PATHOPHYSIOLOGICAL FACTORS OF IRRITABLE BOWEL SYNDROME, AND THE EFFECTS OF PROBIOTIC SUPPLEMENTATION

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<tr>
<td>Bb12</td>
<td><em>Bifidobacterium lactis</em> subsp. <em>animalis</em> Bb12</td>
</tr>
<tr>
<td>Bb99</td>
<td><em>Bifidobacterium breve</em> Bb99</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>GCxGC-TOF</td>
<td>Gas chromatography/time-of-flight mass spectrometry</td>
</tr>
<tr>
<td>FISH</td>
<td>Fluorescent <em>in situ</em> hybridisation</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>HITChip</td>
<td>Human Intestinal Tract Chip</td>
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<tr>
<td>HRQOL</td>
<td>Health-related quality of life</td>
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<tr>
<td>IBD</td>
<td>Inflammatory bowel disease</td>
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<tr>
<td>IBS</td>
<td>Irritable bowel syndrome</td>
</tr>
<tr>
<td>IBS-A</td>
<td>IBS with alternating bowel habit</td>
</tr>
<tr>
<td>IBS-C</td>
<td>IBS with constipation</td>
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<tr>
<td>IBS-D</td>
<td>IBS with diarrhoea</td>
</tr>
<tr>
<td>IBSQ</td>
<td>The Irritable Bowel Syndrome Questionnaire</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>Lc705</td>
<td><em>Lactobacillus rhamnosus</em> Lc705</td>
</tr>
<tr>
<td>LGG</td>
<td><em>Lactobacillus rhamnosus</em> GG</td>
</tr>
<tr>
<td>PI-IBS</td>
<td>Post-infectious IBS</td>
</tr>
<tr>
<td>PJS</td>
<td><em>Propionibacterium freudenreichii</em> subsp. <em>shermanii</em> JS</td>
</tr>
<tr>
<td>PLS/DA</td>
<td>Partial least squares discriminant analysis</td>
</tr>
<tr>
<td>qPCR</td>
<td>Quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>rDNA</td>
<td>Ribosomal deoxyribonucleic acid</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short-chain fatty acid</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>UPLC/MS</td>
<td>Ultra performance liquid chromatography/mass spectrometry</td>
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<tr>
<td>5-HT</td>
<td>5-hydroxytryptamin</td>
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This thesis is based on the following original publications, which are referred to in the text by their Roman numerals I-V:


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ABSTRACT

Gastrointestinal symptoms and impaired quality of life caused by irritable bowel syndrome (IBS) affect up to one fifth of the adult population worldwide. IBS is reckoned as a multifactorial condition with dysregulation at the central and peripheral level, but the exact aetiological and pathophysiological factors of IBS are incompletely understood. No single deviance has been identified in IBS microbiota, but a growing body of evidence indicates the involvement of the microbiota in the pathophysiology of the condition. Clinical studies also suggest that supplementation with certain probiotics may be beneficial in IBS, but there is not enough evidence to make general recommendations. The aim of this thesis was to investigate microbiota- and mucosa-associated pathophysiological factors of IBS, and to evaluate the long-term effects of multispecies probiotic supplementation on symptoms, quality of life, intestinal microbiota and systemic inflammatory markers in IBS.

The intestinal microbiota composition in IBS patients and healthy control subjects was analysed by quantitative polymerase chain reaction (qPCR). Microbiota composition was found to be altered in IBS, as shown by significantly lower counts for the Clostridium coccoides and the Bifidobacterium catenulatum groups. Quantitative differences also appeared in subgroup analysis based on the predominant bowel habit: diarrhoea patients harboured significantly lower numbers of Lactobacillus spp. than the constipation-predominant patients, while higher counts for Veillonella spp. were detected in constipation-predominant patients compared to healthy controls. Analysis of mucosal biopsies by a high-throughput metabolomic approach revealed multiple differences between IBS patients and controls. The most prominent finding was an upregulation of specific lipid species, principally lysophosphatidylcholines and ceramides, in IBS.

The effects of multispecies probiotic supplementation with Lactobacillus rhamnosus GG, Lactobacillus rhamnosus Lc705, Propionibacterium freudenreichii subsp. shermanii JS, and Bifidobacterium breve Bb99 or Bifidobacterium animalis subsp. lactis Bb12 was evaluated in two long-term, randomised, double-blind, placebo-controlled trials. Compared to placebo, the probiotic supplementation significantly reduced the total symptoms of IBS. No effects on bowel habit were seen. Health-related quality of life (HRQOL) is reduced in patients with IBS in comparison with the Finnish population on the whole. The probiotic supplementation improved one IBS-specific domain of quality of life (bowel symptoms), whereas no effects were seen on other IBS-specific
domains or generic HRQOL. The probiotics had no major effects on the predominant microbiota as measured by qPCR, but a microarray-based analysis conducted on a subgroup of patients suggested that the probiotic consumption was associated with a stabilisation of the microbiota. No effects on serum sensitive C-reactive protein (CRP) or serum cytokines were detected.

Taken together, alterations in the microbiota composition and in the mucosal metabolite profile are potential pathophysiological factors of IBS. Multispecies probiotic supplementation alleviated the gastrointestinal symptoms of IBS. The probiotic supplementation improved the bowel symptoms domain of HRQOL, while no effects were seen on generic measures of quality of life. Probiotic supplementation in IBS is associated with a stabilisation of microbiota, but it does not influence systemic inflammatory markers.
1. INTRODUCTION

Irritable bowel syndrome (IBS) is a major cause of abdominal discomfort and gut dysfunction worldwide. The global prevalence of IBS is estimated to be between 10% and 20% of the adult population (for review, see Longstreth et al. 2006), which shows IBS to be the most frequent diagnosis in gastroenterology (Russo et al. 1999). IBS is a benign, non-life threatening condition, but its social and economic burden is significant. From the patient-perspective, IBS is a painful and distressing syndrome that impairs quality of life (Hahn et al. 1999; Gralnek et al. 2000; Amouretti et al. 2006), and increased health-care service utilisation and absence from work by IBS patients account for the considerable financial costs of the condition (for review, see Maxion-Bergemann et al. 2006).

The current view on IBS and other functional gastrointestinal (GI) disorders is that they are complex, biopsychosocial and multifactorial conditions with dysregulation at both the central and peripheral level (for review, see Drossman 2006). The precise aetiology and pathophysiology of IBS remain unexplained, but altered gut motility, enhanced visceral sensation, previous gastroenteritis, mucosal inflammation, abnormal brain-gut communication, psychosocial factors, food intolerance, intestinal microbiota and genetics are identified as key factors. Up-to-date management of IBS is based on a good physician-patient relationship, lifestyle recommendations and pharmacotherapy when required. However, many of the routinely used medications in IBS have only limited evidence for their efficacy, safety and tolerability (for review, see Heading et al. 2006), and almost every second patient taking prescribed medication considers it ineffective (Dapoigny et al. 2004).

Clinical trials suggest that certain probiotic bacteria or combinations of bacteria may have beneficial effects on the IBS symptom complex (Nobaek et al. 2000; Niedzielin et al. 2001; Kim et al. 2003; Kim et al. 2005b; O’Mahony et al. 2005; Whorwell et al. 2006). Though evidence is promising, no general recommendations on the use of probiotics in IBS can be given as yet. In addition to further clinical trials, data on the mechanisms of action of probiotics in IBS are needed. The safety of therapy is of particular importance in a non-life threatening condition such as IBS. Recently, the withdrawal and restricted reintroduction in the US of two serotonergic IBS medicines, alosetron and tegaserod (for review, see Longstreth et al. 2006), has triggered a useful discussion on the safety aspects of IBS therapy. Probiotics are considered safe (for review, see Borriello et al. 2003), and if future scientific data is able to substantiate their efficacy in IBS, they could be one treatment option to be considered.
The purpose of the present study was to investigate the pathophysiological factors involved in the development of IBS and to evaluate the effects of probiotic supplementation in IBS. The intestinal microbiota composition and the mucosal metabolic profile of IBS patients and healthy control subjects were compared. Additionally, the effects of multispecies probiotic supplementation on symptoms, quality of life, intestinal microbiota composition and stability, and systemic inflammatory markers in IBS were evaluated in long-term randomised, controlled trials.
2. REVIEW OF THE LITERATURE

2.1 Irritable bowel syndrome

2.1.1 Definition and disease description

IBS is defined as a functional bowel disorder in which abdominal pain or discomfort is associated with defecation or a change in bowel habit, and with features of disordered defecation (for review, see Longstreth et al. 2006). IBS belongs to the group of functional bowel disorders, which also includes functional bloating, functional constipation, functional diarrhoea and unspecified functional bowel disorder. Functional bowel disorders are one of a total of eight major domains in the large diagnostic group of functional gastrointestinal disorders (Table 1).

<table>
<thead>
<tr>
<th>Functional gastrointestinal disorders</th>
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<tr>
<td>A. Functional oesophageal disorders</td>
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<td>B. Functional gastroduodenal disorders</td>
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<td>C. Functional bowel disorders</td>
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<tr>
<td>D. Functional abdominal pain syndrome</td>
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<tr>
<td>E. Functional gallbladder and Sphincter of Oddi (SO) disorders</td>
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<td>F. Functional anorectal disorders</td>
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<td>G. Functional disorders: neonates and toddlers</td>
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<td>H. Functional disorders: children and adolescents</td>
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The main feature of IBS is recurrent abdominal pain or discomfort that is associated with disordered defecation and changes in bowel habit (for review, see Longstreth et al. 2006). Other symptoms characteristic for IBS and classified as supportive symptoms include: abnormal stool frequency (≤3 stools/week or >3/day), abnormal stool form, defecation straining, urgency, incomplete bowel movements, mucus and bloating. Based on the supportive symptoms, IBS can be subdivided into IBS with diarrhoea (IBS-D), IBS with constipation (IBS-C) and IBS with alternating bowel habit (IBS-A). The syndrome is chronic in nature, but associated with a good prognosis and no
increased mortality in long-term follow-up (Owens et al. 1995). Bowel habit in one patient often changes over time, and symptoms are typically fluctuating (Mearin et al. 2004). Extraintestinal comorbidity is often associated with IBS since specific disorders, such as headache, food allergy, musculoskeletal complaints, fibromyalgia and mood disorders, often overlap with IBS (Sperber et al. 1999; Vandvik et al. 2006; Hillilä et al. 2007). Moreover, IBS often coexists with other functional GI disorders (for review, see Whitehead et al. 2002). A recent study, however, indicates that there are no specific comorbid disorders that are associated with IBS (Whitehead et al. 2007). Instead, a general increase of disease incidence may be typical of IBS since the disorders most common in the general population (i.e. bacterial and viral infections, stroke) are the ones most common in IBS, too. Excess comorbidity may be due to hypervigilance in noticing somatic sensations and to having a lower threshold for consulting a physician.

2.1.2 Epidemiology

Irritable bowel syndrome was the seventh most prevalent diagnosis amongst all physicians in the US based on data collected in the 1970s and 80s (Everhart and Renault 1991). Similarly, more recent data from the US show that IBS accounts for 19% of diagnoses made by GI specialists, and is therefore the most common diagnosis in gastroenterology (Russo et al. 1999). The worldwide prevalence of IBS among adults is estimated to be 10-20% (Österberg et al. 2000; Thompson et al. 2002; Hungin et al. 2003; Hungin et al. 2005). Though the majority of prevalence figures are from Western populations, increasing data reveal that the syndrome is at least as prevalent in such non-Western societies as China, South Korea, India and Malaysia (Jain et al. 1991; Tan et al. 2003; Xiong et al. 2004; Park et al. 2007). It should, on the other hand, be taken into account that at least the urban areas of these countries are rapidly Westernising. The prevalence of IBS on the African continent is poorly known, but data from Kenya and Nigeria show prevalence figures between 8% and 33% (Lule and Amayo 2002; Okeke et al. 2005; Ladep et al. 2007). It is obvious that the definitions, diagnostic criteria and questionnaires employed influence the prevalence rates of IBS, and some studies have consequently shown prevalence figures below 10% (Kay et al. 1994; Andrews et al. 2005). When various diagnostic criteria were compared in a random adult population in Finland, the prevalence of IBS by Manning 2 or Manning 3 (Manning et al. 1978), Rome I (Thompson et al. 1992) and Rome II (Thompson et al. 1999) criteria was 16.2%, 9.7%, 5.6% and 5.1%, respectively (Hillilä and Färkkilä 2004b). The precise incidence of IBS is poorly known, but the incidence of the clinical diagnosis is estimated at 0.2% per year for each decade between 20 and 94 years (for review, see Camilleri et al. 2002b). This may, on the other hand, be an underestimate of the true incidence, since only one in four IBS patients consults a physician (Talley et al. 1995).

In general, there is a clear female predominance among IBS patients (Österberg et al. 2000; Thompson et al. 2002; Hungin et al. 2003; Hungin et al. 2005). Among those seeking health care services, women lead men in IBS diagnoses by a ratio of 2-4:1, whereas the distribution seems
to be less than 2:1 in prevalence data based on community surveys (for review, see Chang and Heitkemper 2002). Possible explanations for the gender differences include social and cultural issues, such as health care seeking behaviour, and sex-related physiological differences in bowel function and pain sensitivity. In contrast to Western societies, IBS seems to be noticeably more predominant among men than women in India (Jain et al. 1991). This has been suggested to reflect cultural differences in health care seeking and accessibility (for review, see Chang and Heitkemper 2002). IBS can affect people at any age, but the condition is most commonly diagnosed between ages 20 and 40, whereas organic GI diseases predominate in those over 60 (for review, see Bennett and Talley 2002). Irritable bowel syndrome may also appear in childhood, but data on prevalence are scarce. In some reports, IBS has been diagnosed in 6-14% of school children (Hyams et al. 1996) and 22%-45% of children aged 4-18 years presenting to tertiary care clinics (Walker et al. 2004; Caplan et al. 2005).

To summarise, the worldwide prevalence of IBS is about 10% to 20%, depending on the diagnostic criteria applied. The condition is more common in women than in men, and it is typically diagnosed in young adults.

2.1.3 Social and economic impact

Although IBS is non life-threatening, it is a painful, bothersome and distressing condition that interferes with daily life and creates considerable direct and indirect costs to society. Health-related quality of life (HRQOL) is significantly lower in subjects with IBS compared to healthy controls (Hahn et al. 1999; Gralnek et al. 2000; Akehurst et al. 2002; Amouretti et al. 2006). Overall, it appears that the more severe the IBS, and especially abdominal pain, the more impaired the quality of life (Coffin et al. 2004; Amouretti et al. 2006). Non-consulting IBS patients have better quality of life than health care consulting patients (Ringström et al. 2007), even though the former group also has slightly impaired HRQOL compared to healthy controls (Koloski et al. 2000). In comparison with other GI conditions, quality of life in IBS is significantly poorer than in those with reflux disease (Gralnek et al. 2000; Frank et al. 2002). Scores are also lower in selected domains compared to patients with diabetes, renal failure, asthma and migraine.

In general, compromised HRQOL appears to be a common observation in patients with GI complaints, and data from the Medical Outcomes Study show that this group of patients scored amongst the lowest of all conditions studied, including conditions with high mortality such as heart failure (Stewart et al. 1989). It should, nevertheless, be kept in mind that symptom severity is a strong predictor of HRQOL in IBS, and patients with mild IBS are thus not likely to score as low as subjects with serious diseases.

The economic impact of IBS arises from the direct consumption of health care resources as well as the loss of productivity by subjects suffering from IBS. Total health care utilisation and, as a result, direct health care costs are higher amongst subjects with IBS than in control
populations without IBS (Levy et al. 2001b; Akehurst et al. 2002), even though the majority of IBS patients do not seek medical help (Talley et al. 1995). A majority of the excess costs result from medical care not directly related to GI problems. A recent review estimates that the total direct costs per IBS patient per year is between USD 348 and USD 8,750, which correlates to a 1.1- to 6-fold higher cost compared to matched non-IBS control groups (Maxion-Bergemann et al. 2006). Compared to other GI problems, the costs per patient for IBS are lower than for inflammatory bowel diseases (IBD), but of the same magnitude as in gastro-oesophageal reflux disease (Levy et al. 2001b). The economic burden of IBS is partly due to loss of productivity: the average annual number of days off work due to IBS is calculated to be between 8.5 and 21.6 in the US and UK (for review, see Maxion-Bergemann et al. 2006). For comparison, this corresponds to an approximately 3-fold higher risk for absence from work in a US IBS population compared to non-IBS patients (Drossman et al. 1993).

Taken together, health-related quality of life is clearly impaired in IBS. The severity of abdominal symptoms correlates positively with HRQOL reduction. IBS causes considerable costs to society in the form of health care utilisation and absence from work.

2.1.4 Diagnosis

The diagnosis of IBS is based on identification of symptoms consistent with the condition and on case-by-case evaluated exclusion of other diseases with similar clinical features (for review, see Longstreth et al. 2006). The first symptom-based diagnostic criteria were published by Manning in 1978 (Manning et al. 1978), and they have been widely utilised in epidemiological and clinical studies. The multinational Rome working committee published its first criteria for IBS diagnosis in 1989 (Thompson et al. 1989), and has since then regularly published updated versions (Thompson et al. 1992; Thompson et al. 1999). The most current version, the Rome III criteria, were issued in 2006 (for review, see Longstreth et al. 2006). The Rome I, II and III criteria appear in Table 2.

Generally, few diagnostic tests are required for subjects who fulfil the symptom-based criteria for IBS and show no alarm signs (for review, see Longstreth et al. 2006). In this patient group, further testing usually provides little or no incremental value, and organic GI disease is rarely identified after symptom-based diagnosis of IBS (for review, see Cash et al. 2002). In cases where further investigations are considered justified, these should be based on the patient’s age, duration and severity of symptoms, psychosocial factors, alarm features and family history of GI disease. Fever, GI bleeding, weight loss, anaemia, family history of colon cancer and onset of symptoms in an older patient are considered alarm features that require closer investigation. The main diseases to be excluded in differential diagnosis are IBD in the young and colon cancer in older subjects in the Western world, and malabsorption or enteric pathogen infections in non-
Western populations (for review, see Camilleri et al. 2002b). The exclusion of coeliac sprue by serology and endoscopic biopsy is recommended, at least in individuals with diarrhoea (Spiegel et al. 2004). Furthermore, microscopic colitis can present with IBS-like symptoms and should consequently be excluded (Niemelä 2001). All in all, a symptom-based diagnosis of IBS is a “safe diagnosis”, where most subjects continue to be symptomatic during follow-up, but where the diagnosis is unlikely to be altered with time (Owens et al. 1995).
**Table 2. The Rome I, II and III criteria for IBS.**

### Rome I criteria (Thompson *et al.* 1992)

At least three months continuous or recurrent symptoms of:

1. Abdominal pain or discomfort which is:
   - Relieved with defecation
   - and/or associated with a change in frequency of stool
   - and/or associated with a change in consistency of stool, and

2. Two or more of the following, at least a quarter of occasions or days: altered stool frequency; altered stool form (lumpy/hard or loose/watery stool); altered stool passage (straining, urgency or feeling of incomplete evacuation); passage of mucus; and bloating or feeling of abdominal distension.

### Rome II criteria (Thompson *et al.* 1999)

At least 12 weeks, which need not be consecutive, in the preceding 12 months of abdominal discomfort or pain that has two of three features:

1. Relieved with defecation; and/or

2. Onset associated with a change in frequency of stool; and/or

3. Onset associated with a change in form (appearance) of stool

The following symptoms cumulatively support the diagnosis of IBS: abnormal stool frequency; abnormal stool form (lumpy/hard or loose/watery stool); abnormal stool passage (straining, urgency or feeling of incomplete evacuation); passage of mucus; and bloating or feeling of abdominal distension.

### Rome III criteria* (Longstreth *et al.* 2006)

Recurrent abdominal pain or discomfort** at least 3 days per month in the last 3 months associated with 2 or more of the following:

1. Improvement with defecation

2. Onset associated with a change in frequency of stool

3. Onset associated with a change in form (appearance) of stool

*Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis.

**Discomfort means an uncomfortable sensation not described as pain. In pathophysiology research and clinical trials, a pain/discomfort frequency of at least 2 days a week during screening evaluation for subject eligibility.
2.1.5 Pathophysiology

IBS is seen as a complex biopsychosocial condition in which a number of major mechanisms at the central and peripheral level interact (for review, see Drossman 2006). These include altered gut motility, enhanced visceral sensation, low-grade mucosal inflammation, abnormal brain-gut communication, psychosocial factors, food intolerance, intestinal microbiota and genetics (Figure 1). The contribution of microbiota to the pathophysiology of IBS will be discussed separately under Section 2.2. The prominence of any particular factor may vary from patient to patient.

Figure 1. Biopsychosocial model of IBS depicting the relationship between pathophysiology, symptom expression and clinical outcome (modified from Drossman 2006).
Abnormal motility

A variety of motor abnormalities have been described throughout the GI tract in IBS. Several distinct patterns of motility that vary in their intensity, type and location normally occur within the human GI tract. Overall, patients and healthy controls differ in quantitative, rather than qualitative, aspects of these motility patterns. In comparison with controls, IBS patients appear to have a delayed gastric emptying (Evans et al. 1997; Caballero-Plasencia et al. 1999), though not all studies support this (Acharya et al. 1983). Small bowel motility is altered in IBS in several ways: typical findings include a shorter duration of postprandial motor activity combined with episodes of clustered, recurring contractions correlating with abdominal pain (Kellow et al. 1990). Furthermore, abnormal duodenal pressure waves that correlate with symptom severity have been observed in IBS-D (Simrén et al. 2000). Small bowel transit time is significantly shorter in IBS-D and longer in IBS-C, compared to controls (Cann et al. 1983).

Both in the small and large bowel, IBS patients show an exaggerated response to a range of provocative stimuli. For instance, hypermotility of the small bowel in IBS patients is seen after infusions of cholecystokinin, a fatty meal, or ileal distension (Kellow et al. 1988), and colonic motor activity is exaggerated after a meal, an anger stressor or cholecystokinin (Welgan et al. 1988; Rogers et al. 1989; Chey et al. 2001). During cholecystokinin administration, abdominal pain coincided with >90% of the large-bowel high-amplitude contractions, suggesting that abnormalities in these vigorous colonic contractions may be one of the causes of pain (Chey et al. 2001). Basal non-stimulated large-bowel motility parameters, such as the myoelectric activity (Bueno et al. 1980) and sigmoid-colonic motor activity (Chey et al. 2001; Houghton et al. 2007a), also appear to be altered in IBS. Similarly to the small bowel transit times, the whole-gut and colonic transit times are shortened in IBS-D and prolonged in IBS-C (Cann et al. 1983; Chey et al. 2001; Dunlop et al. 2005). Besides aberrant gut transit, an impaired transit and tolerance of intestinal gas is suggested to be typical for IBS (Serra et al. 2001). This may be a consequence of altered gut motor function.

Although abnormal GI motor patterns are frequently observed in IBS, the mechanism behind such dysmotility is largely unknown. It has been proposed that disordered functioning of the enteric nervous system and serotonin signalling may be involved. This is corroborated by abnormal serotonin levels and turnover in IBS (Coates et al. 2004; Dunlop et al. 2005) and by the observation that endogenous serotonin (5-hydroxytryptamine, 5-HT) concentrations clearly correlate with the colonic activity index in IBS (Houghton et al. 2007a).

To summarise, various gut motor disturbances are present throughout the GI tract in IBS patients. These are likely to be important in determining the bowel habit, but their relevance in the generation of other GI symptoms is not as clear. The causes underlying these dysmotility patterns are thought to lie in alterations in the function of the enteric nervous system and in serotonin signalling.
Enhanced visceral perception

Visceral hypersensitivity, defined as an increased sensation in response to intestinal stimuli, is one of the most commonly found hallmarks of IBS and other functional gastrointestinal disorders (for review, see Delvaux 2002). The phenomenon is a frequent, but not constant, finding in IBS patients. Evidence of visceral hypersensitivity in humans is principally based on barostat tests that measure the pain sensation caused by gastrointestinal balloon distension. In these study settings, IBS patients perceive the first sensation of pain at lower volumes or pressures than healthy controls or display increased pain scores for a specific stimulus (Whitehead et al. 1990; Kilkens et al. 2005; Nozu et al. 2006; Zuo et al. 2006; Wilder-Smith and Robert-Yap 2007). Furthermore, visceral hypersensitivity in IBS patients and controls is different with regard to external stimuli: duodenal lipid infusion or stress enhances the gut sensitivity to a greater extent in IBS patients compared to healthy subjects (Simrén et al. 2001a; Posserud et al. 2004). As in the case of dysmotility, visceral hypersensitivity in IBS is not limited to the colon, and it should rather be considered as a generalised sensitisation of the GI tract (Trimble et al. 1995). Sensory thresholds may be interrelated with bowel habits, and evidence suggests that diarrhoea-predominant patients are more sensitive to distension compared with healthy controls, while patients with constipation tendency are equally or less sensitive compared with controls (Prior et al. 1990b; Zar et al. 2006; Zuo et al. 2006). Symptom type and intensity do not distinct hypersensitive and normosensitive IBS patients: subjects with enhanced visceral perception present comparable symptoms to those with normal sensitivity level (Kuiken et al. 2005). The threshold for somatic pain in IBS patients has been considered similar or even higher compared to healthy controls (Whitehead et al. 1990; Accarino et al. 1995; Iovino et al. 2006), but but there is also current data to the contrary (Wilder-Smith and Robert-Yap 2007).

The exact cause behind the enhanced visceral pain perception in IBS remains unknown, but studies point out that defects at both the peripheral and central level contribute to the phenomenon. Atypical gut parietal mechanoreceptors, possibly sensitised due to low-grade inflammation, may be key players. Indeed, mechanical balloon distension results in lower pain thresholds in IBS, whereas unspecific electrical stimulation of the receptors does not distinguish between cases and controls (Accarino et al. 1995). Based on studies using specific nitric oxide synthase inhibitors, recent data also advocate a role for intestinal nitric oxide in the development of visceral hypersensitivity in IBS (Kuiken et al. 2006). Mucosal mast-cell mediators histamine and tryptase from IBS patients are able to excite rat nociceptive visceral sensory nerves, while mediators from healthy volunteers are unable to do this, suggesting a role for mast cells in the occurrence of visceral sensitivity (Barbara et al. 2007). In addition to nitric oxide and mast cell mediators, prostaglandin E2 is also a potent modulator of intestinal sensitivity (Haupt et al. 2000). Central processing of visceral stimuli appears to be altered in IBS, as shown by brain imaging studies where different brain areas involved in pain processing are activated in IBS patients vs. controls following painful stimuli (Silverman et al. 1997; Bonaz et al. 2002; Song et al. 2006).
Taken together, visceral hypersensitivity is a key feature of IBS. Hypersensitivity occurs throughout the GI tract, whereas the threshold for somatic pain appears to be unaltered in IBS. The sensitivity defects are most likely a result of the sensitisation of nerve afferent pathways in the GI tract as well as unusual brain processing of nociceptive information.

Gastroenteritis and low-grade inflammation

The term “post-dysenteric irritable bowel syndrome” was introduced nearly 50 years ago by Chaudhary and Truelove (1962), who described that a subset of patients with “irritable colon syndrome” dated the onset of their symptoms to an attack of GI infection. Today, prospective studies show a 4%-31% incidence of post-infectious IBS (PI-IBS) following bacterial gastroenteritis (for review, see Spiller 2007). The relative risk for developing PI-IBS after acute gastroenteritis is estimated to be approximately 12 (Rodríguez and Ruigómez 1999). Most patients do not, however, develop PI-IBS, and the prevalence of IBS is not elevated in countries with high rates of enteric infection, which indicates that a range of risk factors are associated with PI-IBS. Factors associated with increased vulnerability for post-infectious IBS include a longer duration of the initial diarrhoeal disease (Neal et al. 1997; Wang et al. 2004), female gender (Neal et al. 1997; Gwee et al. 1999) and psychological factors, e.g. depression and the presence of adverse life events in the previous three months (Gwee et al. 1999; Dunlop et al. 2003b). An age of over 60 years, in contrast, correlates with a protective effect against developing PI-IBS (Neal et al. 1997). Besides gastroenteritis, the use of antimicrobials for GI infection or other conditions also serves as an independent risk factor for developing functional bowel symptoms (Maxwell et al. 2002).

Even though IBS patients have no identifiable inflammation on routine inspection of intestinal biopsies, there is an increasing amount of data showing that both post-infectious and unselected IBS patients display a low-grade mucosal inflammation (Table 3). An array of immunological cells and markers have been examined, and the levels of lymphocytes, mast cells and enterochromaffin cells are repeatedly found to be altered in the mucosa of IBS patients. Moreover, evidence of systemic immune activation is accumulating, as elevated levels of plasma pro-inflammatory interleukin (IL)-6 and IL-8 have been observed in IBS (Dinan et al. 2006). Peripheral blood mononuclear cells (PBMCs) of IBS patients also produce higher amounts of tumour necrosis factor-α, IL-1β, IL-6 and IL-12 in vitro than cells from healthy controls (O’Mahony et al. 2005; Liebregts et al. 2007b). These findings are supported by genotyping studies indicating that IBS patients are predisposed towards a pro-inflammatory cytokine profile (Gonsalkorale et al. 2003; van der Veek et al. 2005). Gut barrier function is tightly interlinked with inflammation since permeability is increased both by inflammatory cytokines and by bacterial gastroenteritis (Chavez et al. 1999; Spiller et al. 2000). In parallel with studies on cytokine imbalance in IBS, evidence is mounting that there may be an IBS subgroup with increased mucosal permeability (Spiller et al. 2000; Marshall et al. 2004; Dunlop et al. 2006).

Animal studies have given insight into the mechanisms by which prior inflammation may
provide ongoing neuromuscular dysfunction in the gut. While an immunological response is initially required for the induction of neuromuscular changes, it is not necessary for the maintenance of these changes after an infection (for review, see Khan and Collins 2006). Instead, the state of persistently altered GI physiology, observed for instance in IBS, is actively maintained by the production of mediators like transforming growth factor-β and prostaglandin-E2 by intestinal muscle cells (Barbara et al. 2001; Akiho et al. 2005). The involvement of inflammation in neuromotor function is also supported by the finding that, both in normal and in inflamed mucosa, immunocytes lie in intimate contact with nerve fibres, providing an anatomical basis for a functional interplay between immune cells and the enteric nervous system (Stead et al. 1987).

To summarise, gastroenteritis is one recognised risk factor for developing IBS. Routine inspection does not show mucosal inflammation in IBS, but specific analysis of particular immune cells and markers reveals subtle inflammatory alterations in patients. Especially gut lymphocytes, mast cells and enterochromaffin cells are frequently elevated in IBS. Studies in animal models indicate a causal relationship between mucosal inflammation, altered GI motor function and visceral hypersensitivity, with several molecular mechanisms involved in the phenomenon.
Table 3. Studies describing low-grade mucosal inflammation in IBS.

<table>
<thead>
<tr>
<th>Inflammatory markers</th>
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<tr>
<td><strong>Type of IBS</strong></td>
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<tr>
<td>PI</td>
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<td>IBS-D</td>
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<td>PI</td>
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<td>Unselected</td>
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<td>IBS-D</td>
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</table>

Unselected = A mixed group of IBS patients, unselected with regard to bowel habit, and with non post-infectious aetiology; PI = post-infectious IBS; IEL = intraepithelial lymphocyte; MC = mast cell; EC cells = enterochromaffin cells; ↑ = increased in IBS vs. controls; ↔ = unchanged in IBS vs. controls; ↓ = decreased in IBS vs. controls; NA = not assessed.
Brain-gut communication

Perturbations in the brain-gut axis are increasingly recognised as underlying pathophysiological factors in functional GI disorders. The brain-gut axis is considered a model describing the complex bidirectional neural pathways connecting the brain with the gut neuroendocrine centres, the enteric nervous system and the immune system (for review, see Aziz and Thompson 1998). Disturbed brain-gut communication is not an independent pathophysiological factor in IBS, as the brain-gut axis is the key regulator of e.g. gut motor activity and visceral perception, both known to be changed in IBS. Altered communication between the central nervous system (CNS) and the gut is thus tightly interlinked with other established pathophysiological phenomena in IBS.

Overall, brain-gut interactions play an important role in the regulation of many vital functions both in health and in disease. Digestive functions, including motility, secretion, mucosal transport and blood flow are coordinated by the CNS in a top-down manner (for review, see Costa and Brookes 1994). Conversely, signals from the gut play a role in reflex regulation and pain perception in a bottom-up manner (for review, see Randich and Gebhart 1992). The CNS functions as a “filter” with regard to the perception of peripheral afferent signals, and the brain-gut communication is for the most part not consciously perceived: only very few of the signals reaching the brainstem and thalamus are consciously perceived in the cortex (Rosen et al. 1996). The brain-gut axis is stimulated by various stressors, as shown by the fact that acute intestinal inflammation is associated with central sensitisation (Liebregts et al. 2007a), whereas psychological events alter gut function (Mönnikes et al. 1993; Nakade et al. 2007).

Symptoms of IBS are thought to be produced by primary alterations in the CNS, by primary alterations in the periphery, or by a combination of both. Evidence for central alterations in IBS comes from studies using functional brain imaging techniques where different brain areas involved in pain processing are activated in IBS patients vs. controls following painful rectal stimuli (Silverman et al. 1997; Bonaz et al. 2002; Song et al. 2006). Moreover, IBS patients appear to have an altered processing of anticipated pain, since sham distension resulted in similarly low pain scores in IBS patients and healthy subjects, but a differential brain activation pattern (Song et al. 2006). Central processing may also distinguish between different bowel habits, as demonstrated by lower parasympathetic tone and higher autonomic nervous system balance in constipation-predominant vs. diarrhoea-predominant patients (Heitkemper et al. 2001). The role of the central and the autonomic nervous system in IBS pathophysiology is supported by findings of sleep disturbances, and especially an enhancement of rapid eye movement sleep in IBS (Orr et al. 1997). Studies presenting elevated levels of corticotropin-releasing and adrenocorticotropic hormones as well as alterations of the visceral perception in IBS patients following mental stress also point towards disturbed brain-gut interaction (Posserud et al. 2004).

Numerous neurotransmitters are involved in the regulation of the brain-gut axis. Amongst those, serotonin is of particular interest since its effects on gut motility, secretion and sensation as well as on cognition and mood make it of paramount relevance in IBS pathophysiology (for review, see Mawe et al. 2006). Acute lowering of serotonin synthesis reduces the threshold for
painful stimuli and induces a depression-like memory bias both in IBS patients and in control subjects, illustrating the essential role of serotonergic modulation in the brain-gut axis (Kilkens et al. 2004b). In contrast, increased 5-HT activity induced by citalopram is associated with enhanced affective memory performance biased towards positive words (Kilkens et al. 2005). Several elements of serotonin signalling are altered in IBS. The numbers of enterochromaffin cells (the source of 5-HT in the bowel) have been shown to be increased, especially in the post-infectious subgroup (Spiller et al. 2000; Dunlop et al. 2003b). Elevated plasma 5-HT concentrations have been observed in an unselected IBS population (Bearcroft et al. 1998) and in post-infectious IBS (Dunlop et al. 2005), while the opposite is seen in constipation IBS (Dunlop et al. 2005). Moreover, decreased 5-HT levels and turnover, lower 5-HT transporter mRNA concentration, and increased expression of p11, a protein affecting serotonin metabolism, are shown in IBS (Coates et al. 2004; Dunlop et al. 2005; Camilleri et al. 2007). Another important group of molecules affecting the brain-gut are prostaglandins, which appear to exert their effects via peripheral mechanisms in the GI tract rather than via central mechanisms (Dajani et al. 2003).

**Taken together, the brain-gut axis describes the complex and bidirectional interplay between the GI tract and the CNS. Several neurotransmitters, for instance serotonin, modulate the brain-gut interaction. Functional communication between the brain and the gut is crucial for a number of physiological events, including digestion and motility. IBS patients demonstrate alterations in peripheral signalling, central processing of signals and in serotonin metabolism, and these factors are believed to participate in symptom generation.**

**Psychosocial factors**

Psychosocial factors are not believed to cause IBS, but they exert a strong influence on some patients. Psychological stress and emotions produce GI symptoms in almost all individuals, but IBS patients appear to be particularly susceptible to an exacerbation of symptoms by stress (Whitehead et al. 1992). IBS patients also consistently report more lifetime and daily stressful events than control groups (Whitehead et al. 1992; Locke et al. 2004). Psychological symptoms and comorbidity frequently exist in IBS, especially in those seeking health care. Commonly encountered conditions in these patients include depression, somatisation, anxiety disorder, panic disorder and phobic anxiety disorder (Walker et al. 1990; Solmaz et al. 2003). Importantly, psychological comorbidity may be restricted to consulting patients, since community surveys indicate that non-consulting individuals with IBS have psychosocial characteristics similar to the general population (Drossman et al. 1988). A recent prospective study also shows that psychosocial factors are independent risk factors for the development of IBS in a population previously not suffering from the condition (Nicholl et al. 2007). Physical, sexual and psychological abuse as a child or as an adult is associated with the development of IBS, although the issue is somewhat controversial. Certain current studies report a positive association between abuse and IBS and
other functional GI disorders (Koloski et al. 2005; Perona et al. 2005), whereas others have been unable to confirm the findings (Hobbis et al. 2002). It has been proposed that the possible link between abuse and functional GI disorders may be in somatisation and a general tendency to report numerous bodily symptoms (Creed et al. 2005). It should be kept in mind that these phenomena appear to be more common at referral practices compared to primary care or non-clinical settings (for review, see Drossman et al. 1995).

The impact of psychosocial factors lies in their effect on illness experience and treatment outcome. On one hand, psychosocial status is a determinant for health care seeking behaviour (Koloski et al. 2003; Ringström et al. 2007). A topical Finnish study, on the other hand, suggests that health care utilisation is associated with abdominal symptoms rather than psychiatric comorbidity (Hillilä et al. 2007). Psychosocial status is also associated with reduced illness behaviour and coping strategies (Jones et al. 2006).

To summarise, psychological comorbidity and symptoms often coexist with IBS, particularly in referral centres. Psychosocial factors are not, however, considered to be a causative factor of IBS. In clinical practice, attention should be paid to psychosocial factors, since they are an important determinant of illness experience and treatment outcome.

Food intolerance
Food intolerance, sometimes also called food sensitivity, can be subdivided into food allergy, which is mediated by immunological mechanisms, and food intolerance mediated by non-immunological mechanisms (for review, see Ortolani and Pastorello 2006). Both allergic and non-allergic adverse reactions to food have been suggested as playing a role in the development of IBS. A major proportion of IBS patients experience a postprandial worsening of GI symptoms (Ragnarsson and Bodemar 1998; Simrén et al. 2001b), and many patients report adverse reactions to certain food items as well as the use of exclusion diets (Dainese et al. 1999; Monsbakken et al. 2006). Foods rich in carbohydrates and fat as well as coffee, alcohol and spicy foods are frequently considered by IBS patients to be symptom-provoking (Simrén et al. 2001b). On the other hand, perceived food intolerance is not a phenomenon limited to IBS patients since approximately one fifth of the UK population consider themselves food intolerant, though only one to two percent get a positive reaction in a double-blind placebo-controlled food challenge (Young et al. 1994). The reason for the perceived food sensitivity in IBS is likely to be multifactorial, and an involvement of postprandial visceral sensitivity and motor response, carbohydrate malabsorption, IgG antibodies to food, abnormal fermentation and psychological factors has been proposed (for review, see Simrén et al. 2007). The dominant mechanism is, however, suggested to be an exaggerated postprandial GI sensory and motor response.

Positive food skin-prick test is more often encountered in IBS patients compared to controls (Jun et al. 2006). However, specific foods reported to cause intolerance rarely correlate with the
skin-prick test results (Dainese et al. 1999) or with serum immunoglobulins (Ig) (Monsbakken et al. 2006). Moreover, one study shows that challenge with foods reported as symptom-provoking and giving positive skin-prick test results and/or serum IgG antibodies did not exacerbate GI symptoms, suggesting that food allergy is not involved in IBS (Zwetchkenbaum and Burakoff 1988). An earlier study has, in contrast, confirmed the presence of food intolerance in IBS by a similar food challenge trial (Jones et al. 1982). More recent studies have focused on IgG antibodies, which characteristically represent a more delayed response compared with IgE antibodies. Diets where foods giving elevated IgG titres are excluded appear to reduce GI symptom severity and improve rectal compliance, i.e. the ability of the gut to adapt to imposed distension (Atkinson et al. 2004; Zar et al. 2005). It may also be that the local, mucosal response, rather than the systemic response, is of greater importance in the possible food intolerance linked to IBS. Methods investigating the mucosal, immunologic response to antigen or food challenge have turned out to be promising in detecting intestinal food allergy in cases where a skin-prick test or serum IgE test is negative (Bischoff et al. 1997; Lin et al. 2002).

Among adverse reactions to foods in IBS, carbohydrate malabsorption is repeatedly suggested as a typical feature. Some studies indicate malabsorption of fructose and sorbitol in functional GI disorders as assessed by breath hydrogen tests (Rumessen and Gudmand-Høyer 1988; Fernández-Banares et al. 1993), while other data suggest similar absorption capacity in IBS cases and controls (Nelis et al. 1990). It seems that even though malabsorption of fructose and/or sorbitol is as common in healthy individuals as in IBS patients, GI symptoms are more easily produced in IBS (Nelis et al. 1990; Fernández-Banares et al. 1993). The appearance of symptoms after a fructose-sorbitol load in subjects with IBS does not, however, correlate with the presence of hypersensitivity or dysmotility (Evans et al. 1998). The association between lactose maldigestion and IBS seems to be similar to that with fructose/sorbitol: lactose maldigestion is as common in IBS as amongst the general population, but perceived lactose intolerance is significantly higher in IBS (Vesa et al. 1998).

To summarise, current data on food intolerance or allergy in IBS are inconsistent, and it appears that specific intolerance is be uncommon. Instead, an enhanced perception of postprandial events appears to account for the exacerbation of symptoms following meals.

Genetics
Aggregation of symptoms of abdominal pain or bowel disturbances has been described in relatives of IBS patients, and familial studies suggest that a modest genetic contribution is involved in IBS pathophysiology (for review, see Saito et al. 2005). Findings comparing monozygotic and dizygotic twin pairs are controversial as a number of studies show a genetic factor in aetiology (Levy et al. 2001a; Bengtson et al. 2006; Lembo et al. 2007), while others fail to demonstrate a heredity component (Mohammed et al. 2005). It also appears that children of parents with IBS
tend to use health care more frequently for GI complaints than children of parents not suffering from the condition (Levy et al. 2000).

Genotyping studies reveal that IBS, and particularly a diarrhoea-predominant bowel habit, may be associated with different polymorphisms in the serotonin transporter gene (Kim et al. 2004; Pata et al. 2004; Yeo et al. 2004; Park et al. 2006c; Saito et al. 2007). Moreover, a pharmacogenetic study suggests that polymorphisms of the serotonin transporter gene predict the response to 5-HT₃ antagonist therapy (Camilleri et al. 2002a). Besides putative serotonin transporter polymorphisms, little is known about other genetic variants that may affect expression of irritable bowel syndrome. In addition to genetic inheritance, the tendency of IBS to run in families could also be due to social learning (Levy et al. 2000). Parental modelling and reinforcement of illness behaviour seem to contribute to the development of IBS with an effect at least as large as heredity.

2.1.6 Current treatment of IBS

General aspects

Up-to-date treatment recommendations for irritable bowel syndrome are presented in the Rome III consensus document for functional bowel disorders (for review, see Longstreth et al. 2006). A caring and therapeutic physician-patient relationship is one of the cornerstones of treatment. A confident diagnosis, assurance of the benignity of the condition, an explanation for why symptoms occur and suggestions on how to cope with them are key elements. The patient's need for reassurance and knowledge is reflected in health care utilisation, as those patients feeling insufficiently informed will have more health care visits (O'Sullivan et al. 2000b). The type and the severity of symptoms along with possible psychosocial features determine the management regimen (for review, see Longstreth et al. 2006). Overall, patients have more confidence in the effectiveness of education and advice about lifestyle modification than in pharmacotherapy (Whitehead et al. 2004). The lifestyle recommendations most frequently given by physicians include dietary and exercise advice.

Dietary recommendations

Regular and unhurried meals are recommended, and unnecessary restriction of the diet should be avoided. Exclusion of lactose is unlikely to reduce symptoms (Parker et al. 2001), whereas a reduced intake of fructose and sugar alcohols may be helpful (Nelis et al. 1990; Fernández-Banares et al. 1993). Individualised dietary manipulation based on IgG antibodies cannot be recommended until further data are available (for review, see Longstreth et al. 2006), although there are promising figures suggesting that patients may benefit from these exclusion diets (Atkinson et al. 2004; Zar et al. 2005). Increased dietary fibre is inexpensive and safe, and a majority of physicians recommend higher fibre intake as a primary treatment means in IBS patients (Mitchell and Drossman 1987). The clinical evidence for fibre in IBS management is inconclusive, since placebo-controlled trials indicate that the efficacy of bran is no better than placebo (Lucey et al.
1987; Snook and Shepherd 1994; Rees et al. 2005), except for constipation (Cann et al. 1984a). Flatulence may even exacerbate with fibre (Snook and Shepherd 1994). On the other hand, it should be remembered that fibres differ in their properties. Generally, soluble fibre appear to be beneficial, while symptom worsening is typically associated with insoluble fibre (for review, see Bijkerk et al. 2004). Despite this, fibre sources containing insoluble fibre (e.g. psyllium husk) are also recommended in the Rome III criteria (for review, see Longstreth et al. 2006).

Pharmacological treatment
Pharmacotherapy is not necessary for all IBS patients, but when needed, it should be directed towards the predominant symptom (Table 4). Significant methodological inadequacies were recognised in early IBS trials, and the classic publication by Klein (1988) almost two decades ago concludes that not a single study offers convincing evidence that any therapy is effective in treating IBS. In spite of improvement in the design of more recent trials, a current meta-analysis sums up that many of the routinely used therapies for IBS are of dubious efficacy (Lesbros-Pantoflickova et al. 2004). Limited evidence for the efficacy, safety and tolerability of current therapies is particularly relevant for Europe, since alosetron and tegaserod, classified as the best documented medications, are not available in Europe (for review, see Heading et al. 2006). Almost half of the patients taking prescribed medication consider it ineffective (Dapoigny et al. 2004). Finnish guidelines for IBS management follow the Rome criteria, though novel serotonergic medicines are not yet available (Silvennoinen 2002; Hillilä and Färkkilä 2004a).

Diarrhoea is treated primarily with the synthetic opiate derivative loperamide on an as-needed basis. There is good evidence from placebo-controlled studies that loperamide is effective in reducing bowel frequency and loose stools, but it does not improve abdominal pain or global IBS symptoms (Cann et al. 1984b; Hovdenak 1987; Efskind et al. 1996). Cholestyramine is a second-line treatment if bile acid malabsorption is suspected to play a role in diarrhoea (Sinha et al. 1998). Alosetron, a selective 5-HT3 receptor antagonist, can decrease pain, urgency and stool frequency as well as improve global status in women with IBS-D (Camilleri et al. 2001; Chey et al. 2004; Lembo et al. 2004; Krause et al. 2007). Severe adverse effects (ischaemic colitis and constipation) led to its withdrawal from the market, but it was reintroduced in the US with its indication restricted to women with severe IBS-D. A meta-analysis concludes that alosetron positively influences global symptoms, pain and discomfort in non-constipated women, but that the role of alosetron in male patients is unclear (Cremonini et al. 2003).
Table 4. Pharmacotherapy for IBS as advised by the Rome III criteria (modified from Longstreth et al. 2006).

<table>
<thead>
<tr>
<th>Predominant symptom</th>
<th>Medication</th>
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<tr>
<td>Diarrhoea</td>
<td>Loperamide</td>
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<td></td>
<td>Cholestyramine resin</td>
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<tr>
<td></td>
<td>Alosetron&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Constipation</td>
<td>Psyllium husk</td>
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<tr>
<td></td>
<td>Methylcellulose</td>
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<tr>
<td></td>
<td>Calcium polycarbophil</td>
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<tr>
<td></td>
<td>Lactulose syrup</td>
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<tr>
<td></td>
<td>70% sorbitol</td>
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<tr>
<td></td>
<td>Polyethylene glycol 3350</td>
</tr>
<tr>
<td></td>
<td>Tegaserod&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>Smooth-muscle relaxant&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Tricyclic antidepressants</td>
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<td></td>
<td>Selective serotonin reuptake inhibitors</td>
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<sup>a</sup>For severe IBS, women. Available only in the US.
<sup>b</sup>For IBS, women. Unavailable in the European Union.
<sup>c</sup>Selective antimuscarinic agents unavailable in the US.

The recommended treatment for IBS with constipation is increased dietary fibre or commercial fibre analogues. Fibre decreases the whole-gut transit time (Cann <i>et al.</i> 1984a), but it does not improve pain or diarrhoea (Lucey <i>et al.</i> 1987; Snook and Shepherd 1994; Rees <i>et al.</i> 2005). High intake of fibre may also cause increased flatulence in some subjects, and an individual approach to the increase in fibre should be applied (Snook and Shepherd 1994). A number of studies demonstrate the efficacy of the partial 5-HT<sub>4</sub> agonist tegaserod in improving stool frequency and consistency as well as in reducing pain and bloating in IBS-C (Müller-Lissner <i>et al.</i> 2001; Layer <i>et al.</i> 2005; Tack <i>et al.</i> 2005). Similarly to alosetron, tegaserod was withdrawn from the market due to severe adverse effects, but later reintroduced in some markets with tightened indication for women only. Various smooth-muscle relaxants are frequently prescribed for predominant abdominal pain. A meta-analysis evaluates the available evidence for their use in IBS as “inconsistent results from inadequately controlled clinical trials or poor quality cohort studies” (Lesbros-Pantoflickova <i>et al.</i> 2004). Not all smooth-muscle relaxants are effective in IBS, but some agents, including cimetropium bromide (Centonze <i>et al.</i> 1988; Dobrilla <i>et al.</i> 1990), octylonium bromide (Battaglia <i>et al.</i> 1998; Glende <i>et al.</i> 2002) and peppermint oil (Liu <i>et al.</i> 1997; Cappello <i>et al.</i> 2007), appear to be effective, bearing in mind, however, the inadequacies in study designs. Serotonergic modulators, including antidepressants and selective serotonin reuptake inhibitors, may exert their action at both the peripheral and central level of the brain-gut axis since they possess psychotropic properties as well as neuromodulatory and analgesic
properties (for reviews, see Clouse 2003; Kilkens et al. 2003). The evidence for the use of tricyclic antidepressants in IBS is favourable (Greenbaum et al. 1987; Tanum and Malt 1996; Drossman et al. 2003), but due to serious side-effects, these medicines should only be used in subjects with severe abdominal pain.

Investigational medicines
Several compounds affecting various sites of the brain-gut axis are currently being investigated for their potential to alleviate symptoms of IBS. In addition to alosetron and tegaserod, other 5-HT₃ and 5-HT₄ antagonists and agonists have been evaluated. Cilansetron, a 5-HT₃ antagonist, has shown in phase III clinical trials effects similar to alosetron (Chey and Cash 2005). A full 5-HT₄ agonist prucalopride accelerates transit time in healthy subjects and in patients with functional constipation (Bouras et al. 1999; Bouras et al. 2001). Clonidine, an α₂ adrenergic agonist, relaxes the bowel, reduces pain sensation and has shown clinical promise in IBS-D (Camilleri et al. 2003). The κ-opioid agonists fedotozine and asimadoline may reduce pain hypersensitivity in IBS (Dapoigny et al. 1995; Delgado-Aros et al. 2003). The neurokinin-1 and -3 receptor antagonists appear to play a role in disrupted motility and visceral sensitivity, and preliminary experimental and clinical data are promising (Lørdal et al. 2001; Okano et al. 2002). One randomised, double-blind, placebo-controlled trial indicates that pregabalin, an α₂δ ligand used for neuropathic pain, has favourable effects on visceral hypersensitivity by increasing rectal sensory thresholds (Houghton et al. 2007b).

Psychological treatment
Psychological treatments may be useful in patients with moderate to severe symptoms when medical treatments have failed or when there is proof that stress or psychological factors exacerbate symptoms (for review, see Drossman et al. 2002). Interpersonal psychotherapy, relaxation/stress management and cognitive behavioural therapy are the most common approaches that have been considered in IBS. Critical evaluation of the efficacy of psychological treatments is hampered by the fact that trials cannot be double-blind, even though certain studies have included some form of control or placebo group. Generally, psychological treatment is time-consuming and expensive, and in many circumstances it is unavailable, which further limits its employment in IBS management (for review, see Longstreth et al. 2006). Hypnotherapy, one form of cognitive behavioural therapy, is one of the most widely studied psychological treatments in IBS. Hypnosis can improve GI symptoms and quality of life (Houghton et al. 1996; Gonsalkorale et al. 2002) as well as rectal hypersensitivity (Prior et al. 1990a; Lea et al. 2003) in IBS. However, a recent Cochrane review (Webb et al. 2007) identified 25 studies on hypnotherapy in IBS, but included only four in the final review after excluding methodologically inadequate trials. Though hypnosis was found to be superior to usual medical management in those patients who fail standard therapy, the low number of high-quality trials does not allow for any conclusions to be drawn.
Taken together, the treatment of IBS demands a good and caring physician-patient relationship combined with reassurance and education. Individual dietary and lifestyle modifications frequently alleviate the symptoms, and many patients do not need medical treatment. When required, pharmacotherapy should target the predominant symptom. Diarrhoea is treated primarily with loperamide, constipation with dietary fibre or commercial fibre analogues and pain with smooth-muscle relaxants and serotonergic agents. Overall, the efficacy of treatments is limited.

2.2 Human intestinal microbiota

With an approximated area between 250 and 400 m², the GI tract constitutes one of the largest body surface areas. It is inhabited throughout by a complex microbial ecosystem, and the number of microbial cells in the human body is estimated to be 10 times the number of eukaryotic cells (for review, see Savage 1977). Based on findings from cultivation-based studies, it was estimated that about 400 species make up the GI tract microbiota (Moore and Holdeman 1974). However, novel data incorporating information from genome-based approaches suggest that the microbiota includes over 1,200 distinct micro-organisms (for reviews, see Zoetendal et al. 2006; Rajilić-Stojanović et al. 2007). Importantly, the description of the human microbiota diversity is an ongoing process, and complete coverage has not yet been achieved.

2.2.1 Composition and functions

The intestinal microbiota in healthy adults is generally considered highly individual and stable over time (Zoetendal et al. 1998; Vanhoutte et al. 2004). Environmental factors, e.g. antimicrobials, dietary modifications, certain diseases and psychological stress can, however, alter an otherwise stable microbiota (for review, see Thompson-Chagoyán et al. 2007). The individuality of microbiota is emphasised in a recent study showing that 62% of the 395 bacterial phylotypes identified were novel when the microbiota of three subjects were characterised (Eckburg et al. 2005). Furthermore, the inter-individual variation was far greater than variability between one subject’s faecal and mucosal communities. The great majority (97%) of the inhabitants of the GI tract ecosystem are strict anaerobes, while the rest are aerobes or facultative anaerobes (for review, see Noverr and Huffnagle 2004). A limited number of bacterial groups make up the dominant microbiota. Based on molecular methods, the two dominant groups, *Clostridium coccoides-Eubacterium rectale* (28%) and *Clostridium leptum* (25%), represent more than half of the total bacteria (Lay et al. 2005). Within the *C. leptum* group, *Faecalibacterium prausnitzii* is the most commonly found cluster. The most abundant bacterial groups after *C. coccoides* and *C. leptum* are *Bacteroides* (9%), *Bifidobacterium* (4%) and *Atopobium* (3%). The *Lactobacillus-Enterococcus* group represents approximately 2% of the total bacteria.

Bacterial colonisation varies strongly in different parts of the GI tract. The oral cavity is
a heterogeneous environment with different bacterial groups predominating in the mucous membranes, teeth and tongue, and with approximately $10^7$ to $10^9$ bacteria/g in saliva (for review, see Liljemark and Bloomquist 1996). Due to the acidic environment, levels below $10^4$ cfu/g are usually found in gastric contents (for review, see Salminen et al. 1998). The small intestinal bacterial numbers range from $10^4$ to $10^7$ cfu/g, with the highest levels found close to the ileocaecal region. The colon is by far the most vastly populated section, since it harbours as much as $10^{10}$ to $10^{12}$ bacteria/g. The composition of the microbiota differs along the length of the GI tract, and similarly cross-sectionally, since different bacterial communities inhabit the lumen and the mucosa (for review, see Noverr and Huffnagle 2004). It is noteworthy that most of the available knowledge on intestinal microbiota composition is based on faecal samples, and the mucosa-associated and faecal bacterial communities appear to differ from each other (Zoetendal et al. 2002; Ouwehand et al. 2004; Eckburg et al. 2005; Lepage et al. 2005). Strictly speaking, intestinal microbiota and faecal microbiota are thus not synonyms. In this study, however, the term intestinal microbiota is used as a general term for the GI tract ecosystem regardless of the sample material where the microbes have been detected. The mucosal microbiota is considered fairly stable in different locations throughout the colon (Zoetendal et al. 2002; Lepage et al. 2005).

The intestinal microbiota has a considerable influence on host health and disease, both in the GI tract and systemically (for review, see Guarner 2006). Metabolic, protective and trophic effects are considered the key functions of the gut microbial community (Figure 2). Much of the evidence of the role of microbiota in physiology derives from experiments in germ-free animals. These experiments demonstrate major differences in e.g. organ weights, cardiac output, body temperature, intestinal morphology and immunological parameters between conventional and germ-free animals (for review, see Tannock 2001). The metabolic activity of the microbiota results in the production of various potentially beneficial or harmful end products. Carbohydrate fermentation produces short-chain fatty acids (SCFAs), which act as an energy source for colonocytes and regulate water and electrolyte absorption (Scheppach 1994). Among SCFAs, butyrate has gained most attention, and it appears to have several advantageous effects on colonic function, though a topical review calls for more clinical evidence (Hamer et al. 2007). The protective functions of the microbiota refer to the barrier effect, also known as colonisation resistance, which the commensal bacteria provide against potential pathogens (van der Waaij 1982). Considering trophic functions, interactions between the microbiota and the immune system are the subject of considerable investigation. In addition to results from germ-free animals, clinical data in allergic infants imply that gut bifidobacterial composition plays an essential role in the maturation of the immune system (He et al. 2001; Ouwehand et al. 2001; Suzuki et al. 2007). Even if bifidobacteria appear to be central for immunity, the physiological relevance of individual bacterial groups is generally not well-established as studies on germ-free animals primarily demonstrate a difference between the presence and absence of the microbiota. Further indirect evidence on the significance of the microbiota for human
health and physiology is provided by studies showing an aberrant microbiota composition in certain diseases, for example, IBD (for review, see Seksik et al. 2006).

**Figure 2. Main functions of the intestinal microbiota (modified from Guarner 2006).**

Taken together, the characterisation of the microbiota is an ongoing process, and the number of micro-organisms inhabiting the GI tract is currently estimated to be more than 1,200. The adult microbiota is considered highly individual and rather stable over time. The microbiota has several essential functions affecting both the GI tract and systemic physiology.

### 2.2.2 Overview of methods for analysing microbiota

Until recent years, cultivation-based methods were the most widely applied means of studying the GI microbiota (for review, see Furrie 2006). The advantages of plate count analysis include its broad availability, its relative inexpensiveness as well as its potential for quantifying bacterial populations. One serious limitation of plating is that its applicability is restricted to cultivable organisms, whereas it has been proposed that only between 30% and 40% of the GI tract microbiota can be cultured by currently available methods (Hayashi et al. 2002). The development of culture-independent, molecular methods built on microbial nucleic acid sequence information has
contributed to a dramatic increase in the knowledge of intestinal microbiota diversity.

It is well-established that the bacterial ribosomal deoxyribonucleic acid (rDNA) gene contains both highly conserved regions, which are identical for all bacteria, as well as variable regions that can be used for identification of bacterial species or groups, and in some cases even strains (for review, see Furrie 2006). Particularly the 16S ribosomal ribonucleic acid (rRNA) gene sequence is commonly used to identify bacteria. Examples of methods taking advantage of this phenomenon are qPCR, temperature/denaturing gradient gel electrophoresis, fluorescent in situ hybridisation (FISH) and DNA microarrays. Depending on the method used, the composition, genomic diversity or activity of the microbiota can be investigated (for review, see Zoetendal et al. 2006). Each method has its own advantages and disadvantages, but in relation to culturing, the main advantages of molecular biology are the detection of uncultivable species, no requirement for anaerobic handling and, with some methods, the possibility to analyse a large amount of bacterial targets simultaneously (for review, see Furrie 2006). Among the shortcomings are that not all of the methods are quantitative and that a number of techniques are still rather expensive. One field of molecular biology where upcoming research activity is predicted is metagenomics, analysis of the collective genome or “microbiome” of a defined microbial population. As for intestinal microbiota, metagenomics has provided estimates that the combined number of micro-organisms in the GI tract totals up to $10^{13}$ to $10^{14}$ (Gill et al. 2006).

### 2.2.3 Intestinal microbiota in IBS

#### Microbiota composition

The microbiota of IBS patients has been compared to that of healthy subjects both directly, by using conventional culturing methods or DNA-based methods, and indirectly, by measuring microbiota-derived metabolites. An early study applying culturing methods demonstrates significantly lower numbers of coliforms, lactobacilli and bifidobacteria in IBS patients (Balsari et al. 1982). Another study strengthens findings on reduced counts of Bifidobacterium spp. and additionally shows an increase in Enterobacteriacea (Si et al. 2004). In contrast to Balsari et al. (1982) a recent study, similarly using plating methods, found a significantly higher number of coliforms in IBS (Mättö et al. 2005). No differences in the mean cultivable numbers of bacteroides, bifidobacteria, spore-forming bacteria, lactobacilli, enterococci or yeasts were found, but an increased aerobe:anaerobe ratio could be seen.

Amongst culture-independent methods, FISH analysis indicates a higher total bacterial population in IBS patients vs. healthy subjects (Swidsinski et al. 2005). The percent-guanine-plus-cytosine method has revealed community-level differences between healthy controls and IBS patients subtyped according to bowel habit (Kassinen et al. 2007). Subsequent identification of the alterations showed that patients and controls differed particularly in Collinsella aerofaciens, Clostridium cocleatum -related and Coprococcus eutactus -related phylotype assays. A microarray-based analysis with the Human Intestinal Tract Chip (HITChip) has revealed that the total microbiota
of IBS patients is more heterogeneous than that of healthy controls (Rajilić-Stojanović 2007). The microbiota in IBS is also characterised particularly by lower levels of *Bacteroides* and increased levels of the bacilli order. The different IBS subtypes appear to differ with regard to microbiota. Moreover, the predominant bacterial populations in IBS patients show more temporal instability compared to healthy controls (Mättö *et al.* 2005; Maukonen *et al.* 2006) as well as more changes in the clostridial population (Maukonen *et al.* 2006). An abnormal pattern of faecal short-chain fatty acids (Treem *et al.* 1996), characterised by lower levels of total SCFA, acetate and propionate, and higher levels of n-butyrate, also points towards an imbalanced microbiota in IBS.

**Small intestinal bacterial overgrowth**

The possibility that small intestinal bacterial overgrowth could explain the occurrence of IBS symptoms, particularly bloating, is supported by abnormal lactulose breath tests in IBS as well as by an improvement of GI symptoms after eradication of the overgrowth. According to some studies, 30 to 80% of IBS patients may have small intestinal bacterial overgrowth as diagnosed by lactulose breath test (Pimentel *et al.* 2000; Pimentel *et al.* 2003a) or glucose breath test (Lupascu *et al.* 2005). However, not all studies support bacterial overgrowth in IBS (Walters and Vanner 2005). Based on whole-body calorimeter analysis, lactulose ingestion causes increased gas excretion in IBS patients (King *et al.* 1998). Randomised, double-blind, placebo-controlled studies show an improvement in GI symptoms and a normalisation of the lactulose breath test after eradication of overgrowth by antimicrobials (Pimentel *et al.* 2003a; Pimentel *et al.* 2006a). In addition to elevated hydrogen following a lactulose load, methane production has also been associated with IBS, mainly with the constipation-predominant subtype (Pimentel *et al.* 2003b; Chatterjee *et al.* 2007). This is consistent with findings showing that methane is able to slow gut transit time (Pimentel *et al.* 2006b).

To summarise, no single deviance has been identified in IBS microbiota, but various quantitative and qualitative alterations in the gut bacterial composition have, nonetheless, been indicated by a range of techniques. Consequently, an increasing amount of evidence supports the hypothesis of microbiota involvement in IBS pathophysiology.

**2.2.4 The association between microbiota alterations and gut dysfunction**

Though the microbiota appears to play a part in IBS, it is unclear whether alterations in the microbiota are a cause of IBS or a result of e.g. disturbed gut motility induced by the syndrome. Commensal bacteria are central to the development and maintenance of normal gut sensory and motor functions, as is shown by studies in germ-free animals (Husebye *et al.* 2001). An altered microbiota may disrupt this cross-talk between bacteria and the epithelium, and as a result
contribute to sensory-motor dysfunction in IBS (for review, see Barbara et al. 2005). Inflammation induced by bacteria is another mechanistic framework for how commensal microbes may induce IBS symptoms. Studies on IBD imply that the microbiota is able to trigger and perpetuate mucosal inflammation (Linskens et al. 2001). Experimental models subsequently demonstrate that a causal relationship exists between mucosal inflammation, altered GI motor function and visceral hypersensitivity (for review, see Collins 1996). Bacterial lipopolysaccharide is another putative agent involved in the development of visceral hypersensitivity (Coelho et al. 2000). Metabolites produced by the intestinal microbiota may also be active participants in symptom generation since SCFAs have been suggested as playing a part in gut motor functions (Husebye et al. 2001; Fukumoto et al. 2003).

2.3 Probiotics

2.3.1 Definition and health effects

An expert group appointed by the World Health Organization and the Food and Agricultural Organization of the United Nations defined probiotics as ‘live microorganisms which when administered in adequate amounts, confer a health benefit on the host’ (WHO 2001). According to this report, potential probiotic micro-organisms should possess certain fundamental properties: they should survive through the GI tract, be capable to proliferate in the gut, and be safe for use. The most frequently used probiotics are lactobacilli or bifidobacteria, but other micro-organisms from the genera Propionibacterium, Bacillus, Escherichia, Enterococcus and Saccharomyces are also being increasingly used and assessed for future use. Studies investigating the properties of probiotics have revealed that each bacterial strain is unique, and that health effects are consequently strain-specific (for review, see Saxelin et al. 2005). As for clinical health effects, probiotics are, nonetheless, often viewed upon as a group. In the above analyses, the relief of lactose intolerance symptoms, the prevention and treatment of acute diarrhoea in children and the reduction of the risk of antibiotic-associated diarrhoea are generally considered to be the best-documented effects (for reviews, see Ouwehand et al. 2002a; Saxelin et al. 2005; Doron and Gorbach 2006). Interesting fields include the relief of atopic diseases in children and the reduction of the risk of atopic diseases, where particularly Lactobacillus rhamnosus GG (LGG) has demonstrated promising results (Majamaa and Isolauri 1997; Isolauri et al. 2000; Viljanen et al. 2005b; Kalliomäki et al. 2007).

The mechanisms by which probiotics exert their health effects are not fully understood, but the promotion of the host defence systems, modulation of the immune system as well as the competitive exclusion of harmful microbes are thought to be key elements (for review, see Saxelin et al. 2005). However, strains differ in their characteristics and it is therefore likely that selected mechanisms are also strain-specific. The awareness of the strain-specificity of probiotics has paved the way for the idea to combine specific probiotic strains in order to obtain additive
effects. A multispecies probiotic is defined as ‘containing strains of different probiotic species that belong to one or preferentially more genera’ (for review, see Timmerman et al. 2004). It has been suggested that multispecies probiotics may in some conditions be more efficient than single strains due to their synergistic or additive effects as reflected in e.g. enhanced intestinal adhesion, the production of a greater variety of antimicrobial compounds and the ability to colonise several niches of the GI tract. As an example, certain species of Bifidobacterium, Lactobacillus and Streptococcus have been shown to display different effects on gut motility (Massi et al. 2006), indicating that a combination of different species could give a more versatile effect on motility disturbances. Moreover, each strain in a multispecies probiotic comprising LGG, L. rhamnosus Lc705 (Lc705), Propionibacterium freudenreichii subsp. shermanii JS (PJS) and Bifidobacterium breve Bb99 (Bb99) or B. animalis subsp. lactis Bb12 (Bb12) is adherent to mucus in vitro (for review, see Collado et al. 2006), but the presence of LGG more than doubles the adhesion of Bb12 and certain Propionibacterium strains (Ouwehand et al. 2000; Ouwehand et al. 2002b).

A European expert group concludes in its review that the ingestion of lactobacilli and bifidobacteria is considered to be safe in both healthy and immunosuppressed individuals (Borriello et al. 2003). Infections caused by these bacteria are exceptionally rare, and identified cases have been isolated in severely ill patients (Presterl et al. 2001; De Groote et al. 2005; Ledoux et al. 2006). Importantly, data from Finland show that a significant growth in the consumption of LGG at the population level has not led to an increase in the number of Lactobacillus bacteraemias (Salminen et al. 2002). Though particularly lactobacilli and bifidobacteria are regarded as safe, a safety evaluation based on the origin of the strain and on the presence of intrinsic antibiotic-resistance genes should be undertaken when the introduction of new probiotic strains is being considered (for review, see Borriello et al. 2003). However, it should be kept in mind that it is not always possible to determine the origin of a strain.

### 2.3.2 Clinical trials on probiotics in IBS

A number of randomised, placebo-controlled trials on the efficacy of probiotics or combinations of probiotics in IBS have been published (Table 5). One trial using a combination of lactobacilli and bifidobacteria or placebo concluded that no statistical analyses could be conducted due to the small sample size, and was therefore excluded from Table 5 (Saggioro 2004). Furthermore, a few uncontrolled trials with various designs have been published (Brigidi et al. 2001; Bazzocchi et al. 2002; Drisko et al. 2006; Fan et al. 2006), but due to the extremely high placebo response associated with IBS (Patel et al. 2005), no conclusions can be drawn from these studies. Overall, trials are highly heterogeneous concerning symptom questionnaires and outcome measures, which makes it complicated to compare the efficacy of different strains. B. infantis 35624 (O’Mahony et al. 2005; Whorwell et al. 2006) and VSL#3 (Kim et al. 2003; Kim et al. 2005b), a probiotic combination comprising eight different strains, are the only formulations that have demonstrated a consistent and beneficial effect in two trials. L. plantarum 299v has alleviated IBS symptoms in
two studies (Nobaek et al. 2000; Niedzielin et al. 2001), whereas a third one failed to see any effect (Sen et al. 2002), possibly due to a lower administration dose. Similar to IBS trials in general, studies on probiotics suffer from methodological inadequacies, including the use of relatively small sample sizes and the lack of long-term data.

To summarise, the current evidence suggests a role for probiotics in the management of IBS, but further trials are needed before general guidelines for specific probiotics can be designed. The great majority of trials suffer from methodological shortages, and almost no long-term data are available.

2.3.3 Mechanisms of action of probiotics in IBS

Diverse types of mechanisms appear to account for the reduction by probiotics of IBS symptoms. These include effects mediated via modulation of the microbiota, the immune system, gut motility and visceral hypersensitivity or pain sensing.

The microbiota and its metabolites, primarily SCFAs, affect the maturation and maintenance of gut sensory and motor functions as well as the gut barrier function (van der Waaij 1982; Husebye et al. 2001; Fukumoto et al. 2003). Probiotic supplementation can influence the composition and metabolism of the microbiota (Siigur et al. 1996; Kuisma et al. 2003) and decrease intestinal permeability (Isolauri et al. 1993; Lam et al. 2007) and consequently modulate gut function. Observations on microbiota alterations by probiotics are, however, general findings, and the phenomenon has not been demonstrated specifically in IBS. Only one controlled trial on IBS and probiotics has monitored possible alterations in microbiota during L. plantarum 299v supplementation, but it only found an increase in Enterococcus in the placebo group (Nobaek et al. 2000). Uncontrolled studies have reported VSL#3 as increasing faecal β-galactosidase activity and decreasing urease activity (Brigidi et al. 2001; Bazzocchi et al. 2002). Inflammation and altered immune response is recognised as one feature of IBS (Dunlop et al. 2003b; O’Mahony et al. 2005; Barbara et al. 2007; Liebregts et al. 2007b). Certain probiotics possess anti-inflammatory and immunomodulatory effects (Schultz et al. 2003), which could explain their beneficial role in gut dysfunction. The credibility of immunomodulation in relation to other putative mechanisms is increased by the fact that this is the only mechanism that has been shown to correlate with clinical symptom reduction in a human trial on IBS (O’Mahony et al. 2005).

In vitro studies on isolated intestines of guinea pigs have shown that probiotics, especially bifidobacteria, have a relaxing effect on the colon (Massi et al. 2006). L. paracasei NCC2461 also appears to attenuate muscle hypercontractility in a mouse model of post-infectious IBS as evaluated by gut contractility measures (Verdu et al. 2004) and a high-throughput metabolomic approach (Martin et al. 2006). A recent experimental study with a combination of two Lactobacillus strains saw a normalisation of stress-induced gut dysfunction, possibly due to normalisation of the hypothalamus-pituitary-adrenal axis (Gareau et al. 2007). Experimental findings are corroborated
by clinical studies on VSL#3 in IBS, demonstrating that the probiotic reduces colonic motor responses to balloon distension in an uncontrolled setting (Bazzocchi et al. 2002) and retards colonic transit time vs. placebo (Kim et al. 2005a). As for probiotics and pain, a study in rats proves that *L. reuteri* (ATCC 23272) can inhibit visceral pain through effects on enteric nerves (Kamiya et al. 2006). Similarly, experimental hypersensitivity induced by antibiotics or stress may be suppressed by *L. paracasei* NCC2461 and *L. farciminis* (Ait-Belgnaoui et al. 2006; Verdu et al. 2006; Eutamene et al. 2007). It has been suggested that stress-induced hypersensitivity is caused by an increase in permeability (Ait-Belgnaoui et al. 2005) that, equally to hypersensitivity, can be counteracted by selected probiotics (Zareie et al. 2006; Eutamene et al. 2007). On the molecular level, it has been shown that certain *Lactobacillus* strains can induce the expression of pain-suppressing μ-opioid and cannabinoid receptors in intestinal epithelial cells (Rousseaux et al. 2007). The mechanism underlying this discovery remains unknown, but direct contact of the lactobacilli with the epithelium results in the induction of these receptors via the nuclear factor-κB pathway. The applicability of mechanistic data is impaired by the fact that the great majority of strains that have been investigated in mechanistic studies have no clinical substantiation for their efficacy.

**To summarise, probiotics are likely to exert their favourable effects on gut dysfunction and discomfort via several mechanisms. Some mechanisms of action may be specific to certain bacterial strains or species, whereas other mechanisms may be a general feature of probiotic bacteria. However, more clinical data on mechanisms is needed.**
Table 5. Randomised, placebo-controlled studies investigating the effects of probiotic supplementation in IBS.

<table>
<thead>
<tr>
<th>Probiotic</th>
<th>Daily dose (cfu)</th>
<th>Number of patients</th>
<th>Duration of study</th>
<th>Main outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. acidophilus</td>
<td>2.9x10^9</td>
<td>61</td>
<td>2 wk</td>
<td>↔</td>
<td>Newcomer et al. 1983</td>
</tr>
<tr>
<td></td>
<td>2x10^10</td>
<td>18</td>
<td>6+6 wk (cross-over)</td>
<td>IBS score ↓ in 50% of patients in probiotic group</td>
<td>Halpern et al. 1996</td>
</tr>
<tr>
<td>L. rhamnosus GG</td>
<td>1x10^10</td>
<td>24</td>
<td>8+8 wk (cross-over)</td>
<td>↔</td>
<td>O’Sullivan and Morain 2000</td>
</tr>
<tr>
<td></td>
<td>2x10^10</td>
<td>50 (children)</td>
<td>6 wk</td>
<td>Perceived distension ↓</td>
<td>Bausserman and Michail 2005</td>
</tr>
<tr>
<td></td>
<td>6x10^9</td>
<td>104 (children)*</td>
<td>4 wk</td>
<td>IBS patients: frequency of pain ↓</td>
<td>Gawronska et al. 2007</td>
</tr>
<tr>
<td>L. plantarum 299v</td>
<td>2x10^10</td>
<td>60</td>
<td>4 wk</td>
<td>Flatulence ↓</td>
<td>Nobaek et al. 2000</td>
</tr>
<tr>
<td></td>
<td>2x10^10</td>
<td>40</td>
<td>4 wk</td>
<td>Abdominal pain and IBS score ↓</td>
<td>Niedzielin et al. 2001</td>
</tr>
<tr>
<td></td>
<td>6.3x10^9</td>
<td>12</td>
<td>4+4 wk (cross-over)</td>
<td>↔</td>
<td>Sen et al. 2001</td>
</tr>
<tr>
<td>L. reuteri ATCC 55730</td>
<td>2x10^9</td>
<td>54</td>
<td>6 mo</td>
<td>↔</td>
<td>Niv et al. 2005</td>
</tr>
<tr>
<td>L. salivarius UCC 4331</td>
<td>1x10^10</td>
<td>77</td>
<td>8 wk</td>
<td>↔</td>
<td>O’Mahony et al. 2005*</td>
</tr>
<tr>
<td>B. infantis 35624</td>
<td>1x10^10</td>
<td>77</td>
<td>8 wk</td>
<td>Abdominal pain, IBS score and bowel movement difficulty ↓, health worry domain in HRQOL ↑</td>
<td>O’Mahony et al. 2005*</td>
</tr>
<tr>
<td></td>
<td>1x10^9 / 1x10^10</td>
<td>362</td>
<td>4 wk</td>
<td>For 1x10^9 dose only: abdominal pain, IBS score, distension, incomplete evacuation, straining, flatulence ↓, bowel habit satisfaction ↑</td>
<td>Whorwell et al. 2006</td>
</tr>
<tr>
<td>B. animalis DN-173010</td>
<td>1.3x10^10</td>
<td>274</td>
<td>6 wk</td>
<td>At 3 weeks only: responder rate for discomfort score ↑, bloating ↓</td>
<td>Guyonnet et al. 2007</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>NA</td>
<td>54</td>
<td>4 wk</td>
<td>Overall symptoms ↓</td>
<td>Gade and Thorn 1989</td>
</tr>
<tr>
<td>VSL#3</td>
<td>4.5x10^11</td>
<td>48</td>
<td>8 wk</td>
<td>Distension ↓</td>
<td>Kim et al. 2003</td>
</tr>
<tr>
<td></td>
<td>4.5x10^11</td>
<td>25</td>
<td>4 wk (n=31) / 8 wk (n=17)</td>
<td>Flatulence ↓</td>
<td>Kim et al. 2005</td>
</tr>
</tbody>
</table>

cfu = colony forming unit; # = IBS, functional dyspepsia, or functional pain; * = B. infantis 35624 and L. salivarius UCC 4331 formed two groups in the same study setting. NA = data not available; VSL#3 = B. longum, B. infantis, B. breve, L. acidophilus, L. casei, L. bulgaricus, L. plantarum, S. salivarius subsp. thermophilus; ↓ = reduced in probiotic group vs. placebo; ↑ = improved in probiotic group vs. placebo ↔ unchanged in probiotic group vs. placebo
The exact aetiology and pathophysiology of IBS remain unidentified, even though elements such as visceral hyperalgesia, altered motility and disturbed brain-gut communication appear to be central. Current treatments for IBS are considered rather unsatisfactory. Previous data indicate that certain probiotic strains or combinations of strains may be beneficial in IBS. However, the mechanism of action behind clinically efficient probiotics is not well known. The aim of the present study was to investigate the pathophysiology of IBS and the role of probiotic supplementation in the treatment of the syndrome.

The specific aims of the study were:

- **Pathophysiology**: To investigate pathophysiological factors of IBS by comparing the intestinal microbiota composition and the mucosal metabolic profile of IBS patients with the corresponding variables in healthy control subjects (Studies I, V).

- **Probiotic supplementation**: To clarify the effects of long-term multispecies probiotic supplementation on gastrointestinal symptoms and quality of life in IBS (Studies II, IV).

- **Characterisation of probiotic action**: To find out the effects of the probiotic supplementation on intestinal microbiota composition, metabolism and stability, and on systemic inflammatory markers (Studies III, IV).
4. MATERIALS AND METHODS

The description of the study design, duration and outcome measures, as well as the demographic characteristics of the subjects are presented in Table 6. The subjects comprised adult IBS patients (I-V) and healthy control subjects (I, V).

4.1 Subjects

IBS patients were recruited from primary care units (Helsinki and Tampere, Finland) by experienced endoscopists. Most subjects fulfilled the Rome II criteria for IBS, while the rest fulfilled the Rome I criteria (Table 2). IBS patients were predominantly female. Healthy subjects devoid of GI diseases or symptoms were recruited as controls.

4.1.1 IBS patients

The inclusion criteria for IBS patients were: an IBS diagnosis consistent with the Rome I or II criteria for IBS (Thompson et al. 1992; Thompson et al. 1999), an age between 20 and 65 years, a clinical investigation with endoscopy or barium enema of the GI tract performed within one year (I-III) or five years (IV, V) prior to the study, and a normal blood count and serum creatinine, alanine aminotransferase and alkaline phosphatase values within reference limits (IV, V). Patients who were pregnant or lactating, had organic intestinal diseases or severe systemic diseases, previous major or complicated abdominal surgery, severe endometriosis or complicated abdominal adhesions, received antimicrobials during the preceding one to two months, had dementia or were otherwise unable to co-operate adequately were excluded. Subjects participating in Study V additionally had normal gut histology as evaluated by an experienced pathologist. IBS patients in Studies I and III were subgroups of the patients participating in Study II.
### Table 6. Study designs and demographic characteristics of subjects in each study.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design (duration)</th>
<th>IBS patients</th>
<th>Healthy control subjects</th>
<th>Outcome measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Longitudinal follow-up study (6 mo)</td>
<td>27* (7/20)</td>
<td>22 (7/15)</td>
<td>Intestinal microbiota composition</td>
</tr>
<tr>
<td>II</td>
<td>Randomised, double-blind, placebo-controlled (6 mo)</td>
<td>103 (24/79)</td>
<td>-</td>
<td>IBS symptoms HRQOL</td>
</tr>
<tr>
<td>III</td>
<td>Randomised, double-blind, placebo-controlled (6 mo)</td>
<td>55* (16/39)</td>
<td>-</td>
<td>Intestinal microbiota composition Faecal SCFAs and enzymes</td>
</tr>
<tr>
<td>IV</td>
<td>Randomised, double-blind, placebo-controlled (5 mo)</td>
<td>86 (6/80)</td>
<td>-</td>
<td>IBS symptoms HRQOL Intestinal microbiota stability Systemic inflammatory markers</td>
</tr>
<tr>
<td>V</td>
<td>Cross-sectional study in one time point</td>
<td>15 (4/11)</td>
<td>9 (4/5)</td>
<td>Mucosal metabolic profile</td>
</tr>
</tbody>
</table>

*IBS patients in Studies I and III were subgroups of the patients in Study II*
4.1.2 Healthy control subjects

Controls subjects in Study I were recruited by advertisements at the Technical Research Centre of Finland, whereas subjects undergoing colonoscopy for clinical reasons were recruited via endoscopists into Study V. The inclusion criteria for the controls subjects were: overall healthiness and an age between 20 and 65 years. Additionally, subjects in Study V had normal blood count and serum creatinine, alanine aminotransferase and alkaline phosphatase values within reference limits as well as normal gut histology as evaluated by an experienced pathologist.

4.2 Study designs

Interventions were carried out in a randomised, double-blind, placebo-controlled manner with two parallel groups. Patients with ongoing IBS medication (e.g. fibre analogues, antispasmodics, antidiarrhoeals, laxatives) or any other regular medication were allowed to continue the medication throughout the study. Any changes in medication, in health status or in dietary habits as well as antimicrobials and adverse events were recorded. All the subjects were asked to abstain from commercial products containing probiotics during the entire study period.

Intestinal microbiota in IBS patients and healthy controls (Study I)
Twenty-seven IBS patients and 22 healthy subjects participated in this study. During the six-month study period, three faecal samples were collected from all study subjects (0, 3 and 6 months) for the determination of intestinal microbiota composition.

Effect of probiotic supplementation on symptoms and quality of life in IBS patients (Study II)
One hundred and three IBS patients recruited by physicians in primary health care units participated in this intervention. Altogether 86 patients completed the study. The study consisted of a one-week baseline period and a six-month intervention period (Figure 3). The subjects were randomised into the probiotic (n=52) or the placebo (n=51) group according to a computer-generated, blocked randomisation list. During the six-month intervention period, subjects received either probiotic or placebo supplementation daily. GI symptoms and bowel habits were followed using a symptom diary, which the patients filled in once at baseline and then for a period of one week each month throughout the intervention. HRQOL and dietary habits were followed by questionnaires at baseline, and at 3 and 6 months.
Effect of probiotic supplementation on intestinal microbiota in IBS patients (Study III)

Fifty-five IBS patients participated in this study. During a six-month intervention period, the subjects received either probiotic (n=28) or placebo (n=27) supplementation daily. Faecal samples were obtained in three time points: once at baseline and twice during the intervention (3 and 6 months). The composition of intestinal microbiota, the concentration of SCFAs and the activity of bacterial enzymes were determined from faecal samples.

Effect of probiotic supplementation on symptoms, quality of life, microbiota stability and inflammatory markers in IBS patients (Study IV)

Eighty-six IBS patients recruited by a physician in primary health care participated in this intervention. Altogether 71 patients completed the study. The study consisted of a three-week washout period, a five-month intervention period and a three-week follow-up period (Figure 4). The subjects were randomised into the probiotic (n=43) or the placebo (n=43) group according to a computer-generated, blocked randomisation list. During the five-month intervention period subjects received either probiotic or placebo supplementation daily. GI symptoms and bowel habits were followed by a symptom diary, which the patients filled in once at baseline, seven times during the intervention period (every 3 weeks), and once during the follow-up. HRQOL was recorded, and blood samples and faecal samples were collected at baseline, halfway through and at the end of the study. Serum sensitive C-reactive protein (CRP) and selected cytokines were measured from serum samples, and the intestinal microbiota stability was assessed from part of the patients (n=20).
Mucosal metabolites in IBS patients and healthy controls (Study V)
Fifteen IBS patients and nine healthy volunteers recruited by physicians in primary health care participated in this study. Mucosal biopsies from the ascending colon were obtained from each subject during colonoscopy in one time point. The global lipid- and water-soluble metabolic profiles of mucosal samples were determined.

4.3 Administration and doses of probiotics
The intervention studies (II, IV) investigated multispecies probiotic supplementation consisting of four bacterial strains. Probiotic supplementation was given either as a capsule (II) or in a milk-based drink (IV) in a randomised, double-blind, placebo-controlled manner. The supplementation comprised Lactobacillus rhamnosus GG (ATCC 53103), Lactobacillus rhamnosus Lc705 (DSM 7061), Propionibacterium freudenreichii subsp. shermanii JS (DSM 7067), and Bifidobacterium breve Bb99 (DSM 13692) in Study II or Bifidobacterium animalis subsp. lactis Bb12 (DSM 15954) in Study IV. The probiotic capsules and drinks as well as the corresponding placebos were provided by Valio Ltd, Helsinki, Finland.

The capsules used in Study II contained 2-2.25 x 10⁹ cfu of each strain, equalling a total bacterial amount of 8-9 x 10⁹ cfu/capsule. The daily dose was one capsule. Study compliance was assessed by counting the number of capsules remaining in each carton returned by the participants to the study coordinator. The milk-based drink consumed in Study IV contained on average 1 x 10⁷ cfu/ml of each bacterial strain. The daily intake of the drink was 120 ml, equalling a total bacterial amount of 4.8 x 10⁹ cfu/day. The drink was made up of 80% lactose-free milk and 20% fruit juice. The placebo drink was devoid of probiotics, but otherwise identical to the probiotic drink. Study compliance was followed by daily questionnaires.
4.4. Questionnaires

**IBS symptoms and bowel habits (Studies II and IV)**
Patients participating in the intervention studies recorded throughout the study the intensity of GI symptoms as well as bowel habits (frequency and form of stools) in a structured symptom diary. Baseline symptoms were recorded for one week prior to the beginning of the intervention. The primary IBS symptoms recorded were abdominal pain, distension, flatulence and rumbling, and their combined sum made up the total IBS score. The intensity of each symptom was measured on a scale of 0-4, where 0 represented absence of symptoms and 4 severe symptoms.

**Dietary habits (Study II)**
The frequency of use of the major foodstuffs (dairy products, cereals, meat, fruit and vegetables, fats, and beverages) and the consumption of foods generally considered to be symptom-provoking were assessed by a food-frequency questionnaire. The aim of the questionnaire was to obtain a general picture of the patients’ diets, and to detect possible changes in diets during the intervention.

**Health-related quality of life (Studies II and IV)**
The RAND 36-Item Health Survey (Hays et al. 1993), which measures general HRQOL, was employed in Study II. The survey assesses eight health concepts: physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, general mental health, social functioning, energy/fatigue, and general health perceptions. The Irritable Bowel Syndrome Questionnaire (IBSQ) developed by Wong et al. (1998) was used in Study IV. The questionnaire assesses four domains of HRQOL: bowel symptoms, fatigue, activity limitations and emotional function.

4.5 Collection of samples

**Faecal samples (Studies I, II, IV)**
Faecal samples were collected from IBS patients in Studies II and IV, and from healthy control subjects in Study I. Samples obtained from IBS patients during Study II were analysed in Studies I and III. During Study II, three faecal samples (0, 3 and 6 months) were collected from the IBS patients. Samples were immediately stored in anaerobic containers from defecation onwards, frozen within four hours to -70 °C, and stored therein until analysed. Samples from healthy control subjects in Study I were collected at the same time points and according to the same protocol. During Study IV, three faecal samples (baseline, mid-point, and end-of-study) were collected. Samples were immediately frozen at -20 °C, and transferred shortly into -45 °C for storage until required for analysis.
Blood samples (Study IV)
Blood samples were drawn at three time points: baseline, mid-point, and end-of-study (Figure 4). Samples were immediately frozen at -20 °C as serum, and transferred shortly into -45 °C for storage until required for analysis.

Mucosal biopsies (Study V)
Mucosal biopsies from the ascending colon were obtained from IBS patients and healthy controls during colonoscopy after bowel cleansing. The samples were immediately frozen at -20 °C, and transferred shortly into -70 °C until required for analysis.

4.6 Microbiological and biochemical determinations

4.6.1 Intestinal microbiota and its metabolism
The composition of the intestinal microbiota (Studies I, III)
The composition of the microbiota was determined by real-time qPCR. A total of 21 qPCR assays covering more than 300 different species (Table 7) were selected based on previous indications of an association between IBS and the bacteria in question, or due to the predominant nature of the group in the gut ecosystem. Total DNA was isolated from the faecal samples as described earlier (Apajalahti et al. 1998), and qPCR assays were performed in triplicate with an iCycler iQ apparatus (Bio-Rad, Hercules, CA, USA). Additionally, strain-specific real-time PCR assays were developed in Study III for the quantification of each probiotic strain by using the LightCycler fluorescence resonance energy transfer technique (Halme et al. 2002; Mikkola et al. 2006).
The stability of the intestinal microbiota (Study IV)

The stability of the microbiota of a subgroup of patients (n=20) was analysed in three time points with a microarray method, the HITChip. The HITChip is a custom-made Agilent microarray (Agilent Technologies, Palo Alto, CA, USA) designed to cover the diversity of the human intestinal microbiota. The chip contains approximately 5,500 oligonucleotide probes that cover all the currently known approximately 1,000 intestinal microbial species. A detailed description of the HITChip and the precise experimental conditions has been published separately (Rajilić-Stojanović 2007). The scanning of the microarrays was performed with the Agilent Microarray Scanner (Agilent Technologies). The stability of the microbiota was assessed by the similarity index, obtained by constructing scatter plots of the signals for all the HITChip probes for each patient in each time point. The similarity between the time points for each individual patient was quantified by calculating the Pearson correlation index. The resulting value, expressed as a percentage, indicates the degree of preservation of the microbiota composition between the time points.

<table>
<thead>
<tr>
<th>Bacterial group or species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopobium group</td>
</tr>
<tr>
<td>Bacteroides-Prevotella-Porphyromonas</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
</tr>
<tr>
<td>Bifidobacterium spp.</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
</tr>
<tr>
<td>Clostridium cocoides-Eubacterium rectale</td>
</tr>
<tr>
<td>Clostridium difficile</td>
</tr>
<tr>
<td>Clostridium perfringens group</td>
</tr>
<tr>
<td>Desulfovibrio desulfuricans group</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
</tr>
<tr>
<td>Escherichia coli subgroup</td>
</tr>
<tr>
<td>Faecalibacterium prausnitzii</td>
</tr>
<tr>
<td>Helicobacter-Flexispira-Wollinella</td>
</tr>
<tr>
<td>Lactobacillus group</td>
</tr>
<tr>
<td>Ruminococcus productus-Clostridium cocoides</td>
</tr>
<tr>
<td>Veillonella spp.</td>
</tr>
<tr>
<td>B. adolescentis*</td>
</tr>
<tr>
<td>B. bifidum*</td>
</tr>
<tr>
<td>B. catenulatum group*</td>
</tr>
<tr>
<td>B. longum group*</td>
</tr>
<tr>
<td>Fusobacterium spp.*</td>
</tr>
</tbody>
</table>

\*Only in Study I; \#Only in Study III

Table 7. Bacterial groups and species analysed by qPCR in Studies I and III.
Microbiota-derived metabolites (Study III)

Faecal SCFAs and bacterial enzymes were analysed at baseline, and at three and six months. The faecal SCFA content (acetate, butyrate, propionate, valerate, caproate, isobutyrate, isovalerate, isocaproate) and bacterial enzymes (β-glucosidase, β-glucuronidase) were identified, as previously described, with gas chromatography and spectrophotometer, respectively (Goldin et al. 1980; Høverstad et al. 1984).

4.6.2 Serum inflammatory markers

In Study IV, serum sensitive-CRP and selected cytokines were measured at baseline and halfway through the intervention. CRP was measured by a particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany). The serum cytokines interferon-γ, tumour necrosis factor-α and IL-2, 4, 6 and 10 were analysed with the BD Cytometric Bead Array Th₁/Th₂ kit (BD Biosciences, San Diego, CA, USA).

4.6.3 Mucosal metabolic profile

In Study V, a high-throughput metabolomic approach comprising lipidomics and metabolomics was used in order to investigate differences between colonic mucosa from IBS patients vs. healthy controls. Characterisation of lipid molecular species was performed with a lipidomics strategy using ultra performance liquid chromatography coupled to mass spectrometry (UPLC/MS). Additionally, a broad screening of metabolites was conducted by comprehensive two-dimensional gas chromatography coupled to high-resolution time-of-flight mass spectrometry (GCxGC-TOF).

4.7 Statistical analysis

In Study I, the results are shown as means with 95% confidence intervals (CIs) or as medians with interquartile ranges. Comparison between different IBS subgroups as well as between IBS patients and healthy controls were performed by the Kruskal-Wallis test and the Mann-Whitney test.

In Studies II and III, the main results are presented as means with 95% CIs or with the standard error of mean. Analysis of covariance with baseline as covariate was used for comparisons between the probiotic group and the placebo group concerning the main endpoints, IBS symptoms and microbiota.

In Study IV, observations are presented as means, geometric means or medians with 95% CIs, standard deviations (SDs) or interquartile ranges. Analysis of covariance with baseline as covariate was used for comparison between groups in IBS symptoms and CRP, the Hodges-Lehmann estimate for median change was used for the HRQOL data, and the microbiota similarity index comparison was performed with a permutation test with exact p values.

In Study V, partial least squares discriminant analysis (PLS/DA) was used to compare the metabolic profiles between IBS patients and healthy controls. Statistical comparison was made.
using the Mann-Whitney test.

Besides the analyses undertaken in the original articles I-V, additional statistical tests were undertaken for this thesis. The 95% CIs for microbiological data ratios (Studies I and III) and RAND-36 scores (Study II) were obtained by bias-corrected bootstrapping. Statistical comparison between patients with IBS and the age- and gender-matched Finnish population values was conducted by using Monte Carlo-type simulations. A meta-analysis was used to investigate the change in symptom score in a combination of Studies II and IV. The pooled mean effect size ($d$) estimate was calculated using direct weights defined as the inverse of the variance of $d$ for each study. An approximate confidence interval for $d$ is given with a chi-square statistic and with the probability of this pooled effect size being equal to zero.

P values below 0.05 were considered statistically significant. The Graph Pad Prism version 3.02 (GraphPad, San Diego, CA, USA), SPSS version 14.0 (SPSS Inc., Chicago, IL, USA) and STATA version 9.0 (StataCorp, College Station, TX, USA) were used for the statistical analyses.

### 4.8 Ethics

The Human Ethics Committee of the Joint Authority for the Hospital District of Helsinki and Uusimaa, Finland approved the study protocol for the IBS patients in Studies I-III. The study protocol for the healthy subjects in Study I was approved by the Ethics Committee of the Technical Research Centre of Finland. The Human Ethics Committee of the Hospital District of Pirkanmaa, Finland approved the study protocol for Studies IV and V. All subjects gave their written informed consent.
5. RESULTS

5.1 Pathophysiological factors

Intestinal microbiota in IBS patients and healthy controls (Study I)

An extensive individual variation prevailed in the microbiota composition both among the IBS patients and among the control subjects. When all IBS patients were compared with healthy controls, quantitative alterations between patient and control groups were seen for two PCR assays. Based on mean bacterial numbers in the three analysed time points, lower counts for the *C. coccoides* group (10.84 log$_{10}$ vs. 11.25 log$_{10}$; p=0.003) and the *B. catenulatum* group (7.26 log$_{10}$ vs. 7.91 log$_{10}$; p=0.039) were seen in IBS patients vs. healthy controls (Figure 5). When the different subgroups of patients were compared, lower counts for *Lactobacillus* spp. was seen in diarrhoea vs. constipation (6.98 log$_{10}$ vs. 7.60 log$_{10}$, p=0.019), and higher counts for *Veillonella* spp. in constipation IBS compared to the healthy controls (8.30 log$_{10}$ vs. 7.87 log$_{10}$; p=0.045).

Results indicate no alterations in intestinal pathogens in faecal samples of IBS patients, since *C. jejuni* was detected in one patient only, whereas *Helicobacter* spp. or *C. difficile* was not detected in any subjects.

![Figure 5. Ratios between IBS patients (n=21) and control subjects (n=15) for analysed bacterial groups (log$_{10}$ mean with 95% CI of 0, 3, and 6 month sample).](image-url)
Mucosal metabolites in IBS patients and healthy controls (Study V)

In UPLC/MS, a total of 651 lipid peaks were found, and 75 of those were identified. The most significant upregulation among lipids was seen in proinflammatory cell-membrane metabolites lysophosphatidylcholines (p<0.001). Other lipid groups significantly upregulated in IBS patients included lipotoxic ceramides, glyco-sphingolipids and di- and tri-acylglycerols. One hundred and seven mucosal metabolites were identified by GCxGC-TOF. The metabolite contributing most to separation between cases and controls was 2(3H)-furanone, a cyclic ester commonly produced in biochemical pathways, which was almost 14-fold upregulated in IBS patients compared to healthy subjects (p=0.03). Furthermore, elevated levels of creatinine (p=0.004), a molecule involved in smooth-muscle energy metabolism, and reduced concentrations of certain organic carboxylic acids were observed in IBS. A PLS/DA plot combining the lipidomic and metabolomic data appears in Figure 6.

Figure 6. PLS/DA plot on combined lipidomic and metabolomic data in IBS patients (n=15) and healthy controls (n=9). LV = latent variable.
5.2. The effect of probiotic supplementation in IBS

The effect of probiotics on GI symptoms and bowel habits (Studies II, IV)
Multispecies probiotic supplementation alleviated the symptoms of IBS. At the end of Study II, the treatment difference in the baseline-adjusted total IBS score was -7.7 points (95% CI: -13.9 to -1.6) when the probiotic group was compared to the placebo group (p=0.015). In Study IV, the total IBS score had at the end of the supplementation decreased 14 points (95% CI: -19 to -9) in the probiotic group compared to 3 points (95% CI: -8 to 1) in the placebo group (p=0.0083). Results correspond to an average score reduction of 40% for the probiotic supplementation, and less than 10% for the placebo supplementation. Concerning individual symptoms, rumbling (p=0.008; Study II) and distension (p=0.023; Study IV) were most markedly alleviated. The probiotic intervention showed no effect on defecation frequency or stool consistency.

When combining Studies II and IV, the pooled effect size was -0.38 (95% CI: -0.68 to -0.09; p=0.012; Figure 7).

![Figure 7. Effect size meta-analysis plot for change in IBS symptom score in Studies II (n=91) and IV (n=86).](image)

The effect of probiotics on health-related quality of life (Studies II, IV)
The mean RAND-36 HRQOL scores in IBS patients at baseline compared to the scores in the general Finnish population (Aalto et al. 1999) appear in Figure 8. IBS patients have lower scores for physical role functioning, pain, general health, energy and social functioning.

In Study II, there was no effect of the probiotic intervention on HRQOL as analysed by the mean change of the total HRQOL score, the eight individual scales, the RAND-36 Physical Component scale (including physical functioning, role functioning/physical, pain and general...
health) and the RAND-36 Mental Component scale (including energy, role functioning/emotional, emotional wellbeing and social functioning).

In Study IV, the IBSQ divided quality of life into four domains: bowel symptoms, fatigue, activity limitations and emotional function. The probiotic supplementation improved the bowel symptoms domain, as the Hodges-Lehmann estimate for median change from baseline to the end of the study was 0.62 points (95% CI: 0.37 to 0.86) in the probiotic group versus 0.37 points (95% CI: 0.17 to 0.61) in the placebo group (p=0.045). No significant effects were seen in the other domains.

Figure 8. The baseline HRQOL scores (means with 95% CIs) in IBS patients (Study II; n=101). Dotted line shows the scores in the general Finnish population (Aalto et al. 1999) weighted to match the age and gender distribution of the study population.
5.3 Characterisation of probiotic action in IBS

Effect of probiotic supplementation on intestinal microbiota (Studies III, IV)

In Study III, the recovery of the ingested probiotic strains and the counts of altogether 17 bacterial groups or species were investigated. All supplemented probiotic strains (LGG, Lc705, PJS, Bb99) were detected in faecal samples. Intestinal microbiota remained stable during the trial (Figure 9), except for *Bifidobacterium* spp., which increased in the placebo group (from 9.19 log$_{10}$ at baseline to 9.76 at three months and to 9.82 at six months), while the counts decreased in the probiotic group (from 9.58 to 9.43 and 9.11, respectively) (p for difference between groups =0.028). No significant changes in SCFAs or bacterial enzyme activities occurred.

![Figure 9. Ratios between the probiotic group (n=22) and the placebo group (n=21) for analysed bacterial groups (log$_{10}$ mean with 95% CI of 3 and 6 month sample).](image)

In Study IV, the global intestinal microbiota stability was assessed by a similarity index in 20 patients. Following the introduction of probiotics or placebo, the mean logarithmic similarity index between the baseline and the intervention sample (similarity index AB) was 91.8 (SD 3.1) in the probiotic group, while it was 94.5 (SD 1.3) in the placebo group (p=0.026). During the second half of the intervention period, a stabilisation of the microbiota was observed with probiotic supplementation, as the similarity index increased with the probiotic supplementation (1.87 ± 3.13) and decreased with placebo (-2.93 ± 1.68). The difference between the groups (-4.8; 95% CI -6.59 to -2.54) was significant (p=0.0015).
Effects of probiotic supplementation on systemic inflammatory markers (Study IV)

The probiotic supplementation had no effect on serum sensitive-CRP. The ratio of the intervention value to the baseline value was 0.91 (95% CI: 0.73 to 1.10) for the probiotic supplementation and 1.16 (95% CI: 0.85 to 1.47) for placebo (p=0.21). Cytokines were not statistically analysed as such a high percentage of the baseline samples were below the detection limit (interferon-γ: 64%, tumour necrosis factor-α: 100%, IL-2: 89%, IL-4: 79%, IL-6: 64% and IL-10: 99%).
Gastrointestinal symptoms and gut dysfunction caused by IBS affect up to one fifth of the adult population worldwide. The etiopathology of IBS is complex, multifactorial and poorly understood, and the efficacy of current treatment options is considered limited. Earlier studies propose the involvement of intestinal microbiota in the pathophysiology of the condition as well as a beneficial effect of certain probiotics in the alleviation of IBS symptoms. However, the effects of long-term probiotic supplementation are not well-known, and few clinical studies have made an attempt to investigate the possible mechanisms of action of the probiotics.

This study investigated pathophysiological factors of IBS by comparing the intestinal microbiota composition and the colonic metabolite concentration in patients and healthy controls. In addition, the long-term effects of multispecies probiotic supplementation on the symptoms and quality of life in IBS were studied in randomised, double-blind, placebo-controlled clinical trials. In order to characterise the actions of the probiotic, the effects of supplementation on intestinal microbiota composition, activity and stability as well as on systemic inflammatory markers were also assessed.

### 6.1 Methodological considerations

**Subjects**

IBS patients were recruited from primary health care units by experienced physicians. It is advisable to recruit patients broadly and to note if the subjects are from primary, secondary or tertiary care (Irvine *et al.* 2006) since significant differences in treatment response may exist between primary and referred patients (Jones 1999; Longstreth *et al.* 2001). Consequently, the treatment results from this study can be considered applicable to primary health care patients, and possibly non-consulting community patients, since these groups are fairly similar with regards to symptom severity and psychosocial factors (Ringström *et al.* 2007). However, there are no data indicating that the pathophysiology of IBS would differ amongst patients in different health care settings, and the results on pathophysiological factors of IBS could thus have a broader applicability among patients. It is noteworthy that the fact that all patients had undergone endoscopy may have introduced a slight bias to the study population since endoscopy is not recommended routinely as part of IBS diagnosis. In Finland, an endoscopy investigation is recommended in severe
diarrhoea, and in other cases its need should be evaluated on a case-by-case basis (Silvennoinen 2002). All subjects fulfilled the Rome criteria for IBS, and the number of patients needed for the clinical trials (Study II, IV) was based on statistical power calculations, which is in line with current recommendations for trials in IBS (Irvine et al. 2006). The number of patients in the interventions can be considered good in comparison to other interventions within the field of probiotics and IBS. The majority (83%) of patients studied were women, which may reflect the higher prevalence of IBS in women (for review, see Chang and Heitkemper 2002) or the fact that women in general are more prone to seek medical advice for any health problems (Bertakis et al. 2000). The biased gender distribution made it unfeasible to conduct meaningful subgroup analyses. Had it been possible, it would have been valuable to analyse the data separately for men and women since gender differences in the therapeutic benefit to serotonergic agents have been observed in IBS (Cremonini et al. 2003).

Healthy subjects were recruited as controls in Studies I and IV. The subjects in Study I were age- and gender-matched with the patients. Due to ethical issues, the controls in Study V had to be patients referred to colonoscopy for specific clinical reasons, but otherwise as healthy as possible. Subjects with previous intestinal polyps (more than three years since previous finding) or subjects referred to endoscopy due to anaemia were chosen as eligible controls. Overall, it was considered that the controls represented well the general, healthy adult population.

Study designs
The clinical trials were conducted as double-blind, randomised, placebo-controlled studies, which is the recommended trial design in IBS (Irvine et al. 2006). The exceptionally high placebo response, estimated to vary between 16 and 71% (Patel et al. 2005), renders the placebo treatment arm essential in all IBS trials. The current guidelines differentiate between short-term studies with a minimum treatment of four weeks, and long-term studies lasting at least six months (Irvine et al. 2006). Long-term trials should be employed when chronic use of the compound studied is anticipated. Both interventions (II, IV) in the current study are clearly of longer duration than the majority of interventions on probiotics in IBS since most other trials have been on average four to eight weeks long (Nobaek et al. 2000; O’Sullivan and O’Morain 2000; Niedzielin et al. 2001; Sen et al. 2002; O’Mahony et al. 2005; Whorwell et al. 2006).

Selection of probiotic strains
In the clinical trials, a multispecies probiotic comprising LGG, Lc705, PJS and Bb99 (II) or Bb12 (IV) was administered. LGG is considered the most studied probiotic strain worldwide, and its beneficial effects in certain GI disturbances, particularly antibiotic-associated diarrhoea and rotavirus diarrhoea, are well-documented (Huang et al. 2002). However, previous studies show that LGG alone is not particularly successful in alleviating the IBS symptom complex (O’Sullivan and O’Morain 2000; Bausserman and Michail 2005; Gawrońska et al. 2007). No single deviance has been identified in IBS microbiota, but alterations in microbiota composition and some degree
of microbial imbalance appear to be characteristic of IBS (Mättö et al. 2005; Maukonen et al. 2006; Kassinen et al. 2007). This observation combined with the complexity and heterogeneity of IBS gave rise to the hypothesis that a multispecies combination may be more efficient than a single strain. Among the pathophysiological factors of IBS, a dysregulation of peripheral cytokine production and typically a low secretion of IL-10 have been shown (O’Mahony et al. 2005). Probiotic strains differ in their immunological effects, and Bb99, Bb12 and PJS are relatively high inducers of IL-10 production in vitro (Kekkonen et al. 2007). Antimicrobial properties may also be of importance in IBS, and inhibition of pathogen adhesion has been shown for all strains administered (Collado et al. 2007a), while LGG, Lc705 and PJS additionally have antagonising effects against yeasts (Suomalainen and Mäyrä-Mäkinen 1999; Hatakka et al. 2007). It should be remembered that the role of GI yeast in IBS has not been extensively studied, but one study suggests equal levels and prevalence of yeast in IBS patients and control subjects (Mättö et al. 2005). Part of the superiority of multispecies probiotics in certain conditions may be due to synergistic effects between the strains. Especially adhesion can be remarkably modulated by synergism, and the presence of LGG has been shown to more than double the adhesion of Bb12 and certain P. freudenreichii strains (Ouwehand et al. 2000; Ouwehand et al. 2002b). Regardless of several putatively beneficial attributes of multispecies probiotics, their use is not without limitation. Supplementing several strains in a combination makes it impossible to find out whether only some of these strains actually account for the observed health effects. In order to overcome this, trials should optimally include a group receiving the complete multispecies, groups getting each strain alone and groups getting combinations of two to three strains. In practice, this is not feasible in clinical trials. Furthermore, combining strains could also result in unwanted antagonistic effects. As an example, the adhesion of Bb99 is reduced when it is used in combination with other strains (LGG, Lc705, PJS), whereas the adhesion of the other strains is favourably affected by the combination (Collado et al. 2007b). Another inadequacy of the present study is that two different strains of Bifidobacterium were used. However, based on in vitro studies, Bb99 and Bb12 trigger a similar pattern of cytokines (Kekkonen et al. 2007).

**Questionnaires**

A similar IBS symptom diary was used in Studies II and IV. The diary was developed by the research group in Study II, based on our group’s previous questionnaires on GI symptom monitoring and on the literature, and it was pre-tested in healthy volunteers. An advantage of this was that precisely the same diary was used in Studies II and IV, enabling a comparison between the two trials. Validated measurement instruments are recommended, but the lack of validated questionnaires has, on the other hand, been a recognised drawback in several IBS studies (Irvine et al. 2006), including the current one. Paper diaries in general also suffer from recall bias as a substantial proportion of diary entries are made retrospectively (Stone et al. 2002). Electronic diaries are one option to overcome this shortcoming, and we have earlier shown that an electronic diary correlates well with a paper diary in GI symptom monitoring (Kajander et al. 2007).
applied HRQOL surveys were freely available, published questionnaires. The RAND 36-Item Health Survey (Hays et al. 1993) in Study II is a well-tested and validated questionnaire that is used extensively around the world as a tool for assessing generic HRQOL. The IBSQ (Wong et al. 1998) used in Study IV is an IBS-specific questionnaire developed using standard methods, but with no reported data on construct validity as yet. The food-frequency questionnaire used in Study II was developed by our research group and pre-tested in healthy volunteers.

**Microbiological and biochemical analysis**

Quantitative PCR, a widely used microbiological detection method based on the quantity of bacterial DNA present in a sample, was applied in Studies I and III. PCR-based techniques are considered powerful tools for examining the microbial diversity of mixed populations (for review, see Furrie 2006). In Study IV, a novel high-throughput microarray enabling the simultaneous analysis of approximately 1,000 intestinal microbial species was used (Rajilić-Stojanović 2007). The HITChip allows relative quantification of microbial groups, and the results obtained with the chip have been found to correlate fairly well with FISH and denaturing gradient gel electrophoresis. An advantage of the HITChip over qPCR is that no pre-selection of bacterial species or groups to be analysed needs to be done. However, only the samples of a subgroup (n=20) were analysed by the microarray, and consequently the findings should be interpreted with caution.

SCFAs, bacterial enzymes and serum CRP were analysed using well-established methods. The serum cytokines were analysed with the cytometric bead array Th1/Th2 kit, which turned out to be inappropriate for the current study as the great majority of samples were below the detection limit. The method is considered highly sensitive, and studies in e.g. allergic infants have found the method useful (Viljanen et al. 2005a), even though part of the samples were below the detection limit in that population, too. Mucosal metabolites were investigated by two high-throughput metabolomic platforms, UPLC/MS-based lipidomics and GCxGC-TOF-based metabolomics. Metabolomics is a rapidly developing tool, the sensitivity of which is considered high (for review, see Schnackenberg and Beger 2006). One evident shortcoming with metabolomics is that a major part of spectral peaks are still unidentified.

To summarise, the methods used in the current study are considered to be of good quality, to be suitable for the study settings, and to comply with current recommendations. A wide range of diverse methods have been applied, and novel methods have been utilised in parallel with well-established techniques. As for the clinical trials, the durations of the interventions are long, and the number of patients included can be considered sufficient. The studies investigating the pathophysiology of IBS may have gained from having had larger study populations.
6.2 Pathophysiological factors

Alterations in intestinal microbiota in IBS patients vs. healthy controls
The findings of this study are in agreement with previous suggestions of an aberrant microbiota in IBS. Quantitative differences were seen in the B. catenulatum and the C. coccoides groups, both of which were reduced in IBS patients. Moreover, the numbers of lactobacilli were lower in the diarrhoea subgroup vs. the constipation group, whereas Veillonella spp. were increased in the constipation subgroup compared to the controls. Earlier data on bifidobacteria in IBS are conflicting since both reduced numbers of total bifidobacteria (Balsari et al. 1982) and levels similar to healthy controls have been reported (Mättö et al. 2005). Concerning lactobacilli, our findings appear to be in line those of Balsari et al. (1982), who reported lower counts for IBS patients as a group. However, it should be taken into consideration that these earlier studies utilised culture methods instead of molecular DNA-based methods, which makes direct comparison of results difficult. To date, no other studies besides the current one have used qPCR for investigating microbiota composition in IBS patients and healthy controls. It is important to highlight that it remains unknown whether the microbiota alterations are a cause of IBS or a result of disturbed GI function. Recent studies have revealed that commensal bacteria are relevant for normal gut function (for review, see Barbara et al. 2005), and GI symptoms caused by a disruption in microbiota are a common adverse effect of antibiotic treatment (for review, see Coté and Buchman 2006). These observations provide support for the hypothesis that microbiota in general could play a role in IBS symptom generation. Little is yet known about the clinical relevance of individual microbial groups or species on GI health and function, and it is therefore not possible to evaluate the functional consequences of specific alterations in microbiota composition.

Differences in the mucosal metabolic profile in IBS patients vs. healthy controls
This study is the first one to examine global differences in colonic mucosal metabolite concentrations in IBS. The most prominent finding was an upregulation of various lipid species, particularly lysophosphatidylcholines and ceramides, in IBS patients. No similar findings have been reported previously, but interestingly, these molecules or the enzymes involved in their formation have been associated with IBD (Minami et al. 1994; Haapamäki et al. 1999; Homaidan et al. 2002; Sakata et al. 2007). The biological relevance of lysophosphatidylcholines and ceramides in intestinal dysfunction appears to lie in their potential to impair the mucosal barrier (Karlqvist et al. 1986; Otamiri et al. 1986; Sawai et al. 2002) and to enhance pain sensitivity (for reviews, see Malan and Porreca 2005; Park and Vasko 2005). These lipids participate in the transmission of noxious sensory signals by interacting with various receptor systems, but the exact cellular mechanisms behind this remain unknown. As both permeability defects and visceral pain are central to IBS (for review, see Drossman 2006), it is reasonable to hypothesise that the upregulation of lipid species observed in the current study may be of clinical relevance in IBS pathophysiology. Concerning lipids and IBS, it also appears that plasma fatty acid and cholesterol profiles may be disrupted...
in IBS (Kilkens et al. 2004a). The relevance of the findings on non-lipid soluble metabolites is, in contrast, more difficult to grasp. IBS cases and controls were fairly well separated into two distinct groups, but the function of the molecules that contributed the most to the separation in the GI tract is not well established. Similar to a previous experimental study in IBS (Martin et al. 2006), elevated levels of creatinine were observed in the IBS group, which may be a sign of increased energy consumption and muscle contractility (Clark 1994). Furthermore, it is worth noticing that the sample size for the metabolomics study was limited, which implies that the results should be interpreted with extra caution. Overall, it may be that the relevance of a single identified biomarker is not particularly high, while a systematic up- or down-regulation of a certain group of molecules, such as lipids in the current study, could indicate a relevant metabolite type.

Taken together, intestinal microbiota composition is different in individuals with IBS in comparison to healthy volunteers. Particularly the B. catenulatum and the C. coccoides groups are reduced in IBS, but the biological significance of this finding remains unclear. The mucosal metabolic profile in IBS differs from that in healthy controls with regard to both lipid- and water-soluble metabolites. The most prominent finding of colonic mucosa from IBS patients is an upregulation of a number of lipid species.

6.3 The effect of probiotic supplementation in IBS

Effect on IBS symptoms

The multispecies probiotic supplementation significantly alleviated the primary outcome measure, the total IBS score. The IBS score, which incorporates both the frequency and the severity of symptoms, was reduced by approximately 40% with the probiotic supplementation and by less than 10% with placebo supplementation. According to recent guidelines on clinical trial design in functional gastrointestinal disorders (Corazziari et al. 2003; Irvine et al. 2006), global symptom measures that integrate IBS symptoms into a single numerical index are one of the recommended outcomes. There is no consensus on what constitutes a clinically meaningful improvement for IBS, but an approximately 50% improvement in the primary endpoint has been suggested as a reasonable definition of a responder and a 10-15% improvement of the global outcome measure over placebo as a clinically significant therapeutic gain (Corazziari et al. 2003). Based on these recommendations, the improvement over placebo in the current study is evidently clinically relevant, while the improvement in primary endpoint does not entirely reach an improvement of 50%. However, large variations in the responder definition occur, and some studies on alosetron and tegaserod have accepted as little as a 10% improvement in a visual analogue scale (Bardhan et al. 2000) or one step on a seven-step scale (Tack et al. 2005) as clinically relevant. Though several trials on IBS and probiotics report a statistically significant effect on a global symptom score, on individual symptoms or on adequate/satisfactory relief of symptoms, the use of different symptom
questionnaires, outcome measures and responder definitions makes it challenging to compare these results with the ones from the current study. The use of “adequate relief of abdominal pain and discomfort” or “satisfactory relief of IBS symptoms” as primary outcome measures have gained popularity during the last few years (Irvine et al. 2006), and their use in future probiotic trials would ensure better comparability between trials. Even though the effects of probiotics on certain types of diarrhoea are well-established, no effects on stool frequency or consistency were seen in the present study. This may not be unique to the multispecies probiotic since other studies that show beneficial effects with probiotic administration have equally failed to see an effect on bowel habits (Kim et al. 2003; Kim et al. 2005b; O’Mahony et al. 2005).

**Effect on quality of life**

The integration of HRQOL monitoring into treatment trials for IBS is strongly encouraged (Irvine et al. 2006). In Study II, no effect on quality of life was seen as assessed by the generic RAND-36 survey (Hays et al. 1993). It may be that the extent of symptom reduction was not considerable enough to trigger improvements in HRQOL. Alternatively, it could be that the generic questionnaire was unable to detect those HRQOL aspects that are of particular importance in IBS. However, the utilisation of the generic questionnaire made it possible to compare the scores for the IBS patients with Finnish population values. HRQOL in Finnish IBS patients has not been previously reported, and the current data show that quality of life is below population values for several scales in IBS patients, which is in line with international data (Hahn et al. 1999; Gralnek et al. 2000; Amouretti et al. 2006). In Study IV, the employment of the IBSQ (Wong et al. 1998) revealed an improvement of the bowel symptoms domain for the probiotic supplementation vs. placebo. The authors of the IBSQ have predefined a mean change of 0.5 on the 1 to 7 scale as a minimal important change. The present study demonstrated a change of 0.62 points in the probiotic group, but it should be noted that this was based on median, not mean, values. Few studies on IBS and probiotics have incorporated HRQOL measures. *Bifidobacterium infantis* 35624 has shown significant favourable effects on IBS symptoms in two studies, whereas concomitant improvement of HRQOL was seen in one study (O’Mahony et al. 2005) and unchanged HRQOL in another study (Whorwell et al. 2006). Another *Bifidobacterium* strain has been associated with improvement in comparison to placebo in the HRQOL discomfort score (Guyonnet et al. 2007).

To summarise, the multispecies probiotic alleviates the primary symptoms of IBS without affecting bowel habit or characteristics. The probiotic supplementation causes an approximately 40% reduction in the total IBS score, whereas the placebo has an effect of less than 10%. Generic HRQOL is not affected by probiotic treatment, but an IBS-specific domain describing bowel symptoms is positively influenced by the supplementation.
6.4 Characterisation of probiotic action in IBS

Modification of microbiota

The individual strains of the multispecies could all be detected in faecal samples in Study III. No other major modifications of microbiota or its metabolism, apart from an unexpected decrease in bifidobacteria in the probiotic group, could be seen. The probiotic strains were analysed by strain-specific qPCR assays, and as this method may also detect nonviable bacteria (Huijsdens et al. 2002), its usefulness in monitoring probiotic survival may be questioned. Though qPCR in general tends to give higher bacterial counts than cultivation, the two methods have been proven to correlate fairly well (Requena et al. 2002). Our findings are strengthened by an earlier cultivation-based study showing the survival of the strains in the GI tract (Viljanen et al. 2005b). It remains unclear why *Bifidobacterium* spp. were reduced in the probiotic group and increased in the placebo group. Supplementation with bifidobacteria has generally led to an increased (Link-Amster et al. 1994; Bouhnik et al. 1996) or unchanged (Amann et al. 1998) level of the genus in faecal samples. It appeared that only those with originally high counts of *Bifidobacterium* spp. were the ones who suffered the decrease. It can be hypothesised that the individuals harbouring significant levels may be more susceptible to temporal variations in bacterial counts, or that competitive inhibition between the ingested and intestinal bifidobacteria may have occurred. Besides a qPCR-based approach, the effects of probiotic supplementation on microbiota were analysed by a microarray method in Study IV. Instead of looking at each of the approximately 1,000 species on the array separately, a similarity index incorporating the information on all the probes was used. This novel approach shows, for the first time to our knowledge, a general stabilisation of the microbiota by probiotic supplementation. The finding should yet be considered preliminary, since the number of patients analysed was restricted. An overall stabilisation of the microbiota may have clinical relevance particularly in IBS as these patients may experience temporal instability of the microbiota (Mättö et al. 2005; Maukonen et al. 2006).

Immunomodulation

The hypothesis for immunomodulation as one mechanism behind probiotic effects in IBS relies on data showing systemic and mucosal immune activation in IBS (Dunlop et al. 2003b; O’Mahony et al. 2005; Barbara et al. 2007; Liebregts et al. 2007b) and on studies demonstrating immunomodulatory effects of probiotics (for review, see Ezendam and van Loveren 2006). The current study failed, however, to see any effects of the probiotic supplementation on serum sensitive-CRP, and the effects on serum cytokines remained unclear. The majority of samples presented cytokine levels below the detection limit as measured by a cytometric bead array, but it cannot be entirely ruled out that the probiotic may still influence the cytokine profile if another methodology is used. Only one previous study has incorporated the measurement of an immune marker into a clinical trial on probiotics and IBS (O’Mahony et al. 2005). By using PBMCs, this study demonstrated a normalisation of an initially elevated IL-10/IL-12 ratio following ingestion of *B. infantis* 35624. CRP could prove to be an interesting marker for probiotic effects, since one
study in atopic infants indicates modulation of CRP by LGG alone, but not with a multispecies probiotic (Viljanen et al. 2005a). In contrast, two other studies on *L. plantarum* 299v have not seen any effect on CRP in critically ill patients or in surgical patients (McNaught et al. 2002; McNaught et al. 2005). It may be that the mucosal immune system is more relevant than the systemic one when the action of probiotics in IBS is being considered.

As a whole, this study provides data showing a stabilisation of the microbiota of IBS patients by probiotic supplementation. The stabilisation occurred simultaneously with symptom alleviation, and it could at least partly explain the reduced GI symptoms. The precise bacterial groups or species accounting for the stabilisation require further characterisation. This study does not provide support for the idea that systemic immunomodulation is associated with IBS symptom reduction.
The present study investigated pathophysiological factors of IBS and the effect of multispecies probiotic supplementation on GI symptoms, health-related quality of life, intestinal microbiota and systemic immune markers in IBS. The main findings of this study can be summarised as follows:

- The intestinal microbiota composition and the mucosal metabolite profile are altered in IBS patients in comparison to healthy subjects. Management regimens aiming at modulating these pathophysiological findings could hence be of value in IBS.

- Multispecies probiotic supplementation with *L. rhamnosus* GG, *L. rhamnosus* Lc705, *P. freudenreichii* subsp. *shermanii* JS, and a *Bifidobacterium* significantly alleviates the primary symptoms of IBS and appears to improve the bowel symptoms domain of quality of life. The multispecies probiotic can be recommended in mild to moderate cases of IBS as an adjunct therapy to lifestyle modification and possible pharmacotherapy.

- Probiotic supplementation stabilises the intestinal microbiota in IBS, suggesting that the favourable effects of the probiotics may partly be mediated via mechanisms related to the microbiota. The results do not support systemic immunomodulation as a mechanism in symptom reduction.

- Based on this study, subjective symptom recording and monitoring of health-related quality of life remain the main end-points of clinical trials in IBS, and laboratory tests are of only modest, incremental value.
This study was carried out between the years 2002 and 2008 at the Institute of Biomedicine, Pharmacology, University of Helsinki, and at Valio Ltd, Research and Development, Helsinki.

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