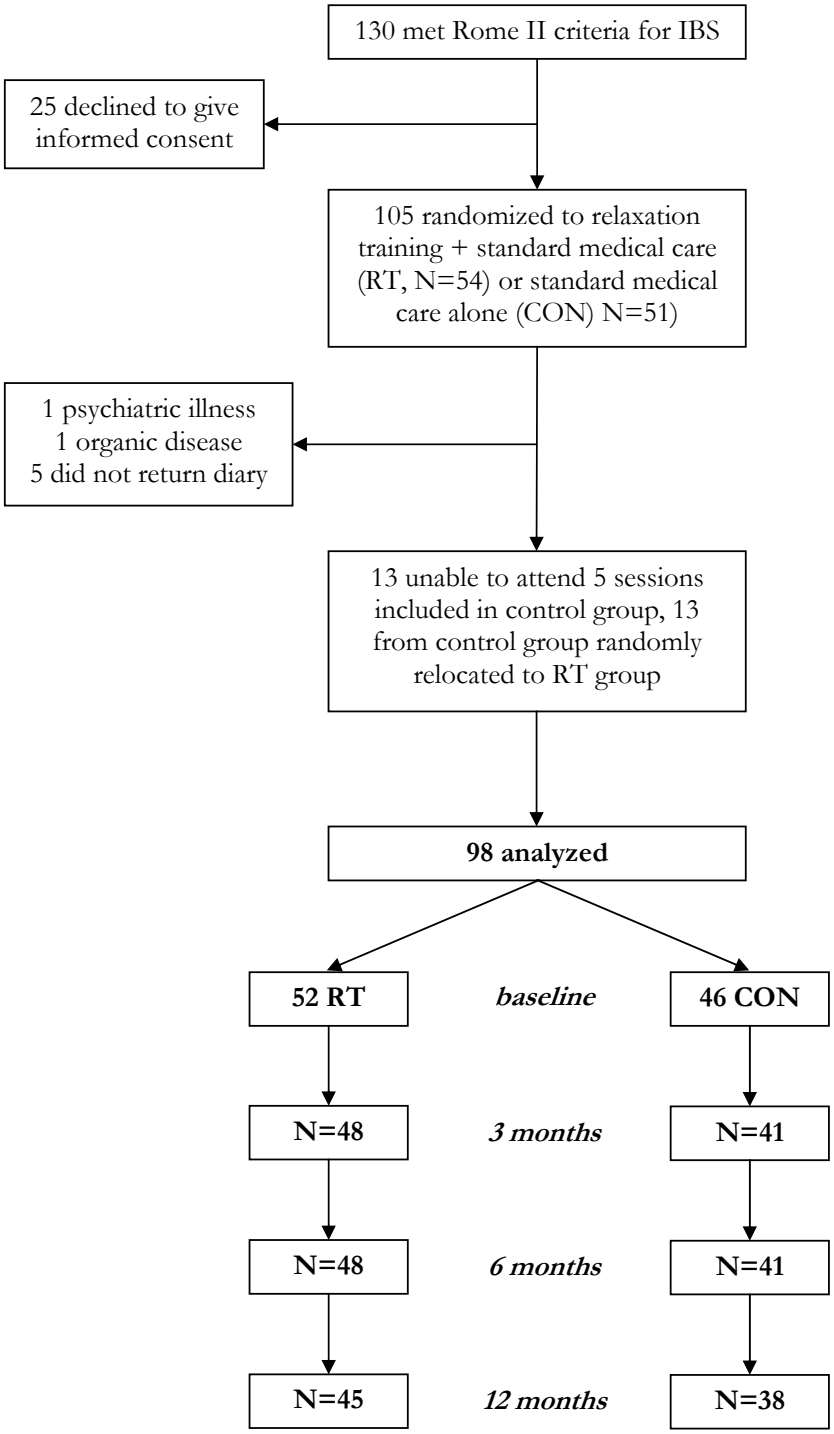

Figure 1. Patient flow during randomisation and number of patients during each phase of the study

A treatment group of 5 or 6 patients was guided by one of three experienced therapists and one of two trainees. Two of three therapists were professional cognitive behavioural therapists and one had nearly finished training. They co-operated with the trainees, who were postgraduate psychologists. Before RT commenced, trainers met each patient individually for 45 minutes to get acquainted to one another and to explain the treatment rationale. Briefly, the therapists explained to patients that abdominal pain involuntarily induces muscle tension. Chronic muscle tension not only maintains abdominal pain, but can also lead to other IBS-associated symptoms, such as borborygmi, indigestion and bloating. By applying relaxation techniques, patients should be able to counteract chronic muscle tension and subsequently experience symptom relief.

RT consisted of weekly 90-min sessions for 4 weeks and one booster session after 3 months. Exercises were audiotaped to facilitate home practice. Training was given according to a written treatment protocol (available on request). Training sessions focused on 1) recognition of muscle tension (progressive relaxation technique), 2) relaxation of muscles (suggestive relaxation technique) combined with breathing retraining, as most IBS patients show evidence of breathing pattern disorders, 3) teaching the patient to elicit a quick relaxation response by prompt recognition of muscle tension and subsequent relaxation, and 4) implementation into daily life. In the booster session, patients shared their experiences and were encouraged to continue using relaxation techniques. All sessions were videotaped and reviewed to monitor therapists' adherence to the treatment protocol. Before randomization, all patients were informed through the consent form that they would be randomized to either RT or standard medical care (CON). On request, patients were notified that, when randomized to standard treatment alone, it would be possible to receive RT after ending of the trial, but only if the efficacy of RT for IBS had been demonstrated.

Outcome measures

Patients used a symptom diary card to rate the severity of abdominal discomfort, abdominal pain, constipation, diarrhoea, bloating, as well as overall symptom severity, daily for 14 days, on a 5-point Likert scale (0 = no symptoms, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe symptoms). The primary outcome measure was the IBS composite score, computed as the sum of the 14-day mean scores for abdominal pain, discomfort, constipation, diarrhoea and bloating (score range 0-20). Secondary outcome measures were: the number of symptom-free days (i.e. overall symptom rating is zero) (score range 0-14); overall symptom rating (i.e., the severity of all symptoms rated together rather than each symptom separately; score range 0-4); quality of life (SF-36)[22]; and medical consumption defined by 1) the number of doctor visits in the previous 3 months and 2) the number of analgesics and laxatives/

antidiarrhoeals used in the previous 14 days. All outcome measures were evaluated at baseline and 3, 6 and 12 months after RT.

Missing data

In case certain questions in the SCL-90 were not answered, subscales of anxiety, depression, somatic symptoms or psychoneuroticism could not be calculated and were regarded as missing. In these cases subscale scores were calculated as ((observed score x the number of scale items) / (the number of scale items - number of missing items)). The same approach was used for missing items on subscales of the SF-36 and the Cognitive Scale for Functional Bowel Disorders. The statistical package dealt with missing subscale scores for all primary and secondary outcome parameters by inserting the mean score of the other patients for that parameter.

Statistical analysis

We aimed to enrol fifty patients per treatment arm, based on: 1) 20 % difference in improvement in IBS composite score (RT versus CON) one year after therapy, which we considered clinically relevant; 2) power of 0.80 and standard deviation of the relative improvement of 47%, based on previous studies by our group, and 3) 20% dropout rate.

Patients' baseline scores were compared to scores in healthy volunteers by one-way analysis of variance (ANOVA). Treatment efficacy with respect to primary and secondary outcome measures was assessed by a mixed model analysis (SPSS for Windows, 11.0). Patients who had missing data were not excluded from the analysis (see above). Confounders of baseline IBS composite scores, time, treatment condition (i.e., relaxation training *versus* standard treatment) and time by treatment interaction were all analyzed as separate contributors to the model. Patient numbers were used as indicator for repeated measurements.

Responders to therapy were identified using Jacobson and Truax' criteria for 'clinical significant change' on the IBS composite score[23]. This change, defined as the extent to which treatment puts an individual outside the range of the patient population or within the range of the non-patient population, was determined by calculation of a reliable change index (RC). This is the difference between pre- and post-test scores divided by the standard error of the difference. An RC larger than 1.96 indicates true change in post-test versus pre-test scores. Differences in responder versus non-responder distributions between groups were calculated by chi-square analysis. Binary logistic regression was used to determine which of the following demographical, clinical and psychological variables could predict therapy success: age, sex, recruitment strategy (outpatient clinic or advertisement), IBS subgroup (diarrhoea, constipation, alternating type), treatment (relaxation or standard medical

care), general health (SF-36), anxiety (SCL-90), depression (SCL-90), somatisation (SCL-90), psychoneuroticism (SCL-90), dysfunctional cognitions (Cognitive Scale for Functional Bowel Disorders), frequency of doctor visits, frequency of analgesic use.

We assessed the efficacy of RT by an intention-to-treat analysis. Data are expressed as mean ± SEM. The level of significance is set at $P<0.05$.

RESULTS

Patients' characteristics

We screened 130 patients of whom 105 provided informed consent. Fifty-four patients were randomized to RT and 51 patients to CON (Fig 1). Seven patients were excluded from the analysis: 1 patient had conversion disorder (diagnosed during the individual session with the therapist), 1 patient had ulcerative colitis (diagnosed after randomization), and 5 patients did not return any of the symptom diaries. Ninety-eight patients were included in the final analysis (RT group, 52; CON group, 46). Sixty-eight patients were recruited through advertisement and 30 through the outpatient department. Some patients did not return 1 or 2 symptom diaries during follow-up, despite regular reminders by telephone to do so (n=9 at 3 months, n=9 at 6 months, n=15 at 12 months, Fig 1). These patients were included in the final analysis (see Missing data). Thirteen patients who were randomized to RT were unable to attend treatment sessions (mostly due to other obligations such as work) and were included in the CON group. These patients were replaced by 13 patients in the CON group (see above).

Table 1 lists baseline demographical and clinical characteristics of both treatment groups and healthy controls. IBS patients had higher symptom scores, impaired quality of life on 6 out of 8 SF-36 subscales, more IBS related dysfunctional cognitions and higher medical consumption. Levels of anxiety and depression did not differ. Baseline IBS composite scores were higher in patients recruited through the outpatient clinic versus patients recruited through advertisement (5.50 ± 0.4 versus 3.85 ± 0.3, P=0.002).

Intention-to-treat analysis

Primary outcome: IBS composite score

IBS composite scores showed a significantly larger reduction in patients who received RT compared to patients who received standard medical care (CON) (time by treatment interaction, P=0.002; Fig 2). Although baseline composite scores were higher in hospital-recruited patients compared to advertisement-recruited patients, the time-by-treatment interaction remained significant after correction for recruitment (P=0.002).

Table 1. Baseline demographical and clinical characteristics of IBS patients and healthy volunteers

Characteristic	Relaxation (n=52)	Control (n=46)	Healthy controls (n=38)
Demographics			
Age (yr)	42.9 ± 1.9	41.7 ± 2.1	39.7 ± 2.4
Female sex (%)	75	72	63
Ethnicity (% Caucasian)	96	89	95
Employment (%)	64	61	-*
Married (%)	84†	70	61
Children (%)	61	52	58
Alcohol use (%)	70	78	94
Current smoking (%)	20	39†	13
Recruitment (% advertisement)	69	70	100
Bowel habit (%)			
Diarrhoea	36	30	0
Constipation	25	48	0
Alternating	31	15	0
Normal or not specified	8	7	100
IBS symptoms			
IBS symptom severity score (0-20)	4.32 ± 0.3	4.41 ± 0.4	0.43 ± 0.1 †
N of symptom free days (0-14)	2.31 ± 0.4	3.02 ± 0.6	0.89 ± 0.3 †
Overall symptom rating (0-4)	1.29 ± 0.1	1.32 ± 0.1	0.13 ± 0.0 †
Psychological profile			
Anxiety (10-50)‡	13.2 ± 0.6	13.8 ± 0.7	12.2 ± 0.6
Depression (16-80)‡	21.6 ± 0.8	23.6 ± 1.2	20.7 ± 1.4
Somatic symptoms (12-60)‡	17.8 ± 0.7	19.0 ± 0.9	15.0 ± 0.6 †
Psychoneuroticism (90-450)‡	119.8 ± 3.8	128.0 ± 5.4	113.3 ± 5.1
Dysfunctional cognitions (31-217)§	108.1 ± 5.1	111.9 ± 5.1	85.6 ± 6.3 †
Quality of life (0-100) **			
Physical functioning	84.2 ± 2.6	79.3 ± 3.3	94.1 ± 1.7 †
Role limitations-physical	58.2 ± 6.0	63.3 ± 6.0	87.2 ± 4.7 †
Bodily pain	63.2 ± 2.6	60.5 ± 3.1	90.3 ± 2.6 †
Mental health	77.3 ± 2.1	73.0 ± 2.6	78.5 ± 2.2
Role limitations-emotional	85.6 ± 4.4	77.0 ± 5.6	91.0 ± 4.4
Social functioning	78.8 ± 2.7	67.1 ± 3.9	90.9 ± 2.4 †
Vitality	61.3 ± 2.2	55.8 ± 2.7	70.8 ± 2.6 †
General health	61.3 ± 2.7	61.6 ± 2.7	75.1 ± 2.4 †
Health change	52.9 ± 3.0	49.5 ± 3.3	53.5 ± 2.5
Medical consumption			
Doctor visits (n/3 months)	1.6 ± 0.1	1.7 ± 0.1	0.7 ± 0.1 †
Analgesics (n/14 days)	2.4 ± 0.7	2.1 ± 0.5	0.6 ± 0.2
Laxative/antidiarrhoeal (n/14 days)	5.4 ± 1.3	4.7 ± 1.2	0.0 ± 0.0 †

Data are presented as mean ± standard error. Numbers in parentheses indicate the range of possible scores for a particular item, with the lower number indicating the best possible score and the higher number indicating the worst possible score. * unknown; † $P<0.01$ versus patient subgroups; ‡ measured using SCL-90 subscales § measured using the Cognitive Scale for Functional Bowel Disorders; ** measured using the SF-36.

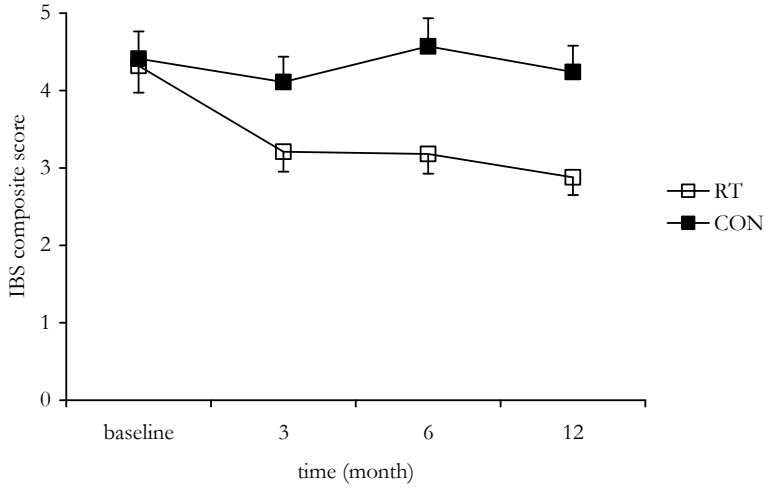

Figure 2. Symptom severity score after 3, 6 and 12 months follow-up in the RT and CON group (time by treatment interaction, P=0.002).

Secondary outcome measures

The number of days without any symptoms (i.e. overall symptom rating was zero) increased significantly more in RT versus CON (time-by-treatment interaction, P=0.027) (Fig 3). Overall symptom rating showed a significantly greater improve-

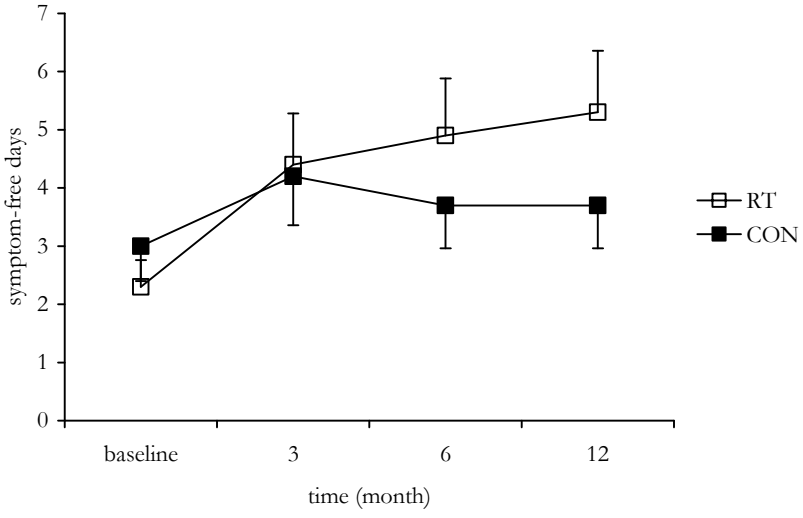

Figure 3. Number of symptom-free days (per 14 days) after 3, 6 and 12 months follow-up in the RT and CON group (time by treatment interaction, P=0.027).

ment in patients who received RT compared to CON (time-by-treatment interaction, P=0.021; data not shown).

Patients in the RT group showed significantly more improvement on the SF-36 General Health (P=0.017) (Fig 4A) and Health Change subscales (P=0.05, Fig 4B). None of the other domains showed significant differences between both groups

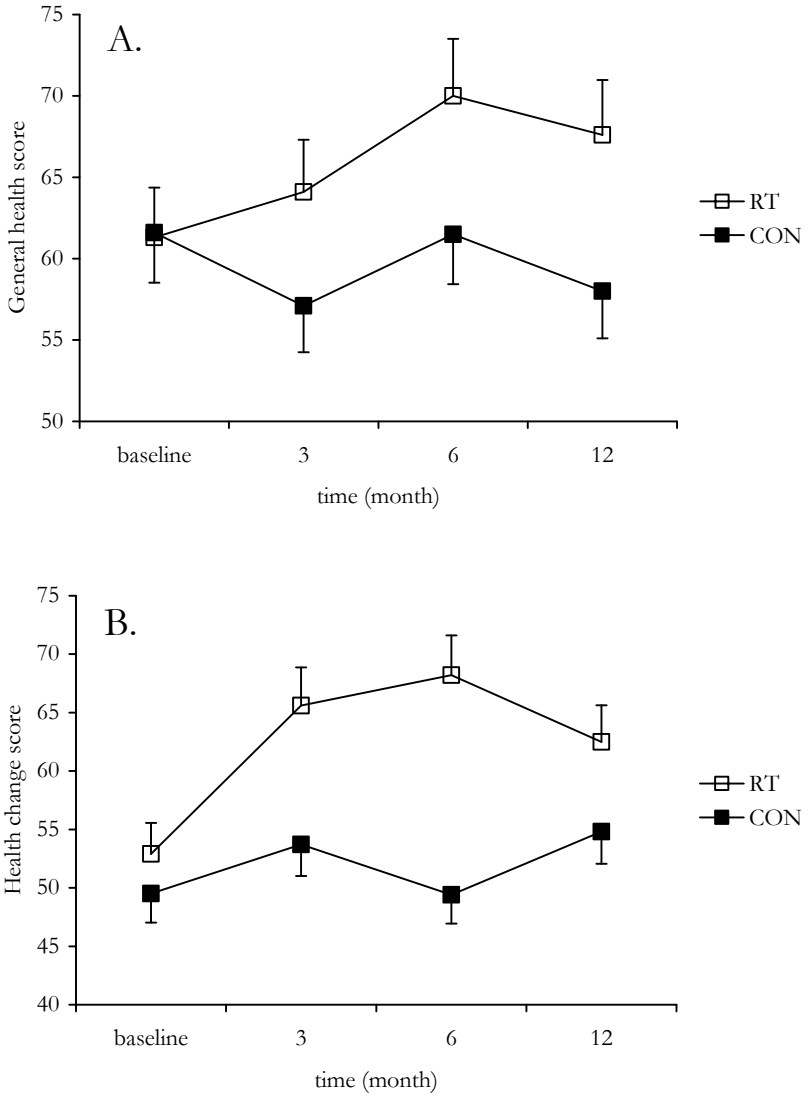

Figure 4. (A.) General Health score (SF-36) after 3, 6 and 12 months follow-up in the RT and CON group (time by treatment interaction, P=0.017). (B.) Health Change score (SF-36) after 3, 6 and 12 months follow-up in the RT and CON group (time by treatment interaction, P=0.05).

Table 2. Medical consumption

	Month of study				
Measure	baseline	3	6	12	P-value*
Doctor visits†					
relaxation	1.6 ± 0.1	2.0 ± 0.2	1.6 ± 0.2	1.4 ± 0.2	0.039
standard medical care	1.7 ± 0.1	1.9 ± 0.3	1.4 ± 0.2	2.0 ± 0.3	
Analgesics ‡					
relaxation	2.4 ± 0.7	2.0 ± 0.7	2.5 ± 0.9	2.7 ± 0.9	0.464
standard medical care	2.1 ± 0.5	1.6 ± 0.5	1.2 ± 0.4	1.9 ± 0.5	
Laxatives/antidiarrhoeals ‡					
relaxation	5.4 ± 1.3	3.7 ± 1.1	2.6 ± 0.9	3.2 ± 1.0	0.496
standard medical care	4.7 ± 1.2	3.4 ± 0.9	2.5 ± 0.8	4.0 ± 1.2	

Data are presented as mean ± standard error. * P-value for time by treatment interaction; † number of doctor visits per 3 months; ‡ number of tablets per 14 days.

(data not shown). Table 2 shows that time by treatment interaction was significant for the number of doctor visits (P=0.039), indicating that patients in the RT group visited their physician less frequently than patients in the CON group. This difference was most pronounced at 12 months post-treatment. No differences were found between the RT and CON groups regarding use of medication.

Response to therapy

According to the Jacobson and Truax' criteria, 8 treated patients (17%) versus 1 control (2%) were significantly improved at 3 months after therapy (P=0.026); 8 treated patients (17%) versus 0 controls were significantly improved at 6 months (P=0.007) and 10 treated patients (23%) versus 1 control (3%) were significantly improved one year after therapy (P=0.009). The number needed to treat (NNT) for long-term improvement was 5 (95% confidence interval (CI) 3.0-15.2). Responders at 1-year follow-up showed similar levels of baseline anxiety (13.4 ± 5.1, range 10-50) as non-responders (12.4 ± 2.6; P=0.41). Binary logistic regression revealed that of all tested demographical, clinical and psychological variables, only treatment condition predicted therapy success (P=0.04). Within the RT group, pre-treatment symptom severity was significantly higher in 12-month responders compared to non-responders (6.90 ± 0.8 versus 3.61 ± 0.3, P<0.001).

DISCUSSION

This is the first randomized controlled trial that has assessed the long-term effect of group-based relaxation training on symptoms and quality of life in a large cohort of

IBS patients. This study shows that RT leads to significant symptom improvement, comparable to symptom reduction obtained with more comprehensive psychotherapies[11-13,18,26]. For example, Creed et al. found that 15 months after psychodynamic interpersonal therapy, which consisted of 8 individual sessions, typical IBS pain scores showed approximately 20% reduction[11]. Boyce et al. found that after 1 year, bowel symptom severity was reduced by 21% in IBS patients who received RT (8 individual sessions) and by 19% in patients who received cognitive behavioural therapy (8 individual sessions)[18]. In both trials, symptom reduction was similar between the treatment group and the group receiving routine clinical care. Our results show that 12 months after five group sessions of RT, IBS composite scores had dropped 34% in the RT group and 12% in the CON group, i.e. a difference of 22%.

Our study extends preliminary data and provides evidence for the efficacy of relaxation training in treating IBS. The first explorative study on this topic suggested that symptom reduction 4 weeks after RT was greater in patients who received treatment (n=8) compared to control patients who only monitored symptoms (n=8)[17]. In our study, symptom improvement increased over time in patients who received RT and was most pronounced after 12 months follow-up, the endpoint of this study. It is unlikely that this increase resulted from symptom fluctuation (a key feature of IBS), because symptom severity remained unchanged in the CON group. In our opinion, routine use of relaxation techniques in daily life, embedded in a clear rationale, provides patients with a useful tool to cope with their symptoms, and this may have a crucial role in the continuation of symptom improvement. The rationale for treatment that was provided to patients may also have contributed to patient compliance in our study: only 16 of 98 patients were lost to long-term follow-up. In a recently published trial, dropout was over 50%, which possibly explains why this study did not find greater efficacy for either relaxation training or cognitive behavioural therapy versus routine clinical care in IBS[18]. Although some of our patients were sceptical towards the concept of RT as treatment for IBS, all were enthusiastic once the rationale had been clarified.

We acknowledge that inclusion of patients in the CON group who were initially randomized to RT but were unable to attend the scheduled training sessions, may have introduced selection bias. Additional analyses, in which these patients were included in the RT group (RT, n=65; CON, n=33), showed similar results for reduction in IBS composite score, overall symptom rating and gain in number of symptom-free days compared to the primary analysis, but statistical significance was not reached (data not shown). In our opinion, this is not surprising as 13 of 65 'RT' patients (20%) in this analysis did not receive treatment. When these 13 patients were excluded from the analysis (RT, n=52; CON, n=33), which has been recommended by some authors[25], the IBS composite score was significantly reduced in the RT group

compared to CON (data not shown), suggesting that RT is indeed beneficial in IBS patients who are treated with RT. Since demographical, clinical and psychological characteristics did not differ between these 13 patients and other patients (data not shown), we believe that adding these patients to the control group (which remained stable during the one year follow-up) did not change outcome in this group.

Whereas some trials included only referred patients[11], we recruited Rome II-positive patients from both the hospital and from the general population, i.e. not only those who seek health care. This strategy was chosen to avoid selection bias, because patients who seek health care represent only a minority of the entire IBS population[27], and symptoms in this subgroup are usually more severe[24,28]. However, inclusion of patients with mild symptom severity may also complicate the interpretation of our results, as less improvement can be expected in this group. Although no additional analysis was performed, it is likely that patients with high symptom severity benefit most from RT simply because their symptom scores can decrease more than low baseline symptom scores. However, our primary finding that, on average, a mixed group of IBS patients having both severe and mild symptoms profits from RT further highlights the potential benefit of this therapy in an individual patient.

We aimed for a reliable distinction between responders and non-responders and therefore used the strict Jacobson and Truax criteria to measure clinical significant improvement[23] in IBS composite score. It is clinically relevant to use outcome measures that represent symptom improvement, since this is the primary outcome of interest in IBS[16]. Most trials have used such endpoints, for instance overall symptom rating[15] and symptom reduction scores[11,17], although some investigators used other outcome measures such as satisfaction with treatment[12]. According to the Jacobson and Truax criteria, significantly more treated patients (23%) than controls (3%) were improved 12 months after therapy. However, the reliable change index (RC) that was utilized to define responders is in part dependent on pre-treatment score as it is calculated by the difference between pre- and post-treatment scores divided by the standard error of the difference in the whole group. As a consequence, significant improvement could not be measured in 12 patients in the CON group and 15 in the RT group due to low pre-treatment scores (data not shown). The higher pre-treatment symptom severity we found in the responder group is therefore associated with the definition of responder according to the Jacobson and Truax criteria. This may underestimate true improvement.

A limitation of our study is the comparison of RT to a standard medical care control group. We cannot exclude that the efficacy of RT is the result of non-specific therapy factors, such as attention and support. A number of control interventions are available for comparison with psychological treatment, but not all of them are appropriate[25]. For instance, a waiting list control group, in which patients do not

receive any treatment until the trial ends, may generate negative expectations with respect to symptom improvement, and these patients may be less inclined to report improvement[25]. Furthermore, the use of a placebo pill might discourage patients who are interested in trying behavioural intervention to participate, while most IBS patients have already tried several drugs to improve their symptoms, without the expected results[25].

We are aware that therapist attention and support might contribute to a positive effect of RT. This may explain the difference in doctor visits between the two groups, since patients in the control group had no additional scheduled interactions whereas patients in the RT group did. Yet, we did not control for this because RT is a minimal intervention and contains elements of patient education as part of the treatment. It is likely that an intervention controlling for attention and support also contains these elements and thereby resembles RT. Controlling for the amount of contact time (5 times 90 minutes in this study) by employing an inert patient-therapist interaction may create an artificial situation. This may further increase the likelihood that patient education or some other form of IBS-related support takes place.

Although using standard medical care as a control intervention has methodological restrictions, such as creating a negative expectation with respect to improvement when assigned to 'more of the same treatment', we expected this effect to be less prominent than in the case of a waiting list control group. Nevertheless, informing these patients that they would not receive any other but their present treatment makes symptom improvement in this group less probable. This may have amplified the differences between treated patients and controls. We attempted to minimize the possible effects of non-specific therapy factors, such as attention and support, by providing highly structured training sessions to patients in the RT group. In addition, all patients in the CON group had free access to medical support from a senior gastroenterologist during the trial period, allowing patients in this group to receive the attention and support they demanded, while we were able to monitor medical consumption. In general, our main objective was to determine the efficacy of group RT as such, inspired by a previous smaller pilot study[17], rather than to assess in detail which aspect of RT is responsible for its beneficial effect (i.e., relaxation, attention, support, group dynamics, etc.).

Finally, it is important to recognize that standard medical care, which was provided to all patients, is essential in treating IBS and cannot be replaced by relaxation training alone. Dietary advice, which is considered the mainstay in IBS treatment, may improve symptoms considerably, especially in patients who report symptom deterioration after a meal. Evidence suggests that some dietary components, such as dairy products and cereals, are involved in abnormal colonic fermentation and increased colonic gas production, leading to postprandial symptom worsening[29].

Furthermore, patient education on the natural course and prognosis of IBS and reassurance with respect to the benign character of IBS symptoms are also essential. These are hallmarks in present-day treatment of IBS and should not be left out.

In conclusion, our study has demonstrated short and long term beneficial effects of RT compared to standard medical treatment, which highlights this treatment as a promising intervention for IBS. RT reduces symptom severity, increases the number of symptom-free days and improves general health satisfaction immediately after therapy. Symptom improvement increases over time until at least 12 months after RT. Patient selection may be important since those patients with high symptom severity are likely to benefit most from RT. The efficacy of RT compared to sham intervention remains to be clarified, but the cost-effectiveness of RT compared to other psychological therapies for IBS deserves further evaluation.

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8

TESTING A BIOBEHAVIORAL MODEL OF IRRITABLE BOWEL SYNDROME

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ABSTRACT

Background: The pathogenesis of irritable bowel syndrome (IBS) is probably multifactorial with dysfunction at different levels of the brain-gut axis. The aim of this study was to evaluate an existing biobehavioral model of IBS symptom generation in a large group of patients.

Methods: In 104 IBS patients, we assessed symptom severity by a symptom diary, age and gender, visceral hypersensitivity using a barostat, autonomic function by measuring arterial baroreflex sensitivity and psychological functioning using questionnaires. Structural Equation Modeling was used to calculate reciprocal and chronological relationships between model variables.

Results: Analysis of the adjusted original model indicated poor fit (Satorra-Bentler scaled chi-square *p*-value .019, comparative fit index (CFI) .842), which was caused by omission of 2 paths (illness behavior-IBS symptoms and trauma-IBS symptoms). The revised model yielded good fit (Satorra-Bentler, *p*=.274; CFI=.967). The trimmed model, obtained by deleting non-significant paths, explained 16.2% of the variance in IBS symptoms. Illness behavior completely mediated the effect of cognitions on IBS symptoms and partly mediated the effect of trauma on IBS symptoms. The fit of this alternative model was significantly better than the fit of the non-trimmed model (Satorra-Bentler, *p*=.43; CFI=.996). The trimmed alternative model explained 16.0% of the variance in IBS symptoms.

Conclusion: The proposed biobehavioral model could not be validated. Whereas visceral hypersensitivity and IBS symptom severity significantly correlate, autonomic function and IBS symptoms do not. Cognitive-behavioral aspects are important in the clinical expression of IBS, with illness behavior playing an intermediate and central role.

INTRODUCTION

Irritable Bowel Syndrome (IBS) is a chronic functional bowel disorder characterized by recurrent abdominal pain and altered bowel habits such as diarrhea and/or constipation[1]. IBS is the most frequent functional gastrointestinal disorder with an estimated prevalence of 6 to 22%[2,3] and substantial economic impact[4,5]. Despite the growing body of literature, the pathophysiology of IBS remains poorly understood and a variety of mechanisms have been proposed in symptom generation. These include enhanced visceral sensitivity[6,7], disturbed intestinal motility[8,9], autonomic dysfunction[10,11], inflammatory processes[12,13], altered immune activity[14,15], altered processing of afferent sensory information[16,17] and psychological disturbances[18,19]. These alterations probably reflect dysfunction at different levels of the brain-gut axis, a conceptual framework which has recently emerged in an attempt to improve our understanding of the etiology, pathogenesis and clinical expression of IBS[20]. Although a biobehavioral model of IBS based on the brain-gut axis would be of great assistance to gain further insight in the relationship between these disturbances, few attempts have been made to construct such a model.

In 1998, Naliboff and colleagues proposed an initial but comprehensive working model of IBS, incorporating the central nervous system, visceral sensory and motor functioning, and cognitive-behavioral systems[21]. This biobehavioral model implies that internal or external stimuli, for example dysenteric illness or sexual or physical abuse, affect visceral sensory and motor function either directly or by an arousal-induced autonomic response ('ANS stress response'), that is, hypervigilance. Furthermore, the model suggests that visceral motor and sensory disturbances subsequently give rise to IBS symptoms, and that prolonged symptom duration will lead to alterations in illness behavior, environmental responses and health beliefs. These biobehavioral changes in turn increase hypervigilance and, ultimately, deteriorate IBS symptoms. Thus, the proposed model represents the clinical manifestation of IBS as interplay between biological and psychological factors, which is in agreement with the current concept of IBS as a multifactorial condition[22,23]. It also provides a verifiable theoretical framework that may improve our understanding of the pathophysiological mechanisms involved in IBS.

The aim of the present study was to evaluate this biobehavioral model of IBS[21] in a large group of patients. We tested the validity of the model using Structural Equation Modeling (SEM), as it allows calculation of reciprocal and chronological relationships between the model variables. Lackner and colleagues have recently shown that SEM is a valid method to test a sequential model of pain processing in IBS[24]. The ratio between the number of observed variables and the number of patients restricted testing possibilities using a model with latent variables and therefore constrained us

to perform a path analysis (as was done by Lackner et al.). To apply a path analysis to the working model proposed by Naliboff et al., we modified the model slightly, that is, we eliminated the feedback loop from IBS symptoms, illness behavior, environmental responses, health beliefs, and vigilance back to IBS symptoms[21] (see Fig 1). Furthermore, as IBS has a female predominance of unknown origin[25] and is less common in the elderly[26], we included age and gender in the model. Based on the proposed model, the existing literature, and the abovementioned statistical restrictions, we built the following hypotheses (Fig 1):

1. Trauma involving the abdomen, e.g., acute gastroenteritis, abdominal surgery, or sexual or physical abuse, will influence IBS symptom severity by modification of autonomic functioning and/or visceral sensitivity[27-29].
2. Autonomic dysfunction (reflected by low baroreflex sensitivity (BRS)-values) is associated with increased visceral sensitivity and hypervigilance[30-32].
3. Hypervigilance will lead to increased IBS symptom severity, either directly or by influencing visceral sensitivity.
4. Dysfunctional cognitions regarding functional bowel disorders lead to hypervigilance and increased IBS symptom severity[33].
5. Illness behavior aggravates dysfunctional cognitions[34].
6. Visceral hypersensitivity will lead to increased IBS symptom severity[6,35-37].
7. In older patients, autonomic functioning (BRS) is impaired[38], while vigilance is increased.
8. Levels of vigilance are higher in female patients[39].

METHODS

Participants

Between March 2001 and July 2002, IBS patients between 18 and 65 years of age were invited to participate in a clinical trial assessing the effect of a brief psychological intervention on IBS symptom severity. This trial included baseline psychological assessment, combined autonomic nerve functioning and rectal sensitivity testing (day 0), and IBS symptom severity measurements (day 1 to 14). All these data were used for the present study.

Patients were recruited through a tertiary referral centre (the outpatient department of Gastroenterology of the Leiden University Medical Center (LUMC)) and through local advertisement. All eligible participants were screened by one of the investigators (PvdV). All patients met Rome II criteria for IBS[1]. Exclusion criteria were organic disease, previous abdominal surgery (except cholecystectomy and appendectomy),

and pregnancy. Use of antispasmodics, laxatives, bulking agents and occasional use of analgesics was permitted. We used the Mini International Neuropsychiatric Interview (Dutch version 5.0.0)[40] to exclude patients with psychotic disorder, or risk of suicide. Informed consent was obtained from each participant. The LUMC ethics committee had approved the study protocol.

Measures

IBS symptom severity

Patients rated the severity of 5 symptoms, i.e. discomfort, abdominal pain, constipation, diarrhea, and bloating, daily for 14 days, on a 5-point Likert scale (0 = no symptoms, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe symptoms) using a symptom diary card. A composite score was computed by summing up the 14-day mean scores for each symptom (range 0-20).

Visceral sensitivity

An electronic barostat (Synectics Visceral Stimulator, Synectics Medical, Stockholm, Sweden) was used to assess visceral perception. This device maintains constant pressure within an infinitely compliant balloon by injecting air when the rectal wall relaxes and aspirating air during rectal contraction[41]. A slow rectal ramp distension procedure was performed (1 mmHg increase/min, maximum 30 mmHg), during which rectal pain perception was quantified on a 100-mm Visual Analogue Scale (VAS)[42] at every even pressure. End points ranged from 'none' to 'intolerable'.

Autonomic function

Autonomic function was assessed by measuring arterial baroreceptor reflex sensitivity (BRS). BRS is defined as the prolongation of the interval between heart beats (milliseconds) induced by aorta and carotid baroreceptor activation when, due to any cause (e.g. stress or pain), arterial blood pressure rises by 1 mmHg. We chose to use BRS rather than more conventional autonomic measures, such as heart rate variability, because the arterial baroreflex not only modulates sympathetic and parasympathetic autonomic outflow, which governs gastrointestinal motor function, but also affects cortical arousal[31,32] and somatic[32,43] and visceral[30] pain perception. Thus, BRS may well be involved in conditions associated with altered visceral sensory and motor function, such as IBS. BRS measurements were performed as described previously[44].

Trauma

A history of trauma involving the abdomen was assessed by asking patients whether they ever experienced 1) sexual abuse, 2) physical violence or abuse involving the abdomen, and/or 3) abdominal illness, e.g. acute gastroenteritis, appendicitis etc. Scores ranged from 0 (no trauma, answer is 'no' to all questions) to 3 (answer is 'yes' to all questions).

Vigilance

We used the Somatosensory Amplification Scale (SAS)[45,46] to determine the extent to which an individual is likely to report enhanced perception of physical symptoms (i.e. lower cognitive perception thresholds). This scale comprises 10 items, with each item being scored on a 0 ('this statement does not apply to me') to 4 ('this statement is fully applicable to me') scale, yielding a total score range from 0 (best score) to 40 (worst score).

Dysfunctional cognitions

The recently developed 31-item Cognitive Scale for Functional Bowel Disorders (CS-FBD) was used to measure patients' levels of dysfunctional cognitions concerning their IBS[47]. Scores for individual items range from 1 (I completely agree) to 7 (I completely disagree), which yields a total score ranging from 31 (best) to 217 (worst).

Illness behavior

Illness behavior was assessed using the 6-item illness behavior subscale of the Illness Attitude Scale (IAS) [45,48]. Scores for individual items range from 0 ('not at all') to 4 ('very much'). The total score was divided by the number of items, yielding an illness behavior subscale score ranging from 0 (best score) to 4 (worst score).

RESULTS

Subjects

We screened 130 patients of whom 26 did not meet Rome II criteria[1], so that 104 patients were included in the analysis. Mean age was 42.0 ± 13.9 years. Seventy-four patients (71%) were female. Thirty-three patients (32%) were recruited through the outpatient department and 71 patients (68%) were recruited through advertisement in a local newspaper.

Preliminary analyses

Descriptive statistics and normality

Means, standard deviations, skewness and kurtosis values for each quantitative variable are displayed in Table 1. We used standard errors of $\sqrt{(6/N)}$ and $\sqrt{(24/N)}$ to evaluate the skewness and kurtosis values, respectively. Two variables showed both a significant positive skewness and kurtosis value: BRS, and vigilance ($z > |3.29|$; $p < .001$). Visceral pain showed a significant positive skewness value ($z = 3.97$; $p < .001$).

Missing data

Table 1 shows the number of patients (n) per variable. Only BRS had a high number of missing values (20, being 19.2%). Little's test of missing completely at random (MCAR) revealed that this assumption was not rejected ($\chi^2 = 77.395$, DF = 72, $p = .311$). Missing values were imputed before the path model analysis using an Expectation Maximization approach (see the Computational Note). Because of the existence of non-normally distributed variables, the corrections of Satorra and Bentler (1988) to the test statistics of the path model were computed (see the Computational Note).

Outliers

We examined model based outliers using linear regression analyses for each of the regression equations derived from the path model (see Fig 1). For each subject in each regression equation, we inspected Cook's distance, a measure of the change in regression coefficients produced by leaving out that subject. No outliers (i.e., a Cook's distance > 1) were detected. The normalized estimate of the multivariate kurtosis was 1.52, indicating no multivariate outliers were present.

Table 1. Descriptive statistics of the quantitative model variables in 104 IBS patients

Variable	n	Mean	SD	Skewness	Kurtosis
Trauma (0-3)	103	0.64	0.67	0.76	0.30
BRS	84	7.93	5.42	1.64	4.35
Visceral pain (0-10)	101	2.50	2.67	0.97	-0.31
Vigilance (0-40)	103	9.68	5.75	1.48	3.87
Cognitions (31-217)	101	110.57	35.56	0.36	-0.28
Illness behavior (0-4)	103	1.88	0.63	0.25	-0.22
IBS symptoms (0-20)	98	4.43	2.52	0.69	0.73
Age	104	41.67	13.83	0.01	-1.05

Score range for each variable is denoted between parentheses when applicable.

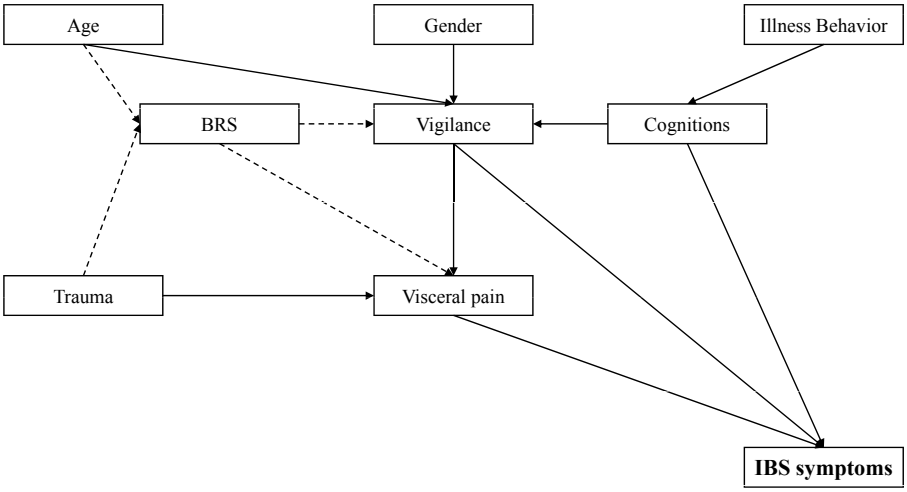

Figure 1. The biobehavioral testmodel of IBS adapted by Naliboff et al. Dashed arrows indicate a negative coefficient. Note the sequential links between a) trauma, visceral pain, and IBS (visceral component); b) trauma, BRS, vigilance, visceral pain, and IBS (central nervous system component); c) illness behavior, cognitions, and IBS (cognitive-behavioral component). The model contains four exogenous variables (i.e., trauma, age, gender and illness behavior).

Model tests

Figure 1 shows the biobehavioral model of IBS that was tested. A dashed arrow is displayed if a negative coefficient was expected for that path. Important features of the model are the sequential links between a) trauma, visceral pain, and IBS (comparable to the 'visceral' component in Naliboff's model); b) trauma, BRS, vigilance, visceral pain, and IBS (the 'central nervous system' component in Naliboff's model); c) illness behavior, cognitions, and IBS (the 'cognitive-behavioral' component in Naliboff's model). The model contains four exogenous variables (i.e., trauma, age, gender and illness behavior), which were assumed to be uncorrelated. The *p*-value of the Satorra-Bentler scaled chi-square was .019 (χ^2 = 39.22; *df* = 23), indicating poor model fit. The robust estimates of the non-normed fit index (NNFI) and comparative fit index (CFI) were .752 and .842, respectively, also indicating a poor fit.

The standardized residual matrix revealed that the ill fit was caused by the omission of two paths, one between illness behavior and IBS symptoms, and one between trauma and IBS symptoms (the corresponding residuals were .274 and .258). The model was revised accordingly. The revised model yielded good fit, indicated by the robust estimates of the test-statistics (Satorra-Bentler χ^2 = 24.40, *df* = 21, *p* = .274; robust NNFI = .943; robust CFI = .967; robust RMSEA = .040). The model explained 18.9% of the variance in IBS symptoms. The path coefficients of this model were examined and those being not statistically significant were deleted in a special way. To control the False Discovery Rate (FDR) in the case of multiple testing, we used a procedure described by Benjamini and Hochberg. Because we hypothesized

a priori the sign of the path coefficients, we computed for each path coefficient a one-sided *p*-value, using the robust estimates of the standard errors. In line with Lackner et al.[24], a family of tests was defined as the path coefficients leading from the exogenous variables to a given endogenous variable. The within-family error rates were controlled using the FDR method. The trimmed model was re-fit and the test statistics yielded a comparable fit as the non-trimmed model (Satorra-Bentler χ^2 = 30.76, *df* = 27, *p* = .28; robust NNFI = .951; robust CFI = .963; robust RMSEA = .037). The standardized path coefficients of this trimmed model are shown in Figure 2. Three of the values of the path coefficients differed a value of .01 with those of the non-trimmed model, the remaining path coefficients were equal. The values of the standardized error variances are displayed in the circles. The trimmed model explained 16.2% of the variance of IBS symptoms.

Ancillary analyses

The biobehavioral model proposed by Naliboff et al. suggests that the effect of illness behavior on IBS symptoms is possibly mediated by environmental response and health beliefs (operationalized as "cognitions" in the present study). The model tests of Figure 1 revealed that a direct path was needed from illness behavior to IBS symptoms. By adding this path to the model, the coefficient of the path from cognitions to IBS symptoms was no longer significant (see Figure 2). This result lead

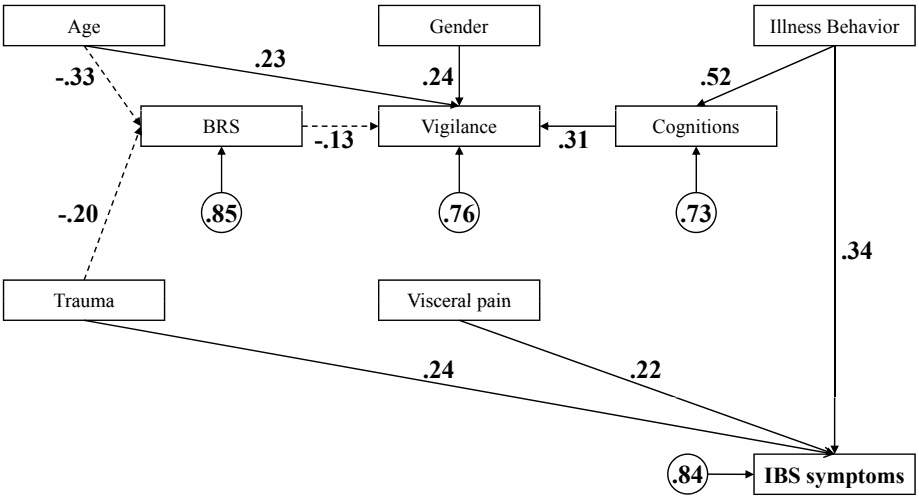

Figure 2. Trimmed model showing the standardized path coefficients after deleting non-significant paths and addition of a path between illness behavior and IBS symptoms and a path between trauma and IBS symptoms. This was necessary due to ill model fit in the initial analysis, in which these paths were omitted. The values of the standardized error variances are displayed in the circles. The trimmed model explains 16.2% of the variance of IBS symptoms.

us to formulate the following alternative hypothesis: the effect of cognitions on IBS symptoms is *mediated* by illness behavior.

We tested if illness behavior met the conditions to be considered as a mediator by means of four linear regression analyses (also see the Computational Note). Cognitions were significantly associated with both illness behavior and IBS symptoms (two-tailed $p < .05$). Illness behavior was significantly associated with IBS symptoms. The effect of cognitions on IBS symptoms was no longer significant (two-tailed $p = .82$) when the effect of illness behavior on IBS symptoms was controlled. The corresponding standardized regression coefficient decreased from .21 to .03 when illness behavior was added to the regression analysis. These findings support the hypothesis that illness behavior mediates the effect of cognition on IBS symptoms completely.

Investigation of the standardized residuals of the trimmed model (Fig 2) revealed a relatively large residual (0.21) between trauma and illness behavior. This result indicated that the model could be improved by adding an additional path from trauma to illness behavior. The addition of this path gave us the possibility to investigate whether the effect of trauma on IBS symptoms was also mediated by illness behavior. We tested this hypothesis by a series of linear regression analyses as mentioned above (also see the Computational Note). Trauma was significantly associated with both illness behavior and IBS symptoms (two-tailed $p < .05$). The effect of trauma on IBS symptoms was no longer significant ($p = .06$) when the effect of illness behavior on IBS symptoms was controlled. The corresponding standardized regression coefficient decreased from .24 to .18 when illness behavior was added to the regression analysis. These findings support the hypothesis that illness behavior mediates partly the effect of trauma on IBS symptoms.

On the basis of the results, we formulated an alternative model to Figure 1. We added three paths, one from trauma to illness behavior, one from trauma to IBS symptoms and one from illness behavior to IBS symptoms. Furthermore, we reversed the direction of the path from cognition to illness behavior. The fit of this model was significantly better than the fit of the non-trimmed model of Figure 2 (Satorra-Bentler $\chi^2 = 20.42$, $df = 20$, $p = .43$; robust NNFI = .993; robust CFI = .996; robust RMSEA = .014). We used the within-family FDR-procedure to remove non-significant path coefficients from this model. The fit of the trimmed model, displayed in Figure 3, was also good (Satorra-Bentler $\chi^2 = 26.93$, $df = 26$, $p = .41$; robust NNFI = .987; robust CFI = .991; robust RMSEA = .019). The model explained 16.0% of the variance in IBS symptoms.

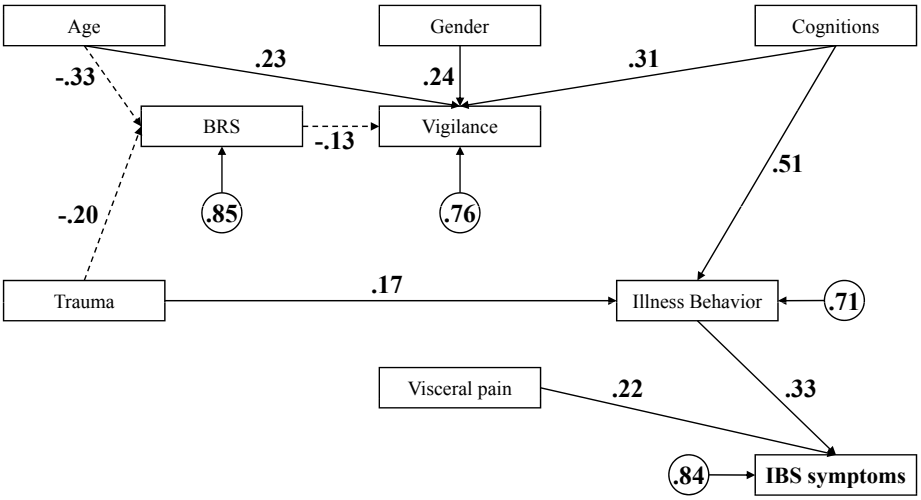

Figure 3. Alternative model to Figure 1 after paths were added between trauma and illness behavior, trauma and IBS symptoms and illness behavior and IBS symptoms and non-significant paths were deleted. Reversal of the path direction from cognitions to illness behavior yielded a significantly better fit than the fit of the non-trimmed model of Figure 2.

DISCUSSION

The biobehavioral model proposed by Naliboff et al. was one of the first attempts to improve our understanding of the pathophysiology and clinical expression of irritable bowel syndrome (IBS). In the present study, this model was operationalized to be able to determine the effect of 1) Autonomic Nerve System (ANS) function, 2) local (visceral) factors, and 3) cognitive-behavioral aspects on IBS symptom severity, as well as the interaction between these domains. Our data do not support the operationalized version of the biobehavioral model presented in Figure 1. In particular, we found no association between ANS functioning (represented by baroreceptor reflex sensitivity) and IBS symptom severity. While the working model indicates that autonomic dysfunction modulates IBS symptoms by increasing visceral sensitivity and/or inducing hypervigilance, these path coefficients were not significant. This leads to rejection of hypotheses 1 and 2 (see Introduction), and raises the question whether ANS-stress responses are involved in symptom generation. However, a growing body of literature highlights ANS alterations in IBS patients[10,11,16,17,49], with most studies suggesting sympathetic predominance or reduced parasympathetic activity. It is likely that altered autonomic functioning is involved in the pathophysiology of IBS, but this probably takes place through different mechanisms than those proposed in the model, for example by modifying intestinal motility[50]. Our finding that ANS functioning was significantly correlated to (hyper)vigilance without affecting IBS symptom severity is supported by a recent study showing that repeated

exposure to aversive visceral stimuli in IBS patients leads to habituation of visceral perception, while central processing of anticipation of visceral pain (i.e., vigilance) remains activated[51].

The relationship between visceral pain during rectal balloon distension and IBS symptoms has been established in the last decades and was confirmed by our model. Hypothesis 6 can thus be accepted. The model also predicts that visceral pain or hypersensitivity would be defined by a history of 'abdominal trauma' (sexual or physical abuse, inflammatory processes), autonomic dysfunction, and vigilance. Yet, none of these path coefficients were significant, thereby rejecting hypotheses 1, 2 and 3. One explanation may be that the level of visceral sensitivity is determined by other factors that are currently unknown, or were not the subject of investigation. A possible candidate is the presence of psychiatric comorbidity, for example depression[52]. Alternatively, it is possible that 1) other measures for assessment of abdominal trauma, ANS function and vigilance, are required, or 2) these domains interact in a different way than proposed in the model.

The working model suggests that illness behavior influences cognitions, which in turn modulate symptom severity. This association was indeed present, but not in the form we anticipated. A better model fit was achieved when the proposed correlation between illness behavior and cognitions was inversed and an additional path from illness behavior to IBS symptoms was added. The alternative model proposes illness behavior as a mediator between cognitions and IBS symptoms and omits the direct relationship between cognitions and symptoms that was initially assumed. This suggests that dysfunctional cognitions on IBS do not affect symptom severity by themselves but are modulated by a patient's approach to his or her symptoms (illness behavior). These findings lead to rejection of hypotheses 4 and 5. Moreover, these results present cognitions as an autonomic or exogenous variable in the model, rather than illness behavior. The final model suggests that more dysfunctional cognitions lead to altered illness behavior and, subsequently, to increased symptom severity. The hypothesized effect of illness behavior on IBS symptoms is thereby confirmed, although the model by Naliboff postulates an indirect association involving environmental response, health beliefs and vigilance.

An interesting finding of this study is that a history of 'abdominal trauma' leads to increased IBS symptoms, but in a different way than we expected. Whereas the working model predicts that a history of abdominal trauma aggravates IBS symptoms by increasing visceral pain perception, the alternative model shows that the effect of trauma on IBS symptoms is mediated by illness behavior. The effect of sexual and/or physical abuse on illness behavior has long been established[53], but the relationship with abdominal illness such as acute gastroenteritis (another form of 'trauma') is less clear. Moreover, it has been shown that long-lasting gut dysmotility and vis-

ceral hyperalgesia develop in mice after transient colonic inflammation[54], suggesting a relationship between abdominal illness (i.e., colonic inflammation) and visceral hypersensitivity. Our sample-size was too small to perform subgroup analyses in patients with post-inflammatory IBS and in those with a history of abuse. However, the relationship between any kind of abdominal trauma and symptom severity in IBS is interesting and deserves further investigation.

Age and gender were expected to affect IBS symptomatology through vigilance (higher in older female patients)[39] and ANS function (impaired in the elderly)[38]. Although the associations with ANS function and vigilance were all significant, age and gender were not related to IBS symptom severity via these paths since no significant path coefficients were found from BRS to IBS symptoms and from vigilance to IBS symptoms. Several mechanisms have been proposed regarding the female predominance in IBS patients, including gender differences in visceral sensitivity, CNS pain processing, gastrointestinal transit time, and specific effects of estrogen and progesterone on gut function[25]. The link with the observed sex differences yet remains to be clarified. Decreased prevalence of functional bowel disorders in older patients has been suggested but, again, very little research addressed this topic and the effect of age on IBS remains largely unknown.

A possible limitation of our study is the adjustment we made to the cognitive-behavioral section in the biobehavioral model proposed by Naliboff and colleagues. The original model suggests that IBS symptoms successively modify illness behavior, environmental responses, health beliefs, vigilance, and visceral motor and sensory function, eventually leading back to IBS symptoms. The model also predicts a direct effect of IBS symptoms on health beliefs and vice versa. As explained in the Introduction, we were coerced to perform a path analysis rather than a structural equation model analysis (including latent variables) due to the ratio between the number of observed variables and the number of patients. In addition, our data were from a cross-sectional design, not a longitudinal design. By eliminating the abovementioned feedback loop, we simplified the model to be able to test its validity, but at the same time denied some of the interactions that may be important in the pathophysiology of IBS. Larger patient samples and a longitudinal design are required to overcome this limitation. Another possible limitation is that 'arousal' and 'environmental responses' were not incorporated in the working model. These were omitted because no accurate measures were available to quantify these domains. Finally, visceromotor activity and viscerosensory activity were operationalized as 'visceral pain' because verification of the proposed interaction would require a much larger sample size and more complex statistical calculations that would exceed the aim of this study.

In conclusion, the biobehavioral model that was proposed by Naliboff and colleagues to improve our understanding of the pathophysiology of irritable bowel syndrome could not be validated in the present study. Although the association between visceral hypersensitivity and IBS symptom severity was undoubtedly present, a relationship between ANS function and IBS symptoms could not be confirmed. Cognitive-behavioral aspects are important in the clinical expression of IBS, with illness behavior playing an intermediate or modulating and not an autonomic role. Internal and/or external stimuli seem to affect IBS symptoms by modulating illness behavior rather than ANS function or visceral sensitivity. Future longitudinal studies in larger patient samples are required to further investigate the mechanisms involved in the pathophysiology of IBS.

Computational Note

The descriptive analyses and linear regression analyses were performed with SPSS, version 11.5. The missing imputation and the path model analyses were performed with EQS, version 6.1. For each path analysis, we used the option METHOD=ML, ROBUST.

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9

SUMMARY AND DISCUSSION

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Irritable bowel syndrome (IBS) is a functional bowel disorder characterized by recurrent episodes of abdominal pain or discomfort accompanied by disturbed bowel habits. It is among the most frequently occurring functional bowel syndromes, with a prevalence ranging from 5 to approximately 20%. Diagnosis is made according to the Rome criteria. Despite the growing body of literature, the pathophysiology of IBS remains poorly understood. A variety of mechanisms have been proposed in symptom generation, including enhanced visceral sensitivity, disturbed intestinal motility, autonomic dysfunction, mucosal inflammation, altered immune activity, altered processing of afferent sensory information, and psychological disturbances. These alterations probably reflect dysfunction at different levels of the brain-gut axis, a conceptual framework which has recently emerged in an attempt to improve our understanding of the etiology, pathogenesis and clinical expression of IBS. The studies presented in this thesis highlight different aspects of the brain-gut axis in order to gain further insight in the pathophysiological mechanisms underlying IBS.

In **Chapter 2**, we studied involvement of baroreflex sensitivity (BRS), a measure of autonomic (dys)function, in IBS patients and healthy controls under baseline conditions and during a gastrointestinal stressor, i.e. rectal balloon distension. As BRS not only modulates sympathetic and parasympathetic autonomic outflow, but also affects cortical arousal and somatic and visceral pain perception, it might play a role in the pathophysiology of IBS. A previous study in rats demonstrated increased sympathetic outflow and decreased BRS during electrical stimulation of abdominal vagal afferents[1]. In contrast, we found an increase in BRS under mild rectal stimulation in healthy subjects and in IBS patients, which persisted in controls during intense stimulation, whereas BRS returned to baseline in patients. The interpretation of these contrasting results is unclear, but the differences may be related to the use of anaesthesia in these rats[1], which affects cortical perception and depresses the arterial baroreflex. More importantly, we demonstrated that resting BRS is significantly larger in IBS patients compared to healthy subjects. This is opposite to our assumption that resting BRS is lower in IBS (as is the case in most chronic diseases[2,3]), which renders the hypothesis that IBS patients are hypersensitive due to diminished baroflex function unlikely. In contrast, a recent study demonstrated decreased BRS in IBS patients compared to controls, both at baseline and during ramp en phasic rectal balloon distension[4]. Differences in balloon distension protocol may, at least in part, account for this discrepancy. Our study does not provide information on the basis of which (the difference between) these results can be explained. One theory is that the frequently experienced viscerosensory stimuli in IBS, such as abdominal pain, may entail a training-effect, possibly materialized in chronic elevated substance P concentrations at the level of the nucleus tracti solitarii (NTS)[5,6]. Such a training-

mechanism can only be further investigated in animal models of visceral afferent stimulation. Alternatively, it may reflect an intrinsic autonomic characteristic in which IBS patients differ from healthy individuals, which may occur at the NTS level, as has previously been shown for the oesophagus[7]. It is tempting to interpret the enhanced baseline baroreflex response in patients as an anticipatory phenomenon and to expect benefits from that anticipation in the form of inhibition of cortical arousal and visceral pain perception during irritating stimuli such as abdominal pain. However, our finding that no differences in BRS values exist between IBS patients and control subjects during rectal distension makes such a hypothesis unlikely. Whether these autonomic changes are either a consequence of IBS or play a role in the pathophysiology should be the focus of future investigations.

Several gut peptides are involved in the regulation of gastrointestinal motor and sensory function. We studied plasma levels of gut peptides released from the upper (cholecystokinin (CCK) and motilin) and lower (peptide YY, PYY) small intestine under fasting and postprandial conditions in IBS patients and controls, the results of which have been presented in **Chapter 3**. Both fasting plasma CCK levels and the incremental postprandial CCK response were elevated in IBS patients compared to controls. These results support previous studies in IBS showing disturbed CCK release and altered organ sensitivity[8], excessive intestinal motor activity[9] and reduced pain thresholds[10] during infusion of CCK. Furthermore, neither fasting nor postprandial CCK levels were significantly different between patients who were classified as either hypersensitive or normosensitive to rectal balloon distension, which renders a contribution of increased CCK secretion to the pathogenesis of enhanced visceral perception less likely. However, CCK infusion has been shown to aggravate symptom severity in IBS patients[11]. It is therefore possible that CCK release after a meal is involved in the exaggerated postprandial colonic motor response that has been demonstrated in IBS patients[12]. Although postprandial CCK concentrations were merely twofold increased in IBS compared with controls, the combination with increased end-organ sensitivity may be responsible for postprandial symptom aggravation in IBS. Against the background of the female predominance in IBS, another interesting finding was that the elevated fasting and postprandial plasma CCK levels were almost completely attributable to female IBS patients. Differences in the effect of CCK on gastrointestinal motility between males and females have been reported (for instance increased sphincter of Oddi motility during CCK infusion in female compared to male dogs)[13], but the interpretation of this finding remains unclear. Fasting and postprandial motilin levels did not differ between patients and controls, which is supported by the literature. Remarkably, fasting motilin levels were significantly elevated in patients with a diarrhoea predominant bowel habit compared to other subgroups. This may be clinically relevant as motilin stimulates human colonic

motility[14] and may therefore play a role in the accelerated colonic transit that has been demonstrated in patients with diarrhoea[15]. Overall, no differences were found in fasting and postprandial PYY-levels, which is in line with previous data. Our observation that patients who were hypersensitive to rectal balloon distension have a greater postprandial PYY response, together with data showing increased numbers of PYY-containing enteroendocrine cells in rectal biopsy specimens from patients with post-infectious IBS[16], may imply a role for this hormone in the development of post-infectious visceral hypersensitivity and/or IBS.

With increasing evidence to suggest a role of mucosal inflammation and immune system alterations in the pathophysiology of IBS, we studied genetically determined immune activity by comparing the prevalence of gene promoter single nucleotide polymorphisms (SNPs) of interleukin 10 (IL-10, anti-inflammatory cytokine) and tumor necrosis factor alpha (TNF-α, pro-inflammatory cytokine) between IBS patients and controls. In **Chapter 4**, we demonstrated that the high producer TNF-α genotype is more prevalent in IBS patients compared to healthy controls, particularly the heterozygous genotype which is associated with a high TNF-α production phenotype (41% versus 26%). The previously demonstrated fivefold increase in TNF-α producing intraepithelial activated macrophages in patients with post-infectious IBS[16], together with the potency of enteric pathogens such as Campylobacter jejuni, Salmonella and Shigella to stimulate macrophage TNF-α production[17], supports a role of this cytokine in persisting bowel symptoms in these patients after infection. Low-producer genotype frequencies for IL-10 were similar between patients and controls. The combined high-producer TNF-α and low-producer IL-10 genotype (i.e., 'high risk profile' for inflammation) was three times more prevalent in patients compared to controls but occurred in only 9% of cases. This implies that other mechanisms and/or cytokines are also involved. Yet, this genotype combination tended to occur more often in patients with a diarrhoea predominant bowel habit compared to the constipation and alternating types (20% versus 4% and 3%, respectively). This is supported by a recent study showing enhanced baseline TNF-α and Escherichia coli lipopolysaccharide-induced TNF-α and IL-6 levels in diarrhoea predominant IBS-patients reporting more than 3 bowel movements per day, urgency, watery stools, and pain associated with diarrhea[18]. While statistical significance was not reached, these data indicate that IBS subgroups may exhibit different cytokine producer genotypes that might be involved in disease expression.

Motor disturbances of the gut have been demonstrated in IBS, but the role of this abnormality in the pathogenesis of IBS and particularly in postprandial symptom deterioration has not been established. With the recent characterization of a rectocolonic inhibitory reflex in healthy individuals, the study presented in **Chapter 5** was performed to investigate this phenomenon in IBS. We found that rectal pain dur-

ing balloon distension after a standard high-caloric meal was increased in patients compared to controls. Rectal distension inhibited colonic motor activity (measured by tone and phasic volume events using barostat) in an intensity-dependent manner in both IBS patients and controls. Most interestingly, the magnitude of this response was comparable between patients and controls under fasting conditions, but was significantly impaired in patients versus controls after a meal, with more postprandial phasic motor activity occurring in patients. A possible explanation for this finding is that exaggerated postprandial colonic motor activity impairs the ability of the colon to relax and thereby attenuates rectocolonic reflexes in IBS patients after a meal. The role of the (impaired) rectocolonic inhibitory reflex in the pathophysiology of IBS awaits further elucidation. Altered reflexes at other locations in the gastrointestinal tract have already been demonstrated in patients with functional bowel disorders. For instance, impaired reflex fundic relaxation following intestinal administration of nutrients has been shown in patients with functional dyspepsia[19]. Our finding that the rectocolonic reflex is impaired in IBS after a meal, together with the increased rectal pain during balloon distension in IBS, is consistent with the hypothesis of a generalized disturbance of postprandial colonic sensori-motor functions in IBS.

Visceral hypersensitivity is one of the few reproducible phenomena in IBS and has been put forward as a biological marker. Processing of afferent visceral information and emotional pain management both occur in similar brain regions, but little is known about the relationship between psychological variables and visceral hypersensitivity. **Chapter 6** explored the prevalence of rectal hypersensitivity, levels of psychological distress and symptom severity in IBS. In addition, we aimed to address which demographical, clinical and psychological variables predict the occurrence of visceral hypersensitivity in IBS. We found that rectal compliance and pain thresholds are reduced and that the intensity of pain perception but not urge is increased in IBS patients when compared to healthy controls. The latter is consistent with previous reports demonstrating decreased perception thresholds in IBS only for noxious stimuli, and not for stool[20]. Furthermore, visceral hypersensitivity (defined by pain perception threshold ≥ 2 standard deviations below the mean threshold in controls) was present in one third of patients. This finding is remarkable, since some report up to 95% percent of IBS patients being hypersensitive to balloon distension[20]. The difference is probably due to the use of different parameters to define visceral hypersensitivity (for instance, inclusion of intensity of sensations and altered viscerosomatic referral in the definition[20] besides reduced perception thresholds). Logistic regression analysis showed that only symptom severity predicts the occurrence of visceral hypersensitivity and that no correlation exists with any of the investigated psychological and demographical characteristics. A recent study in 109 adult IBS patients also demonstrated a significant correlation between symptom

severity and hypersensitivity to rectal balloon distension[21]. In contrast, another recent study in children with IBS and functional abdominal pain did not find an association between symptom severity and rectal pain perception thresholds[22]. Taken together, these data challenge the view that visceral hyperalgesia is a biological marker of IBS, since hypersensitivity may be absent in Rome II positive patients with mild symptoms. They also show that psychological characteristics such as anxiety, somatization, and neuroticism do not correlate with sensory thresholds. In particular, neither vigilance nor dysfunctional cognitions were different between hypersensitive and normosensitive patients, suggesting that symptom perception and management do not differ between these groups. Yet, these findings do not exclude a common neuropsychological basis in the clinical expression of IBS because several studies show that psychological distress is more prevalent among patients who seek health care[23]. Therefore, the role of psychological factors in IBS symptom presentation remains an interesting subject of investigation.

Pharmacotherapy for successful treatment of IBS is often disappointing, but cumulative evidence suggests efficacy of psychological interventions such as cognitive behavioural therapy, dynamic psychotherapy and hypnotherapy in treating IBS. Most of these interventions incorporate a relaxation technique. In **Chapter 7** we presented the results of a randomized controlled trial to determine short and long-term efficacy of relaxation training (RT), a brief psychological group intervention, when added to standard medical care, on symptom severity and psychological wellbeing in IBS patients. We found that RT leads to significant symptom improvement up to 12 months after treatment, with a 34% reduction in IBS composite symptom score in the RT group compared to only 12% in patients receiving standard medical care. Quality of life (general health, health change) also improved significantly more in patients treated with RT compared to those receiving standard treatment. According to the Jacobson and Truax criteria for clinically significant symptom improvement, 12 RT-treated patients (23%) were improved at 12 months after treatment, compared to 1 patient (3%) who received standard medical care. These results are at least similar, if not better, when compared to the beneficial effects of other psychological interventions[24,25]. Although treatment duration is short (4 weeks), consolidation of symptom improvement probably lies in routine use of relaxation techniques in daily life. When embedded in a clear rationale, this provides patients with a useful tool to cope with their symptoms and establishes long-term symptom reduction.

One of the first attempts to conceptualize the multifactorial pathogenesis of IBS comes from Naliboff and colleagues in 1998. They proposed a biobehavioral model which integrates the central nervous system, visceral sensory and motor functioning, and cognitive-behavioral systems into one comprehensive working model. In **Chapter 8**, we tested the validity of an operationalized version of this model using

a path analysis method based on Structural Equation Modeling (SEM). This method allows calculation of reciprocal and chronological relationships between model variables. Initial analysis indicated poor model fit, rejecting the validity of this model when applied to our patient population. In particular, ANS functioning (represented by BRS) was not associated with IBS symptom severity. In view of the convincing evidence showing ANS alterations in IBS patients, it is probable that autonomic dysfunction takes place through different mechanisms than those proposed in the working model. Our finding that ANS functioning was significantly correlated to (hyper)vigilance without affecting IBS symptom severity is supported by a recent study showing that repeated exposure to aversive visceral stimuli in IBS patients leads to habituation of visceral perception, while central processing of anticipation of visceral pain (i.e., vigilance) remains activated[26]. Further evaluation of the model confirmed that visceral pain during rectal balloon distension is related to IBS symptoms (which is consistent with the results presented in **Chapter 6**), but no association with a history of 'abdominal trauma' (sexual or physical abuse, inflammatory processes), autonomic dysfunction, or vigilance was found. We also hypothesized that illness behavior influences cognitions, which in turn modulate symptom severity. The results showed that a better fit was achieved when illness behavior was positioned in the model as a mediator between cognitions and IBS symptoms, suggesting that dysfunctional cognitions do not affect symptom severity by themselves but are modulated by a patient's approach to his or her symptoms (illness behavior). Another interesting finding was that the well-known association between a history of 'abdominal trauma' and increased IBS symptom severity does not involve visceral hyperalgesia, but is also mediated by illness behavior. These data not only suggest a central role for illness behavior in the pathyphysiology of IBS, but also highlight behavioral interventions such as relaxation training as potentially beneficial treatment options.

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SAMENVATTING EN DISCUSSIE

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Irritable Bowel Syndrome (IBS; ook Prikkelbaar Darm Syndroom, PDS) is een functionele darmaandoening die gekenmerkt wordt door episodes van buikpijn of een onaangenaam gevoel in de buik die gepaard gaan met veranderingen in het defaecatiepatroon. PDS behoort tot de meest frequent voorkomende functionele maag-darmaandoeningen en heeft een prevalentie van 5 tot 20 %. De diagnose wordt gesteld met behulp van de Rome criteria. Ondanks het toenemende aantal wetenschappelijke publicaties blijft de pathofysiologie van PDS onduidelijk. Verschillende mechanismen kunnen mogelijk een rol spelen bij het ontstaan van symptomen, zoals toegenomen viscerale gevoeligheid, verstoorde maagdarmmotoriek, stoornissen in het autonome zenuwstelsel, ontsteking van de mucosa, veranderde activiteit van het immuunsysteem, gestoorde verwerking van afferente sensorische informatie, en psychologische problematiek. Waarschijnlijk zijn deze veranderingen representatief voor afwijkingen op verschillende niveaus van de hersen-darm-as. De hersen-darm-as is een model dat gebruikt wordt om de etiologie, pathogenese en klinische expressie van PDS beter te begrijpen. De studies die beschreven staan in dit proefschrift belichten verschillende aspecten van de hersen-darm-as en hebben als doel een beter inzicht te verkrijgen in de pathofysiologische mechanismen die aan PDS ten grondslag liggen.

In **Hoofdstuk 2** onderzochten we de baroreflex sensitiviteit (BRS), een maat voor het functioneren van het autonome zenuwstelsel, bij IBS patiënten en gezonde vrijwilligers tijdens rust en gedurende een 'gastro-intestinale stressor', namelijk ballondistensie van het rectum. Aangezien BRS niet alleen de activiteit van het sympatische en parasympatische deel van het autonome zenuwstelsel moduleert, maar ook op hersenniveau corticale alertheid en somatische en viscerale pijnperceptie beïnvloedt, zou het een rol kunnen spelen in de pathofysiologie van PDS. Een studie in ratten toonde toegenomen sympaticusactivatie en afgenomen BRS aan tijdens elektrische stimulatie van afferente vezels afkomstig uit de abdominale nervus vagus. Wij vonden echter een toename in BRS tijdens een milde viscerale prikkel (rectale ballondistensie), welke tijdens een intensivering van deze prikkel aanhield bij gezonde vrijwilligers maar afnam tot basale waarden bij PDS-patiënten. De interpretatie van deze tegenstrijdige resultaten is complex, maar de verschillen zijn mogelijk het gevolg van het gebruik van anaesthetica bij de rattenpopulatie omdat deze middelen de corticale perceptie beïnvloeden en de arteriële baroreflex onderdrukken. Een van onze belangrijkste bevindingen was dat BRS tijdens de rustfase significant groter is bij PDS-patiënten in vergelijking met gezonden. Dit is in strijd met onze aanname dat BRS tijdens rust lager is, zoals het geval is bij de meeste chronische ziekten. De hypothese dat PDS-patiënten visceraal overgevoelig zijn ten gevolge van een afgenomen functie van de BRS is daarom onwaarschijnlijk. Onze studie geeft geen

informatie op basis waarvan deze bevindingen verklaard kunnen worden. Eén theorie is dat het frequent optreden van viscerale stimuli bij PDS, zoals buikpijn, een trainingseffect behelst, wat op het niveau van de nucleus tracti solitarii (NTS) een chronisch toegenomen concentratie substance P tot gevolg heeft. Zo'n trainingseffect kan alleen onderzocht worden in diermodellen met stimulatie van afferent viscerale vezels. Anderzijds zou het ook een intrinsiek kenmerk van het autonome zenuwstelsel kunnen zijn waarin PDS-patiënten afwijken van gezonden. Mogelijk betreft ook dit een verschil op het niveau van de NTS, zoals reeds is aangetoond voor de oesofagus. Het is verleidelijk om de toegenomen BRS tijdens rust bij patiënten te interpreteren als een vorm van anticipatie en om hiervan een voordeel te verwachten in de vorm van inhibitie van zowel corticale alertheid als somatische en viscerale pijnperceptie tijdens een buikpijnepisode. Echter, onze bevinding dat de hoogte van BRS tijdens rectale ballondistensies niet verschilt tussen patiënten en gezonden maakt een dergelijke hypothese niet aannemelijk. Nader onderzoek moet uitwijzen of deze autonome veranderingen een rol spelen in de pathogenese van PDS of hiervan juist het gevolg zijn.

Verschillende maagdarmhormonen zijn betrokken bij de regulatie van de motoriek en sensibiliteit van de tractus digestivus. Wij onderzochten bij PDS-patiënten en gezonde controles plasmaconcentraties van hormonen die door de proximale (cholecystokinine (CCK) en motiline) en de distale dunne darm (peptide YY, PYY) worden afgescheiden, zowel in de nuchtere fase als na een maaltijd. De resultaten van deze studie staan beschreven in **Hoofdstuk 3**. Plasmaconcentraties CCK waren toegenomen in patiënten ten opzichte van controles, zowel tijdens de nuchtere als de postprandiale fase. Deze bevinding ondersteunt eerdere studies waarin reeds een verstoorde CCK afscheiding, veranderde orgaan sensitiviteit, buitensporige intestinale motoriek en afgenomen pijndrempel bij PDS-patiënten werd aangetoond. Wij vonden echter geen verschillen in nuchtere of postprandiale plasmaconcentraties CCK tussen de groep patiënten die hypersensitief was voor rectale ballondistensie en de groep die normosensitief was. Het lijkt derhalve op basis van deze feiten minder waarschijnlijk dat CCK een belangrijke rol speelt bij de pathogenese van toegenomen viscerale perceptie. Intraveneuze infusie van CCK verergert echter wel symptomen bij PDS-patiënten. Het is dus mogelijk dat toename van de CCK-secretie na een maaltijd van belang is bij de excessieve motorische respons van het colon die al is aangetoond bij deze patiënten. Hoewel de plasmaconcentratie van CCK slechts 2 maal verhoogd was in de patiëntengroep in vergelijking met controles is mogelijk de combinatie met toegenomen eindorgaan gevoeligheid voor CCK mede verantwoordelijk voor toename van de klachten na een maaltijd. CCK wordt gesecerneerd vanuit enteroendocriene cellen. Deze cellen zijn belangrijke nutrient- en chemosensoren van de darm. Het viel op dat de verhoogde nuchtere en postprandiale plasmacon-

centratie CCK voornamelijk viel toe te schrijven aan vrouwen. Dit is een interessante bevinding gezien het gegeven dat ongeveer tweederde van de PDS-patiënten vrouw is. Geslachtsafhankelijke verschillen in het effect van CCK op maagdarmmotoriek zijn weliswaar eerder beschreven (bijvoorbeeld toegenomen sfincter van Oddi motoriek tijdens CCK-infusie in vrouwelijke honden vergeleken met mannelijke), maar het is niet duidelijk hoe deze bevindingen geïnterpreteerd moeten worden. Nuchtere en postprandiale plasmaconcentraties motiline verschilden niet tussen PDS-patiënten en controles, wat vanuit de literatuur al min of meer bekend is. Opvallend was wel dat de nuchtere plasmaconcentratie motiline significant was toegenomen bij patiënten met overwegend diarreeklachten in vergelijking met patiënten met obstipatie of een wisselend ontlastingspatroon. De klinische relevantie van deze bevinding ligt mogelijk in het gegeven dat motiline de colonmotoriek stimuleert en dus een rol zou kunnen spelen bij de versnelde colonpassagetijd bij patiënten met diarree. Overeenkomstig de literatuur werden geen verschillen gevonden in plasmaconcentraties PYY tussen PDS-patiënten en gezonde controles. Patiënten die hypersensitief waren voor rectale ballondistensie hadden echter wel een toegenomen postprandiale PYY-afgifte. Dit, samen met aanwijzingen voor toegenomen aantallen PYY-bevattende enteroendocriene cellen in rectumbiopten van patiënten met postinfectieuze PDS, zou kunnen wijzen op een rol van dit hormoon in het ontwikkelen van postinfectieuze viscerale hypersensitiviteit en/of PDS.

Er zijn steeds meer aanwijzingen dat mucosale ontsteking en veranderingen in het immuunsysteem bijdragen aan de pathofysiologie van PDS. Daarom onderzochten wij bij PDS-patiënten en gezonde controles genetisch bepaalde immuunactiviteit door de prevalentie van genpromoter 'single nucleotide polymorphsims' (SNP's) van interleukine 10 (IL-10, anti-inflammatoir cytokine) en tumor necrosis factor alpha (TNF-α, pro-inflammatoir cytokine) tussen deze groepen te vergelijken. In **Hoofdstuk 4** laten we zien dat het 'high producer' genotype van TNF-α vaker voorkomt bij PDS-patiënten dan bij controles, voornamelijk het heterozygote genotype dat codeert voor een fenotype waarbij veel TNF-α wordt geproduceerd (41 versus 26%). Eerder onderzoek toonde al aan dat het aantal TNF-α producerende intraepitheliale geactiveerde macrofagen bij patiënten met postinfectieuze PDS vijf maal hoger ligt dan in de gezonde populatie. Samen met het gegeven dat darmpathogenen als Campylobacter jejuni, Salmonella en Shigella in staat zijn om TNF-α productie door macrofagen te stimuleren, vormt dit mogelijk een verklaring voor de aanhoudende klachten die sommige van deze patiënten hebben. De prevalentie van het 'low producer' genotype van IL-10 verschilde niet tussen patiënten en controles. Het gecombineerde 'high producer' TNF-α genotype en 'low producer' IL-10 genotype kwam 3 keer vaker voor bij patiënten dan bij controles, maar slechts in 9% van de gevallen. Dit suggereert dat andere mechanismen en/of cytokines mede-verantwoordelijk zijn

bij de pathogenese. Het was wel opvallend dat deze combinatie van genotypes veel vaker voorkwam bij PDS-patiënten die voornamelijk diarreeklachten hadden dan bij patiënten met obstipatie of een wisselend ontlastingspatroon (20% versus 4%). Hoewel dit verschil niet statistisch significant was, is het mogelijk dat verschillen in cytokine producer genotypes tussen PDS subgroepen van belang zijn bij het tot uiting komen van ziekteverschijnselen.

Verstoorde darmmotoriek is een bekend gegeven bij PDS, maar de rol hiervan in de pathogenese van PDS, en met name postprandiale klachtentoename, staat nog niet vast. Omdat recent onderzoek de aanwezigheid van een rectocolische inhibitiereflex heeft aangetoond, verrichtten wij een onderzoek naar dit fenomeen in PDS-patiënten. De resultaten beschreven in **Hoofdstuk 5** laten zien dat pijn tijdens ballondistensie van het rectum na een maaltijd meer aanwezig was bij patiënten dan bij controles. Distensie van het rectum inhibeerde de colonmotoriek (gemeten door afgenomen tonus en fasische contracties met behulp van een barostat) bij patiënten en controles op intensiteitsafhankelijke wijze. De meest interessante bevinding was dat de omvang van deze inhibitiereflex vergelijkbaar was tussen beide groepen in de nuchtere fase, maar in de postprandiale fase significant minder uitgesproken was bij patiënten dan bij controles, waarbij vooral het aantal fasische contracties bij patiënten was toegenomen. Dit kan mogelijk verklaard worden doordat excessieve postprandiale colonmotoriek bij deze patiënten het vermogen van het colon om te relaxeren beperkt en op die manier de rectocolische inhibitiereflex tegenwerkt. De rol van deze bevindingen bij de pathogenese van PDS dient nader onderzocht te worden. Verstoorde reflexen op andere plaatsen in de tractus digestivus zijn reeds aangetoond bij patiënten met diverse functionele maagdarmaandoeningen, zoals een afgenomen fundusrelaxatie tijdens het toedienen van voeding in de dunne darm bij patiënten met functionele dyspepsie. Zowel de gecompromitteerde postprandiale rectocolische reflex als de toegenomen pijnperceptie tijdens ballondistensie van het rectum bij PDS-patiënten ondersteunen de hypothese dat bij patiënten met PDS een gegeneraliseerde postprandiale verstoring van colonmotoriek en –sensibiliteit bestaat.

Viscerale hypersensitiviteit is een van de weinige reproduceerbare fenomenen bij PDS en door sommige auteurs is zelfs gesuggereerd om dit als biomarker voor PDS te gebruiken. Het verwerken van afferente viscerale informatie en verwerking van pijn op emotioneel niveau gebeurt beiden in dezelfde gebieden van de hersenen. Er is echter weinig bekend over de relatie tussen viscerale hypersensitiviteit en psychologische variabelen. In **Hoofdstuk 6** onderzochten we bij PDS-patiënten de prevalentie van viscerale hypersensitiviteit, de ernst van symptomen, en het voorkomen van psychopathologie. Verder analyseerden we welke demografische, klinische en psychologische karakteristieken het optreden van viscerale hypersensitiviteit

voorspellen. Rectale compliantie en pijndrempels tijdens rectale ballondistensie waren afgenomen bij patiënten in vergelijking met controles. Ook de intensiteit van pijnperceptie was toegenomen, maar perceptie van aandrang was hetzelfde in beide groepen. Deze laatste bevinding komt overeen met gegevens uit de literatuur die laten zien dat perceptiedrempels bij PDS-patiënten alleen verlaagd zijn voor noxische prikkels, en niet voor fysiologische. Viscerale hypersensitiviteit, gedefinieerd als een pijndrempel ≥ 2 standaarddeviaties onder de gemiddelde pijndrempel gemeten bij gezonde controles, was aanwezig bij 33% van de patiënten. Dit is een opmerkelijke bevinding, omdat sommige studies vinden dat 95% van de PDS-patiënten hypersensitief is voor ballondistensie. Dit verschil wordt waarschijnlijk veroorzaakt door het hanteren van andere parameters om viscerale hypersensitiviteit te definiëren (bijvoorbeeld door niet alleen een afgenomen pijndrempel maar ook toename in intensiteit van perceptie en een veranderd viscerosomatisch perceptiepatroon tijdens balloondistensie in de definitie van hypersensitiviteit te includeren). Na een logistische regressie-analyse werd duidelijk dat alleen de ernst van de klachten het optreden van viscerale hypersensitiviteit voorspelt en dat er geen relatie bestaat tussen viscerale hypersensitiviteit en demografische of psychologische factoren. Deze gegevens plaatsen vraagtekens bij de opvatting dat viscerale hypersensitiviteit een biologische marker is voor PDS, omdat het afwezig kan zijn bij patiënten met milde symptomen die wel voldoen aan de Rome II criteria. Verder blijkt uit onze resultaten dat psychologische karakteristieken, zoals angst en somatisering, niet correleren met perceptiedrempels voor aandrang en pijn. Met name vigilantie en dysfunctionele cognities waren niet verschillend tussen patiënten met en zonder viscerale hypersensitiviteit, wat suggereert dat perceptie en verwerking van symptomen niet verschilt tussen deze groepen. Een onderliggende neuropsychologische basis voor de klinische uitingsvorm van PDS kan op basis hiervan echter niet worden uitgesloten omdat meerdere onderzoeken aantonen dat psychologische problemen vaker voorkomen bij patiënten die medische hulp zoeken. De betekenis van psychologische factoren bij het manifest worden van PDS-symptomen blijft dus een boeiend onderzoeksterrein.

Medicamenteuze behandeling van PDS-symptomen is vaak teleurstellend. Steeds meer onderzoeken laten echter zien dat psychologische interventies, zoals cognitieve gedragstherapie, dynamische psychotherapie en hypnotherapie, succesvol kunnen zijn bij de behandeling van PDS. Relaxatietechnieken maken onderdeel uit van het merendeel van deze behandelingen. **Hoofdstuk 7** beschrijft een gerandomiseerde, gecontroleerde studie naar de korte en lange termijn effectiviteit van relaxatietraining (RT), een korte psychologische groepsinterventie, toegevoegd aan standaardbehandeling, op klachten en het psychologisch welzijn van PDS-patiënten. De resultaten van deze studie laten zien dat RT een significante klachtenverbetering oplevert tot

tenminste 12 maanden na de behandeling, met 34% afname in symptoomscore in de groep die RT kreeg tegen slechts 12% in de groep met standaardbehandeling. Kwaliteit van leven (algeheel welzijn, verandering in algehele gezondheid) verbeterde ook significant meer in de met RT behandelde patiëntengroep versus de groep die standaardbehandeling kreeg. Volgens de Jacobson en Truax criteria, die klinisch significante klachtenverbetering vaststellen, waren op 12 maanden na de behandeling 12 patiënten in de RT groep (23%) significant verbeterd en 1 patiënt (3%) in de groep met standaardbehandeling. Deze resultaten zijn tenminste vergelijkbaar met, zo niet beter dan die van andere psychologische interventies. Hoewel de behandeling maar kort duurt (4 weken), ligt de verklaring voor het voortduren van deze klachtenverbetering waarschijnlijk in het gegeven dat patiënten wordt aangeleerd om de relaxatietechnieken routinematig in het dagelijks leven toe te passen. Omdat relaxatietraining is gebaseerd op een heldere rationale geeft dit patiënten een hulpmiddel om met hun klachten om te gaan en leidt het tot langdurige klachtenverbetering.

Eén van de eerste pogingen om de multifactoriële pathogenese van PDS door middel van een model inzichtelijk te maken, werd gedaan door Naliboff en collega's in 1998. Zij presenteerden een conceptmodel dat het centrale zenuwstelsel, de viscerale sensoriek en motoriek, en cognitieve gedragsaspecten in één model integreert. In **Hoofdstuk 8** wordt de validiteit van een aangepaste, geoperationaliseerde versie van dit model getest door middel van een padanalyse die gebaseerd is op Structural Equation Modeling (SEM). Deze methode maakt het mogelijk om reciproke en chronologische verbanden tussen modelvariabelen te berekenen. De eerste analyse toonde een matige 'fit', waarmee de validiteit van dit model, als het wordt toegepast op onze onderzoekspopulatie, niet kon worden aangetoond. Opvallend was dat de functie van het autonome zenuwstelsel niet was gecorreleerd met de ernst van de klachten. Er is echter zodanig overtuigend bewijs in de literatuur voor een belangrijke rol van autonome dysfunctie bij PDS, dat de meest waarschijnlijke verklaring voor deze discrepantie is dat autonome veranderingen via andere paden een rol spelen dan in het model wordt gesuggereerd. Tevens vonden we dat autonome dysfunctie wel was gecorreleerd met verhoogde waakzaamheid (hypervigilantie) maar niet met ernst van de klachten. Dit wordt ondersteund door een recente studie die laat zien dat herhaaldelijke blootstelling aan viscerale pijnprikkels leidt tot gewenning wat betreft de perceptie van deze prikkels, maar dat de anticipatie op onaangename stimuli in het centrale zenuwstelsel actief blijft. Verder werd bij de modelanalyse de eerder gevonden relatie tussen pijn tijdens rectale ballondistensie en ernst van PDS-klachten bevestigd (**Hoofdstuk 6**), maar werd geen verband gevonden tussen viscerale hypersensitiviteit en een 'buiktrauma' in de voorgeschiedenis (sexueel of fysiek misbruik, inflammatoire processen), autonome dysfunctie of verhoogde waakzaamheid. We veronderstelden ook dat ziektegedrag cognities beinvloedt, die op

hun beurt de ernst van de klachten moduleren. De resultaten tonen dat een betere 'fit' werd verkregen wanneer ziektegedrag als modulerende factor tussen cognities en PDS-symptomen in het model werd geplaatst, wat suggereert dat dysfunctionele cognities zelf de ernst van de klachten niet beïnvloeden, maar dat dit wordt bepaald door de houding van een patiënt ten opzichte van zijn of haar klachten (ziektegedrag). Een andere interessante bevinding was dat de vanuit de literatuur bekende samenhang tussen een 'buiktrauma' in het verleden en de ernst van PDS-klachten niet via toegenomen viscerale hypersensitiviteit verloopt, maar dat deze relatie ook wordt bepaald door ziektegedrag. Deze gegevens suggereren niet alleen dat ziektegedrag een centrale rol speelt in de pathofysiologie van PDS, maar benadrukken ook gedragstherapeutische interventies zoals relaxatietraining als een potentieel waardevolle behandelingsoptie.

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CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 19 maart 1975 te Lisse. In 1993 behaalde hij het Gymnasium-diploma aan het Fioretticollege te Lisse. In hetzelfde jaar startte hij met de studie Geneeskunde aan de Rijksuniversiteit Leiden. In 1998 behaalde hij zijn doctoraaldiploma en verrichtte hij in het kader van zijn wetenschappelijke stage op de afdeling Maag-, darm- en leverziekten van het Leids Universitair Medisch Centrum onder supervisie van dr. M.K. Vu en dr. A.A.M. Masclee onderzoek naar de invloed van de plaats van toediening van nutriënten in de darm op pancreasenzymsecretie. Hiervoor ontving hij in maart 1999 de studentenprijs van de Nederlandse Vereniging voor Gastro-Enterologie. In augustus 2000 behaalde hij zijn artsexamen. Van oktober 2000 tot september 2004 was hij werkzaam als artsonderzoeker op de afdeling Maag-, darm- en leverziekten van het Leids Universitair Medisch Centrum, deels op basis van een subsidie verleend door de Nederlandse Maag Lever Darm Stichting. Het onderzoek heeft zich gericht op basale en klinische aspecten van het Prikkelbaar Darmsyndroom. De resultaten van dit onderzoek hebben geleid tot het huidige proefschrift. In november 2004 werd aangevangen met de vooropleiding Interne Geneeskunde in het Westeinde Ziekenhuis te Den Haag (opleider dr. P.H.L.M. Geelhoed-Duijvestijn). Sinds januari 2007 wordt de opleiding tot maagdarmleverarts voortgezet in het Leids Universitair Medisch Centrum (opleider prof. dr. D.W. Hommes).

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