Unexplained, self-reported food hypersensitivity

*Explorative studies on mechanisms of abdominal symptom generation*

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Abstract

**Background:** Self-reported food hypersensitivity remains unexplained in most cases. Abdominal symptoms, typically consistent with the irritable bowel syndrome (IBS), are common in patients with such unexplained, self-reported food hypersensitivity. The etiology is obscure.

**Aim:** The overall objective of the present study was to investigate possible mechanisms of postprandial abdominal symptom generation. A main purpose was to explore whether and how ingestion of low-digestible carbohydrates act as abdominal symptom triggers in patients with unexplained, self-reported food hypersensitivity.

**Main results:** The findings can be summarized as follows:

In study I, fructose-sorbitol malabsorption evoked more symptoms in patients with unexplained, self-reported food hypersensitivity than in healthy controls. Alterations in intestinal gas production and secretion of so-called ileal brake hormones (glucagon-like peptide 1 (GLP-1) and peptide YY (PYY)) could not be demonstrated.

In study II, serum levels of chromogranin A (CgA) were found to be lower in patients with unexplained, self-reported food hypersensitivity than in healthy controls.

In study III, lactulose malabsorption evoked more symptoms in patients with unexplained, self-reported food hypersensitivity than in healthy controls. The symptoms could not be fully explained by symptom anticipation, because lactulose induced more symptoms than placebo (glucose). Associated alterations in intestinal gas production and rectal levels of prostaglandin E$_2$ (PGE$_2$) and microbial fermentation products could not be demonstrated.

In study IV, mechanisms of diarrhoea in a mouse model of food allergy were investigated. Changes within the jejunum were demonstrated in the food allergic mice, with development of muscular hypocontractility, increased levels of cytokines IL-4 and IL-6 and high numbers of mast cells.
In study V, fecal levels of short-chain fatty acids (SCFA) were investigated, and the profile was different between patients with unexplained, self-reported food hypersensitivity and healthy controls. Increased proportions of butyric acid were demonstrated in the patient group, particularly in individuals with severe symptoms.

**Conclusion:** Taken together, the results suggest that intolerance to low-digestible carbohydrates plays an important role in abdominal symptom generation in patients with unexplained, self-reported food hypersensitivity. Disturbances of intestinal motility may contribute to gastrointestinal symptom development by increasing the amount of malabsorbed carbohydrates. Altered intestinal fermentation is a potential cause of the patients’ unexplained symptoms.
### List of abbreviations

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<th>Description</th>
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<tr>
<td>ANS</td>
<td>Autonomic nervous system</td>
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<td>CgA</td>
<td>Chromogranin A</td>
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<td>CH₄</td>
<td>Methane gas</td>
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<td>COLAP</td>
<td>Colonoscopic allergen provocation test</td>
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<td>BAFF</td>
<td>B-cell activating factor</td>
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<td>DBPCFC</td>
<td>Double-blind placebo-controlled food challenge</td>
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<td>EAACI</td>
<td>European Academy of Allergy and Clinical Immunology</td>
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<td>EC cell</td>
<td>Enterochromaffin cell</td>
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<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<td>ENS</td>
<td>Enteric nervous system</td>
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<tr>
<td>FODMAP</td>
<td>Fermentable oligo-, di and monosaccharides, and polyols</td>
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<td>GLP-1</td>
<td>Glucagon-like peptide 1</td>
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<td>H₂</td>
<td>Hydrogen gas</td>
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<td>HPA axis</td>
<td>Hypothalamic-pituitary-adrenal axis</td>
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<td>IBS</td>
<td>Irritable bowel syndrome</td>
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<td>IgA, IgE, IgG</td>
<td>Immunoglobulins type A, E and G</td>
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<td>IL-4, IL-6</td>
<td>Interleukins 4 and 6</td>
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<td>IFN-γ</td>
<td>Interferon gamma</td>
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<td>IPEC</td>
<td>Intragastric allergen provocation test under endoscopic control</td>
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<td>LDC</td>
<td>Low-digestible carbohydrate(s)</td>
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<td>MAI</td>
<td>Matallergi og –intoleranse (Food allergy and intolerance)</td>
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<td>MMCP-1</td>
<td>Mucosal mast cell protease 1</td>
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<td>MPT</td>
<td>Mucosal patch technique</td>
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<tr>
<td>mRNA</td>
<td>Messenger RNA (Ribo Nucleic Acid)</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>PGE₂</td>
<td>Prostaglandin E₂</td>
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<td>PYY</td>
<td>Peptide YY</td>
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<td>SCFA</td>
<td>Short-chain fatty acid(s)</td>
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<td>SIBO</td>
<td>Small intestinal bacterial overgrowth</td>
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<td>TGFβ−1</td>
<td>Transforming/tumor growth factor beta 1</td>
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<td>TRPV1</td>
<td>Transient receptor potential vanilloid type 1</td>
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<td>WAO</td>
<td>World Allergy Organization</td>
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List of publications

The thesis is based on the following papers, which will be referred to in the text by their Roman numerals:


The following methodological comment is included as an appendix:

1. Introduction

1.1 Background

Adverse reactions to food have probably always been recognized and feared by man [1]. Food hypersensitivity is common, but whereas up to 35% of the general population in Western countries suspect themselves to be food allergic [2], double-blind placebo-controlled food challenge (DBPCFC) – the “gold standard” diagnostic test – can only verify reactions in response to specific foods in 1-2% [3;4]. The discrepancy between self-reported and medically confirmed food hypersensitivity (fig. 1) poses a great challenge for both patients and doctors, and a better understanding of the phenomenon is clearly needed.

![Figure 1](image_url)

**Figure 1.** Self-reported food hypersensitivity is common in the general population, but remains unexplained in most cases. Illustration by the author.

This thesis will focus on pathogenetic aspects of unexplained, self-reported food hypersensitivity in adults, with emphasis on gastrointestinal symptom development.
1.2 Terminology

In 1995 the European Academy of Allergy and Clinical Immunology (EAACI) published a nomenclature position statement paper regarding the terminology of adverse reactions to food [5]. Adverse reactions to food were divided into toxic (i.e. reactions that occur in any individual exposed to a sufficient dose) and non-toxic (i.e. reactions that depend on individual susceptibility) categories, and the non-toxic reactions into immune-mediated (i.e. food allergy) and non-immune-mediated (i.e. food intolerance) groups. The nomenclature was revised by the EAACI in 2001 [6], and updated by the World Allergy Organization (WAO) in 2004 [7]. According to the revised classification (fig. 2), food hypersensitivity – defined as “objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by normal persons” – should be used as an umbrella term to cover both allergic food hypersensitivity (i.e. reactions where immunological mechanisms are defined or strongly suspected) and non-allergic food hypersensitivity (i.e. reactions where immunological mechanisms are excluded). Allergic food hypersensitivity should embrace both IgE-mediated and non-IgE-mediated reactions.

![Diagram showing the classification of food hypersensitivity](image)

**Figure 2.** Nomenclature of food hypersensitivity, according to the WAO [7]. Illustration by the author.
Although the proposed classification may seem logical and theoretically simple, diagnosing food hypersensitivity can be a demanding and complicated task in clinical practice. In fact, self-reported or perceived food hypersensitivity remains unexplained in most cases, despite extensive medical examinations. This group is conceivably “heterogenous”, but there are currently no convincing diagnostic methods to distinguish between different underlying etiologies. Various names have been applied to describe these “problem patients”, such as ‘pseudo-allergy’ [8], ‘psychological food intolerance’ [9], ‘psychosomatic food adverse reactions’ [10], and ‘subjective food hypersensitivity’ [11]. ‘Unexplained, self-reported food hypersensitivity’, albeit somewhat cumbersome, is presumably an informative and neutral term, and will be used in this thesis.

1.3 Symptomatology

Unexplained, self-reported food hypersensitivity may be regarded as a functional somatic syndrome [12], in which medically unexplained physical symptoms are ascribed or attributed by the patients to intake of certain foods. The clinical presentation may be different from that of patients with medically confirmed food allergy [13]. The symptoms are typically vague and diffuse, multisystemic and chronic, and commonly attributed to intake of staple foods, most often fruits, vegetables, cereals and milk [14;15]. Patients with unexplained, self-reported food hypersensitivity also report reacting to more food items than patients with confirmed food allergy [14]. Psychiatric comorbidity is common [16] and health-related quality of life is considerably impaired [17], at least in a specialist health care setting.

Gastrointestinal symptoms seem to predominate [13], and the patients often complain of bloating, abdominal discomfort and disordered bowel habits (fig. 3).
The abdominal symptoms are typically chronic or recurrent, and in most cases consistent with the criteria for irritable bowel syndrome (IBS) [15]. Vice versa, self-reported food hypersensitivity is common in patients with IBS [20;21]. The IBS diagnosis labels a clinical phenotype, a constellation of symptoms that may have many causes. Although postprandial worsening of symptoms is not included in the current Rome consensus-based criteria for IBS [22], prospective symptom recording has shown that pain is temporally related to eating [23], and fasting may relieve the symptoms [24]. IBS due to possible food intolerance has recently been suggested as a separate entity [25], but whether patients with food-related IBS represent a discrete IBS subgroup or only differ from other IBS patients in terms of symptom interpretation, still remains unsettled.

**Figure 3.** Abdominal symptom profiles in patients with unexplained, self-reported food hypersensitivity. The figures are based upon self-administered questionnaires from 100 consecutive patients referred to Haukeland University Hospital for investigation of self-reported food hypersensitivity and abdominal symptoms between February 2008 and April 2010 [unpublished data]. A: Symptom severity scores (mean values with SD) according to Kane et al. [18]; B: Symptom severity scores (mean values with SD) according to Francis et al. [19]. Illustration by the author.
1.3.1 Role of low-digestible carbohydrates

The occurrence of food-related or postprandial symptoms in patients with functional gastrointestinal disorders typically involves many food items, and is therefore often interpreted as an expression of a generalized and unspecific sensitivity to meals, regardless of composition [20]. However, this may also reflect a propensity to react to categories of nutrients rather than specific epitopes. As such, carbohydrate-rich foods are particularly incriminated by the patients [14;15;20;21]. Indeed, a causal relationship between intake of heavily absorbable carbohydrates and chronic abdominal distress was suspected by physicians in the beginning of the last century – a phenomenon described as ‘Gährungsdyspepsie’ by Schmidt & Strasburger in 1901 [26], and ‘Intestinal carbohydrate dyspepsia’ by Hurst & Knott in 1932 [27]. These conditions were once recognized by highly respected clinicians [28], but fell into disrepute in the early 1970s, seemingly because high fiber diets became fashionable around that time [29].

Low-digestible carbohydrates (LDC) can be defined as carbohydrates that are incompletely or not absorbed in the small intestine, and are totally or partly fermented in the large bowel [30]. The term LDC thus covers both complex carbohydrates (resistant starches and non-starch polysaccharides (dietary fibers)) [31] and a group of heavily absorbable short-chain carbohydrates denoted by Gibson & Shepherd as FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) [32]. The usefulness of the term LDC may be debated, since individual LDC have different physiochemical characteristics and may differ in propensity to induce abdominal symptoms [33]. Nevertheless, the consequence of carbohydrate malabsorption seems to be a general, dose-dependent and additive phenomenon, seemingly reflecting some shared, group-based properties of all fermentable carbohydrates that escape small intestinal absorption [34]. Although there is evidence to suggest that ingestion of LDC is beneficial, LDC malabsorption seems to be poorly tolerated by some individuals [35-37]. The mechanisms behind such “symptomatic LDC malabsorption” or “LDC intolerance” are incompletely understood, and whether
it plays a role in patients with unexplained, self-reported food hypersensitivity remains to be shown.

1.4 Pathophysiology

Although the cause of the abdominal symptoms in patients with unexplained, self-reported food hypersensitivity is unknown, a disturbance in one or more of the control systems that regulate gut behaviour is probably implicated. Numerous signalling networks, residing both inside and outside the digestive tract, must interact in order to control and coordinate the complex actions of the gastrointestinal effector tissues (i.e. glands, musculature and vasculature) (fig. 4).

![Diagram](image)

**Figure 4.** Compartmentalization of gastrointestinal control systems. Illustration by the author.
The gastrointestinal tract is recognized as the largest endocrine [38] and immune [39] organ in the body. The enteric nervous system contains as many neurons as the spinal cord [40], and the gut lumen harbours an exceedingly complex microbial flora [41]. In addition, myriads of neural and humoral signals constitute an important, bi-directional communication route between the brain and the gastrointestinal tract, the so-called brain-gut axis or gut-brain axis [42]. There is an extensive interplay between the neurological, immunological, endocrinological and microbiological components of the gastrointestinal tract. Intake of food affects all of these systems.

1.4.1 Role of the central nervous system

Physicians tend to consider psychological explanations when no organic pathology can be demonstrated. However, no illness exists in a vacuum, and “psychological factors” are always present. It has been stated that “the gastrointestinal tract is the primary battleground for the conflicts between the psyche and the soma” (quoted in [43]). Indeed, digestive problems have been associated with psychological disturbances for many centuries [44] – but what are the links?

There are at least three, not necessarily mutually exclusive, possibilities: Psychological disturbances may 1) cause or exacerbate the illness, 2) be a consequence of the illness, or 3) influence symptom experience and interpretation, and thus determine illness behaviour.

Classical studies of fistulous patients, by Willam Beaumont (1785-1853) on Alexis St. Martin (1794-1880) [45] and by Stewart Wolf (1914-2005) on Tom [46], demonstrated a direct effect of emotions on gastric physiology. Ivan Petrovich Pavlov (1849-1936) discovered a ‘psychogenic secretion’ mechanism of the digestive glands in dogs [47], and Walter Bradford Cannon (1871-1945) evoked inhibition of intestinal motility in cats by scaring them [48]. Modern stress research has revealed important pathways involved in this “top-down” communication. Through the autonomic nervous system (ANS) and the neurohumoral hypothalamic-pituitary-
adrenal axis (HPA axis), stress exerts several well-documented effects on both intestinal and extra-intestinal functions [49]. Emotions modulate the response and specific affective states may elicit different gastrointestinal reactions [50;51], conceivably providing an explanation why intestinal transit tends to be faster in anxious patients and slower in depressed patients [52]. Acute stress induces alterations in gastrointestinal motility and sensitivity that may cause or exacerbate symptoms of functional gastrointestinal disorders; e.g. impaired gastric emptying [53] and accommodation [54] in functional dyspepsia, and accelerated small intestinal transit [55;56] and increased distal colonic motility [57] in IBS. However, the effects of chronic stress, which is often more relevant in a clinical setting, is less well characterized, at least in man.

Extensive activation of cognitive networks, due to so-called cognitive-emotional sensitization, has been proposed to play an important role in the pathophysiology of unexplained, self-reported food hypersensitivity [11]. Conceivably, such central mechanisms may explain the generation of numerous vague and diffuse symptoms from several organ systems [58]. An old case study deserves to be mentioned here, as an example of “central sensitization” [59]: A 54 year old woman with a diagnosis of functional diarrhoea, attributed by herself to the ingestion of pork meat, was examined with x-rays. First, minced pork meat was added to the barium solution, but the patient did not know. She experienced no symptoms and the films were completely normal. Second, the investigation was done only with the barium solution, but in addition, the patient ate a small slice of roast pork. She then experienced strong abdominal pain, and changes in the mucosa and motor activity of the small intestine were seen on the films. A similar study, using balloons to assess gastric and duodenal contractions in a woman with perceived milk intolerance, was reported by Graham et al. [60]. To repeat such experiments with more refined techniques, e.g. transabdominal ultrasonography [61], would be interesting – especially since the concept of cognitive-emotional sensitization is based mainly on theoretical considerations [58].
Philippe Pinel (1745-1826), known as the father of modern psychiatry, stated that “the primary seat of insanity generally is in the region of the stomach and intestines” (quoted in [62]). Psychological disturbances may evolve secondary to abdominal abnormalities, both as direct [63] and indirect [64] consequences. An important example is duodenal ulcer disease, once regarded as a prototypical psychosomatic disorder, in which measures of psychological distress normalize following eradication of Helicobacter pylori infection [65]. Cortical affective information processing can be modulated by signals originating in the gastrointestinal tract and conveyed to the nucleus tractus solitarii, both through the vagus nerves and the sacral spinal nerves [66]. Indeed, electrical stimulation of the vagus nerve is a promising treatment modality for patients with major depressive disorder [67]. Although the view that psychopathology is a direct cause of gastrointestinal perturbations is both provocative and controversial, emerging evidence from animal studies supports the notion [68-71]. However, little is still known about such “bottom-up” influences in man [72;73].

The possibility that psychopathology pertains to illness behaviour rather than the abdominal symptoms per se, has been suggested [74-77]. Far from all subjects suffering from functional gastrointestinal disorders decide to consult a physician [78], and “consulters” seem to represent a self-selected group, in which psychopathology is more common than in “non-consulters”. Accordingly, psychiatric comorbidity is common in patients with unexplained, self-reported food hypersensitivity referred to a specialist health care centre [16], but seemingly not in a community [79] or a primary health care [80] setting. However, differences in assessment of psychopathology may also play a role, since one population-based study demonstrated high prevalences of mood and anxiety disorders in subjects with self-reported allergies using structured psychiatric interviews [81].

Importantly, psychological factors do not seem to be major predictors of neither intestinal nor extra-intestinal symptom severity in patients with unexplained, self-reported food hypersensitivity [82]. Psychological disturbances may, however, be related to a tendency to interpret symptoms as signs of food hypersensitivity. Having difficulties in identifying and expressing feelings, and thus realizing and
communicating psychological problems, conceivably influence attribution style [83]. Hence, psychological problems may be presented as food hypersensitivity [84]. Besides, somatic explanations are often regarded as more reputable than psychological explanations [85]. The belief that the problems are caused by food is probably reinforced and nourished by claims from media, alternative therapists and controversial scientists, as well as going through repeated medical investigations [86]. Simrén et al. [20] showed that IBS patients with anxiety had higher “food scores”, reflecting both the number of foods claimed to produce symptoms and the symptom severity, than IBS patients without anxiety. However, in a population-based study of subjects with IBS, Monsbakken et al. [21] found no correlation between numbers of food items related to abdominal symptoms and degree of psychopathology, as assessed by Hopkin Symptom Check List 10 scores.

*Hence, the role of psychological factors in unexplained, self-reported food hypersensitivity is obviously complex. The abdominal symptoms should not be regarded merely as “gutfelt emotions”, and a biopsychosocial approach is required.*

### 1.4.2 Role of the gut immune system

In 1906 Baron von Pirquet (1874-1929) coined the term ‘*Allergie*’ to designate a ‘changed reactivity’ induced by external agents (*allos* means ‘other’, and *ergon* means ‘work’); originally a wide concept embracing both immunity and hypersensitivity [87]. The conventional Gell-Coombs classification recognizes four distinct types of immunological hypersensitivity reactions, denoted as types I, II, III and IV [88]. Type I responses are immediate reactions involving IgE-antibodies and mast cell degranulation, whereas type IV responses are delayed reactions involving T lymphocytes and cytokine production. Both mechanisms are of major clinical importance, but there is little evidence to suggest that type II (antibody-mediated cytotoxic hypersensitivity) or type III (immune complex-mediated hypersensitivity) responses are implicated in reactions towards foods [89].
Classical IgE-mediated food allergy is characterized by rapid onset of typical symptoms (e.g. anaphylactic shock, asthma, angioedema, urticaria, pruritus, rhinorrhea, vomiting, diarrhoea, abdominal pain), and positive sensitization tests (skin prick tests and/or systemic food-specific IgE antibodies) [90]. Conceivably, mechanisms involving IgE-antibodies and mast cell degranulation may also play a role in patients with unexplained, self-reported food hypersensitivity. As suggested by Lin et al. [91], local IgE-mediated reactions may be implicated. Indeed, an intestinal reaction resembling a typical food allergic response has been visualized in patients with unexplained, self-reported food hypersensitivity after intraduodenal administration of suspected allergens, using both endoscopic [92] and transabdominal [61] ultrasonography, as well as magnetic resonance imaging [93]. The intragastric allergen provocation test under endoscopic control (IPEC) [94], and the colonoscopic allergen provocation test (COLAP) [95], are examples of other potentially diagnostic methods. However, further validation is needed to establish the clinical utility of such provocation tests.

Intriguingly, atopy may predispose for IBS. Atopy has been defined by the WAO [7] as “a personal and/or familial tendency, usually in childhood or adolescence, to become sensitized and produce IgE antibodies in response to allergens, usually proteins”. ‘Atopic IBS’ has recently been suggested as a new entity [96;97]. The view that ‘atopic bowel’ may represent a discrete subgroup has been supported by Lillestøl et al. [98], who demonstrated that among patients with unexplained, self-reported food hypersensitivity, subjects with atopy had higher counts of IgE-bearing mast cells in duodenal biopsies and higher values of intestinal permeability than subjects without atopy. Whether these observations have any clinical relevance requires further investigation, but mast cells coated with IgE antibodies are conceivably more reactive than mast cells without such ‘arming’ [99].

Non-IgE-mediated food allergies are generally much more difficult to diagnose than IgE-mediated food allergies. Apart from celiac disease, such reactions are seldom recognized. Measurement of food-specific IgA and IgG levels to diagnose food hypersensitivity is controversial, as these antibodies appear to reflect a normal rather
than an allergic immune response to dietary antigen exposure [100]. Nevertheless, high levels of food-specific IgGs seem to be associated with gastrointestinal symptoms [101-103]. Lymphonodular hyperplasia – an endoscopic finding of uncertain clinical significance – may indicate delayed food allergy in children [104;105], and possibly also in adults [106;107]. The mucosal patch technique (MPT) [108], and measurement of B cell-activating factor (BAFF) [109] may provide future directions for detecting non-IgE mediated food allergies.

The significance of immune-mediated food hypersensitivity in the pathophysiology of IBS remains elusive. Nevertheless, subclinical, low-grade, chronic inflammation seems to be of importance, especially in post-infectious forms of IBS [110]. The classical study of Chaudhary & Truelove, published in 1962 [111], is often recognized as the first description of post-infectious IBS. However, Stewart coined the term ‘post-dysenteric colitis’ in 1950 [112], and ‘post-acute infectious diarrhea’ was discussed and acknowledged as an etiological factor for functional diarrhoea at the 57th annual meeting of the American Gastroenterological Association in 1956 [113]. Altered numbers of innate and adaptive immune cells, as well as changed levels of cytokines, have been demonstrated in blood samples and gut tissue specimens from patients with both post-infectious and non-postinfectious functional gastrointestinal disorders [114]. Animal studies have revealed important neuroimmune interactions with relevance for symptom generation [115].

Intestinal mast cells may be particularly involved in the pathophysiology of IBS [116]. These bone marrow-derived cells reside in the gut wall and are packed with granulae containing numerous chemical mediators, which are released upon activation. Although the classical activation of mast cells, by allergens crosslinking IgE antibodies bound to cell surface receptors on mast cells, provides a very powerful stimulus, mast cells are also activated by many other stimuli. Mast cells have direct contact with enteric nerve endings [117], and Santos et al. [118] demonstrated that mast cell mediators are released into the gut lumen during periods of cold pain-induced stress. Furthermore, rodent studies have shown that the stressful event of maternal deprivation in early life leads to increased density of gut mucosal mast cells
Mast cell activation may also be implicated in other “hypersensitive” states, such as interstitial cystitis and asthma [120].

Hence, there is evidence to suggest that immunological mechanisms are implicated in the pathogenesis of unexplained, self-reported food hypersensitivity, at least in subsets of patients. As presently used diagnostic tests may be inadequate, further studies are needed.

1.4.3 Role of the enteric nervous system

In the mid-nineteenth century, large collections of nerve cells were discovered within the gut wall by Georg Meissner (1829-1905; plexus submucosus) and Leopold Auerbach (1828-1897; plexus myentericus) [121]. For a long time these ganglia were dismissed merely as parasympathetic relay stations. However, the enteric nervous system (ENS) contains both sensory and motor neurons, as well as interneurons and supportive glial cells, forming circuits that are able to process and integrate information independent of extrinsic innervation [122]. Hence, the ENS acts as a ‘microcomputer’, popularly known as “the gut brain”, “the second brain” or “the brain gone south”.

The ENS participates in the regulation of practically all gastrointestinal functions, and may thus play an important role in many digestive disorders, including adverse reactions to food. Interestingly, the gut seems to react to luminal threats (allergens, bacteria, viruses, parasites, toxins) in a stereotypical manner, suggesting that a general defense mechanism – a final common pathway – is involved in the response towards noxious substances. According to Wood [123], the ENS contains a neural pattern generator – an ‘enteric alarm program’ – that is turned on whenever certain material in the intestinal lumen is sensed and perceived as being foreign or harmful. Upon activation, a protective behaviour occurs, whereby the actions of the glands, musculature and vasculature are orchestrated by the ENS to eliminate the offending stimulus by rapid expulsion of the intestinal content, either rostrally (emesis) or
caudally (diarrhoea). Phylogenetically, this pre-programmed response is probably very old, and resembles other protective mechanisms, such as coughing and sneezing. Importantly, the enteric alarm program may also be activated by the central nervous system [123]. Intriguingly, the ultrasonographic findings by Arslan et al. [61;92;93] suggest that some patients with unexplained, self-reported food hypersensitivity are characterized by inappropriate activation of the enteric alarm program – either induced by peripheral (immunological) or central (cognitive-emotional) mechanisms.

Alterations of gut motor and sensory functions seem to play an important role in the pathophysiology of IBS. Although gastrointestinal dysmotility and visceral hypersensitivity are complex phenomena, two recent studies suggest that ENS abnormalities may be particularly involved. First, studying full-thickness jejunal preparations, Törnblom et al. [124] demonstrated myenteric ganglionitis in patients with severe IBS, indicating that severe IBS may represent a mild form of enteric neuropathy. Second, Akbar et al. [125] observed increased numbers of TRPV1 (transient receptor potential vanilloid type-1)-immunoreactive nerve fibers in mucosal rectosigmoid specimens from IBS patients – a possible neurobiological substrate for visceral hypersensitivity. These interesting findings are still preliminary observations, however, and further clarification is needed.

Hence, unexplained, self-reported food hypersensitivity is probably not primarily a ‘gut brain defect’. However, by means of its integrative functions, the ENS is likely involved in the generation of the patients’ gastrointestinal symptoms.

### 1.4.4 Role of the enteroendocrine system

Since Bayliss & Starling published their discovery of secretin in 1902 [126], a multitude of gut hormones has been characterized. Numerous enteroendocrine cells are dispersed among the absorptive enterocytes, and act as sensory transducers that “taste” the luminal content and translate this information into chemical messages.
These signals regulate several gastrointestinal functions and inform the brain about the state of the gut.

Serotonin producing enterochromaffin cells (EC cells) constitute a major part of the enteroendocrine cell population. Indeed, most of the serotonin in the body is synthesized by these cells. In this regard, the name ‘enteramine’, as suggested by Erspamer in the 1930s, would have been a more appropriate term than ‘serotonin’ [127]. The EC cells have traditionally been conceived as “bottle-shaped” cells which empty their granular contents into the lamina propria. However, recent studies have revealed a neuron-like morphology [128] and ability of luminal secretion [129]. Serotonergic mechanisms seem to play an important role in functional gastrointestinal disorders [130]. Intriguingly, increased plasma serotonin concentrations have been demonstrated in patients with diarrhoea-predominant IBS following ingestion of a carbohydrate-rich meal [131], as well as after cold water intake [132]. However, as pointed out by Camilleri [133], peak serotonin levels were reached well after onset of the postprandial symptoms in both studies. Thus, the role of serotonin in unexplained, self-reported food hypersensitivity remains unclear.

Apart from serotonin, abnormal levels of several regulatory gut peptides have been described in patients with functional gastrointestinal disorders. However, the results are generally inconsistent [134-137], and the clinical importance of the findings is still largely unknown [138]. Although some studies have investigated peptide release after different test meals, few investigators have attempted to relate the endocrine response with the potential of the test meal to induce postprandial symptoms.

Chromogranins are stored and secreted together with amines and peptide hormones from the diffuse neuroendocrine system. These proteins serve as pro-hormones for a range of peptides with regulatory properties [139]. Interestingly, some of the peptides may be released into the gut lumen [140]. Fragments of chromogranins exert antimicrobial effects and may modulate gastrointestinal motility and sensitivity [141], but their potential role in functional gastrointestinal disorders is yet unknown.
Hence, disturbances of enteroendocrine pathways may play a role in patients with unexplained, self-reported food hypersensitivity. Specific nutrients stimulate different hormones, possibly explaining why some foods are better tolerated than others. However, studies addressing these aspects are still scarce.

1.4.5 Role of the gut microbial flora

The idea that the intestinal content can cause a number of physical and psychological ailments is very old [142]. Remedies to clean the bowel were used already in ancient Egypt, probably inspired by the peculiar behaviour of the ibis bird, as stated by Plinius (23-79 A.D.): “The bird which is called the ibis and which is a native of Egypt, by means of its hooked beak, laves the inside of his body by introducing water into the channel, by which it is especially necessary for health that the residuous food should be discharged” (quoted in [143]). Charles-Joseph Bouchard (1837-1915) wrote a book about ‘Auto-intoxication’ in 1894 [144], and the Royal Society of Medicine discussed the role of ‘Alimentary toxæmia’ at a symposium in 1913 [145]. Although these thoughts gradually fell into disrepute [146], new molecular methods to investigate the microbial ecology of the gut have been developed during the last decades [147], enabling scientists to revisit and renew old concepts [148].

The human body contains about 10 times more microbes than human cells, and the gut harbours around 1000 different bacterial species [149]. Regarding its size and metabolic activity, the “microbe organ” is comparable to the liver [150]. Communication between microbiota and their hosts has been denoted as “inter-kingdom signalling”, and constitute a promising research area [151]. The gut microbial flora exerts several protective, structural and metabolic functions [41] and may be incriminated in a number of diseases, including allergy [152], adiposity [153] and autism [154]. However, our understanding of the complex gastrointestinal ecosystem is still in infancy.
Disturbances of the gut microbial flora, so-called “dysbioses” [155], are conceivably implicated in the pathogenesis of functional gastrointestinal disorders [156]. A number of epidemiological studies have demonstrated that gastrointestinal infections and antibiotic usage, factors that disturb the indigenous flora, may increase the risk of developing IBS [157]. Most clinical studies have focused on describing the composition of the fecal microbiota and testing the effect of more or less arbitrary “biotic” therapies (antibiotics, probiotics, prebiotics, synbiotics) in patients with IBS. Less effort has been put on mechanistic studies, i.e. understanding what the microbes do and how this may contribute to symptom generation [158].

The assumed primary function of the gut microbial flora is decomposition of otherwise indigestible food components by means of fermentation [159]. This is a critical part of the digestive process, not only in plant-eating animals (herbivores), but also in humans (omnivores). If nutrients entering the colon are not fermented by bacteria, calories will be lost in feces, together with water and electrolytes. Thus, the role of the colon as a ‘salvage organ’ [160] is dependent on microbes. In return, the microbes are offered stable food supplies and optimal housing conditions for growth and reproduction. Intestinal fermentation yields both gases (e.g. hydrogen, methane, carbon dioxide) and short-chain fatty acids (SCFA; e.g. acetic, propionic and butyric acids), and should normally take place mainly in the proximal colon. Excessive, impaired or altered fermentation, as well as fermentation in other parts of the gastrointestinal tract (i.e. small intestine, rectum), may cause symptoms. Such problems have been denoted as ‘enterometabolic disturbances’ by Hunter [161], and may be involved in the pathogenesis of meal-related, functional gastrointestinal disorders.

_Hence, the “microbe organ” is still largely a terra incognita. Disturbances of the gut microbial flora, and consequently, intestinal fermentation, may play a role in gastrointestinal symptom development in patients with unexplained, self-reported food hypersensitivity._
2. **Aims of the study**

The overall objective of the present study was to investigate possible mechanisms of postprandial abdominal symptom generation. A main purpose was to explore whether and how ingestion of LDC act as abdominal symptom triggers in patients with unexplained, self-reported food hypersensitivity.

The specific aims of the papers included in the thesis were:

I  To assess the role of fructose-sorbitol intolerance in patients with unexplained, self-reported food hypersensitivity, and evaluate whether the abdominal symptoms are related to excessive intestinal gas production or disturbances of ileal brake hormone secretion.

II To explore whether patients with unexplained, self-reported food hypersensitivity have abnormal circulating levels of CgA.

III To evaluate whether abdominal symptoms induced by lactulose ingestion in patients with unexplained, self-reported food hypersensitivity are related to intestinal fermentation, measured as intestinal gas excretion in breath samples and microbial fermentation products in rectal dialysates, or merely reflect symptom anticipation.

IV To investigate mechanisms of diarrhoea in a mouse model of food allergy.

V To explore whether the gut microbial flora has abnormal functions in patients with unexplained, self-reported food hypersensitivity.
3. Materials and methods

An overview of the procedures will be outlined in the following section. Methodological details are described in the separate papers.

3.1 Clinical studies

3.1.1 Patients

Consecutive patients with abdominal symptoms referred to Haukeland University Hospital for investigation of self-reported food hypersensitivity were asked to participate in the study. All patients underwent an extensive, multidisciplinary investigation program by a devoted team of clinical specialists (the "MAI group"; Norwegian abbreviation for 'Matallergi og -intoleranse'; 'Food allergy and intolerance'). Food allergies were excluded by allergological examinations, including careful history-taking and evaluation of immunological sensitization towards suspected food items by using skin-prick tests and measurements of food-specific IgE antibodies. Successive dietary trials were performed by a dietician, and began with an open elimination of the most strongly suspected food item from the diet for 2–3 weeks. If this led to symptomatic improvement, an open provocation test was performed, and if positive, a DBPCFC with the same food item was done. Organic gastrointestinal diseases were excluded by gastroenterological examinations, including upper endoscopy with duodenal biopsies (to diagnose celiac disease), and measurement of intestinal permeability. Colonoscopy was performed when indicated, i.e. if fecal calprotectin was elevated and/or if inflammatory bowel disease was suspected clinically. Initially, tests to diagnose lactase deficiency were performed routinely, but due to the low diagnostic yield [162], the test was soon abandoned. Exclusion criteria were age below 18 years, pregnancy or lactation, prior use (in the past 4 weeks) of antibiotics, confirmed food allergy and organic gastrointestinal diseases.
3.1.2 Controls

Healthy volunteers were recruited among the hospital staff and students at the University of Bergen. They were included if they considered themselves to be healthy, and were not otherwise examined. They were excluded if they had used antibiotics during the previous 4 weeks.

3.1.3 Questionnaires

IBS was diagnosed according to the Rome II criteria as follows [163]:

"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."

Severity of habitual abdominal symptoms was assessed by using questionnaires developed by Kane et al. [18] (study I) and Francis et al. [19] (study III & V). Severity of symptoms following ingestion of carbohydrates was quantified by using scoring scales modified from Farup et al. [162] (study I & III).

3.1.4 Breath tests

Hydrogen and methane breath tests are based on the fact that there is no source for \( H_2 \) or \( CH_4 \) in man other than microbial fermentation [164]. These gases are thus exclusively produced within the gastrointestinal system and a certain proportion (approximately 60% [165]) is excreted into exhaled air from the lungs. As outlined in a recent consensus document [166], certain precautions prior to the procedure are necessary, such as fasting and avoiding use of tobacco. In the present study, exhaled \( H_2 \) and \( CH_4 \) were assessed before and every 15 minutes for 3 hours after ingestion of a carbohydrate solution by using breath collection bags and a gas chromatograph from Quintron Instrument Company (WI, USA). In study I & II, a mixture of 25 g
fructose and 5 g sorbitol in 250 ml tap water was ingested. In study III, mixtures of 10 g lactulose in 120 ml tap water and 10 g glucose in 120 ml tap water were ingested. Osmolality was measured in the carbohydrate solutions as follows (Fiske® Microsample Osmometer, Advanced Instruments Inc., Norwood, MA, USA): fructose-sorbitol – 714 mOsm/kg H₂O; lactulose – 324 mOsm/kg H₂O; glucose – 572 mOsm/kg H₂O.

3.1.5 Blood samples

Blood samples were analysed for GLP-1 and PYY at the Department of Biomedical Sciences, University of Copenhagen, Denmark (study I) and for CgA at the Department of Biological and Medical Psychology, University of Bergen, Norway (study II). The specimens were obtained by drawing blood through an intravenous cannula, allowing the subjects to acclimatize to the study protocol prior to commencing assessments. The importance of such a 'recovery period' has recently been emphasized by Chandarana et al. [167].

3.1.6 Rectal dialysis

In study III, *in vivo* rectal dialysis was employed. The principle behind the method was originally described in 1961 by Wrong et al. [168], and the technique has previously been used to examine a variety of clinical conditions, including assessments of SCFA [169], D- and L-lactate [170] and PGE₂ [171]. As shown by Lauritsen et al. [171], a duration of 4 hours is required for the dialysate to reach equilibrium with the surrounding fluid. The dialysis bags (fig. 5) were made of cellulose membrane tubing (Visking code DVT12000.01.000; molecular weight cut-off 12-14 kDa; Medicell International Ltd., London, UK), prepared by heating the membrane in a solution of 2% NaHCO₃ and 1 mM EDTA at +80°C for 30 minutes. Twelve cm long dialysis bags were tied off and filled with 4 ml Rheomacrodex® (10% dextran (mean molecular weight 40 kDa) in saline; Meda A/S, Norway). Dialysates were analysed for SCFA at the Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Sweden, and for D- and L-lactate and PGE₂ at the Institute of Medicine, University of Bergen, Norway.
3.1.7 Fecal samples

Stool collection may be challenging [172]. In study V, special plastic boxes were used (reg. codes 257077 and 257078, Coperate Express, Oslo, Norway). The upper edge of these boxes is equipped with a rim, making it easy to hold the box with both hands while defecating directly into it. The subjects were carefully instructed on how to perform this procedure, and were told to store the boxes at −20° C immediately after voiding feces. Stool samples were analysed for SCFA at the Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Sweden.
3.2 Experimental animal study

In study IV, an experimental mouse model of food allergy developed by Vaali et al. [173] was employed. A unique feature of this model is that no immunostimulatory adjuvants are used to induce the allergic response (adjuvants have been referred to by Charles A. Janeway Jr. (1943-2003) as “the immunologist’s dirty little secret” (quoted in [174])). The protocol is outlined in figure 6.

**Figure 6.** Murine food allergy model: schematic outline of protocol. Courtesy of Dr. Kirsi Vaali.

Briefly, Balb/c mice are epicutaneously sensitized with ovalbumin (allergic mice) or sham-sensitized with saline (controls). After an immunological maturation period, needed to produce ovalbumin-specific IgE antibodies, both groups are thereafter challenged with intragastric administration of ovalbumin. If successful, the ovalbumin-sensitized mice, but not the sham-sensitized mice, develop diarrhea after
the sixth intragastric challenge, and thereafter repeatedly within 20 to 60 minutes after each intragastric challenge.

In study IV, the mice were sacrificed 1 hour after the tenth intragastric challenge. Segments of jejunum were then obtained and the *in vitro* contractility of the circular musculature towards carbachol was studied in organ bath (fig. 7). Jejunal smooth muscle layer thickness and mucosal mast cell protease-1 (MMCP-1) positive cell density were assayed histologically. Serum MMCP-1 and immunoglobulins were measured by ELISA at the Institute of Medicine, University of Bergen, Norway, whereas mRNA expressions of IFN-γ, IL-4, IL-6 and TGFβ-1 from jejunal and ileal tissue segments were analyzed with quantitative real-time PCR at the Wihuri Research Institute, Helsinki, Finland.

![Figure 7](image)

**Figure 7.** Organ bath arrangement used to assess the *in vitro* contractility of the jejunal circular musculature. Illustration by the author.
3.3 Ethics

The clinical studies (I, II, III, V) were approved by the Regional Committee for Medical Research Ethics and conducted according to the Declaration of Helsinki. The animal study (IV) was approved by the Norwegian Animal Research Authority and conducted according to the European Convention for the Protection of Vertebrates Used for Scientific Purposes.

3.4 Statistics

Data were analyzed using Graphpad Prism version 5 (Graphpad Software Inc., San Diego, CA, USA) and SPSS (version 14, SPSS Inc., Chicago, IL, USA). P-values less than 5% were considered as statistically significant. Details are described in the separate papers.
4. Results

4.1 Study I

Eighteen patients with unexplained, self-reported food hypersensitivity and 15 healthy volunteers of similar age, gender and body mass index were included in the study. Sixteen of the 18 patients (89%) had IBS according to the Rome II criteria and habitual symptom scores were higher in patients than in controls (P < 0.0001). Following ingestion of a mixture of 25 g fructose and 5 g sorbitol, carbohydrate malabsorption, as defined by breath tests criteria, was demonstrated in 61% of the patients and in 73% of the controls. Nevertheless, the patients experienced significantly more symptoms following carbohydrate challenge, and 78% of the patients claimed that the challenge replicated their habitual gastrointestinal complaints. Patients classified as ‘malabsorbers’ experienced more symptoms than patients classified as ‘absorbers’ (P = 0.03). No significant differences in gas excretion or GLP-1 and PYY levels were found between patients and controls or between symptomatic and asymptomatic carbohydrate malabsorbers. A weak correlation between hydrogen excretion and PYY levels was demonstrated in non-producers of methane.

4.2 Study II

The same subjects as in study I were investigated. Serum levels of CgA were significantly lower in patients with unexplained, self-reported food hypersensitivity than in healthy controls at baseline (P = 0.005), and after 60 and 180 minutes following fructose-sorbitol ingestion (P = 0.007 and P = 0.004, respectively). In addition, serum levels of CgA fell significantly from baseline to 180 minutes following fructose-sorbitol ingestion (P = 0.04 for patients and P = 0.01 for controls).
4.3 Study III

Twenty-seven patients with unexplained, self-reported food hypersensitivity and 9 healthy volunteers of similar age, gender and body mass index were included in the study. Twenty-five of the 27 patients (93%) had IBS according to the Rome II criteria and habitual symptom scores were higher in patients than in controls (P < 0.0001). Patients were examined twice, with ingestion of 10 g lactulose and 10 g glucose, given in random order and in a double-blinded fashion. Controls were examined only once, with 10 g lactulose. In patients, symptom scores following lactulose ingestion were significantly correlated to habitual symptom scores (r = 0.6, P = 0.001), and were significantly higher than after glucose ingestion (P = 0.01). Symptom scores following both lactulose and glucose ingestion were significantly higher in patients than in controls (P = 0.0007 and P = 0.03, respectively). Levels of SCFA, lactate and PGE₂ in rectal dialysates were not significantly different after lactulose and glucose, or between patients and controls. Hydrogen excretion was not correlated with symptom scores.

4.4 Study IV

Eight Balb/c mice were sensitized towards ovalbumin (food allergic mice) and 5 Balb/c mice were sham-sensitized with saline (control mice). Both groups were challenged with repeated intragastric administrations of ovalbumin, whereby diarrhoea developed in 5 of the 8 food allergic mice. Hypocontractility of the jejunal circular musculature in response to carbachol stimulation was demonstrated in the food allergic mice with diarrhoea. Food allergic mice had higher jejunal mRNA levels of cytokines IL-4 and IL-6 and increased numbers of jejunal mast cells compared to controls. Jejunal smooth muscle layer thickness and jejunal mRNA levels of IFN-γ and TGF-β1 did not differ between the groups. Serum levels of ovalbumin-specific IgE antibodies were significantly correlated with numbers of jejunal mast cells (r = 0.879, P < 0.0001), as well as serum levels of MMCP-1 (r = 0.863, P < 0.0001).
4.5 Study V

Thirty-five patients with unexplained, self-reported food hypersensitivity and 15 healthy volunteers of similar age, gender and body mass index were included in the study. Thirty-four of the 35 patients (97%) had IBS according to the Rome II criteria. Fecal concentrations and excretions (output) of SCFA were similar in patients and controls, but n-butyric acid comprised a higher ($P = 0.035$) and acetic acid a lower ($P = 0.012$) proportion of total SCFA concentration in patients compared to controls. There were no significant correlations between habitual symptom scores and concentrations or excretions of individual or total SCFA, but the proportion of $n$-butyric acid was significantly higher in patients with severe symptoms compared to patients with moderate symptoms ($P = 0.016$).
5. General discussion

A main purpose of the present study was to explore whether and how ingestion of low-digestible carbohydrates (LDC) act as abdominal symptom triggers in patients with unexplained, self-reported food hypersensitivity. LDC provocation tests were thus used as tools to induce and study the pathophysiology of gastrointestinal symptoms, and this approach proved to be useful. In study I, intake of a heavily absorbable fructose and sorbitol mixture evoked more symptoms in patients than in controls, and although the prevalence of malabsorption was similar in these groups, patients classified as ‘malabsorbers’ experienced more symptoms than patients classified as ‘absorbers’. In study III, ingestion of the unabsorbable carbohydrate lactulose induced symptoms that resembled the patients’ habitual symptoms, both qualitatively and quantitatively, and these post-test symptoms were significantly worse than after ingestion of the easily absorbable carbohydrate glucose. The LDC-induced symptoms were associated with fermentation as assessed by measurements of intestinal gas excretion in breath samples. However, the mechanisms whereby LDC malabsorption causes symptoms were not fully explained. Taken together, study I & III thus suggest that LDC malabsorption is an important prerequisite for LDC-induced symptoms, but the pathophysiology remains unclear.

Tolerance to LDC is influenced by a number of factors, including characteristics of the meal, the individual and the gut microbial flora (fig. 8) [30]. Some of these factors will be discussed in the following text, based on the results of study I-IV and the findings of others.
Disturbances of motility in the upper digestive tract may impair LDC absorption, and aspects of gastrointestinal motor function were specifically addressed in study I & IV.

In study I, the so-called ‘ileal brake response’ was investigated. The aim was to explore whether altered blood levels of the ileal brake hormones glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) could be related to symptoms following ingestion of a poorly absorbable mixture of fructose and sorbitol. The findings did not support this view, however, since similar responses were demonstrated between patients and controls, and among symptomatic and asymptomatic carbohydrate malabsorbers. Study I was originally planned to assess orocecal transit of the fructose-sorbitol mixture as well, by using a scintigraphic method developed by Read et al. [175]. Briefly, 20 MBq $^{99m}$Tc-albumin-colloid (Nanocoll®) was added to the
carbohydrate solution, and radioactivity was measured every 15 minutes for 3 hours with a hand-held gammaprobe (codes 6150AD-17 (probe) and 6150AD-2 (dose rate meter); Automess GmbH, Ladenburg, Germany) positioned over a point 2 cm medial to the right anterior superior iliac spine, corresponding to the surface of the usual place of the cecum. However, this method proved to be inaccurate, and was therefore left out. Although study I failed to demonstrate altered functions of the endocrine L-cells in patients with unexplained, self-reported food hypersensitivity, GLP-1-based treatments for patients with IBS have recently been suggested [176;177]. Furthermore, ileal brake hormones exert effects mainly on upper gastrointestinal motility, and altered secretions of GLP-1 and PYY may therefore be particularly involved in the pathophysiology of functional dyspepsia [178]. L-cell derived peptides may thus play a role in patients with functional gastrointestinal disorders that ought to be further elucidated.

An additional assessment of the blood samples obtained in study I was subsequently published as a ‘Letter to the Editor’ in *Scandinavian Journal of Gastroenterology* (study II). Serum levels of chromogranin A (CgA) were analyzed and found to be significantly lower in patients with unexplained, self-reported food hypersensitivity than in healthy controls. Fragments of CgA exert antimicrobial effects and may modulate gastrointestinal motility and sensitivity [141], but the clinical significance of low systemic levels of CgA remains to be clarified. Follow-up studies with emphasis on EC cell abnormalities [179] have been initiated, and will hopefully gain further insights into the potential role of CgA in patients with unexplained, self-reported food hypersensitivity.

In study IV, *in vitro* contractility of the circular smooth muscle layer was investigated in jejunal segments obtained from mice. The main purpose of the study was to investigate whether food allergy induces a non-allergen-specific disturbance of intestinal motility. A stable derivative of acetylcholine was used to test this hypothesis, since acetylcholine is the principal neurotransmitter involved in intestinal smooth muscle contraction. The study demonstrated that specimens obtained from food allergic mice were less responsive to carbachol than specimens obtained from
control mice. This reduction of smooth muscle contractility was associated with – and possibly a consequence of [180] – increased levels of cytokines IL-4 and IL-6. Hypocontractility of the jejunal circular muscle layer conceivably causes a loss of intestinal tone, leading to a decrease of the intraluminal resistance and an increase of the flow of intestinal content [181]. As such, the finding may have a bearing to the reactions induced by intestinal provocation with suspected allergens observed by Arslan et al. [61;93] in patients with unexplained, self-reported food hypersensitivity. Intriguingly, postprandial loss of tonic motor activity of the intestinal musculature has recently been proposed to play a major role in symptom generation in patients with functional gastrointestinal disorders [182]. Decrease of intestinal tone may favour luminal filling by gas and fluid, and hence cause symptoms [182]. In addition, such changes may impair digestion and absorption of nutrients, especially of those that are heavily absorbable, thereby increasing the entrance of undigested and unabsorbed food residues, particularly LDC, into the colon [183;184].

In study IV, numbers of jejunal mast cells were increased in the food allergic mouse group. Intriguingly, similar findings have been reported in patients with diarrhoea-predominant irritable bowel syndrome [185]. Activation of intestinal mast cells may be implicated in LDC-induced abdominal symptoms in at least two ways. First, psychological stress may accelerate small intestinal transit [55;56] via mast cell degranulation [118], and thereby increase LDC malabsorption. Second, unabsorbed LDC may serve as an osmotic load, drawing fluid into the intestinal lumen, as well as triggering mast cell degranulation [186]. This may explain why LDC accelerate small bowel transit [187;188], and thus influence symptom generation [189]. The intestinal motor response to luminal hyperosmolarity seems to be individual [190], and is conceivably exaggerated in patients with functional gastrointestinal disorders [191]. Subjects with increased numbers of ‘IgE armed’ mast cells may be particularly exposed [98;99]. Induction of airway symptoms by mannitol inhalation in patients with exercise-induced bronchoconstriction (EIB) involves mast cell activation [192]. Whether degranulation of intestinal mast cells plays a similar role in gastrointestinal symptom development following LDC ingestion in patients with unexplained, self-
reported food hypersensitivity, deserves to be investigated. Indeed, IBS has previously been conceptualized as “asthma of the gut” [193].

Abnormalities of gastrointestinal motor function may affect the amount of LDC malabsorbed, and thus lower the threshold at which the symptoms occur [194]. Nevertheless, such alterations may not fully explain why ingestion of lactulose causes more symptoms in patients with unexplained, self-reported food hypersensitivity than in healthy controls, since lactulose is malabsorbed completely in both groups. Disturbances at the level of intestinal fermentation may be implicated. LDC serve as substrates for microbes producing gases and short-chain fatty acids (SCFA), and such fermentation products may be involved in abdominal symptom generation.

In study I & III, intestinal gas production following LDC ingestion was evaluated by measurements of intestinal gas excretion in breath samples. Although this method is a useful technique to assess overall changes in fermentation, it gives relatively little information about qualitative and quantitative changes within the viscera [195]. The lack of correlation between symptom scores and gas excretion in study I & III may reflect such limitations. However, as demonstrated by Morken et al. [196], intestinal gas volumes as quantified by scoring plain abdominal radiographs were not correlated with symptoms following lactulose ingestion, either. Despite certain studies [197;198], the overall impression thus seems to be that excessive intestinal gas production is not the main culprit of the symptoms [199]. Impaired gas transit and enhanced sensitivity to gaseous distension may play a role [200;201]. However, such problems could not explain the response to fructose-sorbitol malabsorption in a study by Evans et al. [202].

Gas is tolerated less well in the small intestine than in the large bowel [203], and small intestinal bacterial overgrowth (SIBO) may cause excessive gas production in the small intestine [204]. Although study I & III were not primarily designed to detect SIBO, the occurrence of so-called 'early positive breath tests', i.e. a rise in hydrogen excretion above baseline of more than 20 parts per million (ppm) within 90 minutes,
was similar in patients and controls in both studies. The validity of this criterion is debated, especially since rapid orocecal transit may cause ‘false’ early positive breath tests [205]. Simultaneous assessment of orocecal transit by use of scintigraphy can be of help to distinguish between SIBO and rapid orocecal transit [206]. Indeed, such a method was employed as a preliminary study in six patients with unexplained, self-reported food hypersensitivity, and confirmed that the large bowel was the source of hydrogen production in all cases [unpublished data]. Unfortunately, however, this combined breath test and scintigraphy assessment had to stop due to logistical reasons. The role of SIBO in patients with IBS is currently debated [207], and even the demonstration of excessive hydrogen excretion (>20 ppm above baseline concentration) in response to 10 g glucose in 3 patients in study III, may be explained by rapid orocecal transit [208].

Methane ($\text{CH}_4$) production in man was first described by François Magendie (1783-1855) in 1816 ([209], quoted in [210]). Methane is primarily produced by *Methanobrevibacter smithii* [211] and can be detected by breath sample measurements in about 35% of the population [212]. Approximately $10^8$ methanogenic microbes per gram dry weight of feces are required to generate enough CH$_4$ to be detected by breath analysis [213]. The overall impression from the present study is that methane is excreted rather independently of LDC ingestion. This probably reflects the fact that methanogenesis takes place mainly in the distal colon, and therefore depends more on endogenous than exogenous substrates [214]. Methane production has been associated with constipation [215], and measurement of exhaled methane has even been advocated as a diagnostic test for constipation-predominant IBS [216]. A direct inhibitory effect of the gas on intestinal transit has been suggested, based on *in vivo* and *in vitro* experiments in dogs and guinea pigs [217]. Reduction of postprandial serotonin levels may be a mediating mechanism [218]. In addition, prolonged intestinal transit may increase methane production [219]. However, conflicting results have been published [220], and methane non-producers and producers seemed to suffer equally from constipation in the present study (fig. 9 A).
Methane production may protect from symptoms following LDC ingestion [221]. An attractive explanation is that methanogenesis reduce the intraluminal gas volume by consuming H₂, according to the reaction 4 H₂ + CO₂ \rightarrow CH₄ + 2 H₂O. However, as demonstrated by Morken et al. [196], methane producers actually have more gas present in their bowels after lactulose ingestion as compared to non-producers. Furthermore, methane producers were not protected from experiencing post-lactulose symptoms in the present study (fig. 9 B). Taken together, the clinical significance of methanogenesis in patients with unexplained, self-reported food hypersensitivity thus remains unclear.

In study III, a dialysis technique was employed to assess the \textit{in vivo} rectal concentrations of prostaglandin E₂ (PGE₂) and microbial fermentation products. The
Rectum has previously been denoted as "a window to IBS" [222], and increased levels of rectal PGE\(_2\) have been incriminated in patients with food-related IBS [223]. The approach of using rectal dialysis was also encouraged by the presence of symptoms suggestive of disturbed distal bowel functions in patients with unexplained, self-reported food hypersensitivity, leading to the idea that the process of colonic fermentation may in part be distally displaced in these individuals. Indeed, preliminary work, using scintigraphy to assess gastrointestinal transit, indicated that a LDC-solution could reach the distal part of the colon rather quickly (fig. 10) [unpublished data].

**Fig 10.** Scintigraphic evaluation of gastrointestinal transit following intake of a solution consisting of 250 ml tap water, 25 g fructose, 5 g sorbitol and 20 MBq \(^{99m}\)Tc-albumin-colloid (Nanocoll®). The patient was a 39 year old male patient with unexplained, self-reported food hypersensitivity and IBS. Gastric emptying half-time was estimated to be around 15 minutes. Radioactivity in the cecum was demonstrated between 90 and 120 minutes, and in the descending colon between 120 and 150 minutes. Courtesy of Dr. Magne Følling.
In retrospect, however, the interval between the ingestion of the carbohydrate solution and the insertion of the dialysis bag may have been too short. Levels of SCFA, lactate and PGE$_2$ in rectal dialysates were not significantly different between the groups investigated in study III, and could thus not explain why lactulose ingestion evoked more symptoms in patients than in controls.

Study V demonstrated alterations of the fecal SCFA profile in patients with unexplained, self-reported food hypersensitivity as compared to healthy controls. The observed SCFA pattern corroborates in part with data from other groups [224;225], but not all [226;227]. The discrepancy between study V and the studies of Tana et al. [226] and Morken et al. [227] may be explained by differences in pre-analytical sample handling. The work of Tana et al. [226] was commented upon in a ‘Letter to the Editor’ in *Neurogastroenterology and Motility*, which is included in the Appendix. In the study of Morken et al. [227], the fecal samples were diluted with tap-water and homogenized before analysis, although this was not explicitly stated in their paper. Tana et al. [226] and Morken et al. [227] both showed increased levels of total SCFA in fecal samples from IBS patients as compared to healthy controls. These studies thus suggest that stool samples from IBS patients have a greater capacity to form microbial fermentation products *in vitro*. Interestingly, this characteristic was once considered as a pathognomic sign of ‘Gährungsdyspepsie’ [26], and the ability of incubated fecal samples to produce excessive gas was used diagnostically [228]. Whether patients with IBS excrete more fermentable substrates than healthy controls would thus be interesting to investigate in future studies.

The fecal SCFA profile observed in study V was characterized by increased proportions of butyric acid, particularly in patients with severe symptoms. This finding may have clinical implications, especially since butyrate has been shown to induce visceral hypersensitivity in rodents [229;230]. Intestinal fermentation of LDC by a microflora skewed to produce butyric acid may thus be a potential cause of LDC intolerance. At the same time, such a fermentation pattern conceivably offer protection against certain organic colorectal diseases [231;232]. Further studies are needed to clarify these aspects.
The gut microbial flora may adapt to dietary changes [233]. Indeed, this is the rationale for the use of prebiotics, defined by Gibson & Roberfroid as "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" [234]. Many LDC possess a potential to change the composition and/or functions of the gut microbial flora, and may thus act as prebiotics [235]. Attractively, continuous intake of LDC may protect against LDC intolerance through colonic adaptation, as has been shown for lactose in individuals with hypolactasia [236]. However, conflicting results have been published [237], and the ability to induce symptomatic adaptation does not apply to all LDC [238-240]. Furthermore, the specificity of prebiotics has recently been questioned [241].

The idea that patients with IBS should eat more LDC has been prevailing for long. Partly motivated by religious beliefs, early health enthusiasts like Sylvester Graham (1794-1851), Sebastian Kneipp (1821-1897), Thomas Richard Allinson (1858-1918) and John Harvey Kellogg (1852-1943) recommended eating more 'roughage' [43]. Coarse foods did not really become popular until the 1970s, however, when Denis Parsons Burkitt (1911-1993) hypothesized that most western diseases are caused by inadequate intake of dietary fiber [242]. Consequently, IBS was conceived as a disorder of fiber deficiency [243]. The effect of fiber on alleviating symptoms of IBS has, however, been extensively investigated and seems limited [244;245]. Soluble fibers, such as ispaghula or psyllium [246] and possibly soluble fibers contained within oatmeal porridge [247], may have positive effects. On the contrary, insoluble fibers seem to worsen the symptoms [248], and recent publications suggest that IBS patients actually benefit from reducing LDC intake [249;250]. Based on this evidence and on the results of the present papers, a study of the potential symptomatic effects of a LDC restricted diet in patients with unexplained, self-reported food hypersensitivity seems worthwhile.
6. Conclusions

1. Intolerance to low-digestible carbohydrates is a common problem in patients with unexplained, self-reported food hypersensitivity.

2. Carbohydrate malabsorption tests replicate habitual abdominal symptoms in patients with unexplained, self-reported food hypersensitivity.

3. Abdominal symptoms following carbohydrate malabsorption in patients with unexplained, self-reported food hypersensitivity are not fully explained by symptom anticipation.

4. Abdominal symptoms following carbohydrate malabsorption in patients with unexplained, self-reported food hypersensitivity are not correlated with intestinal gas production as assessed by breath sample measurements.

5. Patients with unexplained, self-reported food hypersensitivity and healthy controls secrete similar amounts of glucagon-like peptide 1 and peptide YY following fructose-sorbitol ingestion.

6. Patients with unexplained, self-reported food hypersensitivity have lower circulating levels of chromogranin A than healthy controls.

7. Rectal levels of prostaglandin E$_2$ are not significantly different between patients with unexplained, self-reported food hypersensitivity and healthy controls.

8. Abnormal rectal fermentation do not seem to be a cause of symptoms following carbohydrate malabsorption in patients with unexplained, self-reported food hypersensitivity.

9. Non-allergen-specific hypocontractility, increased levels of cytokines IL-4 and IL-6 and high numbers of mast cells were demonstrated in the jejunum of food allergic mice, and may be involved in diarrhoea development.
Patients with unexplained, self-reported food hypersensitivity have a different profile of fecal short-chain fatty acids than healthy controls, indicating altered functions of the gut microbial flora that may be involved in abdominal symptom generation.
7. References


42. Aziz Q, Thompson DG. Brain-gut axis in health and disease. Gastroenterology 1998; 114:559-78.


60. GRAHAM DT, Wolf S, WOLFF HG. Changes in tissue sensitivity associated with varying life situations and emotions; their relevance to allergy. J Allergy 1950; 21:478-86.


68. Hanstock TL, Clayton EH, Li KM, Mallet PE. Anxiety and aggression associated with the fermentation of carbohydrates in the hindgut of rats. Physiol Behav. 2004; 82:357-68.


136. Van Der Veek PP, Biemond I, Masclee AA. Proximal and distal gut hormone secretion in irritable bowel syndrome. Scand J Gastroenterol 2006; **41**:170-7.


176. Hellstrom PM. GLP-1 playing the role of a gut regulatory compound. Acta Physiol (Oxf), In press.


201. Serra J, Azpiroz F, Malagelada JR. Impaired transit and tolerance of intestinal gas in

202. Evans PR, Piesse C, Bak YT, Kellow JE. Fructose-sorbitol malabsorption and
symptom provocation in irritable bowel syndrome: relationship to enteric


204. Lin HC. Small intestinal bacterial overgrowth: a framework for understanding

205. Simren M, Stotzer PO. Use and abuse of hydrogen breath tests. Gut 2006; 55:297-
303.

206. Riordan SM, McIver CJ, Walker BM, Duncombe VM, Bolin TD, Thomas MC. The
lactulose breath hydrogen test and small intestinal bacterial overgrowth. Am.J

207. Vanner S. The lactulose breath test for diagnosing SIBO in IBS patients: another nail


209. Magendie F. Note sur les gaz intestinaux de l'homme sain. Ann Chim Phys 1816;
2:292-6.

210. Di Stefano M, Corazza GR. Role of hydrogen and methane breath testing in


212. Levitt MD, Furne JK, Kuskowski M, Ruddy J. Stability of human methanogenic
flora over 35 years and a review of insights obtained from breath methane

213. Weaver GA, Krause JA, Miller TL, Wolin MJ. Incidence of methanogenic bacteria in
a sigmoidoscopy population: an association of methanogenic bacteria and

substrates for methane production in human colon. Am.J Physiol 1991; 260:G752-
G757.

production in IBS correlates with the severity of constipation. Am.J Gastroenterol


