Gastrointestinal Disorders in Women

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2014

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LUND
UNIVERSITY

DOCTORAL DISSERTATION
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To be defended at Clinical Research Centre, Jan Waldenströmsgata 35, Malmö
Date November 7 and time 13.00

Faculty opponent

Docent Hans Strid, Sahlgrenska universitetssjukhuset.

Organization	Document name
LUND UNIVERSITY	Doctoral Dissertation
	Date of issue
Author(s) Rita J Gustafsson	Sponsoring organization

Title and subtitle: Gastrointestinal Disorders in Women

Abstract: This doctoral thesis has sought to clarify the influence of gender in gastrointestinal (GI) disorders. Women with different hormone profiles were investigated, their colonic microbiota was characterized, and the impact of lifestyle and risk factors for GI disease were examined. In a pilot study, the relation between vaginal and rectal lactobacilli flora and hormone levels was investigated in 20 fertile and 20 postmenopausal women. No correlation was found between the overall levels of *Lactobacillus* species in the vagina and rectum, and no variations in sex hormone levels were found. *L. plantarum* was most often found in the rectal flora of both fertile and postmenopausal women, and *L. crispatus* was found more often in the vaginal flora of fertile women than in that of postmenopausal women. We characterized the mucosa-associated microbiota in the ascending colon in two women with collagenous colitis. After cloning and sequencing of the bacterial 16S rRNA genes, we found that the overall composition of the colonic microbiota was similar to that of a healthy woman and consists of a predominance of Firmicutes and Bacteroidetes. Interestingly, both patients had a high proportion of potentially pathogenic species of *Bacteroides* and clones related to *Clostridium clostridioforme*. Gastroparesis and esophageal dysmotility are common complications of diabetes mellitus in both symptomatic and asymptomatic patients. In a cross-sectional study, we evaluated esophageal and gastric motility, GI symptoms, secondary complications, and plasma biomarkers in consecutive patients with diabetes mellitus. We found an unexpectedly high prevalence of esophageal dysmotility, which presented as a strong association with retinopathy. Furthermore, the majority of patients suffered from GI symptoms that were not associated with objectively measured dysmotility A total of 131 female patients with microscopic colitis (MC) were examined with regard to smoking and alcohol habits compared to population-based controls. The main finding was that current smoking – independently of other lifestyle factors – was associated with an increased risk of developing persistent MC or MC with concomitant irritable bowel syndrome (IBS)-like symptoms, but current smoking was not associated with the development of solely MC without IBS symptoms. Past smoking was associated with transient MC. Taken together, some GI disorders are more common in women. No obvious hormonal explanation could be found, although the rectal lactobacilli flora was not as sensitive as the vaginal lactobacilli flora to hormonal influences. The microbiota in the colon of patients with MC is similar to healthy individuals, but with a higher proportion of *Bacteroides*. Men and women with diabetes mellitus have the same amount of symptoms and dysmotility when examined consecutively. Esophageal dysmotility is more common than gastroparesis in patients with diabetes mellitus, and it is strongly associated with retinopathy. Smoking is an important risk factor in the development of MC independently of other lifestyle factors.

Key words: women, microbiota, sex hormones, lifestyle factors, microscopic colitis, IBS, gastroparesis

Classification system and/or index terms (if any)

Supplementary bibliographical information | Language: English

ISSN and key title: 1652-8220 | ISBN: 978-91-7619-037-1

Recipient's notes | Number of pages | Price

Security classification

Gastrointestinal Disorders in Women

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UNIVERSITY

ISSN 1652-8220
ISBN 978-91-7619-037-1

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2014:108

Printed in Sweden by Media-Tryck, Lund University
Lund 2014

To my father, I only wish you were here!

To Jan-Olof, Maria & Simon,

and to my dear mother.

Above all, don't fear difficult moments. The best comes from them.

Rita Levi-Montalcini

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Paper II: Mucosa-associated bacteria in two middle-aged women diagnosed with collagenous colitis

Rita J Gustafsson, Bodil Ohlsson, Cecilia Benoni, Bengt Jeppsson, and Crister Olsson. World Journal of Gastroenterology. 2012;18(14):1628-1634.

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Paper IV: Smoking- and alcohol habits in relation to the clinical picture of women with microscopic colitis compared to controls

Bodil Roth, **Rita J Gustafsson**, Bengt Jeppsson, Jonas Manjer, and Bodil Ohlsson. BMC Women's Health. 2014;14:16.

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Rita J Gustafsson, Bodil Roth, Mikael Lantz, Bengt Hallengren, Jonas Manjer, and Bodil Ohlsson. Scandinavian Journal of Gastroenterology. 2013;48(12):1414-1422.

Auto-antibodies and their association with clinical findings in women diagnosed with microscopic colitis

Bodil Roth, **Rita J Gustafsson**, and Bodil Ohlsson. PLoS One. 2013;8(6):e66088.

Abbreviations

CC	Collagenous colitis
CFU/g	Colony forming unit per gram
DM	Diabetes mellitus
ER	Estrogen receptor
FSH	Follicle-stimulating hormone
GI	Gastrointestinal
GnRH	Gonadotropin-releasing hormone
GnRH-R	Gonadotropin-releasing hormone receptor
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
LC	Lymphocytic colitis
LH	Luteinizing hormone
MC	Microscopic colitis
MDCS	Malmö Diet and Cancer Study
PR	Progesterone receptor

Introduction

Subjective health complaints are very common in the normal population, and there are gender and age differences in both the prevalence and degree of such complaints (Ihlebaek, Eriksen et al. 2002). Women tend to live longer than men, but somewhat paradoxically report greater levels of morbidity and disability and make greater use of health care services over the course of their lives (Briscoe 1987, Parslow, Jorm et al. 2004). Women are more likely than men to report a digestive condition, but whether women truly experience more troubles with their digestive system than men is difficult to determine. Self-reported health is an important predictor of utilization of some health care services, and the worse self-perceived health of women could partly justify their greater use of health care services such as visits to general practitioners and the use of diagnostic procedures (Bengtsson, Ohlsson et al. 2007, Crimmins, Kim et al. 2011).

Because women tend to visit their doctors more often, they have a greater opportunity to alert their doctors to digestive problem. Some gastrointestinal (GI) diseases are dominated by female gender, including microscopic colitis (MC), gastroparesis, and irritable bowel syndrome (IBS). GI motility differs by gender but also among women based on the hormonal status of the menstrual cycle (Hutson, Roehrkasse et al. 1989, Meier, Beglinger et al. 1995, Gryback, Hermansson et al. 2000, Sadik, Abrahamsson et al. 2003). These clinical observations are supported by animal experiments (Ryan and Bhojwani 1986, Chen, Doong et al. 1995), and it has been shown that female steroid hormones play a role in reducing inflammation in experimentally induced colitis in rats (Gunal, Oktar et al. 2003, Karatepe, Altiok et al. 2012). Furthermore, the predominance of postmenopausal women with MC and reports of the disease resolving itself with the onset of pregnancy (Bohr, Tysk et al. 1996) suggest the possibility of a hormonal influence in disease progression. The microbiota of the human GI tract plays an important role in human health and disease (Fujimura, Slusher et al. 2010), and lactobacilli are considered to be protective organisms (Adams and Marteau 1995). In the past century, men used to be more likely to smoke and consume alcohol compared to women (Waldron 1983, Verbrugge 1985). However, social roles have changed, and women have adopted traditional male lifestyle factors (Emslie, Hunt et al. 2002, Crimmins, Kim et al. 2011). The gender differences in GI diseases could be due to differences in responses to stress, total workload, physical strength, or simply the fact that women have different traditions and thresholds for when and how to complain about their symptoms.

This doctoral thesis has attempted to clarify the influence of gender in GI disorders. To do this, women with different hormone profiles were investigated, their colonic microbiota was characterized, and the impact of lifestyle and risk factors for GI disease was examined.

Cycling female sex hormones

Gonadotropin-releasing hormone (GnRH) plays an important role in the endocrine control of reproduction. GnRH is produced by the hypothalamic neurons and is secreted in a pulsatile manner into the hypophysial portal circulation. When it reaches the anterior pituitary, it activates the GnRH receptor (GnRH-R) on gonadotrophic cells and stimulates the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Conn and Crowley 1994, Sherwood 2010). Circulating FSH and LH stimulate the synthesis and secretion of the gonadal steroid hormones estradiol and progesterone from the ovaries (Naor 2009), and circulating estradiol and progesterone in turn regulate the release of GnRH through a negative feedback mechanism (Figure 1).

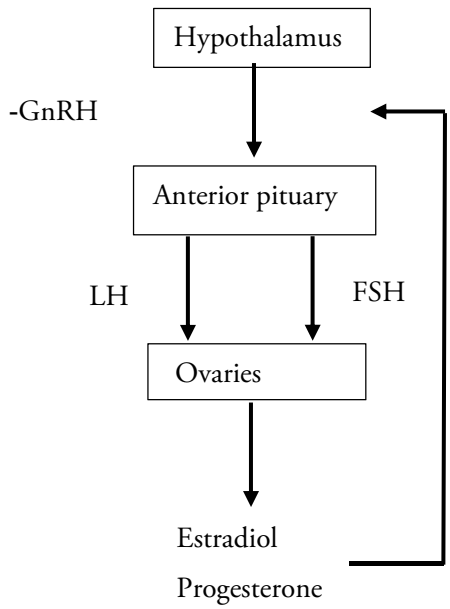

Figure 1: Simplified scheme of the hypothalamo-pituitary-gonadal axis in women.

During a woman's reproductive years, the normal menstrual cycle is characterized by predictable and cyclic changes in estrogen and progesterone levels (Sherwood 2010). The ovarian cycle is divided into the following three phases: the follicular phase, the ovulation phase, and the luteal phase (Sherwood 2010). The follicular phase (days 1–14 of the cycle) has initially low levels of estradiol. Later, increased levels of estradiol exert a positive feedback mechanism on the pituitary secretion of LH, and this elicits the LH surge that precedes ovulation (Gruhn and Kazer 1989). After ovulation, a corpus luteum is formed, and this produces progesterone in addition to estradiol. The presence of progesterone characterizes the luteal phase (days 15–28) that follows ovulation (Figure 2). If conception occurs, human choriogonadotropin produced by the trophoblastic cells in the embryo maintains steroid production in the corpus luteum, a function that is gradually replaced by the placenta. If conception does not occur, the corpus luteum regresses and the sex steroid levels rapidly decline after which menstrual bleeding occurs (Gruhn and Kazer 1989).

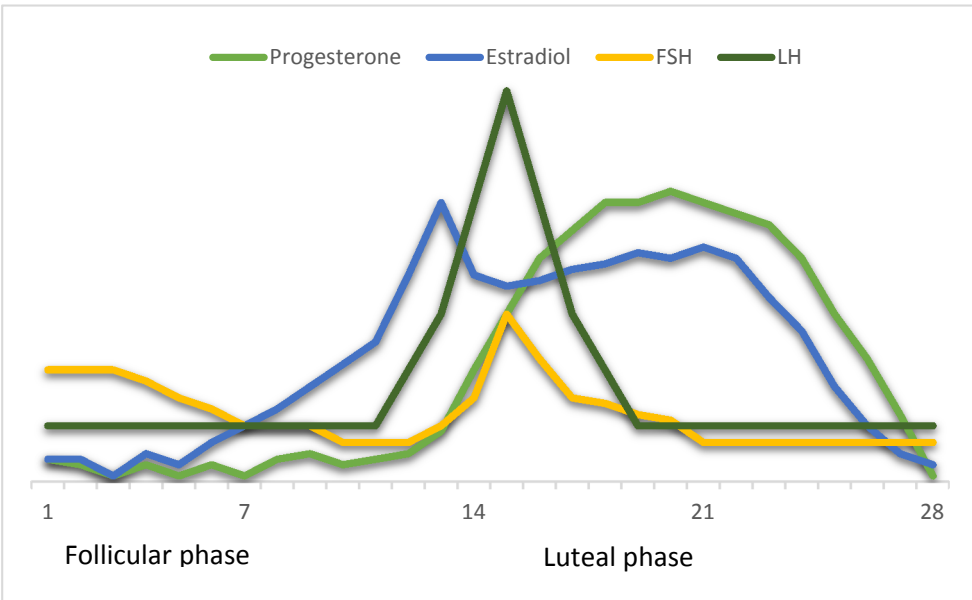

Figure 2: Variations in circulating hormone levels in the normal menstrual cycle.

Perimenopause is a time of markedly fluctuating hormone levels (Sherman and Korenman 1975, Metcalf, Donald et al. 1981, Hee, MacNaughton et al. 1993), and in late perimenopause – about two years before menopause – estradiol levels start to decline because the dominant follicle is no longer capable of maintaining estradiol production (Burger, Dudley et al. 1999, Shifren and Schiff 2000, Burger, Dudley et al. 2002).

Potential hormonal influence on the gastrointestinal tract

GnRH-R are expressed in the GI tract of the rat, and GnRH is produced in the surface epithelium, the glandular epithelium, and in the myenteric plexus (Ho, Nagle et al. 1996, Huang, Yao et al. 2001), but LH receptors (LH-R) have only been described in the enteric plexa (Sand, Bergvall et al. 2013). In the human GI tract, GnRH-R and LH-R have been found in the enteric nervous system (ENS) (Ohlsson, Veress et al. 2007, Hammar, Ohlsson et al. 2012, Hammar, Veress et al. 2012). GnRH-R is a member of the seven-transmembrane, G-protein-coupled receptor family, and LH-R belongs to the G-protein coupled receptor 1 family (Furness, Wootten et al. 2012). GnRH-R are not only expressed on pituitary gonadotrope cells, but also on lymphocytes and cells of the breast, ovary, and prostate. GnRH seems to affect intestinal motility, and antibodies against GnRH have been observed in patients with IBS, motility disorders such as chronic intestinal pseudo-obstruction and enteric dysmotility, diabetes mellitus (DM), and primary Sjögren's syndrome (Borg, Melander et al. 2009, Ohlsson, Scheja et al. 2009, Ohlsson, Sjoberg et al. 2011). Recently, a reduced level of expression of GnRH-containing neurons in the ENS has been found in a subgroup of patients with severe dysmotility (Hammar, Ohlsson et al. 2012).

Both estrogen receptor (ER) and progesterone receptor (PR) are members of the nuclear receptor superfamily of ligand-dependent transcription factors (Inoue, Akahira et al. 2002, Weihua, Andersson et al. 2003). The two most well-studied ERs are ERα and ERβ (Green, Walter et al. 1986, Enmark and Gustafsson 1999). ERα is mainly expressed in the uterus, prostate (stroma), ovary (theca cells), epididymis, bone, breast, liver, and various regions of the brain (Couse, Lindzey et al. 1997). ERβ is expressed in the prostate (epithelium), ovary (granulosa cells), bone marrow, salivary glands, vascular endothelium, and certain regions of the brain (Couse, Lindzey et al. 1997). Both ERα and ERβ are expressed in the GI tract, with ERβ being the predominant ER in the colon and mainly located in epithelial cells (Konstantinopoulos, Kominea et al. 2003). Two isoforms of PR have been identified, PR-A and PR-B (Horwitz and Alexander 1983), and PR expression has been described in the uterus (Press, Udove et al. 1988), ovary (Duffy and Stouffer 1995), prostate (Yu, Liu et al. 2013), breast (Buxant, Engohan-Aloghe et al. 2010), brain (Pichon, Pallud et al. 1992), and throughout the GI tract (Eliakim, Abulafia et al. 2000).

Both ER and PR have many effects on the GI tract, including relaxing the lower esophageal sphincter and decreasing colonic transit (Eliakim, Abulafia et al. 2000). GI symptoms are common during pregnancy, and certain GI disorders can be triggered or worsened by the hormonal changes that occur during a woman's menstrual cycle. There seems to be a link between menstrual cycle and IBS symptoms, and there is a high prevalence of altered bowel function and IBS-like GI complaints among women during

the perimenopausal and postmenopausal periods (Triadafilopoulos, Finlayson et al. 1998, Heitkemper, Jarrett et al. 2003, Zutshi, Hull et al. 2007). During menses (a time of declining/minimal ovarian hormone levels), rectal sensitivity increases (Houghton, Lea et al. 2002) and stools become looser (Heitkemper, Shaver et al. 1988, Jackson, Houghton et al. 1994). In contrast, during the luteal phase, when levels of estradiol and progesterone are high, GI transit increases (Wald, Van Thiel et al. 1981, Turnbull, Thompson et al. 1989) and can lead to firmer stools. However, it must be noted that women with endometriosis suffer from GI symptoms nearly as often as gynecological symptoms, and these do not necessarily reflect bowel involvement (Maroun, Cooper et al. 2009). Estrogen decreases colonic permeability through ERβ-mediated up-regulation of occluding and junctional adhesion molecule-A in epithelial cells (Braniste, Leveque et al. 2009), and because impaired permeability in the GI tract can be a trigger for the development of IBS and MC, this could be an explanation for gender differences in the prevalence of GI diseases.

Houghton et al. reported that men with IBS have lower levels of serum LH than men without IBS, and this suggests a potential protective effect of this hormone (Houghton, Jackson et al. 2000).

Women with inflammatory bowel disease (IBD) report cyclical alterations in symptoms, and there are reports that estrogen plays a protective role regarding the anti-inflammatory activity in exacerbations of IBD (Kane and Reddy 2008). In fact, estrogen has been proven to have an anti-inflammatory effect by acting against promoters of inflammation (Lewis, Johnson et al. 2008, Cerciat, Unkila et al. 2010). Progesterone has also been shown to have protective anti-inflammatory effect on the mucosa of the GI tract (Allport, Pieber et al. 2001, Chen, Shi et al. 2007, Zhao and Zhou 2011).

Lifestyle influences on the gastrointestinal tract

Unhealthy lifestyle factors contribute to the development of various diseases of the GI tract (Hall and Crowe 2011), and alcohol consumption and tobacco use are the most studied. Smoking has been described as a risk factor for developing MC (Munch, Aust et al. 2012, Yen, Pokhrel et al. 2012), post-infectious functional gastrointestinal disorders (FGID) (Parry, Barton et al. 2005), and overlapping syndromes between reflux diseases and FGID (Fujiwara, Kubo et al. 2011). Tobacco smoke contains more than 4.500 chemicals, and many of these, such as nicotine, carbon monoxide, and nitrogen oxide, are toxic or interfere with the immune system (Mehta, Nazzal et al. 2008).

Alcohol has many acute and chronic effects on the function and structure of the GI tract (Bode and Bode 1997). In animal experiments, alcohol leads to increased oxidative stress, hyperpermeability, neuropathy, and dysbiosis, all of which favor and sustain local inflammation (Keshavarzian, Farhadi et al. 2009, Mutlu, Keshavarzian et al. 2009). In cell cultures of monolayers of intestinal cells, ethanol induces disruption of the F-actin cytoskeleton resulting in instability of the assembly of the subunit components of the actin network and a subsequent loss of intestinal barrier integrity (Banan, Fields et al. 2000, Banan, Keshavarzian et al. 2007). The cytoskeleton might be a major target for injury in damaged intestinal epithelium (Miller, Smith et al. 2000), and this is in accordance with experiments showing that ethanol causes mice to develop an inflammatory reaction in the colon that is characterized by infiltration of inflammatory cells into the mucosa and submucosa (Andrade, Vaz et al. 2003). Alcohol also affects sex hormones in postmenopausal women by increasing the conversion of testosterone into estradiol (Gavaler and Love 1992).

Stressful psychosocial conditions can influence the GI tract (Drossman 1996, Van Oudenhove, Vandenberghe et al. 2010) as well as lead to various health-related behaviors such as increased cigarette smoking and alcohol consumption. Women and men respond differently to stress, and women report greater sadness and anxiety/fear (Fischer, Rodriguez Mosquera et al. 2004, Chaplin, Hong et al. 2008) and show greater heart rate responses (Allen, Stoney et al. 1993, Kudielka, Buske-Kirschbaum et al. 2004) than men. The different responses to stress between women and men might have implications for the known differences in vulnerability to stress-related disorders.

The intestinal microbiota

The adult human intestinal tract is a complex and dynamic ecosystem containing an estimated 10^{14} bacteria that has co-evolved with our species and is essential for human health (Bengmark 1998, Gill, Pop et al. 2006, Ley, Turnbaugh et al. 2006). The composition and activity of this ecosystem play important roles in health and disease. Perturbations to the composition of the microbiota, or dysbiosis, has been reported in various diseases and conditions including obesity (Greiner and Backhed 2011), necrotizing enterocolitis (Mai, Young et al. 2011), type 1 and type 2 DM (Larsen, Vogensen et al. 2010, Giongo, Gano et al. 2011), IBS (Carroll, Ringel-Kulka et al. 2011, Saulnier, Riehle et al. 2011), and colon cancer (Sobhani, Amiot et al. 2013).

Culture-dependent and -independent studies of the intestinal bacteria have found up to 13 different bacterial phyla, with Firmicutes and Bacteroidetes being the numerically dominant phyla (Dethlefsen, McFall-Ngai et al. 2007, Mariat, Firmesse et al. 2009).

The type and number of microbial species that persist and colonize the GI tract is determined by a combination of factors, including the inflammatory state of the host, the host's diet, host genetics, and environmental factors (Cerf-Bensussan and Gaboriau-Routhiau 2010, Hansen, Gulati et al. 2010, Musso, Gambino et al. 2010, Buddington and Sangild 2011). The intestinal microbiota differs along the GI tract, and factors such as intestinal motility, pH, redox potential, nutrient supplies, the presence of an intact ileocecal valve, host secretions of hydrochloric acid, digestive enzymes, bile, and mucus all influence the composition of the intestinal microbiota (Wang, Ahrne et al. 2005, Booijink, Zoetendal et al. 2007). The density of the microbial ecosystem also increases along the length of the GI tract. Per gram of intestinal content, the microbial density increases from 10^1–10^4 microbial cells in the stomach and duodenum, to 10^4–10^8 cells in the jejunum and ileum, to 10^{10}–10^{12} cells in the colon and in the feces (Dethlefsen, Eckburg et al. 2006, Booijink, Zoetendal et al. 2007).

The microbial colonization of the GI tract starts during birth when neonates are first exposed to bacteria from the mother and the environment (Adlerberth and Wold 2009). However, there are indications that there is a non-pathological exposure of intestinal bacteria or bacterial DNA to the fetus while it is still in the uterus (Satokari, Gronroos et al. 2009).

The intestinal microbiota in healthy adults remains relatively stable over time even if environmental changes and pathological events may cause temporary variations (Franks, Harmsen et al. 1998, Zoetendal, Akkermans et al. 1998, Vanhoutte, Huys et al. 2004, Costello, Lauber et al. 2009). However, a substantial change in the composition of the intestinal microbiota is seen in both infants and elderly individuals (Adlerberth and Wold 2009, Tiihonen, Ouwehand et al. 2010). In individuals over the age of 65 years, there is physiological changes that affects the composition and functionality of the intestinal microbiota (Woodmansey 2007, Tiihonen, Ouwehand et al. 2010).

Little is known about gender differences in the intestinal microbiota. In a cross-sectional study by Mueller et al. (Mueller, Saunier et al. 2006) on the intestinal microbiota composition of 230 healthy subjects from France, Germany, Italy, and Sweden, gender effects were observed for the Bacteroides-Prevotella group with higher levels in men than in women. Furthermore, a recent study showed that the microbiota in male non-obese diabetic (NOD) mice is distinct from that in females, and it contributes to increased testosterone levels that are associated with protection against type 1 DM (Markle, Frank et al. 2013). Indeed, transferring the microbiota from male mice into female NOD mice results in increased levels of testosterone and reduced susceptibility to type 1 DM in the female NOD mice (Markle, Frank et al. 2013).

Understanding the intestinal microbiota in healthy humans provides the basis for understanding its influences in various important GI diseases. Deviations in the GI microbiota have been noted in patients with IBD (Sartor 2008) and in animal models of intestinal inflammation (Lupp, Robertson et al. 2007).

The microbiota in the digestive tract has a significant impact on the immune system, and early life events are important for establishing the microbiota. The composition of the microbiota is subsequently affected throughout life by different environmental and lifestyle factors. Therefore, studies are now more focused on creating a healthy microbiota that confers maximum tolerogenic and immunomodulatory effects in the GI tract and protects against systemic inflammatory diseases (McLoughlin and Mills 2011).

Lactobacilli

Lactobacilli are a diverse group of Gram-positive, mostly facultative – but under certain conditions strictly anaerobic – non-sporulating lactic acid-producing rods. They are part of the normal human oral, intestinal, and vaginal microflora (Ahrne, Nobaek et al. 1998). Lactobacilli are believed to be safe and beneficial for health and are frequently used as probiotics in dairy products (Borriello, Hammes et al. 2003). The genus *Lactobacillus* currently consists of more than 150 species with substantial genetic and phenotypic differences (Claesson, van Sinderen et al. 2007). Studies have demonstrated a protective role of lactobacilli against urogenital and intestinal infections (Merk, Borelli et al. 2004).

In women of reproductive age, lactobacilli dominate the microbiota in the vagina (Andreu, Stapleton et al. 1995, Burton, Cadieux et al. 2003). The most commonly found vaginal lactobacilli in fertile women are *L. crispatus, L. iners, L. jensenii*, and *L. gasseri* (Vasquez, Jakobsson et al. 2002*)*. The *Lactobacillus* species in the vaginal flora play an important role in maintaining the health of the female vagina by producing lactic acid, hydrogen peroxide (H_2O_2), bacteriocins, and other antimicrobial substances (Boris and Barbes 2000), all of which inhibit the growth of pathogens in the vagina. Loss of lactobacilli in the vaginal microbiota allows for the growth of pathogens and subsequent bacterial vaginosis (Redondo-Lopez, Cook et al. 1990, Hillier 1998). Whether there is a natural decrease in *Lactobacillus* species in postmenopausal women in the intestinal microbiota has not been studied. Lactobacilli are not a dominating bacterial group in the digestive microbiota with the possible exception of the small intestine (Matsuda, Tsuji et al. 2009). The most commonly found lactobacilli in the GI tract are *L. paracasei, L. salivarius, L. rhamnsus, L. fermentum,* and *L. plantarum* (Ahrne, Nobaek et al. 1998). However, the presence of lactobacilli in the digestive tract

does not necessarily imply colonization (Walter 2008), and because lactobacilli are present in fermented food some lactobacilli might be found in the GI tract only transiently. However, which *Lactobacillus* species are transient and which are true inhabitants of the GI tract has not yet been clearly established (Walter 2008).

Subjective symptoms versus objective findings

Health conditions usually present with a mixture of subjective symptoms and objective findings, and these do not always correlate with each other. This is especially the case in functional disorders of the GI tract where health care professionals consider dysmotility a more morbid condition than IBS, but IBS patients often describe their symptoms to be as bad as those of patients with dysmotility (Bengtsson, Hammar et al. 2011). Despite the patients' complaints, Tornblom and colleagues found objectively identifiable transit alterations in only one of five patients with IBS, and the proportion of patients with an abnormal colonic transit time was higher in men than in women with IBS (Tornblom, Van Oudenhove et al. 2012).

Psychological aspects such as stress, emotions, or personality can influence the severity of GI symptoms (Drossman 1996), and the interpretation of subjective complaints is probably influenced by personal concepts. Functional GI disorders present mostly with subjective symptoms (Drossman 2006). To have a disease that is not "visible", but to still have substantial symptoms, can lead to intense frustration in many patients (Bertram, Kurland et al. 2001) and can be highly detrimental to the patient's quality of life (Chang 2004, Bengtsson, Ohlsson et al. 2007). Such diseases also involve social costs such as excess sick leave and frequently seeking health care services (Cash, Sullivan et al. 2005). Obtaining confirmation through an official diagnosis is important for patients to be able to accept and deal with their illness (Faresjo, Grodzinsky et al. 2006).

Many of the patients with signs of gastroparesis are asymptomatic and, conversely, many patients with symptoms can still have normal gastric emptying (De Block, De Leeuw et al. 2002, Rey, Choung et al. 2012). Sadik et al. (Sadik, Abrahamsson et al. 2003) found that transit abnormalities in patients with severe and unexplained GI symptoms are more prevalent in men, and this might reflect a difference for sensing abnormalities between women and men. Symptoms in men might only become obvious when the transit disturbance becomes severe, and this should be taken into consideration when motility and symptoms are analyzed. Women are also more likely than men to have other concomitant GI disorders as well as other disorders involving chronic pain (Riedl, Schmidtmann et al. 2008).

Microscopic colitis

MC includes both collagenous colitis (CC) (Lindstrom 1976) and lymphocytic colitis (LC) (Lazenby, Yardley et al. 1989), which have indistinguishable clinical presentations but are separated by histopathological characteristics. Both CC and LC can coexist and can interchange with each other (Fraser, Warren et al. 2002).

The first description of CC was in 1976 by Lindström (Lindstrom 1976). He described microscopic inflammatory changes within the subepithelial collagen band of the macroscopically normal colon of a women suffering from diarrhea and called the condition CC. Later, in 1980, Read et al (Read, Krejs et al. 1980) introduced the term MC to describe patients with idiopathic chronic diarrhea, normal endoscopic findings, and microscopic evidence of an inflammatory infiltrate in the colonic mucosa. The conditions were further delineated in 1989 when Lanzeby et al. (Lazenby, Yardley et al. 1989) showed that an increased number of colonic intraepithelial lymphocytes was the most characteristic feature of MC and suggested the term LC.

The colonic mucosa appears normal or almost normal on visual inspection by colonoscopy, but microscopic examination of mucosal biopsies reveals diagnostic histopathological changes (Olesen, Eriksson et al. 2004). Chronic, inflammatory infiltrate in the lamina propria is a mandatory finding for a diagnosis of MC (Tremaine 2000). CC is further characterized by a thickened subepithelial collagen band of ≥ 10 μm (Bohr, Tysk et al. 1996, Baert, Wouters et al. 1999, Tagkalidis, Bhathal et al. 2002), and LC is characterized by an increased number of intraepithelial lymphocytes in the surface epithelium (≥ 20 lymphocytes/100 epithelial cells) (Robert 2004, Lazenby 2005, Thijs, van Baarlen et al. 2005, Temmerman and Baert 2009).

MC is characterized by chronic, watery (secretory) diarrhea without bleeding and is often associated with fecal urgency. The natural history of MC is widely variable; the onset is often gradual, but 40% of MC patients have a sudden onset (Bohr, Tysk et al. 1996). A majority of patients with CC naturally enter symptomatic remission after 3–4 years (Goff, Barnett et al. 1997, Bonner, Petras et al. 2000, Sveinsson, Orvar et al. 2008). For patients with LC, resolution of diarrhea and normalization of histology in over 80% of patients has been reported, and 63% of LC patients have only a single attack (Olesen, Eriksson et al. 2004). In contrast, prospective studies show a 60% relapse rate after cessation of budesonide in patients whose symptoms do not resolve spontaneously (Miehlke, Madisch et al. 2005, Bonderup, Hansen et al. 2009). In some patients, the course of the disease can be complicated due to a lack of response to medication, and surgery with a diverting ileostomy or colectomy is an option for these patients (Jarnerot, Tysk et al. 1995, Pardi, Loftus et al. 2001, Pardi and Kelly 2011).

The disease usually occurs in middle-aged individuals but can occur in all ages, including children (Benchimol, Kirsch et al. 2007, Pardi and Kelly 2011). Women are more frequently affected than men, particularly for CC (Bohr, Tysk et al. 1995, Agnarsdottir, Gunnlaugsson et al. 2002, Olesen, Eriksson et al. 2004, Pardi, Loftus et al. 2007). The reason for the female predominance is unknown, but a possible contribution of hormonal alterations or an ascertainment bias in women has been suggested (Storr 2013). In one report, patients that became pregnant after a diagnosis of MC lost their clinical symptoms, and the patients remained symptom-free even after childbirth (Bohr, Tysk et al. 1996). Population studies have shown an increasing incidence of MC (Bohr, Tysk et al. 1995, Fernandez-Banares, Salas et al. 1999, Agnarsdottir, Gunnlaugsson et al. 2002, Olesen, Eriksson et al. 2004, Pardi, Loftus et al. 2007, Williams, Kaplan et al. 2008), but whether this is a result of an increased awareness along with higher rates of colonoscopies with biopsies or it is a true increase in incidence has yet to be verified.

The etiology of MC is largely unknown, but it is most likely multifactorial involving luminal factors such as dietary antigens, drugs, and bile salts as well as bacterial products and toxins (Jarnerot, Bohr et al. 1996, Munch, Aust et al. 2012). Other theories involve autoimmunity (Giardiello, Lazenby et al. 1989, Pardi and Kelly 2011) and genetic inheritance (Fine, Do et al. 2000, Abdo, Zetler et al. 2001, Jarnerot, Hertervig et al. 2001, Fernandez-Banares, Esteve et al. 2005, Koskela, Karttunen et al. 2008). Smoking seems to be a risk factor for MC, and active smokers tend to develop the disease about 10 years earlier than non-smokers (Vigren, Sjoberg et al. 2011, Yen, Pokhrel et al. 2012, Fernandez-Banares, de Sousa et al. 2013).

Treatment of MC has evolved rapidly in recent years, and clinical trials and meta-analyses have established budesonide as the treatment of first choice for both acute and long-term treatment of CC and LC (Chande 2008, Gentile, Abdalla et al. 2013, Storr 2013).

Diabetes mellitus

The global prevalence of DM is 2.8% for all age groups, and this rate is expected to double by the year 2030 (Wild, Roglic et al. 2004). There are approximately 400,000 known diabetic patients in Sweden, and this is a prevalence of 4% (TNBoHa 2009). Type 1 DM mainly debuts early in life and is diagnosed shortly after rapidly appearing symptoms, but it can also have a slower onset and be diagnosed later in life. Type 2 DM accounts for the majority of diabetes cases within the general population. Because the symptoms progress very slowly, individuals can live with undetected type 2 DM for many years. A predisposition for both type 1 DM and type 2 DM can be inherited

(O'Rahilly, Barroso et al. 2005), but the incidence of DM is also strongly correlated with lifestyle factors such as smoking and diet, and obesity is considered a main risk factor for the onset of type 2 DM (Lazar 2005).

Complications of diabetes mellitus

DM is associated with a number of complications, but the most devastating impact of DM is undoubtedly its long-term vascular complications. These complications are wide-ranging and are at least partially due to chronic elevations of blood glucose levels. These circulatory complications can be divided into two main categories. "Microvascular disease" occurs when the small blood vessels are damaged and includes complications such as retinopathy, nephropathy, and neuropathy. "Macrovascular disease" occurs when the arteries are damaged, and this leads to accelerated cardiovascular disease, myocardial infarction, cerebrovascular disease, and strokes (Forbes and Cooper 2013).

Gastrointestinal complications of diabetes mellitus

GI complications from DM have become more common as the rate of DM has increased, and these complications seem to be more common in patients with longstanding DM. GI motility is dependent on the coordination between the intrinsic and extrinsic nervous system, the interstitial cells of Cajal, and the smooth muscle cells of the GI tract, and abnormal GI motility – including esophageal dysmotility and gastroparesis – is the most common source of GI complications and symptoms in diabetic patients. Early identification and appropriate management are important for improving both diabetic care and quality of life of the affected patients.

Esophageal dysmotility is usually associated with connective tissue abnormalities resulting in dysphagia (Sheehan 2008). In diabetic patients with GI symptoms, esophageal dysmotility is common (Faraj, Melander et al. 2007), but there is no correlation between GI symptoms and esophageal dysmotility. It has been shown that esophageal dysmotility in patients with DM might have an effect on glucose homeostasis (Ohlsson, Melander et al. 2006).

Gastroparesis is characterized by delayed gastric emptying in the absence of mechanical obstruction of the stomach (Parkman, Hasler et al. 2004). Gastroparesis can be caused by any condition affecting neuromuscular dysfunction of the GI tract, and the most frequent condition is idiopathic or secondary to DM (Hasler 2007). Other causes

include previous gastric surgery and neurological and rheumatologic disorders. Systematic analyses indicate that gastroparesis can be demonstrated in 25%–55% of type 1 DM patients (Nowak, Johnson et al. 1995, Kong, Horowitz et al. 1999) and in 30% of patients with type 2 DM (Kong, Horowitz et al. 1999).

The pathogenesis of diabetic gastroparesis is multifactorial and still poorly understood. Loss of the normal migrating motor complexes is demonstrable in patients with DM (Hasler 2007). Other factors involved in the pathogenesis include loss of expression of neuronal nitric oxide synthase, absent or dysmorphic interstitial cells of Cajal, smooth muscle fibrosis, and abnormal macrophage-containing immune infiltrates (Ordog, Takayama et al. 2000, Camilleri, Bharucha et al. 2011, Grover, Farrugia et al. 2011). The incidence of gastroparesis is reported to be higher in women than in men (Horowitz, Wishart et al. 1996, Jones, Russo et al. 2001, Rayner, Samsom et al. 2001, Jung, Choung et al. 2009). This gender bias can be explained by female hormonal changes (Baron, Ramirez et al. 1993, Baschetti 1997, Knight, Parkman et al. 1997), and several animal and human studies have demonstrated that estradiol-17β causes delayed gastric emptying (Chen, Doong et al. 1995, Gonenne, Esfandyari et al. 2006). In addition, hyperglycemia stimulates pyloric contraction and inhibits antral contraction, and this also delays gastric emptying (Fraser, Horowitz et al. 1991).

Diabetic gastroparesis can cause severe symptoms and can result in nutritional deficiencies, impaired glucose control, and a poor quality of life, and these occur independently of other factors such as age, tobacco use, or type of DM (Talley, Young et al. 2001). Symptoms attributable to gastroparesis are reported by 5%–12% of patients with DM (Jones, Russo et al. 2002), and the primary symptoms include postprandial fullness (early satiety), nausea, vomiting, and bloating (Parkman, Camilleri et al. 2010). Nevertheless, most diabetic patients with delayed gastric emptying are asymptomatic or report only mild foregut symptoms (Camilleri, Bharucha et al. 2011).

Before evaluating a patient for gastroparesis, it is essential to rule out obstruction through the use of esophagogastroduodenoscopy or a barium study of the stomach. Food retained in the stomach after a 12-hour fast is suggestive of gastroparesis. Hyperglycemia slows gastric emptying, so it is important to assure a relatively constant euglycemic state of the patient (Hornbuckle and Barnett 2000). To confirm a diagnosis of gastroparesis, the rate of gastric emptying of solid food needs to be determined, e.g. by gastric-emptying scintigraphy (Parkman, Hasler et al. 2004). Interestingly, the gastric emptying time differs between women and men – with women having slower emptying than men especially in premenopausal age (Gill, Murphy et al. 1987, Hermansson and Sivertsson 1996) – and this is true both in healthy subjects and in patients with DM (Jones, Russo et al. 2001, Samsom, Vermeijden et al. 2003). This difference is considered so significant that some advocate the use of different reference

values for premenopausal women than for other patients (Stanghellini, Tosetti et al. 1996).

Irritable bowel syndrome

IBS is a common GI disorder with an estimated prevalence between 5% and 20% in the general population (Hungin, Whorwell et al. 2003, Hillila and Farkkila 2004), and it accounts for approximately 30% of all referrals to gastroenterologists and 3% of all visits to general practitioners (Simren, Castedal et al. 2000). Women are 1.5 to 3 times more likely to be affected by IBS than men, but the mechanism behind this phenomenon has yet to be completely explained (Drossman, Whitehead et al. 1997, Mayer, Naliboff et al. 1999, Zaman 2002, Quigley, Bytzer et al. 2006). Williams et al. (Williams, Black et al. 2006) showed that women were more likely than men to receive IBS as a diagnosis despite the fact that men sought medical care for their abdominal symptoms, which fulfilled the criteria for IBS, more frequently than women. Even though IBS is present in all age groups, its prevalence seems to decline with advanced age (Rey and Talley 2009), although older women seem to be more likely to seek medical care for IBS than younger women (Williams, Black et al. 2006). In a recent study in patients with posterior laryngitis, men reported more GI symptoms compared to women (Pendleton, Ahlner-Elmqvist et al. 2013).

The clinical expression of IBS and severity vary (Longstreth 2005), especially between women and men, and IBS is often sub-classified according to the predominant stool pattern experienced by the patient into diarrhea-predominant, constipation-predominant, or alternating bowel habit (Drossman, Morris et al. 2005). Women report more abdominal pain and constipation, but men typically report more diarrhea (Drossman, Morris et al. 2005).

IBS has a significant impact on quality of life, and psychosocial factors have long been regarded as important predictors for seeking health care in patients with IBS. However, more recent studies have concluded that bowel symptoms are the major predictor for patients with IBS to seek health care (Osterberg, Blomquist et al. 2000). Women with IBS tend to have a lower quality of life than men (Simren, Abrahamsson et al. 2001), and women with IBS often describe their symptoms as being as severe as patients with dysmotility disorders who present with objective and measurable changes during GI examinations (Bengtsson, Hammar et al. 2011).

The pathophysiology of IBS still remains uncertain. However, it is commonly viewed to be the result of interactions among various factors, including stress, biological processes, and characteristics of the patient's internal and external environment

(Roisinblit 2013). Patients with IBS have more intense reactions to stress in terms of motility of the GI tract, pain perception, emotional response, and stress hormone levels (Chang 2011). Inflammatory pathogenesis has been suggested in the etiology of IBS, and an increasing number of inflammatory markers such as cytokines, mast cells, and lymphocytes have been found in IBS patients (Ford and Talley 2011). In addition, an association between phenotypes of IBS and 5-hydroxytryptamine-related genes, noradrenaline-related genes, and cytokine genes has been found (Fukudo and Kanazawa 2011).

The intestinal microbiota has an indirect influence on GI motility, epithelial and intestinal immune cells, and GI sensitivity (Barbara, Stanghellini et al. 2005), and the fecal microbiota in patients with IBS differs from that of healthy controls (Malinen, Rinttila et al. 2005, Kassinen, Krogius-Kurikka et al. 2007, Rajilic-Stojanovic, Biagi et al. 2011, Saulnier, Riehle et al. 2011, Jeffery, O'Toole et al. 2012). An imbalance of the microbiota might contribute to the pathophysiology of IBS, and there is evidence for a possible link between exposure to environmental agents and the development of IBS (Thabane, Kottachchi et al. 2007). In this case, altered gut flora, low grade inflammation, and changes in gut motility and permeability have been suggested as mechanisms for the IBS symptoms in these patients (Ghoshal and Ranjan 2011). Risk factors associated with the development of postinfectious IBS include the type of pathogen, female gender, younger age, and psychological comorbidities (Thabane, Kottachchi et al. 2007, Spiller and Lam 2012).

The diagnosis of IBS is made according to the Rome III criteria (Drossman 2006) (Figure 3) that characterize multiple physiological determinant s that contribute to a common set of symptoms rather than to a single disease entity. In fact, MC and IBS have similar symptoms with MC not only leading to diarrhea but also causing constipation and abdominal pain (Olesen, Eriksson et al. 2004, Barta, Mekkel et al. 2005, Roth and Ohlsson 2013).

Recurrent abdominal discomfort or pain at least three days per month during the last three months, and with symptom onset at least six months ago and associated with two or more of the following: - Relief/symptom improvement with defecation, and/or - Onset associated with a change of stool consistency, and/or - Onset associated with a change in form (appearance) of stool
Symptoms that cumulatively support the diagnosis of IBS: - Abnormal stool frequency (more than three bowel movements/day or fewer than three bowel movements/week) - Abnormal stool form - Abnormal stool passage - Passage of mucus - Bloating or feeling of abdominal distension

Figure 3: Rome III diagnostic criteria.

Aims

The overall aim of this thesis was to identify possible etiologies of GI disorders in women. The aims of the individual studies were the following:

Paper I To determine whether there is a correlation between sex-hormone levels and the lactobacilli in the gut that could explain the high incidence of MC in postmenopausal women.

Paper II To characterize the mucosa-associated microbiota in the ascending colon in two women histologically diagnosed with CC by cloning and sequencing of the bacterial 16S rRNA genes.

Paper III To evaluate esophageal and gastric motility, complications, GI symptoms, and plasma biomarkers in a cross-sectional study of patients with DM.

Paper IV To examine patients suffering from MC regarding smoking and alcohol habits – compared to population-based controls from the same geographic area – in terms of the clinical expression of the disease and other simultaneous lifestyle factors.

Material and Methods

Ethics

All studies were approved by the Committee of Research Ethics at Lund University (approval numbers 2007/158, 2009/565, and 2011/209). All of the participants gave their written, informed consent before participating.

Subjects and samples

Paper I

Subjects

Twenty healthy fertile women (28–49 years, average 40 years) at two different phases of the menstrual cycle (day 7 and day 21) and 20 healthy postmenopausal women (52–85 years, average 60 years) were recruited among staff personnel from Skåne University Hospital Malmö and relatives and friends. A baseline clinical examination, including routine blood samples, was performed, and a gynecological examination was carried out, including a Pap smear, to ensure the health of the participants. Exclusion criteria included abnormal vaginal bacterial flora, bacterial vaginitis and other vaginal infections, the use of hormonal contraceptives, and estrogen replacement therapy. The fertile women were asked about their use of hygienic products between the two occasions. All women reported current or previous use of proton pump inhibitors, non-steroidal anti-inflammatory drugs, and antibiotics.

Samples

Blood samples were collected and centrifuged, and sera were stored at –20 °C until analysis. Smears from the vagina and rectum were collected with cotton-tipped swabs that were placed in transport medium on ice and immediately used for the cultivation of lactobacilli.

Paper II

Subjects

Two female patients, 51 years and 60 years old, with a known diagnosis of MC took part in the study. Both were non-smokers, and neither patient was taking any medication at the time of the study. Celiac disease had been excluded in both patients. The patients were asked to avoid fiber-rich food some days before colonoscopy, and intestinal cleansing was carried out with Phosphoral® (Clean Chemical Sweden AB), which is a salt preparation with osmotic effects.

Samples

During colonoscopy, two biopsies from the right colon were collected and placed in tubes with TE buffer (10 mmol Tris-HCl and 1 mmol ethylenediaminetetraacetic acid (EDTA), pH 8.0). The biopsies were frozen immediately in liquid nitrogen and stored at –80 °C until further analysis.

Paper III

Subjects

During their scheduled routine clinical follow-up, consecutive patients with DM at least 18 years of age at the Department of Endocrinology, Skåne University Hospital, Malmö, and at one primary health care center in Malmö, were invited to participate in the study. Types and duration of DM and the presence of diabetic complications were noted by the patients' physicians. Diabetic complications included retinopathy (based on fundus photography), angiopathy, microalbuminuria (measured as the albumin/creatinine ratio), albuminuria, peripheral neuropathy (examined by patellar and Achilles tendon reflexes, vibration sense test, and monofilament test), autonomic neuropathy according to established clinical criteria (sexual dysfunction, profound sweating, and orthostatic blood pressure), drug treatments, concomitant diseases, and body mass index (BMI). Exclusion criteria were severe cardiac disease or severe renal failure requiring dialysis. The patients were referred to esophageal manometry and gastric emptying scintigraphy, and only the patients who performed both tests were allowed to participate in the study. Out of 122 patients who agreed to participate, 84 patients (69%, 42 men/42 women) completed the study and were included in the analysis. Thirty-eight patients (45%, 12 men/26 women with a median age of 51.3 years) had type 1 DM, and 46 patients (55%, 30 men/16 women with a median age of 64.7 years) had type 2 DM.

Paper IV

Patients with MC

Women under the age of 73 years who had been treated for MC at any outpatient clinic of the Department of Gastroenterology, Skåne, between 2002 and 2010 were identified by searching for the ICD-10 classification of the two forms of MC – CC and LC (K52.8) – in the outpatient records. Study participants were also identified in the local register at the Department of Pathology, Skåne University Hospital, Malmö. Of the patients identified, only the 240 patients who had their diagnoses verified by colonic biopsy were invited to participate in the study. Altogether, 158 (median age 63 years, range 22–73 years) of the 240 patients accepted and were recruited to the study.

Controls

The Malmö Diet and Cancer Study (MDCS) is a population-based prospective cohort study that invited all women in Malmö born between 1923 and 1950 to participate. Recruitment for the MDCS was carried out between 1991 and 1996, and 41% of the eligible subjects participated. Altogether 17,035 women completed the baseline examination (Manjer, Malina et al. 2001). Of these, 737 women (median age 56 years, range 45–73 years) who had been selected as controls in a previous study on breast cancer (Almquist, Bondeson et al. 2010) were used as the control group.

Questionnaires

Paper III

At the time of inclusion, the patients completed a questionnaire regarding the following 15 symptoms related to complications of the digestive tract: loss of appetite, difficulty swallowing, meal-related cough, early satiety, nausea, vomiting, weight loss, abdominal fullness, bloating, regurgitation, constipation, diarrhea, evacuation incontinence, symptomatic postprandial hypoglycemia, and postprandial perspiration. The questionnaire had been used previously with this category of patients (Ohlsson, Melander et al. 2006, Faraj, Melander et al. 2007).

Paper IV

Rome III

The patients completed a shortened version of the Rome III questionnaire that only asked about IBS symptoms (Drossman 2006). This questionnaire has been translated and validated into the Swedish language (Magnus Simrén and Anna Rydén). Patients who fulfilled the Rome III criteria were classified as also suffering from IBS, but because their diagnosis was MC we have – in accordance with the presence of MC in IBD – referred to this as IBS-like symptoms (Roth and Ohlsson 2013).

Visual Analogue Scale for Irritable Bowel Syndrome (VAS-IBS)

The VAS-IBS is a short psychometrical test developed to measure the treatment response and well-being during the previous two weeks in patients suffering from IBS (Bengtsson, Ohlsson et al. 2007). The questionnaire includes nine items about GI symptoms and psychological well-being. The seven items of abdominal pain, diarrhea, constipation, bloating and flatulence, vomiting and nausea, perception of psychological well-being, and the intestinal symptoms' influence on daily life use a scale from 0 mm to 100 mm with 100 mm representing the best health. The two items of urgency and feeling of incomplete evacuation of bowel passage are answered by "yes/no". The questionnaire was completed by the patients themselves.

The Malmö Diet and Cancer Study questionnaire (MDCS)

The MDCS baseline examination included a self-administered questionnaire about marital status, education, employment, smoking habits, wine consumption, physical activity, medical conditions, and medication (Manjer, Elmstahl et al. 2002). This questionnaire was also completed by the patients at the time of inclusion in the present study.

Methods

Paper I

Cultivation and identification of lactobacilli

Vaginal and rectal samples were treated in an ultrasonic bath for 2 min and diluted before plating on Rogosa agar plates. The plates were incubated anaerobically at 37 °C for 72 h. Two or three colonies were randomly picked and characterized by Randomly

Amplified Polymorphic DNA (RAPD) as described by Quednau et al. (Quednau, Ahrne et al. 1998). Cultures having the same RAPD pattern within the same sample were regarded as belonging to the same species. Species identification was performed by multiplex PCR as described by Song et al. (Song Y-L 2000) and slightly modified by Vasquez et al. (Vasquez, Jakobsson et al. 2002). If this was not applicable, then identification was by partial 16S rRNA sequencing.

Sex hormone analyses

Serum estradiol, progesterone, FSH, and LH levels were analyzed in the fertile women at day 7 and day 21 of their menstrual cycle and were measured once in the postmenopausal women at the Department of Clinical Chemistry, Skåne University Hospital. The levels of the different hormones were classified into groups. Estradiol and progesterone were analyzed by a one-step competitive immunoassay with alkaline phosphatase, enzyme marking, and magnetic separation. FSH and LH were analyzed by a two-step immunometric assay using alkaline phosphatase, enzyme marking, and magnetic separation.

Paper II

DNA extraction and amplification

A single biopsy was transferred to a 1.5 mL tube and the total DNA was extracted with 190 µL Buffer G2 (DNA Tissue Kit; Qiagen, Gmbh, Hilden, Germany) and 10 µL of Proteinase K (Qiagen).

PCR amplification and cloning

Universal bacterial primers were used to amplify the bacterial 16S rRNA genes. Amplification was performed on an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany). The amplicons were then cloned into competent *Escherichia coli* cells. Colonies were selected randomly and recultivated on LB agar containing ampicillin and then harvested and stored in freezing buffer at –80 °C.

Sequencing

Selected clones were single-strand sequenced by MWG Biotech (Ebersberg, Germany) using the ENV1 primer as the sequencing primer. Sequences were edited using Bioedit Sequence Alignment editor 7.0.5.3. Sequences were identified by comparing them to sequences from the Ribosomal Database Project using the option “seqmatch”. Sequences were checked for chimeric artifacts by using the Bellerophon server and by

creating phylogenetic trees of both the 5′ and 3′ ends of the sequences. DNAdist calculations were performed using the "similarity table" option in the Phylip DNAdist program.

Paper III

Esophageal manometry

Standardized esophageal manometry was performed with an intra-luminal solid-state transducer system (Gaeltec Ltd, Isle of Skye, Scotland). The polygraph ID converter in the PolyGram NET software package (Medtronic- Synmed, Stockholm, Sweden) was used to digitize the analog signal. All pressure values were expressed in mmHg and referenced against the atmospheric pressure. The manometry catheter was introduced through the nose and fluoroscopically positioned in the distal esophagus with the patient sitting in an upright position. With the catheter in place, all participants were instructed to swallow 10 mL of a barium contrast medium (60% w/v). At least five barium swallows were recorded. The video fluoroscopic image and the manometry registration were mixed using a video output card (Medtronic) (Faraj, Melander et al. 2007, Samsom, Bharucha et al. 2009).

The diagnosis of esophageal dysmotility is confirmed if at least one of the five following criteria is fulfilled (Spechler and Castell 2001):

- Absence of peristaltic contractions in the esophagus
- Mean peristaltic contraction amplitude <30 mmHg or >200 mmHg in the esophagus
- More than 10% of the peristaltic waves in the esophagus are simultaneous and non-propulsive
- Speed of the peristaltic wave <3 cm/s or >6 cm/s in the distal esophagus
- Resting pressure in the lower esophageal sphincter <10 mmHg or >30 mmHg

Gastric-emptying scintigraphy

A test meal was prepared by adding tin colloid labeled with 30–50 MBq of 99m Tc to an egg that was whipped in a glass cup in a hot water bath until coagulated. The egg and a slice of toasted white bread were cut into pieces smaller than 1 cm × 1 cm and served with 100 mL of 37 °C water. The meal was ingested within 5 min. Immediately

after consuming the meal, a large-field, double-headed gamma camera (Philips Skylight, Philips Medical Systems, Best, The Netherlands) was placed anteriorly and posteriorly parallel to the upper abdominal wall. The radioactivity was measured in continuous 1 min frames for 70 min. A region of interest representing the stomach was created, and the activity of the first frame was set to 100%. The gradual decrease in radioactivity – measured as the number of radioactivity decays per minute (counts/min) – was plotted against time. The time elapsed to reach a 50% decrease in radioactivity in the region of interest (T50) was identified. The radioactivity measurements were corrected for the half-life of 99m Tc and for attenuation by using the geometrical mean values of the decay curves obtained from the two gamma camera heads. A T50 that was >2 standard deviation of the value for healthy control subjects (after 70 min) was considered abnormal and was classified as gastric dysmotility (Hanson and Lilja 1987).

Statistical analyses

Statistical analyses were performed with SPSS versions 17–20 (Statistical Package for the Social Sciences) for Windows.

In paper II, Shannon and Simpson's indices were used for diversity calculations, and the Simpson's indices were expressed as 1/D.

Variables were analyzed for normal distribution by Kolmogorov-Smirnov test (paper III, IV). In paper IV, all distributions differed significantly from a normal distribution so the factors were categorized and the values were given as medians (interquartile ranges). Differences between groups were calculated with the Mann–Whitney *U*-test (paper III, IV). Fisher's exact test was used for categorical variables (paper I, III, IV). Correlations were performed using Spearman's rank correlation test (paper I, III, IV) or Pearson's test (paper III). Multiple logistic regression analysis was performed to determine associations with esophageal dysmotility as the dependent variable (paper III). In paper IV, the factors being studied (the independent variables) were initially examined using an unconditional logistic regression to calculate odds ratios with 95% confidence intervals (OR, 95% CI). Analyses to adjust for confounding factors were then performed. The Kruskal–Wallis test was used to calculate differences in VAS-IBS between subgroups of smoking and alcohol habits among all included patients in paper IV.

All values in the papers were expressed as medians (interquartile ranges (IQR)) or means ± standard deviations (SD).

We considered p-values <0.05 to be statistically significant.

Results

Paper I

In fertile women, the colony forming units per gram (CFU/g) of vaginal smear varied from 8.3×10^4 CFU/g to 1.8×10^8 CFU/g at day 7 of the menstrual cycle and from 4.0×10^2 CFU/g to 4.0×10^7 CFU/g at day 21 of the cycle. In postmenopausal women, the vaginal smear varied from 1.7×10^2 CFU/g to 3.0×10^7 CFU/g. The rectal smear in fertile women varied from 1.8×10^2 CFU/g to 1.9×10^7 CFU/g at day 7 and from 2.2×10^3 CFU/g to 4.8×10^6 CFU/g at day 21, and in postmenopausal women it varied from 1.0×10^3 CFU/g to 4.9×10^6 CFU/g.

In vaginal smears, lactobacilli were found and isolated from 11 out of the 20 fertile women and from 11 out of the 20 postmenopausal women. In rectal smears, lactobacilli were found and isolated from 15 out of 20 fertile women and from 10 out of 20 postmenopausal women ($p = 0.071$). Altogether, 39 isolates from vaginal smears and 67 isolates from rectal smears were further characterized.

L. crispatus was found significantly more often in the vaginal flora of the fertile women than that of the postmenopausal women ($p = 0036$). The vaginal flora of the postmenopausal women was more often colonized by *L. gasseri*. The most commonly occurring *Lactobacillus* species in the rectal flora of both the fertile and postmenopausal women was *L. plantarum*. In eight women – six fertile and two postmenopausal – the vaginal and rectal smears presented with the same *Lactobacillus* species. Seven fertile women were colonized in the vagina with the same *Lactobacillus* species on both day 7 and day 21 of the menstrual cycle, and three of those presented the same species in the rectal smears. *L. gasseri* and *L. ruminis* dominated both the vaginal and rectal flora in two postmenopausal women (Figures 4 and 5).

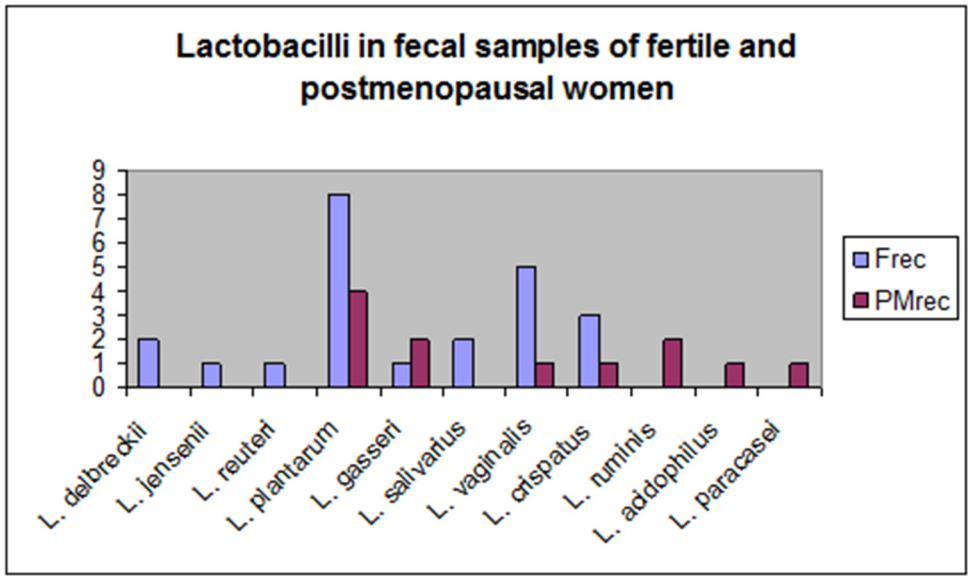

Figure 4: Presence of lactobacilli in rectal samples of fertile (Frec) and postmenopausal (PMrec) women.

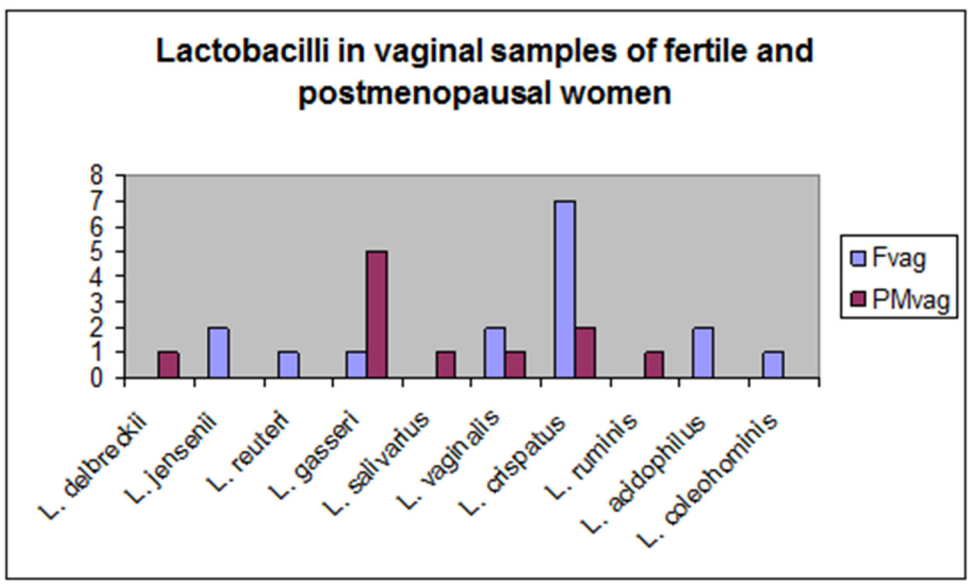

Figure 5: Presence of lactobacilli in vaginal samples of fertile (Fvag) and postmenopausal (PMvag) women.

No statistically significant differences were found in the number of bacteria in the rectal flora in the fertile women between day 7 and day 21 of the menstrual cycle, and no differences were found between fertile and postmenopausal women. In addition, no correlation was found between rectal microbial flora and sex hormone levels.

Sex hormone levels in the serum of both fertile and postmenopausal women were within the normal ranges. In fertile women, the presence of *L. crispatus* in the vaginal ecosystem of women with high and medium levels of estradiol reached statistical significance compared to postmenopausal women ($p = 0.036$). However, one woman with the lowest estradiol level was also colonized with *L. crispatus*.

Paper II

The clones could be divided into 44 different phylotypes, and the microbiota was dominated by Firmicutes and Bacteroides. Seven phylotypes were found in both patients, and these constituted 47.5% of the total number of clones that were similar to *Bacteroides cellulosilyticus, B. caccae, B. thetaiotaomicron, B. uniformis*, and *B. dorei* within the Bacteroidetes. Sequences similar to *Faecalibacterium prausnitzii* and *Clostridium citroniae* were also found in both patients.

Paper III

The majority of patients had GI symptoms – although they entered the study independently of symptoms – and abdominal bloating was the most prevalent symptom followed by regurgitation and abdominal fullness. Gastrointestinal symptoms did not correlate to objective findings. In fact, only the experience of postprandial hypoglycemia tended to be associated with gastroparesis ($p = 0.054$), and no symptoms were associated with esophageal dysmotility.

Among patients suffering from diarrhea (16%), further examination with colonoscopy and extended laboratory analyses could not diagnose IBD, MC, or any other organic disease in these patients. Thus, the diarrhea was classified as secondary to a dysmotility complication or as functional diarrhea.

Out of 84 patients, 53 patients (63%) presented with esophageal dysmotility. These patients had longer durations of DM compared to those without dysmotility ($p = 0.043$). Interestingly, there was a strong association between esophageal dysmotility and retinopathy as determined by Fisher's exact test ($p > 0.001$). When testing for an

independent association among all variables, retinopathy was the only variable associated with esophageal dysmotility (OR = 10.15, 95% CI = 2.16–47.62, p = 0.003).

Only 11 of the 84 patients (13%) had gastroparesis, and this was not associated with esophageal dysmotility. Age was negatively correlated with gastric emptying rate (p = 0.004).

Paper IV

Patients were divided into persistent MC (MC1, n = 78) and transient MC (MC2, n = 53). There was an increased risk for both former and current smokers to develop MC based on calculations on the whole group (OR = 1.88, 95% CI = 1.04–3.39 and OR = 2.71, 95% CI = 1.50–4.91, respectively). When calculated with respect to both smoking and alcohol habits, only the group of smoking without concomitant alcohol intake was associated with an increased risk of developing MC (OR = 2.80, 95% CI = 1.19–6.63).

When dividing the patients into subgroups, past smoking was associated with increased risk of developing transient MC (OR = 2.67, 95% CI = 1.15–6.23), whereas current smoking was associated with increased risk of developing persistent MC (OR = 3.18, 95% CI = 1.57–6.42).

The IBS criteria were fulfilled in 43 patients (52.4%) with CC and in 25 patients (51.0%) with LC, and concomitant symptoms of IBS were associated with smoking (OR = 4.24, 95% CI = 1.92–9.32). The group of patients who only smoked with no intake of alcohol had the lowest values (most symptoms) on all VAS-IBS scales but reached statistical significance only on the scales of *bloating and flatulence* (p = 0.011) and *the gastrointestinal symptoms' influence on daily life* (p = 0.012). There was no difference between persistent MC and transient MC with regard to concomitant IBS-like symptoms.

Alcohol intake had no association with MC or IBS.

Discussion

The main finding, or at least one of the main findings within this thesis, was the strong association between retinopathy and esophageal dysmotility in patients with DM (paper III) that occurred independently of gender. We also found that current smoking in women with MC patients was associated with an increased risk of developing persistent MC and that past smoking was associated with transient MC (paper IV). Smoking was also associated with the presence of MC with concomitant IBS-like symptoms (paper IV). We found no correlation between sex hormone levels in fertile or postmenopausal women and the overall levels of *Lactobacillus* species in the vagina and rectum (paper I). The colon microbiota of the two patients with MC showed similarities to the microbiota of a healthy individual (paper II).

The association between retinopathy and esophageal dysmotility in patients with DM found in paper III is in agreement with a previous study that identified a correlation between diabetic gastroparesis and retinopathy (Hyett, Martinez et al. 2009). A case report in 1999 described the striking biopsy findings of diabetic microangiopathy in a female patient with long-standing insulin-dependent DM and chronic diarrhea (De Las Casas and Finley 1999). Diabetic retinopathy is characterized by a spectrum of lesions within the retina, including changes in vascular permeability, capillary microaneurysms, capillary degeneration, and excessive formation of new blood vessels (neovascularization) (Forbes and Cooper 2013). Clinically, diabetic retinopathy is separated into non-proliferative and proliferative disease stages. In the early stages of diabetic retinopathy, hyperglycemia can lead to loss of retinal pericyte and thickening of the basement membrane, and this contributes to changes in the integrity of blood vessels within the retina and alters the blood-retinal barrier and the vascular permeability (Forbes and Cooper 2013). In this initial stage of non-proliferative diabetic retinopathy, most people do not notice any visual impairment. Hypothetically, the same microangiopathies that contribute to retinal damage can also affect relevant nerve and muscle function of the stomach and can contribute to the evolution of diabetic gastropathy.

The development of systemic sclerosis is analogous to that of diabetic retinopathy. Systemic sclerosis is a collagenous disease characterized by GI disorders in which the esophagus is the most frequently affected GI section (Sallam, McNearney et al. 2006). The pathophysiology of systemic sclerosis involves autoimmunity with vasculitis and

widespread damage to small blood vessels. This leads to fibrosis and subsequent destruction of the smooth muscle layer in the bowel wall (Forbes and Marie 2009).

Gastrointestinal symptoms are common in patients with DM, and the prevalence of GI symptoms is higher in women compared to men (Spangeus, El-Salhy et al. 1999, Oh, Choi et al. 2009). In paper III we consecutively included patients with DM independently of any GI symptoms. Among these patients we found that the majority had GI symptoms that were not associated with any clinical measurements of dysmotility. Objective data might not always correlate with the subjective perception of symptoms, and this has been demonstrated in studies in patients with DM that found a high prevalence of GI symptoms that had little or no correlation with objective findings (Bytzer, Talley et al. 2001, Kong and Horowitz 2005).

There is a high prevalence of GI symptoms in the general population, and it is associated with a considerable decrease in health-related quality of life. A large body of evidence shows that women complain more frequently of GI symptoms than men, and a recent study identified female gender as an independent risk factor associated with a higher prevalence of GI symptoms (Tielemans, Jaspers Focks et al. 2013). In our study, however, we found no differences between genders with regard to GI symptoms. Whether female gender is really a risk factor for GI disorders has yet to be elucidated, especially because the prevalence of GI disorders might not differ significantly between genders when efforts are made to objectively assess patient complaints (Sadik, Stotzer et al. 2008, Pendleton, Ahlner-Elmqvist et al. 2013). Another explanation for gender differences in GI complaints might be due to differences in the attitude towards, and in the self-perception of, personal health. For example, female IBS patients experience their symptoms as severely as patients with GI dysmotility who are dependent on nutritional support and opioid analgesics (Bengtsson, Hammar et al. 2011).

Interestingly, we found that the presence of esophageal dysmotility was more common than gastroparesis in both type 1 and type 2 DM. This is in contrast to an earlier study by our group that found that delayed gastric emptying was slightly more common than esophageal dysmotility in patients with type 1 DM who presented with GI symptoms (Faraj, Melander et al. 2007). In the present study, we included consecutive patients independently of symptoms. We found a higher incidence of type 2 DM, and this could explain the differences that we found.

Sex differences have been demonstrated in the mucosal immune system with women having a higher baseline level of immune activation compared to their male counterparts that predisposes them to inflammation-associated diseases that are exacerbated following menopause (Sankaran-Walters, Macal et al. 2013). Autoimmunity is a suggested etiology of MC. In a recent study, however, women with MC had only a slightly increased prevalence of some autoantibodies (Roth, Gustafsson

et al. 2013). This could be explained by other concomitant autoimmune diseases, a high frequency of smokers among this group, and the composition of the cohort being primarily middle-aged women. Furthermore, we showed that thyroid disorders are more common in female patients with MC than in controls, but we did not find significant differences in subclinical thyroid disorders between female patients with MC and controls (Gustafsson J, Roth et al. 2013). Interestingly, the majority of patients in the former study were diagnosed with thyroid disorders before the diagnosis of MC, and they were all taking levothyroxine at the time of their diagnosis. Several medications have been linked to the onset of MC (Beaugerie and Pardi 2005, Fernandez-Banares, Esteve et al. 2007), and whether levothyroxine can contribute to the development of MC needs further studies.

In a recent interview study, women said that they were more likely to experience GI symptoms as they aged (Gamble, Skinner et al. 2013). In older women, the increased risk for GI dysfunction could be due to hormonal, immunological, and/or vascular changes (Olesen, Eriksson et al. 2004, Pardi, Loftus et al. 2007) or concomitant diseases and medications. In accordance with the general population that claims to be aware of how the food they eat will impact on their health, the women in the study claimed that their experiences over time had led them to try different remedies, many of which involved foods or beverages that contained ingredients that play important roles in gut fermentation by influencing the gut microflora (Gamble, Skinner et al. 2013). The concept of ingesting fermented food products containing health-promoting bacteria was first introduced by Metchnikoff (1908). Probiotic bacteria are defined as living organism that exert beneficial effects on the host when ingested (Schrezenmeir and de Vrese 2001), and the species that are often used as probiotics belong to the genera *Lactobacillus* (Holzapfel, Haberer et al. 1998).

The vaginal lactobacillus flora varies in relationship to hormonal levels, and women and men differ strongly from each other with respect to sex steroids. Some GI disorders tend to have their first onset during the years of menopause (Storr 2013). Therefore, it seems necessary to discuss whether endocrine factors such as hormonal changes in women could contribute to the higher prevalence of GI disorders in middle-aged women. However, a recent study found no differences in the exposure to factors that influence sex hormones, such as oral contraceptives and hormonal replacement therapy, between patients with MC versus controls but instead showed that patients with MC reached menarche and menopause earlier than controls (Roth, Manjer et al. 2013). It could, of course, also be the fall in levels of both estrogen and progesterone at menopause that could play a role in the pathophysiology of MC. Sex hormones play a role in pain modulation, and pain inhibition is more effective in the ovulatory phase of the menstrual cycle – when estradiol levels are high and progesterone levels are low – than in the follicular phase when both estradiol and progesterone levels are low (Rezaii,

Hirschberg et al. 2012). Postmenopausal women have low estrogen levels, and this could explain the differences by gender.

In paper IV, we found smoking to be associated with MC. There is still a debate over the effect of cigarette smoking on estrogen levels, but this could be due to too much confidence being placed on the responders rather than the methodology. For example, the Endogenous Hormones and Breast Cancer Group (Endogenous, Breast Cancer Collaborative et al. 2011) analyzed 13 prospective studies and showed that smokers of more than 15 cigarettes per day had higher levels of estrogen than non-smokers. In contrast to this, different results were obtained by Soldini et al. (Soldin, Makambi et al. 2011) who divided 293 women into active smokers, passive smokers, and non-smokers based on a combination of self-reporting and serum cotinine concentrations. Interestingly, in many cases smoking status differed from the levels of estimated cotinine. The authors concluded that smoke exposure decreased estrogen levels and that future studies should include serum cotinine concentrations. Yet another study showed that the effects of estrogen are diminished in women who smoke (Krolik and Milnerowicz 2012), and both past and current smoking have been reported to be risk factors for developing MC (Vigren, Sjoberg et al. 2011, Munch, Aust et al. 2012, Yen, Pokhrel et al. 2012). In our study, almost half of the study patients who had stopped smoking had done so during the observation period and after the diagnosis of MC had been made. This is in agreement with previous studies that describe such health events as 'teachable moments' that motivate changes in smoking behavior (McBride, Emmons et al. 2003, Dohnke, Ziemann et al. 2012). The association between past smoking and transient MC that was found in our study might depend on the fact that the patients regained their health when they stopped smoking. Cigarette smoking is strongly associated with atherosclerosis and can increase the susceptibility of blood vessels to vasospasms due to oxidative stress. In fact, one study reported that atherosclerotic arteries might be due to supersensitivity to the constrictor effect of superoxide anions that are found in cigarette smoke (Sugiyama, Kugiyama et al. 1998). Current smoking is a risk factor for recurrence of ischemic colitis (Sherid, Sifuentes et al. 2014), and GI symptoms and the presence of microscopic intestinal mucosal inflammation in women with MC could be a secondary reaction to ischemia.

The colonic mucosa of patients with severe MC tends to heal after fecal diversion (Stroehlein 2007). This has led to the most widely supported hypothesis that a noxious agent in the lumen, probably originating from the bacterial microflora, might have a major pathogenic role in chronic intestinal inflammation. Our pilot study (paper I) found no relation between the numbers or occurrences of species of lactobacilli found in the vagina and rectum of the study participants. However, this was a small pilot study. Recently, Petricevic et al. (Petricevic, Domig et al. 2013) analyzed 30 postmenopausal women and found that 40% harbored the same lactobacilli in both the vagina and rectum. In a larger study of 531 fertile women ranging in age of from

14 years to 35 years, 43% of those having *L. crispatus* in the vagina also had this species in the rectum (Antonio, Rabe et al. 2005).

Our understanding of how the bacterial flora in MC might be altered is poor, but Helal et al. (Helal, Ahmed et al. 2005) have recently found an association between *E. coli* and LC (Helal, Ahmed et al. 2005). In paper II, we found that the overall composition of the colonic microbiota in patients with CC was similar to that of healthy individuals with Firmicutes and Bacteroidetes being the dominant phyla. However, in our study the proportion of clones belonging to *Bacteroides* was much higher than presented in other studies. *Bacteroides* play an important role in the human gut by mediating mucosal and systemic immunity, but they sometimes cause opportunistic infections (Jiang, Dupont et al. 2010). In our study, the most dominating clones within *Bacteroides* belonged to the *B. fragilis* group that are regarded as the most virulent *Bacteroides* species (Wexler 2007). Furthermore, in both patients in paper II clones related to *Clostridium clostridioforme* were found. Strains of *C. clostridioforme* and closely related species have been shown to be involved in a variety of infections (Finegold, Song et al. 2005). It is difficult to draw any conclusions from an analysis of only two patients, but an abnormal microbiota could play a role in the pathogenesis of CC even if this is not a primary cause.

There is a high comorbidity between GI symptoms and stress, anxiety, and depression. Several studies have demonstrated that IBS patients as a group have an increased level of anxiety and depression, and this is especially the case in those who seek help from a gastroenterologist because of their symptoms (Simren, Abrahamsson et al. 2001, Koloski, Talley et al. 2003). It is, therefore, necessary to discuss whether a higher prevalence of anxiety and depression in women is one reason, or even the main reason, for the higher prevalence of GI complaints among women or whether gender has a significant impact on GI disorders independently of psychiatric conditions. MC induces GI symptoms that partly overlap with IBS predominately in middle-aged women. Cigarette smoking tends to increase under stressful conditions, and the main findings in the study of female MC patients in paper IV was that smoking was associated with an increased risk of developing persistent MC and MC with concomitant IBS-like symptoms independently of other lifestyle factors. However, we also showed that smoking was not associated with the development of only MC in the absence of IBS symptoms.

Alcohol is known to have a number of deleterious effects on the intestinal mucosa, and it has also been reported that alcohol affects hormones in postmenopausal women by increasing the conversion of testosterone into estradiol. The women in our study (paper IV) mainly drank red wine, which contains phenolic compounds that have been shown to affect the composition of the human gut microbiota. Because we found that only women in the smoking without concomitant alcohol intake group had an increased risk

of developing MC, it would be interesting to analyze whether wine consumption is harmful to the GI tract and whether there is a protective effect against the effects of smoking when combining smoking and alcohol. In analogy with another study of rheumatoid arthritis where alcohol has a protective effect (Maxwell, Gowers et al. 2010)

Taken together, MC, IBS, and GI dysmotility are common diseases in the general population that are considered to be more frequent in women. However, the fact that they are more common in women than in men has no obvious hormonal explanations, and the rectal microflora is not as sensitive to hormonal influences as the vaginal flora. Women and men with DM report similar levels of GI symptoms, gastroparesis, and esophageal dysmotility when examined consecutively. Furthermore, esophageal dysmotility is much more common than gastroparesis, and this is strongly associated with retinopathy. There are no obvious changes in microflora and hormonal influences are not involved in the pathogenesis of MC, thus other etiologies such as medications, other illnesses, and intestinal ischemia should be further investigated.

Strengths and Limitations

One of the strengths of this thesis is that the studies have sought to clarify the influence of gender in GI disorders by highlighting differences in a variety of factors such as sex hormones, colonic microbiota, and the impact of lifestyle factors.

The strength of paper IV is that we systematically examined patients with MC in the whole population of the southernmost part of Sweden and compared these patients to a well-defined control group from the same geographic area.

There are several limitations to the studies in this thesis. One limitation is the small number of examined patients in papers I, II, and III. However, paper I was designed as a pilot study that aimed to generate a larger study if positive results were obtained. Another limitation is that established bacterial growth was found in only half of the smears. This might partly be explained by the lack of *L. iners*, which differs from other *Lactobacillus* species due to its peculiar culture requirements. Future studies should include different culture methods.

In paper II, only two patients were examined due to the high cost of the method. In fact, the cost is still a critical issue in the evaluation of 16S rRNA gene sequence analysis as a diagnostic tool in clinical laboratories. In paper III, the patients were only allowed to participate in the study if both manometry and scintigraphy were performed. Most withdrawals were due to an inability to swallow the manometry catheter, and only 84 patients out of 122 (69%) who agreed to participate were able to complete the study.

Another limitation in paper III was that the examinations were only performed once. Knowing that GI motility varies from day to day (Lartigue, Bizais et al. 1994), this might lead to some level of uncertainty in the analysis.

One limitation in paper IV was the use of an external control group and another was the fact that the women in the MC group were elderly with many concomitant diseases and drug treatments that generated several confounding factors. However, it is very difficult to recruit healthy volunteers to clinical studies. The response rate of our control group was 41%, and it can be assumed that these subjects are healthier than those who did not agree to participate. Future research should involve prospective studies looking at persons with only MC. Another limitation was that GI symptoms were examined only once at varying time intervals after the diagnosis of MC. However, this was a cross-sectional study and patients and controls were not enrolled during the same time period.

Future perspectives

To further study whether the association found between esophageal dysmotility and retinopathy is due to microangiopathy in the GI tract, future studies should examine the GI tract with full-thickness biopsy. However, this requires laparoscopic surgery with anesthesia and this is not currently ethically justified. In the meantime, further studies confirming our histopathological findings of microangiopathy as a possible pathogenesis for GI dysmotility are important.

We still know very little about gender-specific differences in GI disorders, particularly when it comes to symptoms, the influence of social and psychological factors, and the ramifications of these differences for treatment and prevention. Future research with a focus on clinical investigations of gender differences is needed in order to understand differences between men and women in terms of clinical signs, diagnostic procedures, and therapeutic needs. Perhaps we could better understand the differences by examining the general population consecutively and over a longer period of time.

The MDCS is a prospective population-based study designed to investigate the relationship between diet and other lifestyle factors on the risk of developing cancer (Berglund, Elmstahl et al. 1993). All women born between 1923 and 1950 (aged 44–74 years, mean age 58 years) and all men born between 1923 and 1945 (aged 45–73 years, mean age 59 years) living in the city of Malmö were eligible for participation. Baseline examinations were performed between March 1991 and October 1996. It would be of interest to further analyze women in the MDCS who later developed MC or IBS. This could be a great opportunity to examine both women and men at baseline

before the onset MC or IBS. This would provide better indications of whether or not the development of MC and IBS is related to environmental changes, changes in nutrition, drug exposure, or concomitant diseases.

An interesting question to ask is why men get MC. In my clinical experience, I have only seen a small number of men with MC, and the majority were diagnosed with LC where the pathogenesis has been mostly drug-induced. Most of the men with CC had a history of hormone therapy after prostate cancer, and testosterone has been shown to be effective in stimulating metabolic activities and intestinal contractions in the epithelium (Sukhotnik, Shiloni et al. 2005, Gonzalez-Montelongo, Marin et al. 2006, Akcora, Altug et al. 2008).

It is still unclear how smoking affects the GI tract and through what mechanisms smoking affects the GI tract. Although smoking is the most clearly defined environmental risk factor for the development and progression of IBD, the mechanism behind this association is poorly understood. This might be due to the chemical complexity of tobacco smoke. A related question is why smoking affects Crohn's disease and ulcerative colitis in different ways and how smoking affects MC. The risk of Crohn's disease in current smokers is more elevated in women (Persson, Ahlbom et al. 1990), but it is not clear why this is so. Future experiments should focus on both smoking and alcohol use when analyzing the data because smoking and alcohol use are associated behaviors.

In addition to the effects of smoking, future research should investigate how alcohol affects the GI tract. Alcohol can be used to induce colonic mucosal inflammation in mouse models (Andrade, Vaz et al. 2003), and regular and moderate consumption of red wine has been shown to have a noteworthy effect on the growth of select gut microbiota (Queipo-Ortuno, Boto-Ordonez et al. 2012). Wine consumption in women has increased during the last decades (Lissner, Sjoberg et al. 2008), and on average women weigh less than men so for the same amount of alcohol a woman's blood alcohol concentration will tend to be higher and put her at greater risk for harm. It is also possible that other biological differences, including hormones, might contribute to the effects of alcohol on the GI tract.

It is important in the future to establish methodological procedures for the diagnosis of MC. Histological criteria have been established, but clear guidelines for their use are needed. We have shown clinical differences between subgroups of MC (chronic, relapsing disease and a transient single attack), but not when MC is divided into CC and LC. As for IBD, a diagnosis of the disease should not be made until at least two attacks of the disease have occurred. This will allow IBD to be differentiated from other causes of diarrhea such as infection or side effects from drugs (Henriksen, Jahnsen et al. 2006).

Conclusions

No correlation was found between the overall levels of *Lactobacillus* species in the vagina and rectum and variations in sex hormone levels. The most often occurring *Lactobacillus* species in the rectal smears of both fertile and postmenopausal women was *L. plantarum*. Furthermore, *L. crispatus* was more often found in the vaginal flora of the fertile women than in the postmenopausal women.

The overall colon microbiota of two patients with a histologically diagnosed case of CC had a predominance of Firmicutes and Bacteroidetes, and this was similar to the colon microbiota of a healthy woman. However, it is difficult to draw conclusions from this study because only two patients were analyzed. One noteworthy finding was that in both patients a high proportion of potentially pathogenic species of *Bacteroides* and clones related to *C. clostridioforme* were found.

Esophageal dysmotility was more common than gastroparesis in patients with DM, and this was independent of gender, symptoms, and type of diabetes. There was a strong association between esophageal dysmotility and retinopathy.

In female patients with MC under the age of 73, smoking was associated with an increased risk of developing persistent MC and MC with concomitant IBS-like symptoms independently of alcohol consumption and other lifestyle factors.

Populärveteskaplig sammanfattning

Mag-tarmsjukdomar hos kvinnor

Är mag-tarmbesvär, olika för män och kvinnor?

Förekomsten av mag-tarmbesvär är utbrett bland befolkningen. Sjukdomar i mag-tarmkanalen presenteras med en blandning av subjektiva symtom och objektiva fynd som inte alltid korrelerar med varandra. Funktionella mag-tarm besvär har mestadels endast subjektiva symtom. Att ha en sjukdom som inte ”syns”, men ändå har betydande symtom är frustrerande för patienten och livskvalitén påverkas avsevärt. Kvinnor är mer benägna än män att rapportera mag-tarmbesvär, men om kvinnor verkligen har mer bekymmer med mag-tarmsjukdomar är svårt att avgöra. Kvinnor och män skiljer sig åt på många sätt, och en del av orsaken är troligen relaterade till grundläggande fysiologiska egenskaper. Kvinnor i alla åldrar har oftare behov av sjukvård än män trots att kvinnor lever längre, man talar då om den så kallade könsparadoxen. Upplevelsen av hälsa är mycket individuell och vissa med kroniska sjukdomar upplever sig ha god hälsa medan andra som är friska upplever ohälsa. Fler kvinnor än män upplever ohälsa oavsett de har en tydlig diagnos eller inte. Ohälsosam livsstil såsom rökning och alkohol är riskfaktorer för olika sjukdomar och tillstånd i mag-tarmkanalen. Dagens medelålders kvinnor har anammat många livsstilsfaktorer från männen. Rökning är till exempel vanligare bland kvinnor än män i denna åldersgrupp. Alkoholvanorna har ökat bland kvinnor i medelåldern. Mikrobiotan i mag-tarmkanalen spelar en viktig roll för människans hälsa, och framförallt lactobaciller som anses vara skyddande organismer. Tarmfloran påverkas av olika livstilsfaktorer och även av individens ålder.

Vissa mag-tarmsjukdomar domineras av kvinnligt kön som t.ex. mikroskopisk kolit (MC), gastropares och colon irritabel (IBS). Mikroskopisk kolit är ett samlingsnamn för en grupp kroniska inflammatoriska diarrésjukdomar, där de två vanligaste är kollagen kolit, som beskrevs första gången 1976 av Malmöpatologen Claes G Lindström och lymfocytär kolit, som beskrevs första gången 1989 av Lanzeby. Namnet MC har den fått eftersom man inte hittar några förändringar synliga för blotta ögat vid undersökning av tarmen med ett så kallat koloskop (det vill säga en böjlig slang med kamera som man för in i tjocktarmen). Först när provbitar (biopsier) från tarmen

undersöks med mikroskop ses att slemhinnan är inflammerad. Vid kollagen kolit ses ett förtjockat bindvävsskikt precis under ytan på tarmslemhinnan. Vid lymfocytär kolit är den dominerande bilden en ökad mängd immunceller (så kallade lymfocyter) i det yttersta skiktet av slemhinnan. För de flesta som får mikroskopisk kolit är sjukdomen inte lika aktiv efter det första skovet (den första sjukdomsperioden). Det gäller särskilt för lymfocytär kolit, där upp till 60 procent endast får ett enstaka skov. Men sjukdomen är oftast kronisk, vilket betyder att man har den hela livet, även om man är besvärsfri långa tider. Medelålders individer drabbas oftast av MC, men det kan även förekomma hos yngre individer. Kvinnor är oftare drabbade än män, och detta framförallt för CC. Således sammanfaller medelåldern för insjuknandet hos kvinnor med åren under och efter menopaus.

Gastropares är en magsjukdom som innebär att kroppen smälter maten långsammare än normalt. I ett friskt matsmältningssystem förs födan från magsäcken och vidare genom matsmältnings-kanalen genom starka muskelsammandragningar. Vid gastropares arbetar magmusklerna dåligt (eller inte alls), vilket gör att magsäcken inte töms som den ska. Många diabetiker drabbas av gastropares.

IBS är en vanlig mag-tarmsjukdom som drabbar mellan 5 % och 20 % av befolkningen och svarar för cirka 30 % av alla remisser till specialister i mag-tarmsjukdomar, och 3 % av alla besök till allmänläkare. I åratal har vi känt att fler kvinnor än män har IBS, närmare bestämt drabbas kvinnor 1,5-3 gånger mer än män. Det finns studier som tyder på att mag-tarmkanalens muskelrörlighet är lite långsammare hos kvinnor än hos män, och det är ännu mer sant när IBS är närvarande. Mag-tarmkanalens rörlighet påverkas även av hormoner.

Denna avhandling syftar till att försöka klargöra påverkan av könen i mag-tarmbesvär, genom att belysa höjdpunkter i olika fält, könshormoner, tjocktarmsflora och effekterna av livsstils- och riskfaktorer.

Den första studien var en pilotstudie för att studera om det kan finnas en korrelation mellan könshormoner och antal lactobaciller i tarmen som skulle kunna förklara att fler kvinnor efter menopaus drabbas av MC. Man vet att menscykeln påverkar vaginalfloran och att laktobacillhallten i vaginan sjunker efter menopaus. När lactobacillhalten sjunker i vaginan ökar förekomsten av andra bakterier, med risk för infektioner. Tillförsel av hormonet östrogen motverkar dessa effekter. En nedgång av dessa skyddande bakterier i tjocktarmen skulle kunna göra den postmenopausala kvinnan mer känslig, inte bara för överväxt av sjukdomsalstrande bakterier i vagina utan också i tjocktarmen. I detta syfte analyserades laktobacillfloran i vaginan och ändtarmen från 20 fertila kvinnor under två faser av menscykeln och en gång från 20 kvinnor i menopaus. Vi fann ingen skillnad i rektalfloran i de olika faserna av menscykeln, eller postmenopausalt, och vi fann ingen korrelation till könshormoner.

I den andra studien analyserades slemhinneprover från två kvinnor med CC, med en avancerad gentest metod för att karakterisera bakteriefloran i tarmen. Vi fann att den totala sammansättningen av tarmfloran liknande en frisk, med dominans av bakterierna Firmicutes och Bacteroidetes. Intressant var att både patienter hade en högre andel av potentiellt patogena arter av Bacteroides, jämfört med tidigare rapporter av friska individer. Dock analyserades endast två patienter och det är då svårt att dra några slutsatser, men ytterligare studier behövs.

I den tredje studien valde vi att utvärdera matstrupens och magsäckens rörlighet, förekomst av komplikationer och mag-tarm besvär hos patienter med DM som kom på återbesök till sjukhuset eller vårdcentralen. I vår oselekterade population av patienter med DM hittade vi en oväntad högre förekomst av esofagusdysmotilitet, d.v.s. rubbningar i matstrupens rörlighet, än gastropares d.v.s nedsatt magsäcksrörlighet. Intressant var att esofagusdysmotilitet presenterade en stark koppling till retinopati. Vidare, led en stor del av patienterna av mag-tarm symtom, som inte var förknippade med objektivt uppmätta dysmotiliteter. Vi fann ingen skillnad mellan män och kvinnor.

I det fjärde arbetet undersökte vi 131 kvinnliga patienter med MC beträffande rökning och alkoholvanor och jämförde de med populationsbaserade kontroller. Den viktigaste slutsatsen i denna studie var att rökning var associerad med en ökad risk för att utveckla ihållande MC och MC med åtföljande IBS -liknande symtom, oberoende av andra livsstilsfaktorer, medan rökning inte var i samband med utvecklingen av enbart MC, utan IBS symtom. Tidigare rökning var i motsatsen associerad med övergående MC, vilket skulle kunna förklaras med att rökning har en övergående effekt på tarmen. Samtidigt alkoholintag verkade skydda mot rökningens negativa effekt.

Sammanfattningsvis, kan den höga förekomsten av kvinnor med MC inte förklaras enbart av skillnader i könshormoner, och tarmfloran är inte lika känslig som vaginalfloran för hormonsvägningar. Rökning är en riskfaktor för utveckling av ihållande MC, och framförallt för förekomsten av IBS- liknande symtom. Objektiva fynd kan inte alltid korreleras till subjektiva symtom, och hos patienter med DM fann vi ingen skillnad mellan förekomst av objektiva fynd och subjektiva symtom mellan män och kvinnor. Att kvinnor oftare diagnostiseras med MC, gastropares eller IBS kan delvis bero på olika sök mönster hos könen snarare än hormonella skillnader.

Riassunto popolare

Malattie gastrointestinali nelle donne

I disturbi gastrointestinali, differiscono fra gli uomini e le donne?

L'incidenza dei disturbi gastrointestinali é molto diffusa nella popolazione. Le malattie del tratto gastrointestinale si presentano con un misto di sintomi soggettivi e constatazioni oggettive che non sempre correlano l'uno con l'altro. Disturbi gastrointestinali funzionali si presentano per lo più solo con sintomi soggettivi. Avere una malattia che non è "visibile", ma con sintomi significativi è frustrante per il paziente e la qualità della vita ne è significativamente influenzata. Le donne sono più propense degli uomini a riferire disturbi gastrointestinali, ma se le donne abbiano davvero piú problemi legati alle malattie gastrointestinali é difficile da determinare. Donne e uomini differiscono in molti modi, e parte della ragione è probabilmente legata alle caratteristiche fisiologiche di base. La probabilità di avere bisogno di cure mediche é maggiore nelle donne di tutte le età rispetto agli uomini anche se le donne vivono più a lungo, si parla del cosiddetto paradosso di genere. La percezione di salute è molto individuale, esistono infatti individui con malattie croniche che percepiscono di avere una buona salute e altri individui che pur godendo di buona salute lamentano diversi disturbi. Più donne che uomini percepiscono di avere problemi di salute, indipendentemente dal fatto che sia stata accertata loro una malattia o meno. Uno stile di vita malsano, come ad esempio fumo e alcol, costituisce un importante fattore di rischio per varie malattie e disturbi del tratto gastrointestinale. Le donne di mezza età di oggi hanno abbracciato molti fattori di stile di vita in passato tipici del degli uomini. Per esempio, fumare è più comune fra le donne rispetto agli uomini in questa fascia di età. Inoltre il consumo di alcol é aumentato tra le donne di mezza età. La flora batterica del tratto gastrointestinale svolge un ruolo importante nella salute del corpo umano e particolarmente i Lattobacilli sono considerati organismi protettivi. La flora intestinale è influenzata da diversi fattori, fra i quali lo stile di vita e l'età dell'individuo.

Alcune malattie gastrointestinali sono piú rappresentate nel genere femminile, come ad esempio la colite microscopica (MC), la gastroparesi e la sindrome dell'intestino irritabile (IBS). La colite microscopica è un nome collettivo per un gruppo di malattie

diarroiche croniche infiammatorie, le due più comuni sono la colite collagenosa, che fu descritta per la prima volta nel 1976 dal patologo di Malmoe Claes G Lindström e la colite linfocitaria, che fu descritta per la prima volta nel 1989 da Lanzeby. L´origine del nome MC deriva dal fatto che durante l'esame endoscopico dell'intestino con un cosiddetto colonoscopio (cioè, un tubo flessibile di lunghezza variabile contenente una piccola telecamera) ad occhio nudo non è evidenziabile alcuna alterazione della parete intestinale. Durante l'esame vengono presi dei campioni (biopsie) di tessuto del colon che una volta esaminati al microscopio rivelano una infiammazione caratteristica. Nella colite collagenosa si trova uno spesso strato di proteine (collagene) appena sotto la superficie della mucosa intestinale. Nella colite linfocitica, l'immagine dominante é una quantità aumentata di cellule immunitarie (chiamate linfociti) nello strato più esterno della mucosa. Per la maggior parte delle persone con la colite microscopica, la malattia non è così attiva dopo la prima recidiva (il primo periodo di malattia). Ciò è particolarmente vero per la colite linfocitica, dove fino al 60 per cento può avere solo una ricaduta occasionale. Ma la malattia è di solito cronica, il che significa che é presente per tutta la vita, anche se si è senza sintomi per lunghi periodi. Individui di mezza età sono i più colpiti, ma può verificarsi anche in individui più giovani. Le donne sono più spesso colpite rispetto agli uomini, e questo soprattutto per la colite collagenosa. Quindi l'età media di insorgenza nelle donne coincide con gli anni durante e dopo la menopausa.

Gastroparesi significa una paralisi dei muscoli dello stomaco che provoca ritardo nello svuotamento del cibo dallo stomaco. Di solito, forti contrazioni muscolari spingono il cibo attraverso il tratto digestivo. Ma nella gastroparesi i muscoli della parete dello stomaco lavorano poco o nulla. Questo impedisce allo stomaco di svuotarsi in modo corretto. Molti diabetici soffrono di gastroparesi.

IBS è un disturbo gastrointestinale comune che colpisce tra il 5% e il 20% della popolazione e rappresenta circa il 30% delle visite ambulatoriali da medici specialisti in gastroenterologia ed il 3% di tutte le visite effettuate dai medici di medicina generale. È ben noto che le donne soffrono di IBS piú degli uomini, in particolare, le donne sono colpite 1,5-3 volte più degli uomini. Ci sono studi che suggeriscono che il movimento muscolare dell'intestino è un pò più lento nelle donne rispetto agli uomini, e questa differenza è stato evidenziata in misura ancora maggiore nelle pazienti affette da IBS. La motilità gastrointestinale è anche influenzata dagli ormoni.

Scopo di questa tesi è di chiarire l'influenza del genere nei disturbi gastro-intestinali, evidenziando vari campi quali gli ormoni sessuali, la flora del colon e gli effetti dei fattori di rischio legati allo stile di vita.

Il primo lavoro è stato uno studio pilota per valutare se ci possa essere una correlazione tra gli ormoni sessuali e il numero di lattobacilli nell'intestino, che potrebbe spiegare il

perché più donne in postmenopausa sono affette da MC. Sappiamo che il ciclo mestruale influisce sulla flora vaginale diminuendo il contenuto di lactobacilli vaginali dopo la menopausa. Quando esso diminuisce nella vagina aumenta la presenza di altri batteri, e quindi il rischio di infezione. La fornitura di ormoni estrogeni contrasta questi effetti. Una diminuzione di questi batteri protettivi nel colon potrebbe rendere la donna in postmenopausa più sensibile, non solo per la crescita eccessiva di batteri patogeni nella vagina, ma anche nel colon. A tal fine, abbiamo esaminato il contenuto di lactobacilli nella vagina e nel retto da 20 donne fertili durante due fasi del ciclo mestruale, e da 20 donne in menopausa. Non abbiamo trovato alcuna differenza nella floran rettale e durante le diverse fasi del ciclo mestruale, o in post-menopausa, e non abbiamo trovato alcuna correlazione con gli ormoni sessuali.

Nel secondo studio abbiamo analizzato campioni di mucosa da due donne con colite collagenosica, con un metodo di test genetico avanzato per caratterizzare la flora batterica nell'intestino. Abbiamo scoperto che la composizione complessiva della flora intestinale é simile ad un'individuo sano, con la predominanza dei batteri Firmicutes e Bacteroidetes. È interessante notare comunque, che entrambe le pazienti avevano una più alta percentuale di specie potenzialmente patogene di Bacteroides, rispetto alle precedenti segnalazioni in individui sani. Tuttavia avendo analizzato solo due pazienti è difficile trarre conclusioni, sono quindi necessari ulteriori studi.

Nel terzo studio, abbiamo scelto di valutare la motilità esofagea e gastrica, la presenza di complicazioni legate al diabete e di problemi gastrointestinali nei pazienti con diabete mellito (DM) venuti ad una visita di controllo al centro ospedaliero o sanitario. Nella nostra popolazione non selezionata di pazienti con DM, abbiamo trovato inattesamente una maggiore incidenza di dismotilità esofagea, vale a dire disturbi della motilità esofagea, rispetto alla presenza di gastroparesi cioè ridotta la motilità gastrica. È interessante notare che, la dismotilità esofagea ha presentato una forte connessione con la retinopatia. Inoltre, un gran numero di pazienti soffriva di sintomi gastrointestinali non associati a dismotilità obiettivamente misurate. Non abbiamo trovato alcuna differenza fra uomini e donne.

Nel quarto lavoro, abbiamo valutato l´abitudine al fumo e all´alcol di 131 pazienti di sesso femminile con MC e li abbiamo confrontati con controlli basati sulla popolazione. Il principale risultato di questo studio é che il fumo è stato associato ad un aumentato di rischio di sviluppare MC persistente e MC con concomitanti sintomi di IBS, indipendentemente da altri fattori di stile di vita, mentre il fumo non è stata associato con lo sviluppo di solo MC senza sintomi di IBS. Al contrario, il fumo in passato è stato associato allo sviluppo della MC transitoria, che potrebbe essere spiegato dal fatto che il fumo ha un effetto transitorio sull'intestino. Mentre l'assunzione di alcol sembra avere un effetto protettivo contro l´effetto dannoso provocato dal fumo.

In sintesi, l'alta prevalenza di donne con MC non può essere spiegata con le differenze di ormoni sessuali, e la flora intestinale non è sensibile come la flora vaginale alle fluttuazioni ormonali. Il fumo è un fattore di rischio per lo sviluppo della MC persistente, in particolare con la presenza dei sintomi di IBS. Constatazioni oggettive non sempre possono essere correlate a sintomi soggettivi, e nei pazienti con DM non abbiamo trovato alcuna differenza tra l'incidenza dei riscontri oggettivi e dei sintomi soggettivi tra uomini e donne. Il fatto che alle donne vengano diagnosticate MC, gastroparesi e IBS piú spesso che agli uomini può essere in parte giustificato dalla diversità nei modi di cercare cura sanitarie, piuttosto che da oggettive differenze ormonali.

Acknowledgements

First of all, I would like to thank all the patients, nurses and friends who kindly gave their time and participated in the studies. Without their effort and generosity this work would not have been possible

When finally reaching a long-term goal, I believe it serves a purpose to pause and reflect upon the process that has taken place, with failures, successes, facilitating factors, obstacles and how they have been mastered. I wish to express my sincere gratitude to all those who have encouraged and supported me during the work with this thesis. In particular I would like to express my appreciation to:

Professor **Bodil Ohlsson**, my excellent main supervisor, former clinical tutor and friend, thank you so much for giving me the motivation to continue my research work. You have been an unending source of inspiration, support, knowledge, enthusiasm, generosity, and encouragement. I´m honored that you accepted to be my supervisor and guided and pushed me forward through this work. I couldn´t have done it without you.

Professor **Bengt Jeppsson** my co-supervisor for supporting me and encouraging me and for sharing his extensive knowledge in the microbiota of the gastrointestinal tract.

Cecilia Benoni, my co-supervisor who first introduced me to the world of microscopic colitis. Who always believed in me and encouraged me.

Bodil Roth, my co-supervisor for helping me in recruiting women with MC, and keeping track of data and for her friendly support and discussions.

My co-authors, thanks for your time, support, guidance and valuable contribution: **Crister Ohlsson** for your help in the lab, it has been great working with you and I have learned so much, **Siv Ahrné** for interesting discussions and excellent guidance within the field of Lactobacilli, **Martin Stjernquist** for sharing your knowledge in gynecology, **Jonas Manjer** for your valuable statistical support, **Bengt Littorin**, **Kerstin Berntorp** and **Anders Frid** for the help including clinical follow-up patients, **Ola Thorsson**, **Rolf Olsson** and **Olle Ekberg** for the help in performing and interpreting the gastrointestinal motility tests.

Ingrid Palmquist and **Agneta Enander** for superb assistance with collecting the smears and blood samples, and for valuable aid in other practical matters.

My colleague and friend **Lorenza Bonelli** for helping me with the Italian language revision.

Jan Lillienau head of the department of Gastroenterology in Malmö and Lund, who gave me the opportunity to work on this thesis.

My **colleagues at the department of Gastroenterology, SUS Malmö**, everyone remembered, none forgotten for warm friendship and never-ending support.

My **friends,** everyone remembered, none forgotten, for all good times and long talks, with special thanks to **Ruzica Mitrovic** for always being there when I need you.

My family friends **Helena Fork**, who also is my godmother, for endless support and encouragement and **Thomas Fork**, also a colleague, for valuable and stimulating discussion.

My loving **mother Maria-Pia** for always being there for me. Grazie mamma per avere sempre creduto in me, e per sempre avermi detto che se voglio posso fare tutto!

I would like to give my **father Sigurd** a thought in loving memory: You always believed in me, and I am sorry you did not experience this day!

My dear twin brother **Enrico**, my amazing sister **Sabina,** and **Renzo** my younger brother (who never stop reminding me that), for being the best siblings ever, for encouragement, laughs, and for sharing so much fun with you and the whole of your nice families.

My wonderful children **Maria** and **Simon**, you are the strength in my life and you means all to me!

And my husband **Jan-Olof** the love of my life, you´re simply the best.

Those who are not mentioned by their names are not forgotten.

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