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Peripheral and central factors in the pathophysiology of irritable bowel syndrome

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ABSTRACT

Peripheral and central factors in the pathophysiology of irritable bowel syndrome

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Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder characterized by abdominal pain and/or discomfort together with abnormal bowel habits. The pathophysiology is complex and incompletely understood. Potential important factors are altered brain-gut interactions, visceral hypersensitivity, psychosocial factors, disturbed GI motility, inflammatory changes, and bacterial overgrowth. Our aim was to investigate some of the different pathophysiological factors in IBS.

Altered rectal perception was found in 62% of IBS patients. These subjects more frequently reported moderate or severe abdominal pain, bloating, diarrhea, satiety, and anxiety. Symptoms of abdominal pain and bloating were associated with altered rectal perception in a multivariate analysis. Moderate or severe symptoms overall were also associated with female gender and anxiety.

Stress decreased visceral sensory thresholds in controls, probably due to distraction. In IBS patients, sensory thresholds remained stable during stress, indicating a disability to suppress signals from the bowel during stress in these patients. Compared with controls, IBS patients had altered neuroendocrine hormones both in the basal state and in response to stress.

In an experimental setting, investigation of memory and attention showed that compared with patients with organic GI disease, IBS patients were faster at identifying words, especially words representing GI symptoms and negative affects. There were no group differences regarding levels of anxiety or depression, but in IBS patients these levels were correlated with memory processing of GI words.

Small intestinal bacterial overgrowth investigated with proximal jejunal cultures, was present in 4% of IBS subjects, which was not different from healthy controls. However, mildly elevated counts of bacteria were more common in IBS patients than in controls. Patients with bacterial overgrowth tended to have fewer phase IIIs, and enteric dysmotility was twice as common in these subjects. There was no relation between mildly elevated counts of bacteria and small bowel motility.

Conclusions: The pathophysiology of IBS is complex and multifactorial. Altered visceral perception is associated with symptom severity, and stress induces an altered visceral and neuroendocrine response in IBS patients, which could explain why stress is sometimes associated with the onset and worsening of symptoms. IBS patients seem to be hypervigilant regarding GI symptoms through memory processing connected to psychological state. Small intestinal bacterial overgrowth is not common in IBS, but of uncertain relevance, a proportion of IBS patients have elevated counts of bacteria in the proximal jejunum.

Keywords: irritable bowel syndrome; visceral perception; rectal perception; visceral hypersensitivity; gastrointestinal symptoms; hypervigilance; selective attention; stress; small bacterial overgrowth; rectal barostat; small bowel motility; gastrointestinal dysmotility.

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To Sofia and Signe

"Imagination is more important than knowledge"
(Albert Einstein)

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LIST OF PAPERS

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IV. **Small intestinal bacterial overgrowth in patients with irritable bowel syndrome.**
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CONTENTS

ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
AML	Ascending method of limits
cfu	Colony forming units
CRF	Corticotropin releasing factor
fMRI	Functional magnetic resonance imaging
GHBT	Glucose hydrogen breath test
GI	Gastrointestinal
GSRS	Gastrointestinal Symptom Rating Scale
HAD	Hospital Anxiety and Depression scale
HPA	Hypothalamic-pituitary-adrenal
HRQOL	Health-related quality of life
IBS	Irritable bowel syndrome
IBS-A	Alternating type IBS
IBS-C	Constipation predominant IBS
IBS-D	Diarrhea predominant IBS
IQR	Interquartile range
LHBT	Lactulose hydrogen breath test
MDP	Minimal distending pressure
MMC	Migrating motor complex
NS	Non-significant
RIA	Radioimmunoassay
SD	Standard deviation
SEM	Standard error of the mean
SIBO	Small intestinal bacterial overgrowth
STAI	Spielberger State Trait Anxiety Inventory
vs.	Versus

INTRODUCTION

Irritable bowel syndrome (IBS) is characterized by abdominal pain and/or discomfort together with disturbed bowel habits in the absence of any detectable organic cause [1]. The term irritable bowel first appeared in the 1940's [2] but reports of patients with symptoms similar to IBS can be found in the literature as early as 1818 [3].

IBS affects up to 20% of the population in Western countries [4-7] with a 2-3:1 female predominance [8, 9]. It is probably the most common disorder encountered by gastroenterologists [10], and also the most common gastrointestinal disorder seen by primary care physicians [11]. Due to the high prevalence and many times incapacitating symptoms, IBS is the cause of both individual suffering and considerable socioeconomic costs [8, 12-14]. There are no reliable structural or biochemical markers found in these patients why various diagnostic criteria have been developed, such as the Manning [15], Rome I [16], Rome II [17] and finally the most recent, Rome III criteria [1].

Despite being a common disorder, the pathophysiology of IBS is not completely understood. Psychological factors, disturbed gastrointestinal motility and altered visceral sensitivity have traditionally been viewed upon as being the most important pathophysiological factors. However, consistent and uniform patterns of abnormalities and associations with symptoms have been hard to establish as investigators have reported contradicting results and varying prevalence figures regarding specific abnormalities. At present, IBS is believed to stem from a dysregulation of the brain-gut axis, involving abnormal interaction and function of the enteric, autonomic and central nervous systems [18, 19]. The brain-gut axis regulates and modulates visceral motility, secretion, sensitivity and immune function through a complex pattern of feedback signaling [20]. Different alterations probably dominate in various subgroups of patients based on gender, predominant bowel habit, psychological state, and so on. IBS is a multifactorial disorder and there are still many unresolved issues regarding the relationship between different pathophysiological factors and symptom patterns [21]. Some pathophysiological findings, relevant to this thesis are discussed below.

1. PSYCHOSOCIAL FACTORS

1.1 Psychiatric comorbidity

Several studies have reported a high prevalence of mood and anxiety disorders in patients with IBS [22], and psychiatric comorbidity has been associated with greater chronic symptom severity [23]. This was previously thought to be true only in patients seeking healthcare and not otherwise [24, 25]. However, a more recent study showed an association with psychological factors and IBS in a community-based sample [26]. Several psychiatric characteristics such as neuroticism, somatization, hypochondriasis, anxiety, and depression have been linked to post-infectious IBS [27-30].

1.2 Stress

Patients often describe a correlation between stressful life events and the onset or exacerbation of their gastrointestinal symptoms [31]. Also, stress influences disease outcome [32], and IBS patients seem more susceptible to the stressful events of daily life [33]. Accordingly, they exhibit a more pronounced emotional and more intense subjective response to experimental stress [34, 35].

Various experimental stressors induce changes in gastrointestinal motor function, including slowing of gastric emptying, a decrease in the number of migrating motor complexes, and enhanced colonic motor activity [36-42]. Except for an exaggerated colonic motor response, there are no consistent findings indicating that IBS patients respond much different from healthy subjects [43]. Experimental stress has also been reported to alter visceral perception [34, 44-49] However, previous studies on stress and visceral perception have shown contradictory results, both regarding the effect on healthy subjects, as well as whether or not there is an altered response in IBS patients. Both psychological and physical stress can increase gut secretion, epithelial permeability and alter the intestinal barrier [50]. Moreover, stress has been proposed to be able to augment or reactivate inflammatory responses in the gut [51]. This could explain why psychological factors influence the risk of developing post-infectious IBS [52].

A dysfunctional neuroendocrine stress response has been proposed to be an etiological mechanism in IBS [18]. Differences between IBS patients and healthy subjects regarding levels of hormones involved in the stress response,

such as cortisol, epinephrine, and norepinephrine have been reported [53] In addition, corticotrophin releasing factor (CRF), which is believed to play an important role in the stress response [54], induces an altered neuroendocrine response [55], as well as a more profound enhancement of colonic motility in IBS patients compared with healthy controls [55]. Furthermore, these effects are suppressed by the administration of a CRF receptor antagonist [56]. Also, CRF has been shown to increase rectal sensitivity in healthy subjects [57]. Thus, alterations in the neuroendocrine response to stress may be of importance in the pathophysiology of IBS [58], but this needs to be further investigated.

1.3 Cognitive factors

Symptom severity in IBS patients is connected to GI-specific anxiety [59, 60], and people with gastroenteritis who interpret their symptoms negatively are more likely to develop post-infectious IBS [27, 61]. Accordingly, IBS patients with depression often report more severe abdominal pain, which could be explained by a tendency to engage in more catastrophic thinking [62]. Furthermore, the placebo response to any therapeutic intervention in IBS is usually considerable [63], and cognitive behavioral treatment has proven to be effective [64].

Selective attention refers to a tendency to selectively process certain stimuli. The term hypervigilance has also been used in a similar meaning, indicating increased attention towards certain stimuli. Hypervigilance regarding gastrointestinal stimuli is thought to be a contributing factor in IBS, and partly explain visceral hypersensitivity through cognitive bias and a tendency to label visceral sensations negatively [65, 66]. Accordingly, IBS patients exhibit a similar cerebral activation pattern during anticipated and actual visceral stimuli [67].

Two studies in IBS patients have used different types of memory tests to assess the presence of selective processing of negatively charged information and came to opposite conclusions [68, 69]. A similar method was also used by Gibbs-Gallagher et al [70], who showed selective attention for words representing gastrointestinal sensations in IBS patients compared with healthy controls and patients with asthma. A recent study further assessed the presence of selective processing of GI symptom related cues using a modified Stroop task and found selective processing of words representing GI symptoms when these were

presented subliminally [71]. However, it is perhaps not surprising that IBS patients are hypervigilant towards the origin of their symptoms, in the same way that patients with asthma selectively processed respiratory words [70]. Therefore, a comparison between IBS patients and other groups with gastrointestinal symptoms would seem more relevant, in order to determine if GI directed selective attention is of specific importance in IBS.

1.4 Health related quality of life

Health related quality of life (HRQOL) in patients with IBS is worse than in the general population [72] and as low as in medically more severe disorders [73, 74]. The impact on HRQOL has also been shown to be greater in patients with functional GI disorders, including IBS, than in patients with organic GI diseases[75, 76]. Reductions in HRQOL are strongly correlated with severity of GI and psychological symptoms, and female IBS patients report reduced HRQOL when compared with men [77]. Disease-specific fears and concerns (e.g., fear of underlying cancer) in patients with IBS may influence disease outcome [78]. However, a recent study showed that a negative colonoscopy was not associated with a decreased belief in the serious nature of IBS symptoms or improved HRQOL [79].

2. VISCERAL SENSITIVITY

Increased visceral sensitivity in patients with IBS was first observed in the colon by Ritchie in 1973 [80]. Visceral hypersensitivity, usually assessed with balloon distensions, has since then been one of the most commonly reported pathophysiological alterations [81], and it seems to be a feature found throughout the entire gastrointestinal tract [82-87]. Rectal hypersensitivity has been proposed to be a biological marker for IBS [82], even useful to discriminate the disorder from other causes of abdominal pain [88].

Experimental manipulation of psychological state has shown that stress, distraction, relaxation and hypnotherapy affect sensory thresholds to visceral distensions [34, 44, 49, 89-91], and some investigators argue that increased visceral sensitivity in IBS patients is mainly due to psychological factors and response bias due to anticipation [65, 92]. However, others have not found support for the presence of psychological response bias in IBS patients [93], and

physiological stimuli, such as nutrients, have also been shown to affect visceral perception in IBS [94-97].

Even though several studies have shown that IBS patients are hypersensitive to visceral stimuli as a group, visceral hypersensitivity is not present in all IBS patients [81]. Differences between different subgroups based on predominant bowel pattern, as well as females and males have been reported [98-100], and the relevance of visceral sensitivity for symptoms remains unclear [81]. Hypersensitivity to gastric distension has previously been shown to be associated with specific symptoms in patients with functional dyspepsia [101], and it is well known that there is a significant overlap between IBS and other functional disorders [4, 102]. Results from studies assessing the relationship between visceral sensitivity and IBS symptoms are divergent [82, 103-107], and a recent study of perceptual response to rectal stimulation in IBS patients showed that disease activity remained stable over time despite normalization of rectal perceptual responses due to habituation following repeated testing [66]. Therefore, the relevance of visceral hypersensitivity for symptoms in IBS remains to be proven.

3. GASTROINTESTINAL MOTILITY

3.1 Small bowel motility

There is evidence of disturbed small intestinal motility in IBS patients as a group, but no uniform motility pattern has been found, and consistent correlations between motility findings and symptoms have been hard to demonstrate [108]. The periodicity of the migrating motor complex (MMC; interval between MMC cycles) has been found to differ depending on predominant bowel habit [109-111], though not consistently [112, 113]. Increased frequency of clustered activity has been found in some [109, 110, 114], but not all studies [112, 113]. Likewise, correlations between IBS symptoms and certain motility patterns, such as clustered activity (e.g. “discrete clustered contractions”) and prolonged propagated contractions have been reported by some [109, 110], but not by others [112, 113]. IBS patients have demonstrated enhanced perception of physiological motility, such as the activity front [115]. Also, altered contraction amplitude and postprandial contraction frequency has been observed [113, 116], and there appears to be a relationship

between postprandial jejunal motor abnormalities and increased sensitivity to small intestinal distensions [117]. High resolution analysis of individual pressure waves in addition to conventional manometry, possibly discloses more abnormalities in IBS patients [114].

Severe small intestinal motor dysfunction is known to cause small intestinal bacterial overgrowth [118, 119], which has been proposed to be common in IBS [120]. One study has reported decreased numbers and shorter duration of phase III in IBS patients with small intestinal bacterial overgrowth according to abnormal lactulose hydrogen breath tests [121]. However, the importance of bacterial overgrowth in IBS is controversial, which will be discussed further on.

3.2 Colonic motility

A primary role for colonic dysmotility in the pathophysiology of IBS has been hard to demonstrate [122]. Most studies of colonic motility using intraluminal pressure recordings have not been able to find different patterns in IBS patients compared to controls or when comparing different IBS subtypes [123, 124]. This has to some extent been contradicted in more recent studies of colonic motility [125, 126]. An increased frequency of high-amplitude propagating contractions with an association with episodes of pain has been observed in non-constipated IBS patients [125]. There are some data supporting altered gastrointestinal reflex activity, shown by an abnormal postprandial recto-sigmoid tone [127], or as an attenuated rectal tone in response to colonic distension [128, 129]. Exaggerated colonic motor response to stimuli such as food, stress and emotions [41, 42, 130], could also be of relevance to the symptomatology in IBS.

3.3 Gas handling

IBS patients often complain of bloating and abdominal pain, as well as visible abdominal swelling [131], which has also been documented objectively [132]. These symptoms have been proposed to be caused by a combination of altered intestinal motility and sensitivity, and not by increased volumes of gas or abnormal gas composition [133]. However, increased colonic fermentation has been demonstrated in IBS patients [134] and treatments that alter colonic flora have been shown to improve abdominal bloating, pain and flatulence [135, 136]. Patients complaining of bloating have been found to have impaired clearance of intestinal gas, and when confronted with a gas overload, which is well tolerated

by healthy subjects, they develop gas retention, symptoms, and abdominal distension [137]. Altered gastrointestinal reflex activity is thought to be involved in abnormal gas handling [138], and the small bowel is the probable region responsible for ineffective gas propulsion [139]. Intraluminal lipids impair intestinal gas clearance because of upregulated inhibition of small bowel transit [140, 141], while mild physical activity enhances intestinal gas clearance and reduces symptoms in patients complaining of abdominal bloating [142].

4. BRAIN-GUT AXIS

The brain-gut axis is a model describing bidirectional pathways linking emotional and cognitive centers in the brain with visceral afferent sensation and intestinal function. Central nervous system (CNS) communication with the gut is mediated via the autonomic nervous system (ANS) and hypothalamic-pituitary-adrenal (HPA) axis by modulation of the enteric nervous system (ENS). Several observations have led to the hypothesis of a dysfunctional brain-gut axis in the pathophysiology of IBS [18, 143, 144].

4.1 Central nervous system

Brain imaging techniques have been used to study brain response during different stimuli including visceral distensions. Despite contradicting results, several studies of visceral pain processing indicate differences between IBS patients and healthy controls in activation of different regions [145]. Existing studies suggest that IBS patients may have increased affective and attentional responses to actual or anticipated visceral stimuli, indicating hypervigilance, as well as a failure to activate pain inhibition systems [67, 146-149]. Differences within the IBS group have been demonstrated. The cerebral activation pattern differs between IBS subgroups based on predominant bowel habit [148], and female and male IBS patients show differences in activation of brain regions with females showing greater activation of regions that could be part of a pain facilitation circuit, while males show increased activity in regions that could be involved in pain inhibition [150, 151]. Interestingly, changed cerebral activation has also been associated with treatment response in IBS [152]. In addition to brain imaging, IBS patients have also shown different results compared to healthy controls in studies using electroencephalography [153, 154], and evoked potentials [155, 156].

4.2 Hypothalamic-pituitary-adrenal axis

The HPA axis has important effects on GI motility, sensation and immune function [157]. Activation of this system takes place in response to both physical and psychological stressors [18]. Alterations in the HPA axis have been reported in patients with IBS, although the results have not been consistent. IBS patients are proposed to have an exaggerated CRF response [18, 55] supported by a recent study showing that IBS patients responded favorably to a CRF antagonist [56]. Higher basal cortisol levels have been reported in subjects with IBS compared with control subjects [158, 159], but there are also studies showing decreased cortisol levels and blunted cortisol responses to CRF challenge [53, 55]. Interpretation of these inconsistent findings is difficult due to the fact that the HPA axis is subject to complex feedback and feed-forward influences that respond differently depending on psychiatric co-morbidities[160], time of day [161], as well as gender [162]. However, published studies support the concept of a dysregulated HPA axis in IBS.

4.3 Autonomic nervous system

CNS communication with the gut is partly mediated trough the sympathetic and parasympathetic pathways of the ANS. Several studies have reported autonomic dysfunction in IBS [163-167]. The results are again inconsistent, but increased sympathetic and decreased parasympathetic activity in IBS patients compared with controls are the most frequently reported differences [168-172]. Studies assessing plasma and urine levels of catecholamines have found increased levels of norepinephrine in IBS patients, which could indicate an increased sympathetic tone [34, 158, 173]. Interestingly, activation of the sympathetic nervous system has been reported to increase visceral sensitivity [174]. Anxiety and depression influences autonomic function in IBS [175], and there also seem to be discrepancies between different subtypes of IBS [168, 171, 176], as well as gender [177].

4.4 Enteric nervous system

The ENS is structurally and chemically similar to the CNS and is not fully understood or easily explained [178] why only a few key points possibly involved in the pathophysiology of IBS will be mentioned.

Since nerve fibers do not enter the GI lumen, the ENS needs sensory transducers of which the enterochromaffin (EC) cells are the best characterized [178]. EC

cells synthesize and store most of the serotonin (5-HT) found in the body, and secretes this in response to mucosal stimuli. The 5-HT then acts through different 5-HT receptors and its action is terminated by a 5-HT transporter (SERT). Serotonin has diarrheogenic effects and diarrhea predominant IBS patients are suggested to have increased postprandial release and/or decreased reuptake of 5-HT [179, 180], whereas impaired release is found in patients with constipation predominant IBS [179, 181].

Mucosal biopsies from IBS patients contain increased numbers of mast cells that could cause visceral hypersensitivity [182, 183] through mediators that sensitize enteric mechanosensitive nerve endings [184]. Colonic mast cells in diarrhea predominant also release more histamine [183] which might enhance intestinal secretion [184]. Mast cell mediators have been reported to increase in response to stress which could also explain some of the reported gastrointestinal effects of stress [185].

The presence of IBS-like symptoms in patients with degenerative enteric neuropathy has led to the suggestion that IBS resembles early stages of such a disease [186], and full-thickness biopsies in a study with a small number of IBS patients have shown inflammatory changes and neuropathy [187].

5. INFLAMMATORY CHANGES

Transient or chronic inflammation is thought to be a possible cause of persistent gut dysfunction [188], and a larger proportion than expected of inflammatory bowel disease (IBD) patients have symptoms compatible with IBS [189]. Inflammation is associated with the production of mediators that can induce changes in visceral perception, motility, and secretion [190, 191]. The mucosal immune system seems to be activated, at least in a subset of patients with IBS [192], and histopathological studies have shown subtle morphologic changes involving inflammatory cells, mast cells, enteroendocrine cells and enteric nerves [182, 187, 193].

5.1 Post-infectious IBS

As many as 30% of IBS patients believe that their problems started with a gastrointestinal infection [194, 195]. Post-infectious IBS is defined as a new

onset of IBS symptoms meeting Rome criteria following an episode of acute gastroenteritis [52]. The incidence of post-infectious IBS varies from 7% to approximately 31% in different studies [27-29, 196-201]. Patients with post-infectious IBS have an increased numbers of T cells and serotonin containing enteroendocrine cells in the intestinal mucosa [28, 202, 203]. Risk factors for developing post-infectious IBS include female gender, high age, psychological factors, severity of disease and antibiotic treatment [27, 30, 199-201, 204].

5.2 Small intestinal bacterial overgrowth

5.2.1 Basic concepts

The stomach and proximal small bowel normally contain relatively small numbers of bacteria in adults. The concentration of gut bacteria increases from 10^{0-4} colony forming units (cfu)/ml in the duodenum and the jejunum, to 10^{0-5} in the proximal ileum, 10^{5-8} in the terminal ileum, and 10^{10-12} cfu/ml in the cecum [205, 206]. The flora in the upper small bowel consists mainly of Gram positive bacteria, the numbers of Gram negatives are low and anaerobes are rare [207]. Small intestinal bacterial overgrowth (SIBO) is a condition caused by an abnormal number of bacteria in the small intestine due to predisposing conditions, such as failure of the gastric acid barrier, which mainly causes overgrowth of Gram-positive bacteria, and impairment of the MMC pattern with failure of intestinal clearance, which causes colonization of Gram-negative bacteria [118, 119, 208, 209]. Gram-negative bacteria cause symptomatic overgrowth with symptoms such as abdominal discomfort, bloating, diarrhea, and malabsorption [210-212]. Bacterial overgrowth with Gram-positive bacteria is frequently found in healthy elderly people and has not been correlated with the symptoms mentioned above [213-216].

5.2.2 Diagnostic tools

Opinions regarding the preferred diagnostic test for SIBO are conflicting. Aspiration and direct culture of jejunal contents is by many regarded as the gold standard [217], even though the reach of the instrumentation leaves cases with isolated or distal overgrowth undiagnosed [120, 218]. SIBO is usually defined as a total growth of $\geq 10^5$ cfu/ml [219, 220]. However, this definition includes Gram positive flora including upper respiratory flora. As growth of colonic type bacteria (mainly Gram negatives, strictly anaerobes and enterococci) correlates to symptoms of SIBO [210-212], a definition of SIBO as $\geq 10^5$ colonic type bacteria seems more clinically relevant [119]. Indirect, non-invasive tests such

as ^{14}C-xylose breath test and hydrogen breath tests using lactulose or glucose have been widely used in diagnosing SIBO. The glucose hydrogen breath test (GHBT) and ^{14}C-xylose breath test have been considered as fairly reliable tools [219], whereas the accuracy of the lactulose hydrogen breath test (LHBT) is questionable due to both low sensitivity and specificity in comparison with culture of small bowel aspirate [221, 222].

5.2.3 SIBO in IBS

SIBO has been proposed to be an important factor in IBS [120]. Evidence of a positive effect of antibiotics on IBS symptoms has been found in some studies [136, 223-227], and SIBO has been reported to be present in 38-84% of IBS patients [225-228]. However, these results were obtained using hydrogen breath tests and have been heavily criticized because of the weakness and interpretation of these tests, as well as because of studies with contradictory results [136, 229-232]. So far, there are no studies where the prevalence of SIBO in IBS has been assessed systematically using bacterial cultures of aspirate from the small bowel.

AIMS OF THE PRESENT STUDIES

The incomplete understanding of the complex and multifactorial pathophysiology of IBS raised the following questions:

1. Is altered rectal perception in IBS patients associated with the presence and severity of gastrointestinal and/or psychological symptoms?

2. What are the effects of acute mental stress on hormonal stress response and visceral sensitivity in IBS patients, and are there any differences between patients and healthy subjects?

3. Are patients with IBS hypervigilant regarding the gastrointestinal tract and/or negative material compared with patients with organic gastrointestinal diseases?

4. What is the prevalence of small intestinal bacterial overgrowth in IBS patients according to jejunal cultures? Is bacterial overgrowth in these patients predicted by small bowel motility alterations or symptom profile?

SUBJECTS AND METHODS

All the participants in the studies gave informed consent and the studies were conducted according to the Declaration of Helsinki and approved by the ethics committee of the University of Göteborg. In this chapter the methods used are presented and commented on. For further details see the separate papers (I-IV).

1. SUBJECTS

The investigations were performed during 1999 to 2006 in IBS patients with healthy controls as a comparison group, except for paper III, where IBS patients were compared with patients with organic GI diseases. All patients were recruited from our outpatient clinic, whereas healthy volunteers were recruited through advertisements. The IBS diagnosis, as well as classification into IBS subgroups according to predominant bowel habits (constipation-predominant (IBS-C), diarrhea-predominant (IBS-D), and alternating-type (IBS-A)), was based on the Rome II criteria [17] (Table 1). Organic disorders were ruled out with appropriate testing and investigations determined by presenting symptoms. Healthy subjects had no history of gastrointestinal symptoms and completed a bowel symptom questionnaire to ensure this. In general, all medications known to affect the GI tract were discontinued at least 48 hours before the studies (I, II, IV).

Table 1. Rome II criteria [17].

At least 12 weeks, which need not be consecutive, in the preceding 12 months of abdominal discomfort or pain that has two out 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 IBS, that can also be used to subclassify patients

1. Fewer than three bowel movements a week
2. More than three bowel movements a day
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

Diarrhea-predominant: one or more of 2, 4, or 6 and none of 1, 3, or 5
Constipation-predominant: one or more of 1, 3, or 5 and none of 2, 4, or 6

Paper I

A total of 109 consecutive patients with IBS and 29 healthy subjects were included for rectal sensitivity testing using a barostat. The results from the control group were used to define a group of IBS patients with altered perception. Thirty-three of the IBS subjects underwent their barostat procedure as part of a baseline evaluation before entering a probiotic treatment trial. Repeated rectal sensitivity testing was then performed after six weeks of treatment, as well as six weeks after the treatment-period. The probiotic treatment did not have any effect vs. placebo, why the follow-up testing at 12 weeks was used to evaluate the stability of our sensitivity and symptom assessments.

Paper II

In all, 27 IBS patients and 32 healthy controls participated in the study. Of these, 18 patients and 22 healthy subjects were enrolled in the main part of the study and underwent one rectal barostat procedure. The rest of the included subjects took part in supplementary sessions aiming to clarify results obtained in the main part. These subjects underwent three barostat procedures each.

Paper III

Thirty-six patients with IBS and 40 patients with an organic GI disease were recruited for the study. The patients with an organic GI disease constituted a mixed group regarding disease activity, consisting of 22 patients with IBD and 18 patients with celiac disease. Subjects were not included if they were younger than 18 or older than 60, had uncorrected visual impairment, were not fluent in Swedish, or suffered from any additional severe medical conditions.

Paper IV

A total of 162 IBS patients and 42 healthy volunteers were included. None of the subjects had been treated with antibiotics within two weeks before the study. Jejunal cultures were obtained from 162 patients and 26 controls. Glucose hydrogen breath tests (GHBT) were performed in 54 patients and 20 controls. Lactulose hydrogen breath tests (LHBT) were performed in 46 patients and 21 controls. Small bowel manometries were analyzed in all patients, and a more detailed analysis was performed in 7 patients with small intestinal bacterial overgrowth and 74 patients without bacterial overgrowth.

2. QUESTIONNAIRES (I, II, III)

Self-administered questionnaires were used in studies I-III to assess GI symptoms and psychological state. The results were compared between IBS patients and healthy subjects (I, II), and patients with organic GI disease (III). In study I, correlations between GI symptom severity and psychological symptoms and visceral sensory parameters were assessed. Study III assessed correlations between symptom severity and psychological state and cognitive test results.

The Hospital Anxiety and Depression Scale (HAD) (I, II, III) was developed for use in medical outpatients rather than psychiatric patients [233]. In the construction of this scale, symptoms that might equally arise from somatic as from mental disorders were excluded, which means that the scale scores are not affected by bodily illness. The HAD-scale is a reliable instrument, with 'cut-off' scores, screening for probable cases of clinically relevant anxiety and depression in patients attending a general medical clinic, and has also been shown to be a valid measure of the severity of these mood disorders. This self-assessment scale consists of 14 items, 7 each for anxiety and depression. It uses a 4-point Likert scale (0-3), and a maximum score of 21 per subscale. A score of 8-10 on each scale denotes a borderline case, and ≥11 a likely case of anxiety or depression.

The Spielberger State Trait Anxiety Inventory (STAI) (II) is a self reporting instrument used to measure both anxiety resulting from acute stressors (state anxiety) (STAI-S) as opposed to the patient's intrinsic level of anxiety irrespective of any particular acute stressors (trait anxiety) (STAI-T) [234]. It is the most widely used state and trait anxiety scale. Adequate reliability and validity estimates have been established in several studies. The STAI-S consists of 20 items asking respondents to report how they feel at a particular moment in time, and STAI-T consists of 20 statements asking respondents to report how they generally feel. The STAI-S is usually administered along with the STAI-T in the same scaling format scoring a 4-point Likert scale (1-4) ranging from 'not at all' to 'very much so' (STAI-S) and 'almost never' to 'almost always' (STAI-T).

The Gastrointestinal Symptom Rating Scale (GSRS) (I, III) was initially constructed as an interview-based rating scale designed to evaluate a wide range of gastrointestinal symptoms [235] and was then modified to become a validated

self-administered questionnaire[236], originally consisting of 15 questions, but an additional item assessing eating dysfunction was later added [237]. An IBS specific version (GSRS-IBS) composed of 13 questions has recently been developed to assess the pattern and severity of IBS related GI symptoms [238]. Both versions of the GSRS use a 7-graded Likert scale (1-7) ranging from 'no discomfort' to 'very severe discomfort'.

Comments. A combination of the two GSRS versions, consisting of 19 questions, was used in paper III, in which we only calculated a total GSRS score. In paper II we used the GSRS-IBS, grouping the questions into five domains or syndromes; pain, bloating, constipation, diarrhea, and satiety (Figure 1). A mean total score, representing over all symptom severity, was calculated, but each domain was also analyzed separately as a mean score from the included questions. Three different symptom severity cut-offs were used; mild (≥3), moderate (≥4), and severe (≥6).

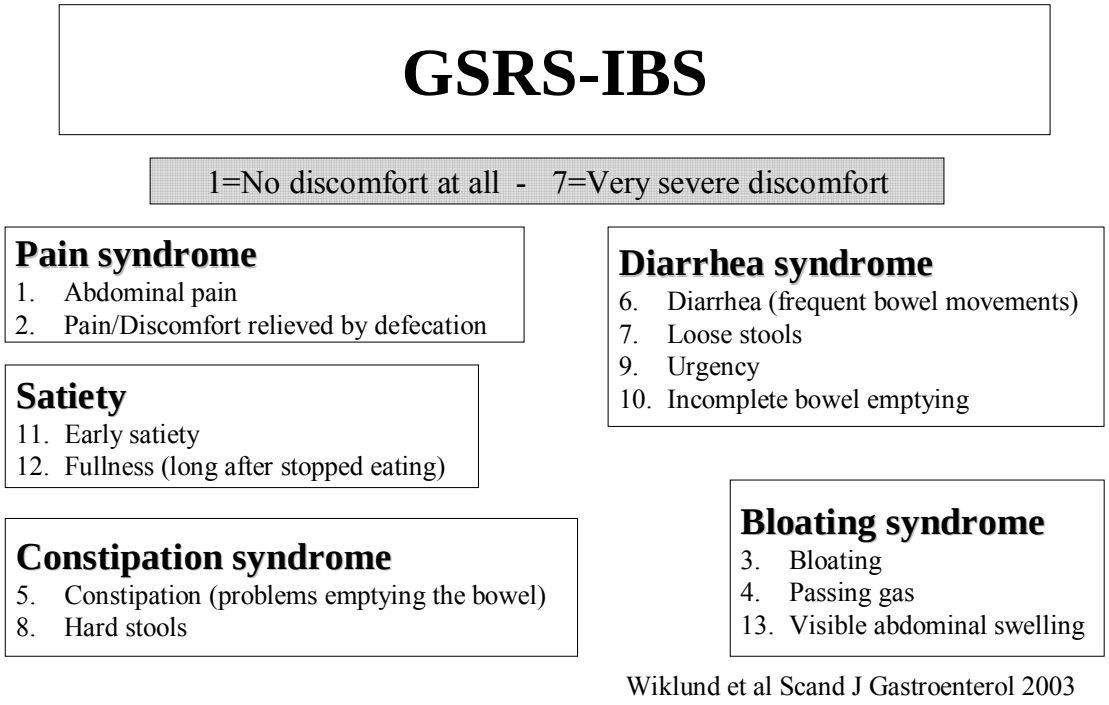


Figure 1. The individual symptoms included in the GSRS divided into five different domains [238].

3. RECTAL BAROSTAT PROCEDURES (I, II)

Rectal sensitivity was tested with balloon distensions using a computer driven electronic barostat (Dual Drive Barostat, Distender Series II; G&J Electronics Inc., Toronto, Canada). The principle of the barostat is to maintain a constant pressure within an air-filled bag positioned in the lumen of the studied organ [239]. Perception can be studied by using phasic distension stimuli to determine sensory pressure thresholds [83] (Figure 2).

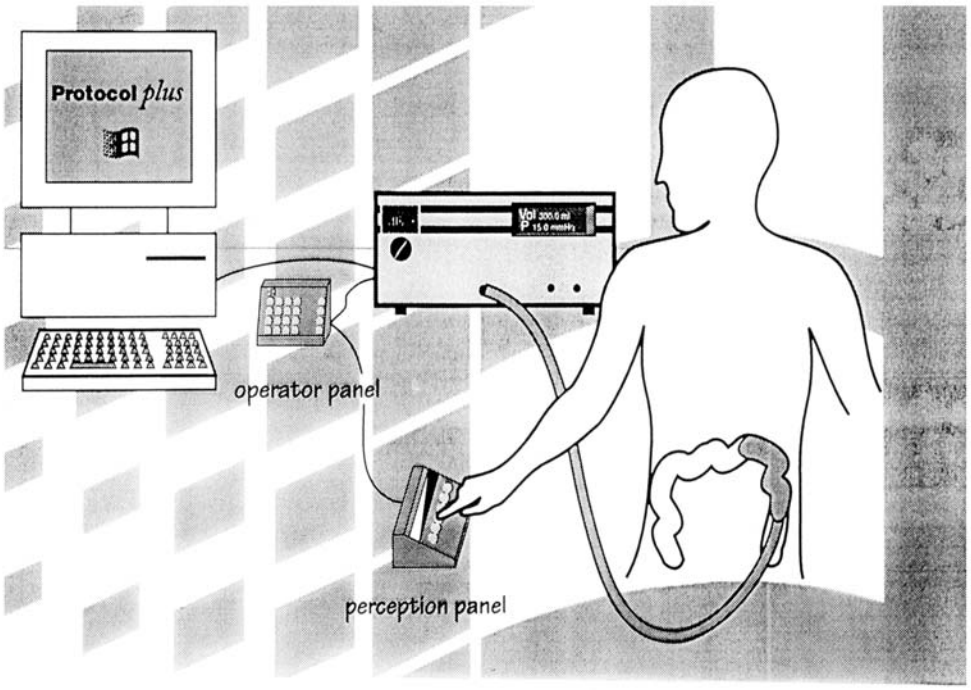


Figure 2. A schematic picture showing the principle of the barostat.

Subjects received a cleansing tap water enema (500 – 1000 ml) and were then placed in a left lateral decubitus position in a hospital bed. A highly compliant polyethylene balloon attached to a double lumen polyvinyl tube (Salem Sump Tube, 18F; Sherwood Medical, Tullamore, Ireland) was inserted into the rectum. The balloon catheter was connected to the barostat and two distensions were performed to unfold the balloon before leaving it at an operating baseline pressure. Maximal inflation resulted in a spherical balloon shape.

Slightly different distension protocols were used in paper I and II as described below. However, both protocols consisted of phasic isobaric distensions (45 ml/s) lasting 30 s followed by 30 s at the operating pressure. During the last 10 s of each distension subjects were prompted to rate perceived sensations on a

keypad graded 1-5 representing: (1) no sensation; (2) rectal fullness; (3) urge to defecate; (4) discomfort; and (5) pain. Sensory thresholds (the lowest pressure needed to provoke a sensation; mmHg) for these sensations were determined for each subject.

DISTENSION PROTOCOLS

Paper I – One distension sequence with distensions performed with stepwise increments starting (ascending method of limits; AML) at the operating pressure and increasing 5 mmHg until the subject reported pain or when a pressure of 70 mmHg was reached (Figure 3). Subjects completed VAS for discomfort after every distension and VAS for pain when they reported pain, i.e. the last distension.

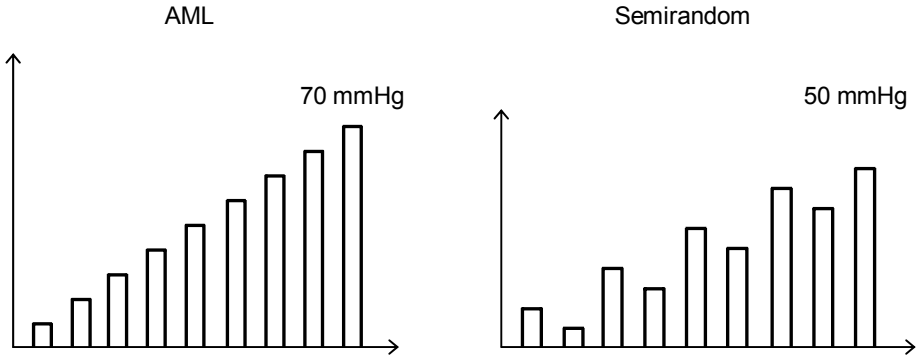


Figure 3. The different types of distension protocols used in paper I and II.

Paper II – A distension protocol consisting of distensions with semirandomly ascending pressure (15 – 10 – 25 – 20 – 35 – 30 – 45 – 40 – 50 mmHg) [82]. If the subject reported pain prior to the last distension (50 mmHg), the sequence was interrupted. The next distension was carried out only if it was set at a lower pressure than the distension that had caused pain according to the semirandom sequence (Figure 3). The sequence was repeated three times, in series I, IIA and IIB experiments, and twice in series IIC experiments (Figure 4). Subjects completed VAS for unpleasantness and pain after each distension sequence.

Comments. Two series of experiments were performed in paper II as shown in figure 4. The main part (series I) consisted of three distension sequences, and the second sequence was carried out with ongoing stress. In series II, a new set of subjects were studied during three separate sessions (A, B and C). This was done in attempt to separate the effects of stress from

the effects of repeated distensions on rectal sensitivity [240]. The setup of each session was as follows: A) Three consecutive distention sequences (1, 2 and 3) separated by 20 min resting periods (providing results on effects of multiple distensions alone), B) three distension sequences (1, 2 and 3) separated by 20 min resting periods; the second sequence following immediately upon a 10 min stress period (eliminating possible effects of distraction), C) two distension sequences (1 and 2) separated by 20 min rest, 10 min stress and an additional 20 min rest (giving information on possible late effects of stress).

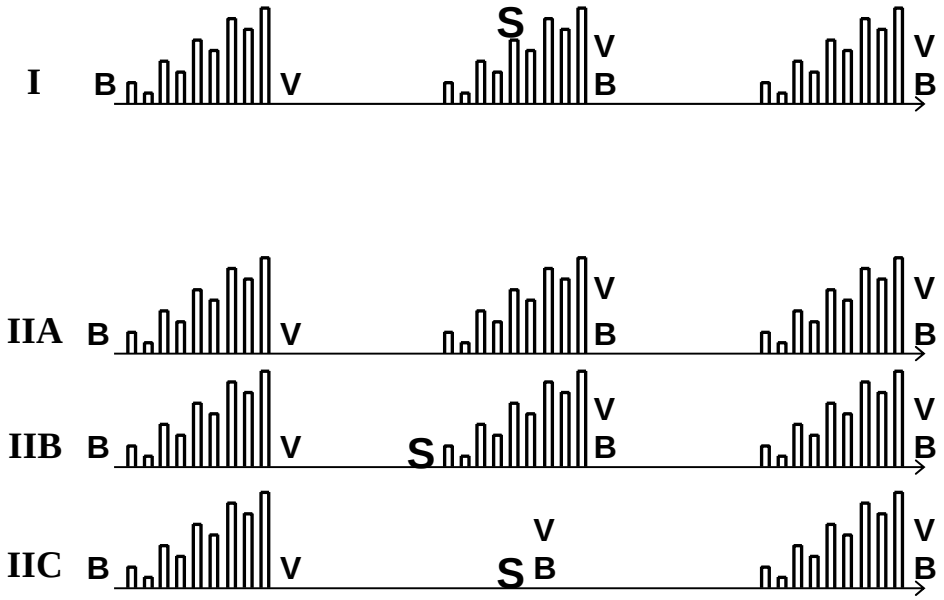


Figure 4. The experimental protocol in paper II. Blood samples (B), VAS (V), stress (S).

4. ALTERED RECTAL PERCEPTION (I)

Rectal sensitivity was assessed using three different aspects of perception: sensory thresholds, perceived intensity of sensations, and viscerosomatic referral [82]. The 95th percentile of the referral area for non-painful sensations in healthy controls was used to define abnormal viscerosomatic referral in IBS patients. IBS patients with at least one abnormality (abnormal sensory thresholds for discomfort and/or pain, abnormal perceived intensity, or abnormal viscerosomatic referral) were considered to have altered perception.

Sensory thresholds for discomfort and pain were determined as previously described. The 5th percentile in healthy controls for the discomfort and pain pressure thresholds was used to define abnormal sensory thresholds in IBS patients.

Perceived intensity of sensations during barostat distensions was evaluated using VAS assessing the intensity of perceived unpleasantness and pain on two separate scales. Since the distension protocol was interrupted when subjects reported pain, only one VAS value for pain was obtained. Perceived unpleasantness was however rated following each distension yielding several values depending on how many distensions each subject tolerated. Most subjects underwent at least four distensions before reporting pain, why a mean from the first four distensions was calculated. The 95th percentile in healthy controls of the VAS for unpleasantness was used to define abnormal perceived intensity in IBS patients.

Viscerosomatic referral of rectal distensions was investigated using schematic body maps as shown in figure 5 (approximate scale 1:4). After the distension protocol, summarizing the whole sequence, subjects were asked to mark the location of perceived pain and non-painful sensations produced by the distensions.

Comments. VAS and viscerosomatic referral area for pain were not included in the definition of altered perception since a substantial proportion of healthy subjects never sensed or reported pain, which would result in falsely low cut-off. The sensory pressure threshold for pain was included in the definition since the threshold was automatically set to 70 mmHg in subjects that did not report pain, which if anything would lower the sensory cut-off.

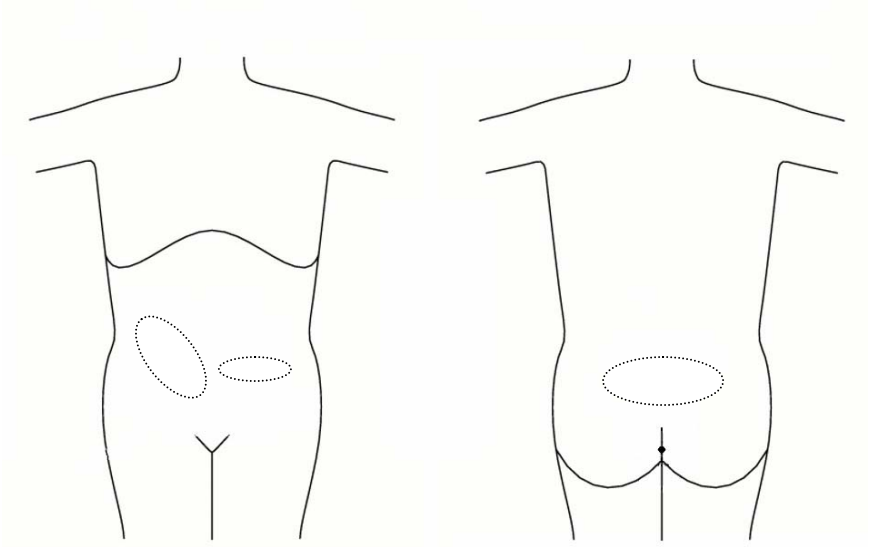


Figure 5. The schematic body maps used to assess visceral referral. Subjects were told to mark the corresponding area of their sensations as shown in this example.

5. STRESS PROCEDURE (II)

The stress period in paper II lasted approximately 10 min during which stress was provoked using a color word conflict test (Stroop test) [241, 242] and mental arithmetic in an alternating fashion. In the Stroop test, subjects were asked to rapidly identify colors in which words representing colors were printed (for example, the word "red" printed in green, the correct answer being green). To induce performance pressure, subjects were under the impression that they were being timed and that accuracy was monitored. Also, approximately once every minute, subjects were told that correct answers were incorrect.

Levels of experienced stress and arousal were evaluated using 100 mm visual analogue scales (VAS) [243, 244]. In addition, the subject's heart rate was monitored continuously using a pulse oximeter (Oscar/oxy, Datex; Dansjö/Omega, Solna, Sweden), recording a value every 10 s.

Comments. The Stroop test is a validated test to study various stress-induced effects [242] and has been used in previous studies investigating IBS patients [173, 245].

6. BLOOD SAMPLES (II)

In paper II, plasma levels of CRF, ACTH, cortisol, epinephrine, and norepinephrine were analyzed to detect differences between IBS patients and healthy controls during rectal sensitivity testing with three distension sequences and acute mental stress. All experiments were started at 1 pm to control for circadian variations. Blood samples were drawn from an intravenous cannula at baseline before the first distension sequence, as well as after the second and third distension sequences. The samples were immediately centrifuged at 3800 g at 4°C for 10 minutes. The supernatant was then aspirated and stored at –20°C (CRF, ACTH, and cortisol) or –80°C (epinephrine and norepinephrine) until analysis. Radioimmunoassays (RIA) for CRF were performed in duplicate according to Ekman and colleagues [246]. Concentrations of ACTH were determined with reagents from Euro-Diagnostics (Malmö, Sweden). Cortisol was measured using a commercial RIA (Orion Diagnostica AB, Sweden). Analysis of epinephrine and norepinephrine were performed by high performance liquid chromatography according to Holly and Makin [247].

7. COGNITIVE TESTING (III)

Study III consisted of three tasks assessing memory and attention.

Word association – subjects were instructed to freely write down as many words as possible representing four legged animals (as a control) and then signs of disease, during a time period of 1 minute for each category of words.

Word recognition – identifying words representing positive or negative affects, GI symptoms, or non-GI symptoms shown on a computer screen (tachistoscope). A total of twelve words (three from each category) were displayed one at a time in random order. The words were shown during increasing time, starting at 10 ms and adding 5 ms at each step, until the subject correctly identified the word. They were encouraged to guess and after a correct answer they moved on to the next word.

Word recall – subjects were told to memorize words from a slide show with 30 words representing positive and negative affects, and GI symptoms. Ten words from each category were displayed one at a time in random order on a computer screen, for three seconds each. After all the words had been presented, subjects engaged in a 15 minutes distraction task during which they read a part from a short-story ("The Overcoat" by Nikolaj Gogol). Subjects were then asked to write down all of the words that they could recall and were given five minutes to do this.

Comments. This part was designed based on descriptions [70] of previous studies on selective attention in IBS patients. The words that the subjects recalled after the reading task were divided into the three categories, and correct or incorrect (false). A total (correct and incorrect) number was also calculated for each category.

The Swedish words used in the study are shown in appendix A. The GI symptom words were taken (primarily) from the Gastrointestinal Symptom Rating Scale (GSRS) [236]. The adjectives describing emotional states (positive and negative affects) were taken from the Swedish version of the Mood Adjective Check List (MACL) [248]. We had the intention to match our word lists for frequency of occurrence in published media. However, this was not entirely possible due to the fact that we had to use some expressions made up by two words, for example "loose stools", that are not included in Swedish

frequency dictionaries. The words used in the word recognition test were matched for length and number of syllables.

8. SMALL BOWEL MANOMETRY (IV)

Following an overnight fast, antroduodenojejunal motility was assessed using a stationary water-perfused (0.3 ml/min) eight-channel assembly for pressure recording (Zinetics, Salt Lake City, Utah, USA). The manometry catheter was placed under fluoroscopic guidance, leaving the tip in the proximal jejunum, with pressure recording side ports situated at 2, 17, 30, 32, 34, 45.5, 47 and 48.5 cm from the tip of the catheter. Thus, three ports were situated 1.5 cm apart in the antrum (A1-3), three in the descending part of the duodenum 2 cm apart (D1-3), one in the distal duodenum close to the ligament of Treitz (T), and one in the proximal jejunum (J; Figure 6). The catheter was connected to pressure transducers and recordings were made with a polygraph (PC Polygraph, Synetics, Stockholm, Sweden). The information was transformed to a computer via a fiberoptic interface. Individual recordings were displayed on a computer screen and stored for later analysis. Fasting (interdigestive) motility was recorded for three hours. A standard meal (500 kcal) was then given, and the recording continued for another hour.

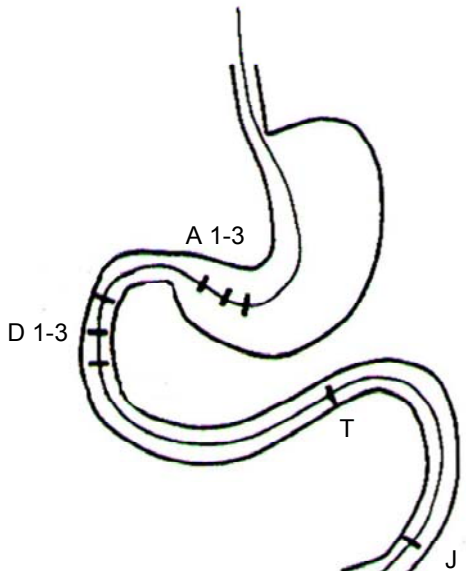


Figure 6. A schematic picture of the manometry catheter with eight pressure recording ports: three in the antrum (A 1-3), three in the descending duodenum (D 1-3), one close to the ligament of Treitz (T), and one in the proximal jejunum (J).

The manometric data were reviewed in regard to the characteristics of phase III, migrating motor complex (MMC), motility indices, postprandial motor pattern and presence of enteric dysmotility. Analysis of the different phases of interdigestive motility was performed by direct visual inspection on the computer screen using a commercially available program (Polygram, version 5.06 X1, Synetics Medical, Stockholm, Sweden). The area under the curve was used as motility index, expressed as mmHgxs, and calculated for the last 30 min of phase II (late phase II) and for 30 min after the subjects had finished their meal. The following recording points were used for calculation of motility index in the four segments studied: A3, D2, T and J (Figure 6). The propagation velocity of phase III from proximal to distal duodenum and from distal duodenum to jejunum was analyzed with a computerized calculation after manual marking on the computer screen (cm/min).

The presence of enteric dysmotility was diagnosed by a more conventional evaluation of the condensed manometric data, performed by two of the investigators (MS and HA) reaching a consensus [249]. Specifically, findings compatible with neuropathy (normal amplitudes but an abnormal contraction pattern) and/or myopathy (low amplitudes) were sought for [250, 251], as well as specific motor patterns falling outside the normal range found in healthy controls previously investigated at our laboratory [114](Table 2).

Comments. The true pathological meaning and the relevance for symptoms of some of the included alterations – for instance clusters – are still debated [252].

Table 2. Signs of enteric dysmotility using criteria from Kellow [251] modified based on normal values from healthy controls at our lab [114].

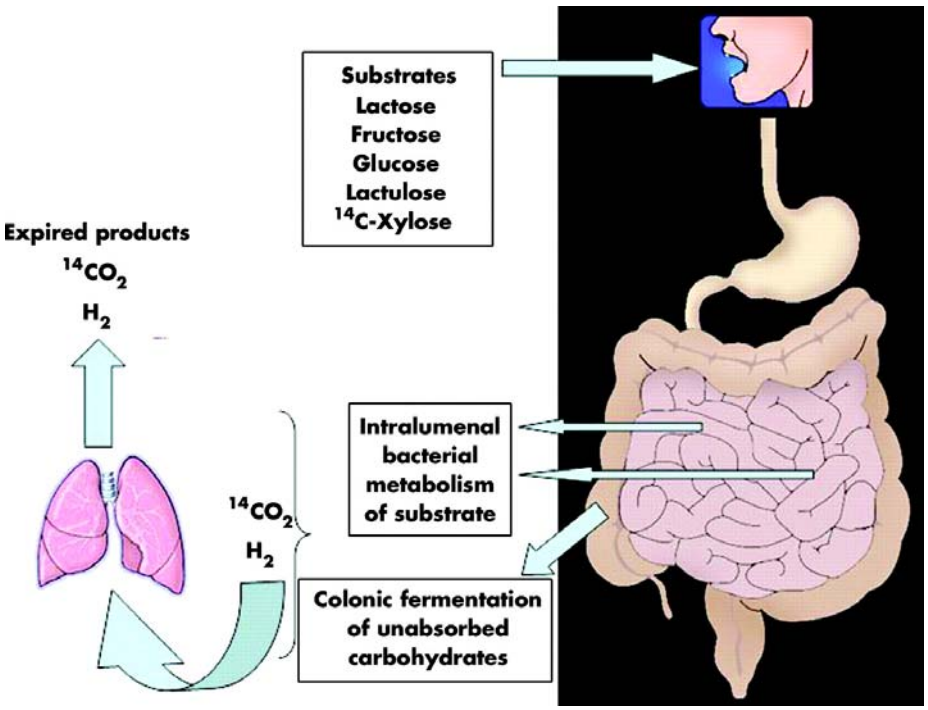
Migrating motor complex (MMC)
> MMCs per 3 h of recording
Phase III duration ≥10 min
Phase III propagation ≤1 cm/min
Simultaneous or retrograde phase III
Elevation of basal line >30 mmHg for >3 min
Contraction amplitude
<20 mmHg
Postprandial pattern
No established fed pattern
Presence of specific contractile patterns
Isolated bursts
Sustained incoordinated pahsic activity
Multiple, simultaneous, prolonged (>8 s) phasic contractions
Postprandial discrete clustered contractions >30 min duration
Postprandial phase III-like activity
Frequent long clusters (>30 s)

9. JEJUNAL CULTURES (IV)

Jejunal juice was aspirated via the central lumen of the manometry catheter and collected in a sterile plastic tube. The samples were sent to a microbial laboratory within two hours, and cultured for aerobic and anaerobic bacteria on blood agar plates with 4% defibrinated horse blood in aerobic and anaerobic atmospheres of N_2 and 10% CO_2. Selective cultivation of Gram-negative strains was performed on Drigalski agar under aerobic conditions. Yeast fungus was cultured on Sabouraud's agar. The minimum incubation time was 48 hours. Identification of the microorganisms was based on colony characteristics, Gram staining, biochemical and chromatographic tests. Quantification was performed by counting the number of colony-forming units (cfu/ml). Culture-verified small intestinal bacterial overgrowth (SIBO) was defined as growth of colonic bacteria in a density of $\geq 10^5$ cfu/ml. For explorative analyses, we also looked at lower cut-off levels, including non-colonic bacteria, as well as bacterial counts $\geq 95^{th}$ percentile in our healthy volunteers, to represent increased counts of small bowel bacteria.

10. HYDROGEN BREATH TESTS (IV)

The principle of breath tests is shown in figure 7. After an overnight fast and at least one day of low-fiber diet, the subjects presented at the laboratory. Hydrogen concentrations were measured in parts per million (ppm) with a GMI exhaled H_2 monitor (GMI Medical Ltd., Inchinistan Estate, Renfrew, UK). A H_2 breath sample was obtained at baseline before the intake of a solution containing 50 g glucose dissolved in 300 ml of water or 10 g lactulose (15 ml of a 670 mg/ml syrup solution). H_2 in end-expiratory breath samples was then continuously analyzed every 15 min for 120 (glucose) or 180 (lactulose) min. The measurements were plotted graphically and analyzed.



Simrén M, Stotzer PO. Gut 2006;55:297-303. Reproduced with permission from the BMJ Publishing Group.

Figure 7. Schematic drawing showing the principle of breath tests.

The breath tests were considered to indicate SIBO based on the following criteria: 1) a >15 ppm increase in H_2 15-120 min after ingestion of glucose in at least two breath samples [219], 2) two distinct H_2 peaks (>20 ppm increase) 15-180 min after ingestion of lactulose – that is, an early peak consisting of two consecutive hydrogen values >20 ppm above the baseline value, clearly distinguishable from the later "colonic" peak [253] (Figure 8). For comparison we also used the recently proposed criteria for a positive LHBT: rise in H_2 > 20 ppm by 90 or 180 min [226].

Comments. Definitions of normal and abnormal results of breath tests are variable. Glucose is usually absorbed in the proximal small bowel and an increase in H_2 is supposed to represent fermentation of bacteria in the small bowel. Lactulose passes unabsorbed through the GI tract and gives rise to a H_2 peak when it reaches bacteria in the colon. Without a clear pattern with two peaks, SIBO cannot be distinguished from colonic fermentation. In fact, lactulose can be used to measure orocecal transit time, which is just above 90 min in healthy controls [217].

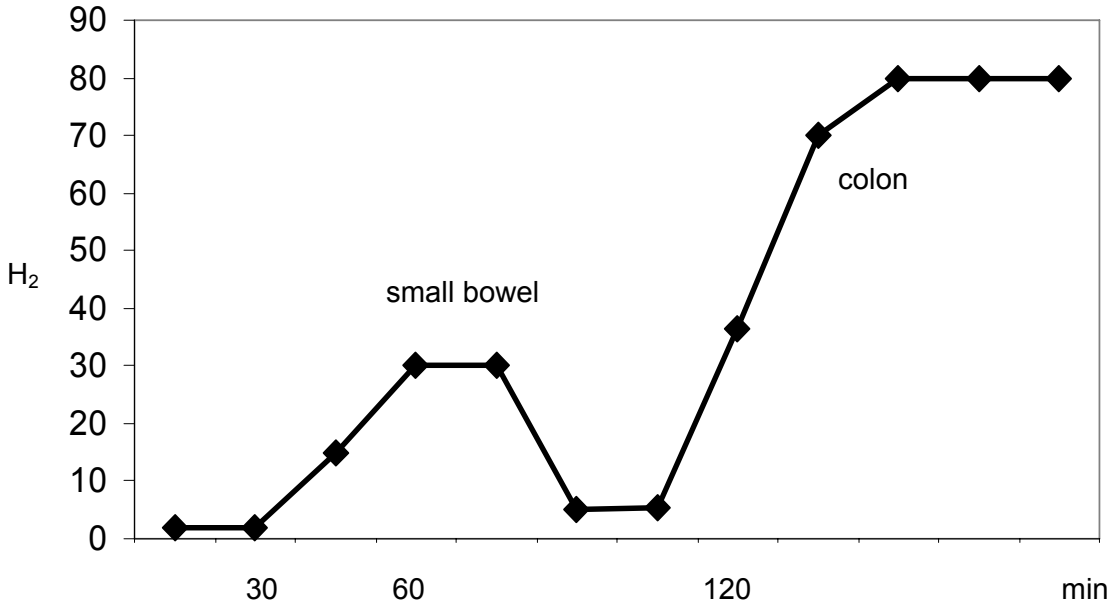


Figure 8. A positive LHBT indicating small intestinal bacterial overgrowth with an early peak due to small bowel bacteria.

11. STATISTICAL METHODS

Results are presented as mean and standard deviation (SD) (I, III, IV) or standard error of the mean (SEM) (II), and median and interquartile range (IQR) (I, II, III,). In general, significance was accepted at the 5% level.

The following statistical methods were used: paired and unpaired Student's *t* test (I, II, III), Mann-Whitney *U* test (I, II, III, IV), Wilcoxon signed rank test (II, IV), Chi squared test (I, III, IV), ANOVA (II, IV), Spearman's rank correlation (I, II, III), and forward stepwise multiple logistic regression (I). In paper I and IV, the 5^{th} or 95^{th} percentile in the healthy control group were assessed to serve as a reference limit of different variables

RESULTS

1. PSYCHOLOGICAL FACTORS (I, II, III)

1.1 Anxiety and depression (I, II, III)

(I) The HAD scores for IBS patients were 6(3-10) for anxiety and 4(2-9) for depression. According to the HAD scales, 24% patients had a score compatible with clinically significant anxiety, and 14% a score compatible with clinically significant depression (score ≥11).

(II) IBS patients had higher scores on HAD than healthy subjects for both anxiety (7(7-10) vs. 5(3-6); $p<0.001$) and depression (4(2-7) vs. 1(1-2); $p<0.001$). STAI showed differences between patients and controls for both state anxiety (36(32-43) vs. 30(25-34); $p<0.01$) and trait anxiety (41(34-49) vs. 30(26-34); $p<0.001$). Only three and two patients suffered from clinically significant anxiety and depression, respectively.

(III) The scores on HAD were similar in both patients with IBS and patients with organic GI disease for both anxiety (6(4-9) vs. 6(3-8); NS) and depression (4(1-6) vs. 2(1-5); NS). The prevalence of HAD scores indicating probable cases of anxiety (score ≥11) were similar in the two groups, seven IBS patients (19%) and six patients with organic GI disease (16%). Only one subject (a patient with celiac disease) had a score indicating clinically relevant depression.

Comments. The patients in our studies had higher scores on HAD and STAI compared with healthy controls, but still the prevalence of anxiety and depression was relatively low compared with many other reports. This should be kept in mind when comparing our results with other studies.

1.2 Stress (II)

Compared to controls, patients reported higher VAS ratings of stress in association to all three distension sequences ($p=0.1$; $p<0.05$; $p=0.08$). The stress procedure increased the ratings of perceived stress compared with before and after stress in both patients ($p<0.05$; $p<0.01$) and controls ($p=0.07$; $p<0.01$). Patients demonstrated significantly lower ratings of arousal than controls both before and after, but not during stress ($p<0.05$). Higher ratings of arousal were also reported during the stress period compared with before and after by both patients ($p<0.01$; $p=0.001$) and healthy subjects ($p<0.01$; $p<0.001$).

2. VISCERAL PERCEPTION

2.1Sensory thresholds (I, II)

I) As a group, IBS patients had lowered sensory thresholds to rectal distensions compared with healthy subjects for defecatory urge (22±7.1 vs. 26±8.3 mmHg; p=0.01), discomfort (32 ±12 mmHg vs. 43±16 mm Hg; p<0.0001) and pain (44 ±15 mmHg vs. 58±12 mm Hg; p<0.0001). The 5th percentile in healthy controls for the thresholds for discomfort and pain was 23 mmHg and 31 mmHg, respectively (Table 3). Using these cutoffs, 39% of the patients had lowered thresholds for discomfort and/or pain.

II) IBS patients had lower sensory thresholds to rectal distensions compared to healthy subjects for discomfort (34±12.3 vs. 44±8.2 mmHg; p<0.01) and pain (42±8.8 vs. 50±1.1 mmHg; p<0.001). (Figure 9)

Comments. Different distension protocols were used in study I (AML) and II (semirandom staircase). The maximum distension pressure was 70 mmHg and 50 mmHg, respectively. If subjects did not report pain before reaching the maximum pressure, their pain threshold was automatically set to the maximum pressure. This explains the difference in pain threshold between healthy controls in study I and II. It has been argued that perceptual response bias is especially pronounced in AML [65, 254, 255] while a semirandom protocol, being less predictable would be less prone to response bias. We obtained similar sensory thresholds in the two studies despite different distension protocols, and a recent study also using a semirandom distension protocol, reported that 45% of IBS patients were hypersensitive to rectal distensions using the 5th percentile as a cut-off [104]. Therefore we believe that AML is a valid method to study visceral sensitivity [256, 257].

2.2 Perceived intensities (I, II)

I) IBS patients reported significantly greater unpleasantness VAS ratings in response to the rectal distension compared with controls (13(6-26) vs. 5(2-11) mm; p<0.0001). The 95th percentile in healthy subjects was 24 mm (Table 3). With this cut-off 37% of the patients had increased unpleasantness ratings in response to rectal distensions.

II) Perceived unpleasantness during distensions were greater in patients than in controls (60(39-73) vs. 29(13-49) mm; p<0.01).

Comments. Results from VAS in paper I and II were different because of different approaches. In paper I subjects scored perceived intensities of unpleasantness for each distension, and a mean of the first four distension was calculated. In paper II subjects reported perceived intensities after each distension sequence, summarizing the whole sequence, including distensions that caused discomfort and pain.

2.3 Viscerosomatic referral (I)

IBS patients reported significantly larger viscerosomatic referral areas for non-painful sensations compared with controls (5.6 ± 7.1 cm^2 vs. 1.8±2.4 cm^2; $p<0.0001$). The 95th percentile for the viscerosomatic referral area for non-painful sensations in healthy controls was 8.8 cm^2 (Table 3). Using this cut-off, 28% of the patients had enlarged viscerosomatic referral areas for non-painful sensations during rectal distensions.

2.4 Altered perception (I)

When adding the different aspects of perception (discomfort and pain thresholds, perceived intensity, and viscerosomatic referral), 62% of all patients were found to have altered perception to rectal distensions according to at least one of the three assessments. There was no age difference between the patients with altered rectal perception and those with normal perception (mean age 41± 12 vs. 44± 14 years; NS).

Table 3. The four parameters used to assess the rectal perception. The cut-offs used for defining abnormal rectal perception are shown: the 5th percentile for the discomfort and pain thresholds, the 95th percentile for the area of referred non-painful sensations and VAS unpleasantness. **** $p<0.0001$ vs. healthy controls

	Controls (n=28)		**IBS (n=109)**
	mean ± SD	5th percentile	mean ± SD
Discomfort threshold (mmHg)	43±16	23	32 ±12 ****
Pain threshold (mmHg)	58±12	35	44 ±15 ****
	mean ± SD	95th percentile	mean ± SD
Referral area (non-painful sensations) (cm^2)	1.8±2.4	4.8	5.6 ± 7.1 ****
	median (IQR)	95th percentile	
VAS unpleasantness (mm)	5(2-11)	24	13(6-26) ****

2.5 Psychological symptoms (I)

HAD anxiety scores tended to be higher in patients with altered perception compared with those with normal perception (7.7±4.7 vs. 6.0±3.7; p=0.06), and clinically significant anxiety was more common in patients with altered perception (31% vs. 12%; p<0.05). HAD depression scores were higher in the group with altered perception (6.3±4.6 vs. 4.4±3.9; p=0.03), but the presence of clinically significant depression was similar in patients with altered versus normal rectal perception (16% vs. 10%; NS). HAD anxiety was not significantly correlated with any of the rectal perception variables (data not shown), and HAD depression was only weakly, but significantly correlated with the rectal pain threshold (r=0.24; p=0.01).

2.6 Stress (II)

In paper II subjects underwent three distension sequences; before (1), during (2) and after (3) stress. Sensory thresholds were increased in healthy controls during stress compared with before and after stress (2 vs. 1 and 3) for rectal fullness (p=0.003; p=0.03), urge to defecate (p=0.007; p=0.003) and discomfort (p=0.1; p=0.002). IBS patients were found to have similar sensory thresholds before and during stress, and instead decreased thresholds after stress (2 vs. 3). This pattern was seen for all sensations (rectal fullness p=0.02; defecatory urge p=0.03; discomfort p=0.008; pain p=0.02; Figure 8). No major effects of stress on perceived intensities of unpleasantness and pain during distensions were observed in any of the groups (data not shown).

Comments. Few controls reported pain at the maximum pressure of 50 mmHg, making comparisons for pain inconclusive. Repeated distensions without acute mental stress had little effect on sensory thresholds in both groups. When stress was administered immediately before, instead of during distension sequence 2, no major differences in thresholds were observed between the three distension sequences in controls. In patients, thresholds again tended to decrease during the last distension sequence (2 vs. 3) for rectal fullness (22±4.3 vs. 15±5.0; p<0.01), discomfort (36±11 vs. 32±11; p<0.05) and pain (43±8.3 vs. 39±10; p=0.08). These results indicate that the decreased sensitivity in healthy subjects during stress was an effect of distraction which was not observed in IBS patients. Distraction has previously been shown to decrease visceral sensitivity in healthy subjects [46, 89] but not in IBS patients[46], perhaps due to selective attention regarding GI sensations [70] which prevents them to suppress signals from the GI tract.

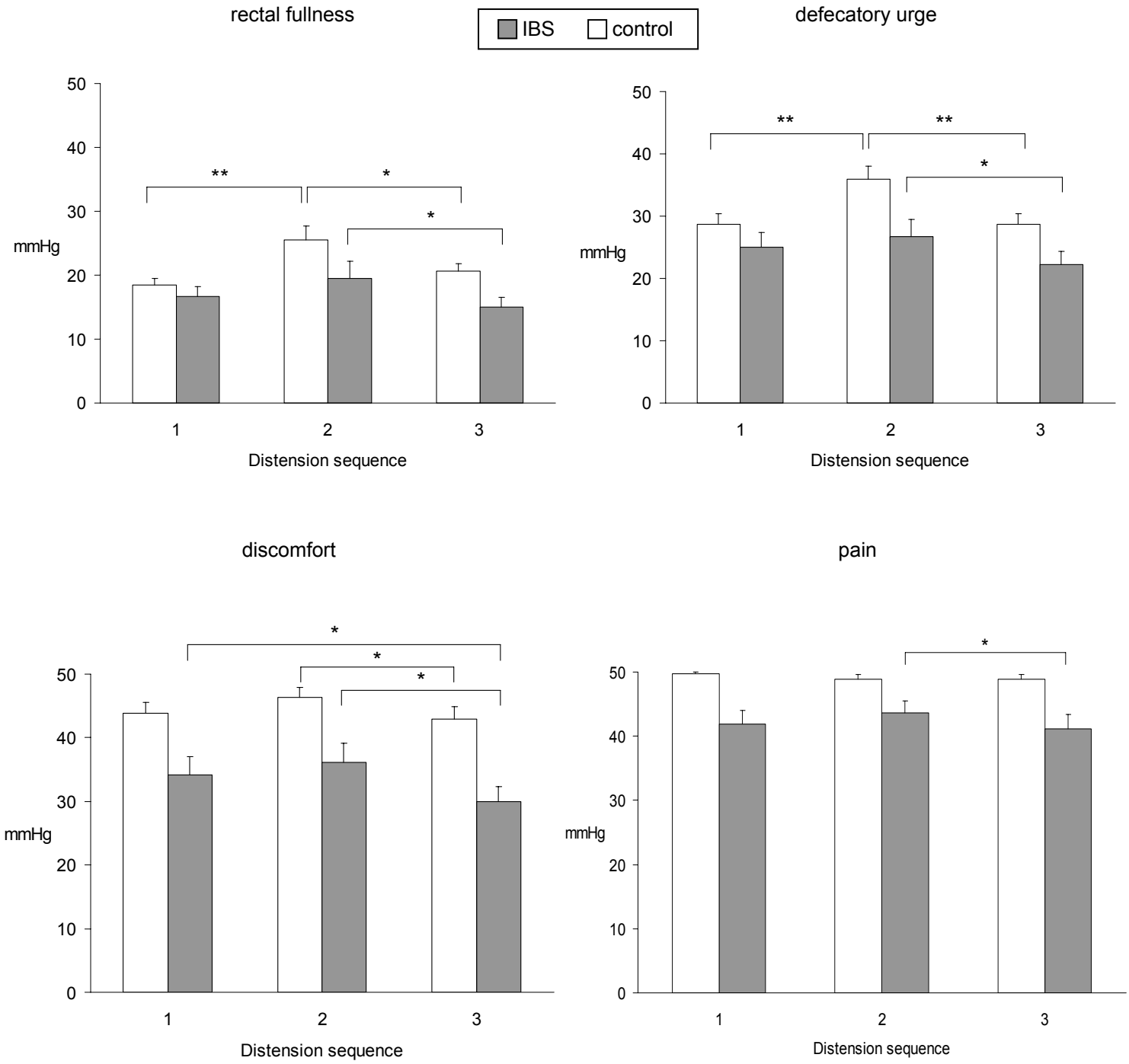


Figure 8. Sensory thresholds (mean (SEM)) before (1), during (2), and after stress (3).
* p<0.05 ** p<0.01

2.7 Gender (I)

Female IBS patients had significantly lower sensory thresholds than male patients for all the studied sensations (Table 4). However, we could not detect any gender differences for the VAS ratings (14(6-26) mm vs. 11(3-25) mm; NS) or the areas for referred painful (4.0±4.6 vs. 3.8±4.8 cm^2; NS) or non-painful sensations (5.8±8.0 vs. 5.1±4.3 cm^2; NS). As mentioned earlier, 62% of all patients were found to have altered rectal perception. The proportion of subjects

with altered perception was the same for female and male patients (61% vs. 66%; NS). Also, a similar gender distribution was observed in the group of patients with altered rectal perception and those with normal perception (69% vs. 73% females; NS).

Table 4. The different rectal perception variables compared between male and female IBS patients. * p<0.05 ** p<0.01

	Females	**Males**
threshold first sensation (mmHg)	15±4.2 *	17±4.7
threshold urge to defecate (mmHg)	21±6.6 **	25±7.5
discomfort threshold (mmHg)	30±11 **	36±13
Pain threshold (mmHg)	42±14 *	49±15
Referral area, non-painful (cm^2)	5.8±8.0	5.1±4.3
Referral area, pain (cm^2)	4.0±4.6	3.8±4.8
VAS unpleasantness (mm)	18±16	17±16

2.8 IBS subgroups (I)

No differences in sensory thresholds were seen between IBS subgroups based on the predominant bowel habit. The different subgroups also had similar perceived intensities and referral areas during rectal distensions (data not shown). This resulted in a similar distribution of predominant bowel pattern between the patients with altered rectal perception and those with normal perception (49% vs. 46% IBS-A, 18% vs. 15% IBS-C, 34% vs. 39% IBS-D; NS).

2.9 Multivariate analysis (I)

We used a stepwise multiple logistic regression analysis to identify factors independently associated with altered rectal perception. In the first analysis we entered the different GSRS-IBS domains along with demographic factors and HAD scores univariately associated with having altered rectal perception at p<0.1. GSRS-IBS Pain syndrome (OR, 1.73; 95% CI 1.14-2.64; p=0.01) and GSRS Bloating syndrome (OR, 1.48; 95% CI 1.01-2.17; p=0.04) were the only factors in this analysis significantly associated with having altered rectal perception (R^2=0.22). In a second analysis we replaced the GSRS domains with the individual symptoms from GSRS-IBS univariately associated with having altered rectal perception at <0.1. Abdominal pain (OR, 1.53; 95%CI 1.07-2.19; p=0.02) and bloating (OR, 1.54; 95% CI 1.07-2.22; p=0.02) was the only factors significantly associated with having altered rectal perception (R^2=0.23).

3. GASTROINTESTINAL SYMPTOMS (I)

When using moderate discomfort as the cut-off, the most commonly reported symptoms were bloating (82%) and being bothered by passing gas (76%), followed by pain/discomfort relieved by defecation (70%), abdominal pain (67%) and incomplete bowel emptying (65%). Combining the individual symptoms into the 5 domains on the questionnaire (Figure 1) and evaluating the mean scores of the items included, symptoms related to pain and bloating were the most prevalent, being present (mean score≥3) in 86% and 89%, respectively (Figure 9). Almost 2/3 of all patients reported on average moderate or severe symptoms (mean score≥4) of abdominal pain (63%) and bloating (59%), while about 1/3 reported on average moderate or severe symptoms of diarrhea (37%), constipation (31%) and satiety (30%). Severe symptoms of bloating was reported by 16% of all patients, but with this exception, the prevalence of severe symptoms was for each symptom cluster <10%. Moderate GI symptoms overall (mean score ≥4) were reported by 31%, but no patients were found to have severe GI symptoms overall (mean score ≥6).

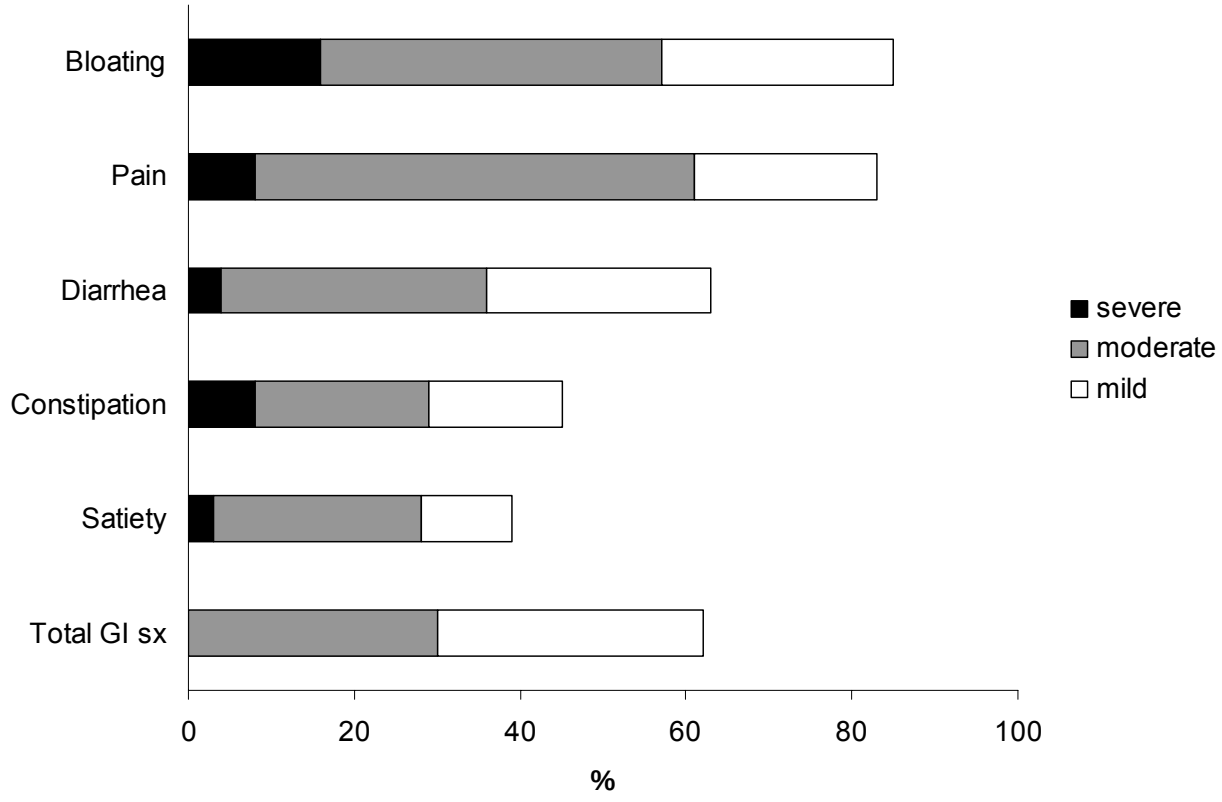


Figure 9. Frequency of patients reporting on average at least mild (score ≥3), moderate (score ≥4) and severe (score ≥6) symptoms divided into the different GSRS-IBS domains, as well as a mean total score indicating symptom severity overall (Total GI sx).

3.1 Altered visceral perception

The mean scores on the different domains on GSRS-IBS were higher, indicating more severe symptoms in the patients with altered vs. normal rectal perception for all domains except for constipation. Figure 10 shows the percentage of patients having at least moderate symptom severity (mean scores on GSRS-IBS domains≥4) in the groups with altered vs. normal rectal perception. Compared to patients with normal rectal perception, patients with altered perception more frequently reported at least moderate symptoms of abdominal pain (73% vs. 44%; $p<0.01$), bloating (73% vs. 36%; $p<0.0001$), diarrhea (47% vs. 21%; $p<0.01$), and satiety (39% vs. 13%; $p<0.01$). Only 8% of the normosensitive patients had moderate GI symptoms overall (average GSRS-IBS score ≥4), compared to 46% of the patients with altered perception ($p<0.0001$) (Figure 10). The prevalence of severe pain and bloating (mean score≥6) was also significantly higher in patients with altered rectal perception compared with patients with normal rectal sensitivity (14% vs. 0%; $p<0.05$ and 23% vs. 5%; $p<0.05$).

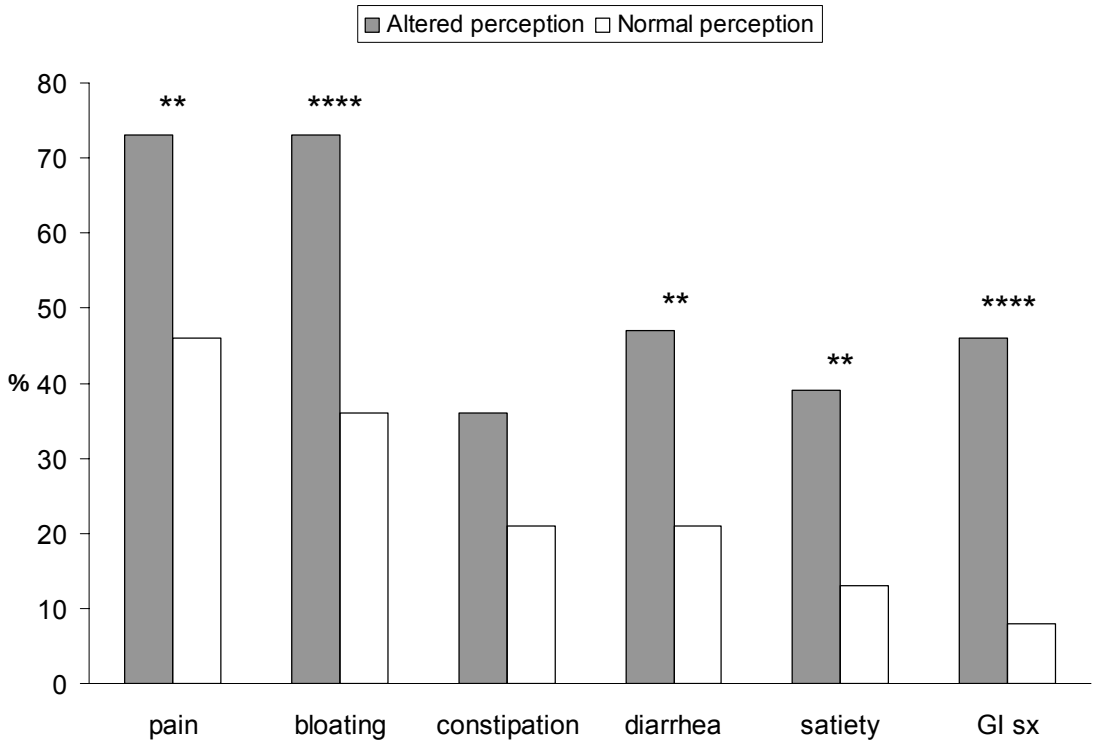


Figure 10. The proportion of patients with altered (n=67) vs. normal (n=42) perception reporting at least moderate symptom severity (score ≥4), including GI symptoms overall (GI sx). ** $p<0.01$ **** $p<0.0001$

3.2 Psychological symptoms

GSRS-IBS domain scores were compared between IBS patients with and without HAD scores indicating anxiety or depression. IBS patients with anxiety were found to have higher GSRS-IBS scores for pain (4.6±1.2 vs. 3.9±1.2; $p=0.01$), bloating (4.8±1.4 vs. 4.2±1.3; $p<0.05$), diarrhea (4.1±1.3 vs. 3.3±1.3; $p<0.01$), satiety (3.2±1.8 vs. 2.4±1.4; $p=0.06$) and GI symptoms overall (4.0±1.0 vs. 3.3±0.9; $p=0.001$). No significant differences in GSRS scores were found between patients with and without depression. A higher proportion of the patients with anxiety reported at least moderate symptoms of pain (85% vs. 56%; $p<0.01$), constipation (46% vs. 25%; $p<0.05$), and diarrhea (54% vs. 32%; $p<0.05$). Moderate GI symptoms over all were more common in patients with HAD scores indicating anxiety or depression compared with those with normal HAD scores (58% vs. 23%; $p=0.001$ and 53% vs. 28%; $p=0.07$).

3.3 Gender

When comparing the GSRS-IBS domain scores between female and male IBS patients, females were found to have more severe abdominal pain (4.2±1.2 vs. 3.7±1.1; $p<0.05$), bloating (4.6±1.3 vs. 3.8±1.1; $p<0.001$), constipation (3.1± 1.9 vs. 2.3± 1.4; $p<0.05$) and GI symptoms overall (3.7± 1.0 vs. 3.1± 0.6; $p<0.01$). A higher proportion of female than male IBS patients reported at least moderately severe (mean domain score ≥ 4) abdominal pain (70% vs. 47%; $p<0.05$) and bloating (67% vs. 41%; $p<0.05$), as well as total GI symptom severity of at least moderate severity (41% vs. 9%; $p<0.001$).

3.4 Correlations

Table 5 summarizes the correlations between reported symptom severity scores (GSRS-IBS domains) and rectal discomfort and pain sensory thresholds, VAS for unpleasantness, viscerosomatic referral, and HAD scores for anxiety and depression. Pain and bloating was significantly correlated with all three assessments of rectal perception as well as with HAD anxiety and depression. The strongest correlation was seen between overall GI symptom severity and rectal pain threshold ($r=0.43$; $p<0.0001$). No significant correlation with constipation was detected. Among the GSRS domains the strongest correlations were seen between pain and satiety ($r=0.52$; $p<0.0001$), and pain and bloating ($r=0.47$; $p<0.0001$). The correlations between rectal perception and the severity of gastrointestinal and psychological symptoms were similar in female and male IBS patients (data not shown).

Comments. In a subgroup of patients (n=33) rectal sensitivity was repeated after 12 weeks and the questionnaires were completed again. The GI symptom severity was unchanged compared with baseline, and so were the anxiety and depression scores. Of the rectal sensitivity parameters only the pain threshold was significantly altered compared with baseline (37±14 vs. 33±12; $p<0.05$), i.e. a tendency towards increased sensitivity at 12 weeks. The GI symptom severity (GSRS total) at 12 weeks was negatively correlated with the discomfort ($r=-0.46$; $p<0.01$) and pain thresholds ($r=-0.51$; $p<0.01$), and positively correlated with VAS for unpleasantness ($r=0.45$; $p<0.01$) and the viscerosomatic referral area for non-painful sensations ($r=0.46$; $p<0.01$), indicating a somewhat stronger association compared with baseline.

Table 5. Correlations between the different rectal perception variables, psychological symptom severity assessed with HAD and the different domains on GSRS-IBS.
* p<0.05 ** p<0.01 *** p<0.001 **** p<0.0001

	GSRS-IBS domains					
	Pain	**Bloating**	**Constipation**	**Diarrhea**	**Satiety**	**Total GI sx**
Discomfort threshold	r=-0.37****	r=-0.35****	r=-0.12	r=-0.17	r=-0.28**	r=-0.40****
Pain Threshold	r=-0.41****	r=-0.22*	r=-0.13	r=-0.26**	r=-0.36****	r=-0.43****
VAS unpleasantness	r=0.30**	r=0.28**	r=0.18	r=0.27**	r=0.24*	r=0.40****
Viscerosomatic referral area	r=0.21*	r=0.25*	r=0.07	r=-0.10	r=-0.14	r=0.18
HAD Anxiety	r=0.25*	r=0.21*	r=0.16	r=0.22*	r=0.21*	r=0.33**
HAD Depression	r=0.25**	r=0.19*	r=0.19	r=0.19	r=0.28**	r=0.33**

3.5 Multivariate analysis

In order to find independent factors for gastrointestinal symptom severity we performed a forward stepwise logistic regression entering all factors (demographic, HAD scores, rectal perception variables) univariately associated with having at least moderate gastrointestinal symptoms overall (GSRS total ≥ 4) at $p<0.1$. Female gender (OR, 7.86; 95% CI 1.69-36.4; p=0.008), HAD anxiety (OR, 1.19; 95% CI 1.04-1.34; p=0.009), the rectal pain threshold (OR, 0.93; 95% CI 0.89-0.98; p=0.004), and the area of referred pain (OR, 1.18; 95% CI 1.03-1.34; $p<0.01$) was found to be independently associated with overall gastrointestinal symptom severity ($R^2=0.48$).

4. NEUROENDOCRINE BLOOD SAMPLES (II)

4.1 Basal state

Basal levels of CRF in plasma were significantly lower in patients than in controls ($p<0.05$). However, no significant group differences were observed for levels of ACTH or cortisol (Figure 11). Patients had higher basal levels of norepinephrine compared with controls ($p=0.01$), but there was no significant difference in basal levels of epinephrine (Figure 12).

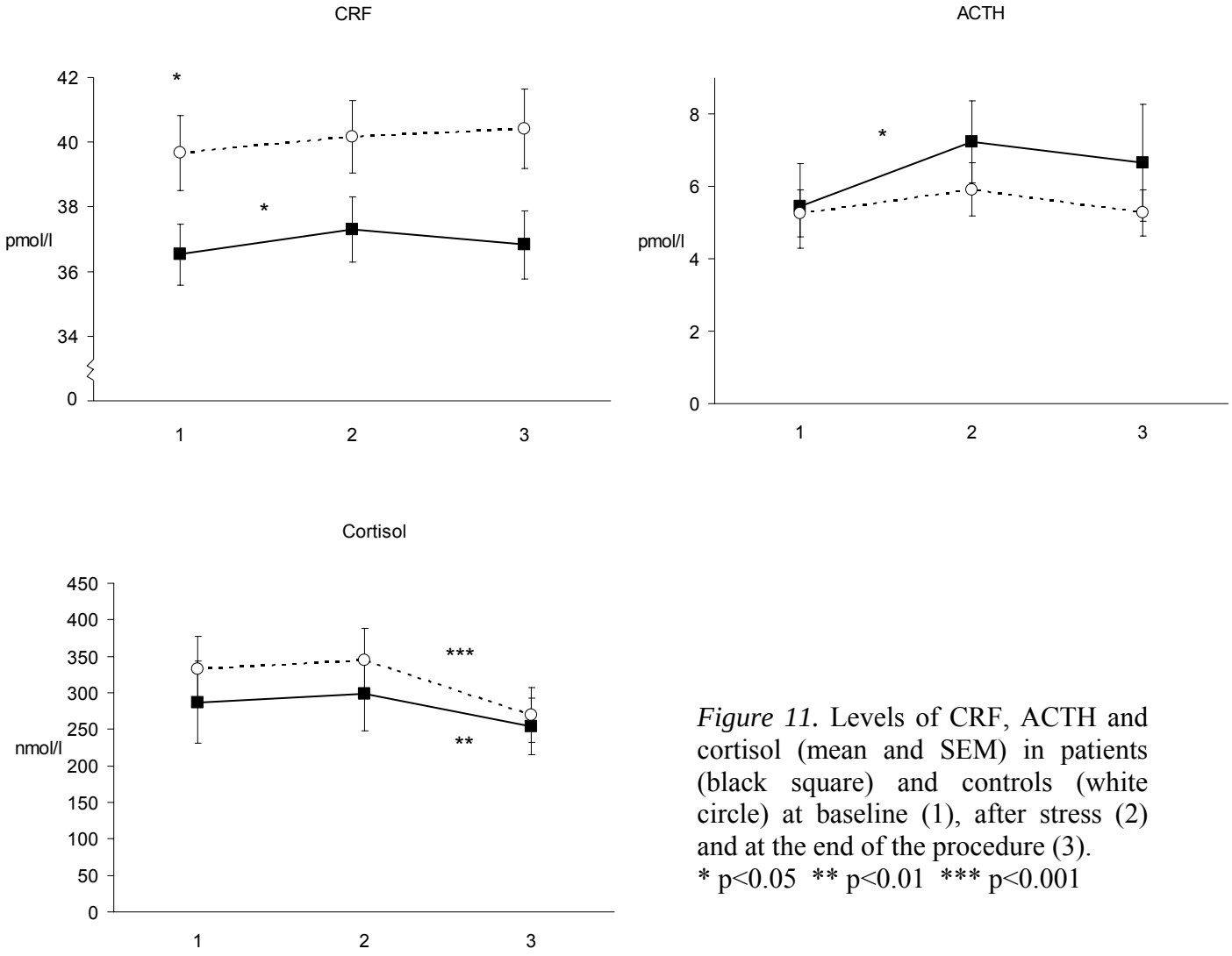


Figure 11. Levels of CRF, ACTH and cortisol (mean and SEM) in patients (black square) and controls (white circle) at baseline (1), after stress (2) and at the end of the procedure (3).
* $p<0.05$ ** $p<0.01$ *** $p<0.001$

4.2 Stress

IBS patients but not controls demonstrated a small but significant rise in CRF during stress (p=0.01). Accordingly, patients but not controls demonstrated a marked rise in ACTH (p<0.05). No significant stress induced increase in cortisol could be observed in any of the groups. However, the cortisol levels in both groups were significantly higher in connection to the stress period compared with at the end of the experiment (Figure 11). Healthy subjects responded to stress with increased levels of epinephrine and norepinephrine (p=0.01; p<0.01), but no marked rise in catecholamines was observed in patients in response to stress (Figure 12).

Comments. The increase in CRF observed in patients was significant but small. Peripheral levels of CRF are probably not representative for central signaling and IBS patients are proposed to have an exaggerated CRF response [18, 55, 258]. An intravenous infusion of CRF (1 μg/kg) produced an ACTH response comparable to our results [258]. The increase of ACTH is seen within minutes, whereas the peak of the cortisol response is delayed 45-60 minutes [55, 258, 259]. This could perhaps explain why we did not observe a marked cortisol response. It is possible that anticipation anxiety and the barostat procedure itself caused an activation of the HPA-axis in some subjects. This is especially important since IBS patients could have more pronounced anticipatory anxiety as indicated by the ratings of perceived stress, as well as a study showing increased salivary cortisol levels in IBS patients before a rectal barostat procedure compared to the same time on an ordinary day [260].

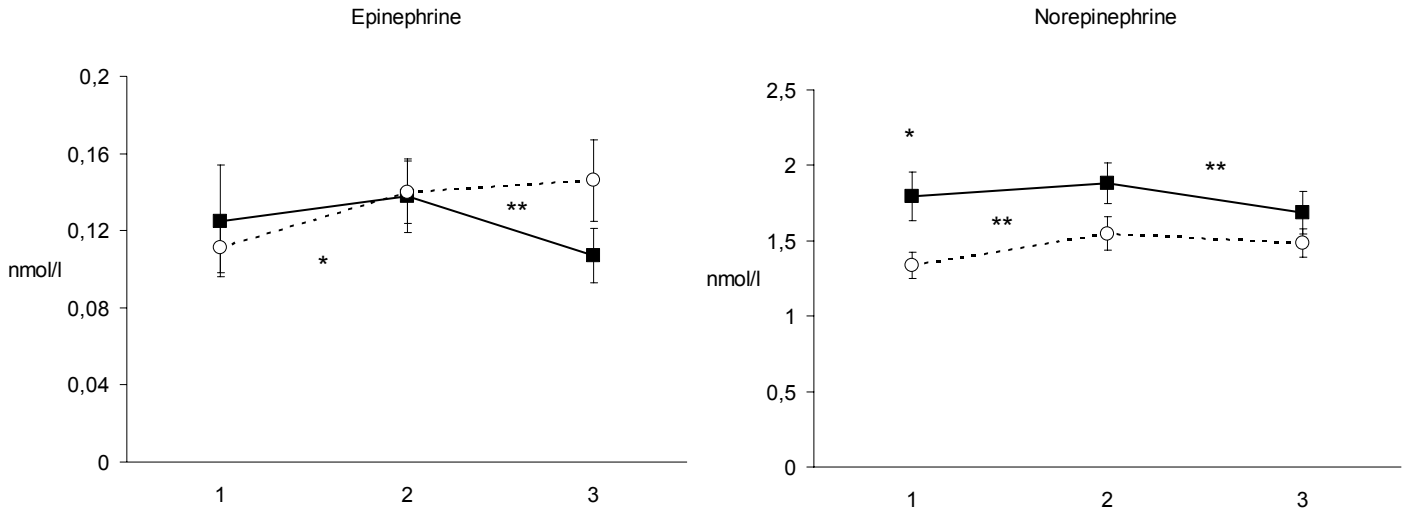


Figure 12. Levels of epinephrine and norepinephrine in patients (black square) and controls (white circle) at baseline (1), after stress (2) and at the end of the procedure (3).
* p<0.05 ** p<0.01

5. HYPERVIGILANCE (III)

5.1 Word recognition

During the word recognition test, GI symptoms was the category of words that most subjects recognized the fastest, 52% of the IBS patients and 40% of the patients with organic GI disease (NS). For IBS patients the mean time needed to identify GI symptoms was shorter than for non-GI symptoms ($p=0.05$), as well as positive ($p<0.0001$) and negative affects ($p=0.008$). Patients with organic GI disease were also significantly faster at recognizing GI symptoms compared to positive ($p=0.008$) and negative affects ($p<0.0001$), but not non-GI symptoms. IBS patients were, compared with the organic GI disease group, significantly faster at recognizing words representing GI symptoms (21±8 vs. 26±12 ms; $p=0.04$) and negative affects (27±19 vs. 34±18 ms; $p=0.03$). IBS patients also tended to be faster than patients with organic GI disease at identifying positive affects (24±10 vs. 29±17 ms; $p=0.08$) and non-GI symptoms (22±7 vs. 27±19 ms; $p=0.2$). (Figure 13)

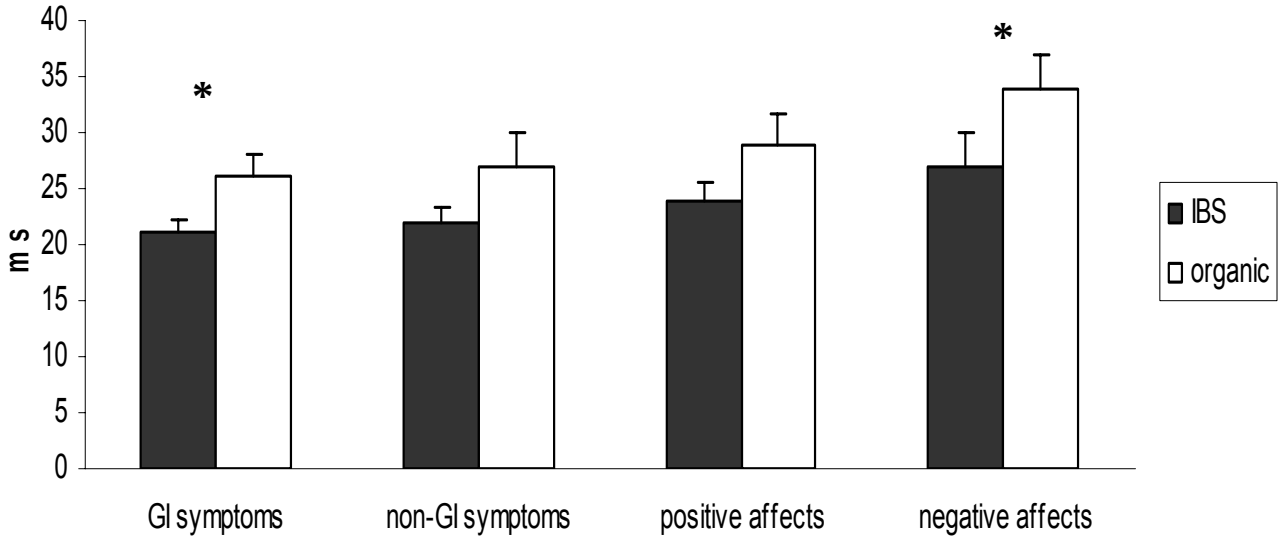


Figure 13. Results from the word recognition test (mean and SEM) in IBS patients (IBS) and patients with an organic GI disease (organic). * $p<0.05$

5.2 Word recall

There were no differences between IBS patients and patients with an organic GI disease regarding the numbers of remembered words from each category, and a similar pattern of recognizing more GI words than positive words, and more

positive than negative words was observed in both IBS patients (3.1±1.5 vs. 2.3±1.3 vs. 1.6±1.3; p=0.008; p=0.001) and organic GI disease patients (3.2±1.5 vs. 2.0±0.8 vs. 1.5±1.3; p<0.0001; p=0.01). However, IBS patients tended to recall more false GI words compared to patients with organic GI disease (1.3±1.1 vs. 1.0±1.2; p=0.06).

5.3 Correlations

Table 6 summarizes the correlations between the main test results (not including false and total word counts on the memory test) and age, GSRS score, as well as HAD scores for anxiety and depression. Fairly strong correlations were seen in both groups between the results on the word recognition test and age, showing that the older the subject, the more time they required to identify the correct words. The HAD scores for both anxiety and depression were correlated with the number of correctly remembered GI words by IBS patients on the memory test (r=.42; p=0.01 and r=.37; p=0.03). In IBS patients, the HAD anxiety scores also seemed to be correlated with the number of false GI words (r=.44; p=0.007) and total GI words (r=.56; p<0.0001). In organic GI patients, no correlations were seen with HAD depression scores, but HAD anxiety scores were correlated with the number of recalled negative affects, both correct (r=.41; p=0.01), false (r=.30; p=0.08) and total (r=.54; p=0.001). In IBS patients, but not in organic GI disease patients, the total number of recalled GI words on the memory test was also significantly correlated with the total GSRS score (r=.35; p=0.04).

Comments. There were no group differences on the word association test and both groups had similar overall test performances showing that they were homogenous regarding cognitive function. There were no significant group differences regarding demographic factors that could explain observed differences in tests results. Both groups also had similar HAD scores for both anxiety and depression, indicating that differences among the groups in psychological well-being was not a confounding factor. IBS patients had more severe symptoms according to the GSRS (median total score 57(47-64) vs. 38(27-49); p<0.0001). The GSRS assesses a range of symptoms from the entire GI tract, and IBS patients often report symptoms from several regions [8], which probably distinguishes them from patients with organic GI diseases. On the other hand, the GSRS is a self administered questionnaire and selective attention towards gastrointestinal symptoms would probably result in a higher score. The difference in symptoms could explain why IBS patients recalled more incorrect GI words from the memory task, especially since the total numbers of recalled GI words were correlated with the GSRS score. However, no such correlations were seen with the results from the word recognition task.

Table 6. Correlations between main test results (except false and total recalled words on the memory test) and age, HAD and GSRS in IBS patients (IBS) and patients with an organic GI disease (organic). * p<0.05 ** p<0.01 **** p<0.0001

	Age		**HAD anxiety**		**HAD depression**		**GSRS**	
	IBS	organic	IBS	organic	IBS	organic	IBS	organic
Word recognition								
Symptoms	r=.42*	r=.43**	r=-.13	r=-.36*	r=.12	r=-.24	r=-.02	r=-.11
GI symptoms	r=.36*	r=.43**	r=.05	r=-.23	r=.05	r=-.19	r=-.15	r=-.11
Negative affect	r=.48**	r=.44**	r=.08	r=-.23	r=.11	r=-.27	r=-.07	r=-.06
Positive affect	r=.25	r=.53****	r=.07	r=-.17	r=.06	r=-.04	r=.13	r=-.11
Word recall								
Correct GI words	r=.13	r=.10	r=.42*	r=.06	r=.37*	r=.04	r=.26	r=-.04
False GI	r=-.04	r=.15	r=.44**	r=.16	r=.13	r=.18	r=.09	r=-.13
Correct negative affects	r=.02	r=-.27	r=-.01	r=.41*	r=-.10	r=.07	r=.22	r=-.11
False Negative	r=.25	r=-.03	r=.41*	r=.30	r=.11	r=.09	r=.07	r=-.16
Correct positive affects	r=-.09	r=-.23	r=.11	r=.21	r=.06	r=-.15	r=.20	r=-.33*
False Positive	r=-.14	r=.29	r=.08	r=-.16	r=.09	r=-.31	r=.05	r=-.33

6. SMALL INTESTINAL BACTERIAL OVERGROWTH (IV)

6.1 Jejunal cultures

Seven patients (4%) had jejunal cultures showing bacterial overgrowth with $\geq 10^5$ cfu/ml of colonic bacteria. In addition, three patients had cultures with 10^5, $5x10^5$ and 10^6 cfu/ml of S. aureus. This was not significantly different from the control group where one healthy volunteer (4%) had a culture with $5x10^5$ cfu/ml of Enterococci. Patients (n=7) were treated with antibiotics (ciprofloxacin 500 mg twice a day for 10 days). Cultures after treatment showed decreased levels of bacteria in five patients, and four patients still fulfilled the standard definition for SIBO. Three patients reported ≥25% symptom improvement compared with that before treatment (Table 7).

The 95^{th} percentile in our control group was $5x10^3$ cfu/ml of any bacteria (respiratory and oral flora excluded). Mildly elevated counts of small bowel bacteria were more common in patients compared with controls. Cultures with $\geq 10^4$ cfu/ml were found in 24% of the patients compared with 4% in controls (p=0.02), and cultures with $\geq 5x10^3$ cfu/ml were observed in 43% vs. 12% (p=0.002).

Table 7. The amounts and different types of bacteria (cfu/ml) found in the cultures of the patients with SIBO before and after antibiotic treatment, as well as the effect of treatment (responder = ≥25% symptom improvement).

Subject	Before treatment	After treatment	Responder
IBS-C	$5x10^5$ E. coli	-	No
IBS-A	10^6 mixed G- flora 10^5 Enterococci $5x10^5$ Clostridium	10^6 Klebsiella 10^6 Enterococci $5x10^3$ S. aureus	No
IBS-C	10^6 mixed G- flora	$5x10^5$ Serratia 10^5 Enterococci	No
IBS-D	$5x10^5$ Enterobacter 10^5 S. aureus	-	Yes
IBS-D	$>10^7$ Klebsiella	$5x10^5$ Klebsiella	No
IBS-A	10^7 E. coli	10^3 E. coli	Yes
IBS-C	$>10^7$ Enterococci $5x10^5$ G- mixed flora	10^6 Klebsiella	Yes

6.2 Hydrogen breath tests

No healthy volunteer and only one patient had an abnormal GHBT possibly indicating SIBO (Figure 18). The culture from this patient showed only respiratory tract flora (10^3 cfu/ml). In the LHBT, seven patients (15%) and four controls (20%) had an abnormal test using the double peak definition (NS). Of these, three had bacterial counts of $5x10^3$, 10^4 and $5x10^4$ cfu/ml, respectively, but none had growth of colonic type bacteria $\geq 5x10^3$ cfu/ml. A 20 ppm H_2 rise within 90 minutes was observed in 35 % of the patients and 45% of the controls (NS). A 20 ppm H_2 rise within 180 minutes was observed in 78% of the patients and 70% of the controls (NS) (Figure 14).

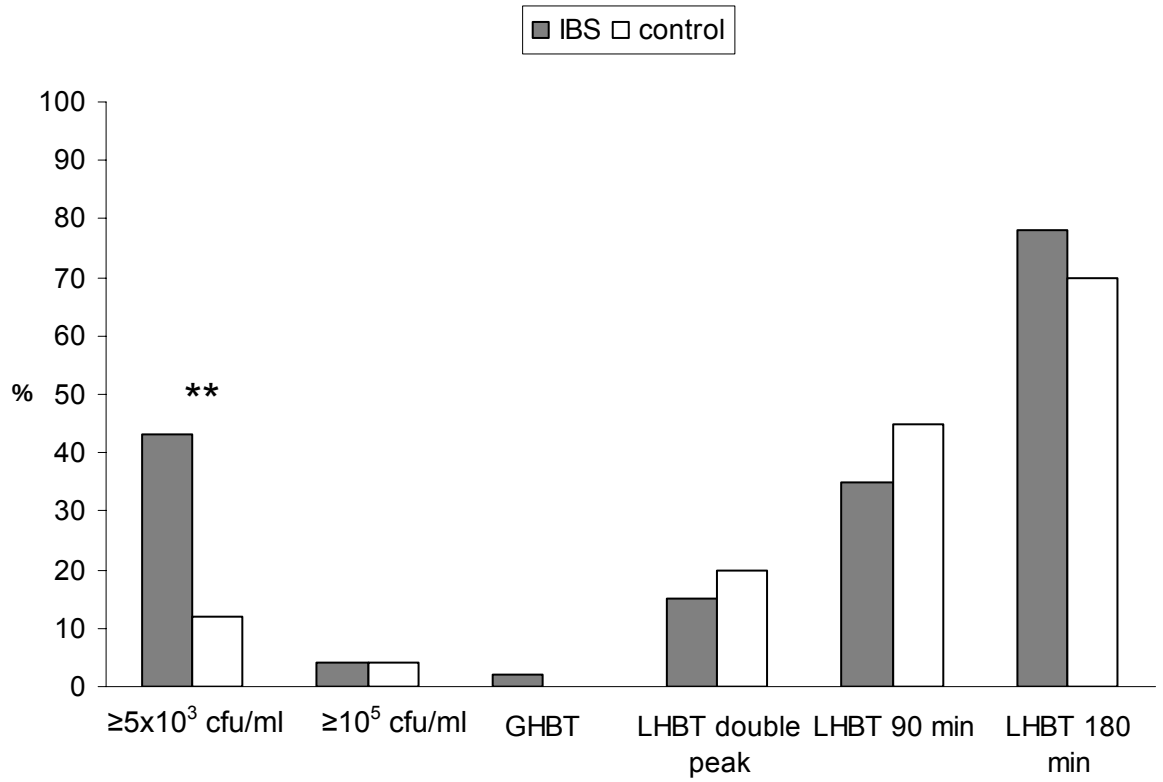


Figure 14. Proportions of subjects with tests indicating altered small bowel flora according to different tests; jejunal culture ($\geq 10^5$ and $\geq 5x10^3$ cfu/ml), glucose hydrogen breath test (GHBT), lactulose hydrogen breath test (LHBT) with two distinct H_2 peaks (double peak) or a single peak within 90 or 180 min. ** $p<0.01$

6.3 Small bowel motility

Conventional manometric evaluation discovered motor abnormalities suggesting enteric dysmotility (Table 2) in 86% (6/7) of the patients with culture proven SIBO ($\geq 10^5$ cfu/ml of colonic bacteria) compared with 39% of the patients without SIBO (p=0.02). A more extended analysis of small bowel motility showed that patients with culture proven SIBO tended to have fewer phase IIIs compared with those without SIBO (0.6 [0-1.8] vs. 1.2 [0-4] / 3 h; p=0.08). Otherwise, no group differences were observed for the remaining analyzed motility parameters, such as phase III duration, propagation velocity, MMC cycle length or motility index. The manometry results from the patients with mildly elevated bacterial counts ($\geq 5x10^3$ cfu/ml) were not different from those with lower bacterial densities.

6.4 IBS subgroups

Over all, bacterial counts did not differ significantly between the different IBS subgroups (figure 15). Of the seven patients with culture proven SIBO, three had IBS-C, two had IBS-D and two had IBS-A. Being a responder to treatment, as well as bacterial counts after treatment was also unrelated to IBS subtype.

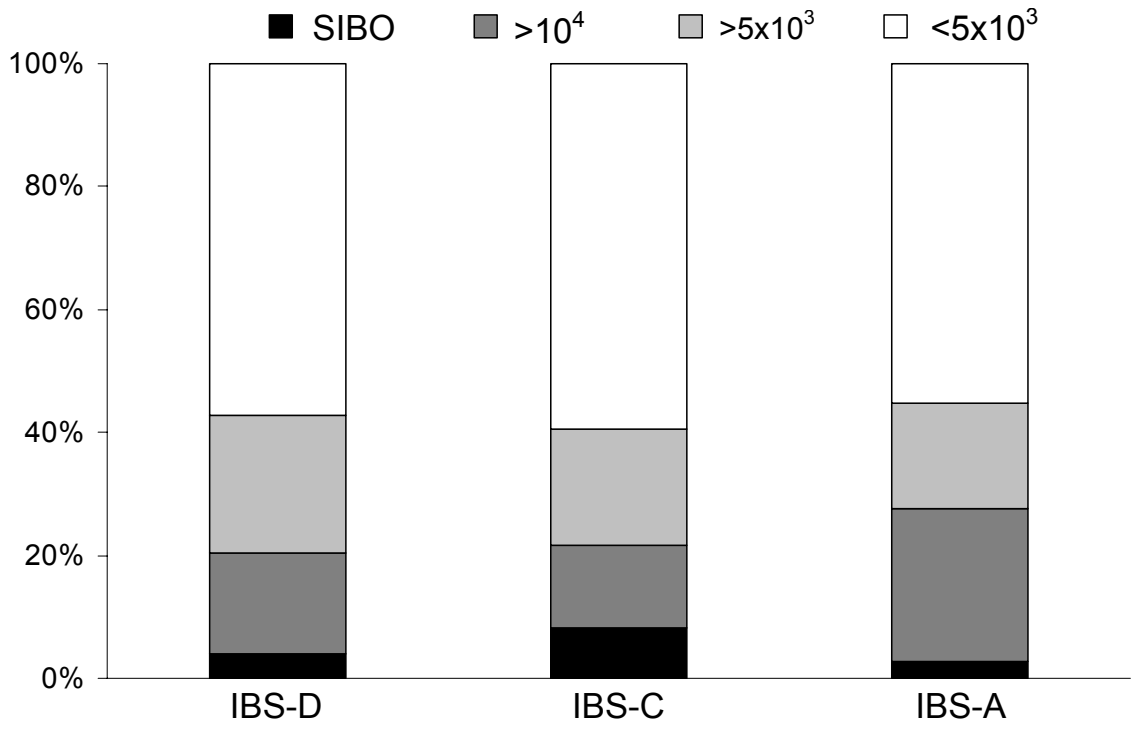


Figure 15. The proportions of patients with diarrhea predominant IBS (IBS-D), constipation predominant IBS (IBS-C), and alternating type IBS (IBS-A) with bacterial overgrowth (SIBO), $>10^4$ cfu/ml, $>5x10^3$ cfu/ml and $<5x10^3$ cfu/ml.

DISCUSSION

The main results in the present studies will be discussed briefly below and compared with other findings in the literature.

1. VISCERAL PERCEPTION (I, II)

We confirmed that as a group, IBS patients have lower sensory thresholds for rectal distensions in comparison with healthy controls, which has been demonstrated repeatedly [81]. Female IBS patients were found to have significantly lower sensory thresholds, which has been reported previously [98]. Interestingly, differences in brain activation have been seen between male and female IBS patients [151], where female patients showed greater activation of regions that could be part of a pain facilitation circuit, while males showed increased activity in regions that could be involved in pain inhibition. There is also evidence supporting a role for sex hormones on rectal sensitivity [261]. Based on these data, both central and peripheral factors seem to be of relevance for the gender effect on visceral sensitivity in IBS patients.

In the majority of studies sensory pressure thresholds have been used to define the presence or absence of visceral hypersensitivity. We chose to widen the concept of visceral hypersensitivity and also include viscerosomatic referral and perceived intensity during rectal distension, according to Mertz et al [82], and confirmed that altered rectal perception is common in patients with IBS [82]. Atypical or enlarged areas of viscerosomatic referral have previously been interpreted as evidence of increased or abnormal pain sensitivity in patients with IBS [82, 240, 262].

Similar to Kuiken et al [104] we found that the proportions of patients with different predominant bowel habits based on the Rome II criteria [17], were similar in groups with normal and altered rectal perception. Consequently, all subgroups had similar rectal sensory parameters, and we found no strong association between symptoms of constipation or diarrhea and the individual rectal sensory parameters, or the presence or absence of altered rectal perception. However, some previous studies have reported differences in sensitivity between diarrhea and constipation predominant IBS patients, although the results are divergent [99, 263, 264].

There are studies suggesting that reduced tolerance to rectal distension in IBS patients is primarily attributable to psychological rather than biological processes [65]. However, we could only demonstrate relatively weak associations between psychological symptom severity and rectal perception. This is probably due to patient selection. Psychological symptom severity in our studies was moderate and the patients were perhaps more representative of the IBS population in general and, may differ from IBS patients in highly specialized centers. Furthermore, a variety of other psychological factors not assessed in this study, such as illness specific coping, somatization, and/or gastrointestinal symptom-specific anxiety may influence perception of experimental pain stimulus as well as symptom reporting [59, 265, 266].

Our study on the effects of stress demonstrates that acute mental stress modulates rectal perception in both healthy controls and IBS patients. In controls sensory thresholds for rectal balloon distensions were higher during stress. However, in patients thresholds did not change significantly during stress, but were decreased after stress. Stress has been found to both increase [34]and decrease [46] rectal sensitivity to balloon distensions in IBS patients, whereas it seemed to have no effect on healthy controls. A more recent study using anorectal electrostimulation also reported enhanced rectal sensitivity in response to stress in IBS patients but not in controls [49]. Different methodological approaches in the studies mentioned must, however, be taken into account when interpreting these and our results.

Distraction during visceral distensions seems to decrease visceral sensitivity in healthy subjects [46, 89] but not in IBS patients [46]. Inconsistent results in the stress studies mentioned above may have occurred because stress coexisted with distraction in varying amounts. We did not observe any effects of stress on the sensory thresholds when the stress stimulus was administered immediately before the second distension sequences (II B). This indicates that the decreased rectal sensitivity observed in healthy subjects was probably due to an effect of distraction, most probably affecting descending inhibitory pathways [267]. In IBS patients, rectal sensitivity was unaltered during stress perhaps indicating that they could not suppress or “turn off” signals from their bowel during the mental task, as was observed in controls. Accordingly, IBS patients are presumed to have selective attention or hypervigilance regarding gastrointestinal

sensations [254]. Moreover, patients reported altered perception of stress and arousal which could indicate differences in cognitive processing of incoming stimuli [146, 147].

Patients were more sensitive during the last distension sequence (after two distension sequences and stress). This pattern was not seen when they underwent distension sequences without stress, or only two distension sequences with stress in between. Therefore, a sensitizing effect of repeated distensions [240] and/or response bias [255] is less likely to solely explain this. Instead a mechanism involving mast cell degranulation induced by mechanical stimulation and stress [185], leading to sensitization of mechanosensitive nerve endings [184, 268] could be a more probable explanation.

2. PSYCHOLOGICAL FACTORS (II, III)

IBS patients have previously been reported to have an enhanced emotional response to experimental stress [41, 130]. We observed that IBS patients, compared to healthy controls, reported higher levels of perceived stress overall, and the difference was even more pronounced during experimental stress. In contrast, IBS patients reported lower ratings of arousal before and after, but not during stress. Our findings are in line with reports from previous studies also reporting increased perception of stress but reduced arousal in IBS patients [34, 49, 98]. An enhanced emotional response to experimental stress corresponds to an increased susceptibility to stressful events in IBS [33].

Our investigation of hypervigilance is the first study to evaluate the presence of GI directed selective attention or hypervigilance in IBS patients compared with patients with organic GI disease. Compared to patients with organic GI disease, IBS patients showed signs of hypervigilance towards GI sensations and negative states of emotion as they more easily identified these types of words in an experimental situation using a tachistoscope. However, IBS patients also tended to be faster at recognizing words representing positive emotional states and non-GI symptoms, which could indicate that they were generally more vigilant or attentive, which has been suggested previously [153].

No major group differences were observed on our memory test, but IBS patients tended to recall more incorrect GI words, and also showed a correlation between the total number of recalled GI words and total GSRS score, representing overall symptom severity. Interestingly, positive correlations were seen between HAD scores for anxiety and depression and the number of recalled GI words in IBS patients but not patients with organic GI disease, in whom scores for anxiety were instead correlated with the number of recalled negative affects. These results are in line with those from previous assessments using the Visceral Sensitivity Index (VSI) showing a connection between gastrointestinal-specific anxiety and symptom severity [59, 60].

Our word recall task was very similar to that of a previous study reporting that IBS patients selectively recall words describing GI sensations compared to healthy controls and asthma patients [70]. In line with their results, we also found that IBS patients more easily remembered words representing GI symptoms compared with other word categories. However, IBS patients could be expected to have an increased familiarity with GI words, naturally facilitating the memory process, which is supported by the fact that we observed a similar memory pattern in patients with an organic GI disease, despite significantly lower GI symptom severity scores. A recent study, with a slightly different approach using a modified Stroop test, reported that IBS patients selectively process words representing GI symptoms compared with neutral words when they are presented subliminally. These results somewhat resemblances our findings in the word recognition task, even though their control group consisted of healthy volunteers, and they did not assess psychological factors or GI symptom severity. In line with one previous study [69], the IBS patients in our study did not selectively recall negative affects, and there was no difference compared with organic GI patients. Gomborone et al previously reported that IBS patients, similar to patients with depression, also selectively recognize emotionally negative words when compared with both healthy controls and patients with IBD [68]. However, the IBS patients in their study had a mean HAD score of 18.0, while the mean score in the IBD group was 10.9, compared to 11.0 and 9.2, respectively, in our study, which could be a probable explanation to our conflicting results.

3. NEUROENDOCRINE BLOOD SAMPLES (II)

We report that IBS patients have changes in their neuroendocrine stress response, as well as basal hormone levels. Basal levels of CRF were lower in patients than in controls but the meaning of this is unclear. IBS patients are instead proposed to have elevated levels of CRF [18, 269], at least in some cases, as seen in patients exposed to severe stress and/or with mood and anxiety disorders [160]. Most patients in our study did not fulfill criteria for depression or anxiety, which might explain why they demonstrated low CRF levels. Moreover, peripheral levels do probably not reflect central levels or signaling which also depends on receptor regulation. Instead, the observed stress induced increase in ACTH is an indirect measure of CRF activity, indicating an exaggerated response compared with healthy controls. This pattern has been reported previously in IBS patients following a CRF infusion [55, 258], indicating a sensitized HPA axis [270]. However contradictory results exist [259]. Despite an increase in ACTH we did not observe an increase in cortisol. There are studies showing decreased basal cortisol levels and blunted cortisol responses in IBS [53, 55, 259], but again, contradictory results exist [158, 258] [159]. Observed differences are probably due to differences in methodology as well as varying psychiatric co-morbidity. The different patterns of HPA axis dysregulation may develop in response to different types of pathological stress [18]. Despite conflicting reports, published studies support that the HPA axis in IBS patients may be altered.

In accordance with other investigators we found that IBS patients had elevated basal levels of norepinephrine [34, 158, 173] indicating increased sympathetic tone. However, in contrast to controls, IBS patients did not respond with increased epinephrine or norepinephrine levels during stress, but the reason for this is unclear.

4. GASTROINTESTINAL SYMPTOMS (I)

We have demonstrated that IBS symptom severity in general, and pain and bloating in particular, is associated with altered rectal perception, supporting the relevance of visceral hypersensitivity for gastrointestinal symptoms in IBS patients. Mertz et al found an association between perception thresholds and

temporal changes in symptom severity was found [82], but a recent study reported that symptoms remained stable over time despite a normalization of rectal perception [66]. A number of recent studies have reported relationships between visceral hypersensitivity and some, but not all, of the characteristic symptoms in IBS [104-107, 271]. However, many of these studies were limited by the use of non-validated questionnaires, a focus mainly on the difference between IBS-C and IBS-D, or by limited sample sizes. Furthermore, none of these considered psychological factors, which seems to be important, since this has been shown to affect symptom severity [272]. We found that patients with anxiety had higher severity scores for several IBS symptoms, and both patients with anxiety and depression more frequently reported moderately severe symptoms overall, but only anxiety was found to be independently associated with overall symptom severity.

We observed that pain and bloating were the gastrointestinal symptoms most clearly related to alterations in rectal perception. The association between abdominal pain and rectal hypersensitivity is in line with a large scale study on patients with functional dyspepsia, where gastric hypersensitivity was found to be associated with pain, but also with belching and weight loss [101]. Also, some previous studies in IBS patients have found an association between pain and rectal hypersensitivity [104, 107], whereas the association between bloating and rectal hypersensitivity has, to the best of our knowledge, not been reported before. However, it has been proposed that visceral hypersensitivity might be of relevance for this symptom, especially in IBS patients complaining of bloating without objectively demonstrable abdominal distension [273]. Since bloating is considered to be one of the most bothersome symptoms by many IBS patients [131, 274], a more thorough knowledge of the mechanisms behind this symptom is certainly needed. Based on our findings and some preliminary findings from the Manchester group [275], visceral hypersensitivity might be one of the factors responsible for this bothersome symptom, together with altered gas handling within the GI tract [137, 273].

There are reports of increased symptom severity in female compared with male IBS patients [77, 276, 277], which could partly be explained by greater visceral sensitivity in women as previously reported [98]. Our results also indicated increased visceral sensitivity and increased symptom severity in females, but the correlation between symptom severity and rectal sensitivity was similar in male

and female IBS patients. However, female gender was independently associated with overall gastrointestinal symptom severity.

To further strengthen the association between visceral hypersensitivity and gastrointestinal symptom severity in IBS, future studies should address the effect of therapeutic interventions on sensitivity and symptoms. Ideally, these should improve in parallel to support the concept of visceral hypersensitivity being an important factor for symptom severity. There are some treatment studies supporting an association between change in GI symptom severity and change in visceral sensitivity [103, 278-280]. However, there are also examples of studies where clinical improvement in IBS patients could be obtained with psychological [103] or pharmacological agents [281, 282], without change in colorectal sensitivity. Taken together, the available data, including our own results, implicate that the severity of gastrointestinal symptoms seems to be determined by several factors. We report evidence for contribution of visceral sensitivity, gender and anxiety, but there are most likely other contributing factors that were not evaluated in our study.

5. SMALL INTESTINAL BACTERIAL OVERGROWTH (IV)

Only a small subset of IBS patients (4%) in our study had small intestinal bacterial overgrowth as diagnosed by jejunal cultures showing growth of colonic bacteria in a concentration of $\geq 10^5$ cfu/ml. This was not different from control subjects without gastrointestinal symptoms. Culture of intestinal content is the gold standard for detecting bacterial overgrowth, and colonic bacteria in a density exceeding 10^5 cfu/ml is the definition most clearly associated with gastrointestinal symptoms [210-212]. No previous study has evaluated the growth of small bowel bacteria in a large sample of patients with IBS. However, one study of the micro-flora of the proximal jejunum included seven IBS patients as part of a mixed patient group with gastrointestinal symptoms compatible with SIBO. No major differences between patients and healthy controls were found, but specific information regarding the IBS patients was not provided [283]. The bacteria found in the cultures of subjects with bacterial overgrowth (7 patients and 1 control) were predominantly of the Enterobacteriacea species (E. coli, Klebsiella and Enterobacter) which can be

found at low counts in healthy individuals. The origin may be ingested food or oral carriage, and they should therefore be considered as transient [119].

It has been argued that direct culture is not sufficient to detect bacterial overgrowth, as sampling is restricted to one location in the proximal small bowel, missing isolated or more distal overgrowth [120]. Furthermore, it is a common belief that cultures are often false negative, especially concerning obligate anaerobes [206]. Studies using hydrogen breath tests, especially LHBT, have reported abnormal tests suggesting SIBO in as many as 84% of IBS patients [225-228, 284]. Studies on IBS patients showing symptom improvement after antibiotic treatment [223, 225, 226, 285], have been used to further support the conclusion that SIBO is a pathophysiological factor in IBS [120].

We investigated a subset of our subjects with hydrogen breath tests and did not find any support for a high prevalence of SIBO in IBS or a difference between patients and controls. These results are supported by others [136, 229, 230] and are very similar to those of Walters et al [230] reporting results in IBS patients and controls showing comparable proportions of abnormal LHBT and increasing proportions of abnormal tests using the criteria suggested by Pimentel et al [226]. A study combining the LHBT with scintigraphy, enabling anatomical location of the lactulose bolus [221], showed that a late H_2 rise is due to physiological variations in transit reflecting colonic fermentation and not due to bacteria in the small bowel [231]. Abnormal colonic fermentation has been reported in IBS [134] and therapies that modify the gut flora may improve symptoms in some patients [135, 224] by altering the colonic flora [223, 231]. This is further supported by a recent study showing symptomatic improvement in patients without evidence of SIBO, with a correlation between symptom improvement and hydrogen excretion [136].

Pimentel et al. has reported infrequent and short-duration phase III in patients with SIBO (according to LHBT) during a 4-hour small bowel manometry [286]. Impaired phase III of the MMC has been associated with colonization by Gram-negative bacteria [118, 208]. Our patients with culture verified SIBO tended to have fewer activity fronts, and a majority had enteric dysmotility as defined by the criteria in table 2. However, signs of dysmotility were also seen in several of the patients without SIBO, making it impossible to predict the presence of

overgrowth just by analysis of the small bowel motility patterns. The duration of our manometry recordings were for practical reasons relatively short, possibly diminishing the clinical value of our manometries, and a longer recording would possibly discover a larger proportion of patients with motor abnormalities. However, the lack of group differences is unlikely to be explained by this.

Few of the cultures were completely negative, possibly indicating contamination with oral flora which is known to be common [287]. However, cultures with bacterial densities of $\geq 5x10^3$ and $\geq 10^4$ cfu/ml were more common in patients compared with controls. The relevance of this finding is unclear since these counts are still within the normal range, and there were no differences when looking at only colonic bacteria. No manometric abnormalities were more common in the patients with mildly elevated bacterial counts. Except for impaired motility, failure of the gastric acid barrier is also known to predispose to bacterial colonization in the small bowel [119]. No medications known to affect the gastrointestinal tract were allowed within 48 hours before the study. However, prior to this we did not control for use of acid suppressive therapy or H. pylori status which can affect bacterial density [119]. The use of acid suppressive drugs could be more frequent in the IBS population considering the high overlap with functional dyspepsia [4]. This could be one explanation for alterations in the gut flora in patients with IBS, even though our study did not assess this in detail.

However, enteric bacteria in IBS deserves further investigations as it can induce mucosal inflammation, perhaps also with systemic effects [288, 289] which could explain some of the inflammatory changes observed in IBS patients. This needs to be assessed in further studies looking at both systemic and local inflammatory activity, including possible correlations to symptoms.

SUMMARY AND CONCLUSIONS

1. As a group, IBS patients are hypersensitive to rectal distensions, and altered rectal perception is not just a reflection of the patient's psychological state. Altered rectal perception is an important pathophysiological factor in IBS as it is associated with gastrointestinal symptom severity, particularly pain and bloating.

2. Gastrointestinal symptom severity is determined by several factors. Besides visceral sensitivity, other contributing factors include gender and anxiety.

3. Acute mental stress modifies visceral perception in both healthy controls and IBS patients. Controls were able to focus on the mental challenge which resulted in decreased visceral sensitivity, whereas the sensory thresholds remained stable in IBS patients, indicating selective attention towards gastrointestinal stimuli and/or inadequate descending pain inhibition pathways. A combination of stress and repeated rectal distensions resulted in increased rectal sensitivity in IBS patients. This together with an increased susceptibility to stress could explain why IBS patients often report a worsening of symptoms in relation to stressful events.

4. IBS patients demonstrate neuroendocrine alterations indicating a dysfunctional HPA stress response and increased sympathetic tone.

5. Small intestinal bacterial overgrowth, diagnosed with culture of jejunal aspirate, is not a common feature in IBS patients. However, the patients that have bacterial overgrowth have minor uncharacteristic small bowel motility alterations, which may be a predisposing factor in these cases.

6. Compared with controls, a significant subset of IBS patients tend to have increased counts of small bowel bacteria, which cannot be explained by altered small bowel motility. The clinical relevance of this finding is unclear.

7. Compared with patients with organic GI diseases, IBS patients are hypervigilant regarding information representing gastrointestinal sensation, and possibly also negatively charged information. The memory process of GI words in IBS patients is correlated with anxiety scores. These findings could be an expression of a psychosomatic component in the pathophysiology of IBS.

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APPENDIX A

Word recognition test

Symtom	GI symtom	Positiv affekt	Negativ affekt
influensa	magknip	harmonisk	likgiltig
astma	diarré	aktiv	orkeslös
tandvärk	halsbränna	avslappnad	uppjagad

Word recall test

GI symtom	Positiv affekt	Negativ affekt
gasbesvär	glad	rastlös
rapningar	pigg	oföretagsam
uppkördhet	uppmärksam	olycklig
hungerkänsla	rofylld	matt
lös avföring	skärpt	spänd
magont	belåten	jäktad
illamående	intresserad	nedslagen
sura uppstötningar	energisk	nervös
förstoppning	lugn	trött
trängande avföringsbehov	avspänd	bekymrad