From Department of Clinical Sciences, Danderyd Hospital
Karolinska Institutet, Stockholm, Sweden

INFLAMMATORY BOWEL DISEASE: DETERMINANTS RELATED TO GUT MICROBIOTA

Ali Kiasat

Karolinska Institutet

Stockholm 2023

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2023

ISBN 978-91-8016-916-5

Inflammatory bowel disease: Determinants related to gut microbiota
Thesis for Doctoral Degree (Ph.D.)

By

Ali Kiasat

The thesis will be defended in public at, Karolinska University Hospital, Solna, Clarence Crafoord A5:04, June 9th, 2023, 9:00 a.m..

Principal Supervisor:
Associate Professor, MD, PhD, Ulf O Gustafsson
Karolinska Institutet
Department of Clinical Sciences, Danderyd Hospital

Co-supervisors:
Associate Professor, MD, PhD, Anna Löf Granström
Karolinska Institutet
Department of Women's and Children's health and Department of Clinical Sciences, Danderyd Hospital

MD, PhD, Richard Marsk
Karolinska Institutet
Department of Clinical Sciences, Danderyd Hospital

Opponent:
Professor, MD, Pär Myrelid
Linköping University
Department of Biomedical and Clinical Sciences

Examination Board:
Professor, MD, Annika Bergquist
Karolinska Institutet
Department of Medicin, Huddinge

Professor, MD, Karl Franklin
Umeå University
Department of Surgical and Perioperative Sciences

Professor, MD, Marie Carlson
Uppsala University
Department of Medical Sciences

To Mithra, Darya and Ava

Popular science summary of the thesis

Ulcerative colitis (UC) and Crohn's disease (CD) are the two most prevalent forms of chronic inflammatory bowel disease (IBD). In Sweden, the prevalence of IBD is 0.65%, and it is increasing. This disease can result in intestinal strictures, significantly impaired bowel function, cancer and premature death. Early detection and optimized treatment can decrease the need for major surgery and related complications. However, there are no reliable markers for detecting IBD or evaluating treatment outcomes.

The cause of IBD is not yet fully understood, but research suggests that lifestyle and environmental factors may disrupt the bacterial flora in the colon. This, in turn, can lead to inflammation resulting from the body's immune response to the intestinal bacterial flora. Bile acids (BA) and short chain fatty acids (SCFA) are found in both the gastrointestinal tract and blood and have been associated with various bacteria and colon inflammation. These substances could potentially serve as biomarkers for IBD.

Various surgical procedures have been linked to both new-onset IBD and disease severity. However, further epidemiological studies are needed to map the associations between surgical procedures and subsequent IBD development. Such studies would provide valuable insights into the study of gut dysbiosis.

The aim of this thesis was to investigate the relationship between appendectomy and bariatric surgery and later development of IBD, as well as to identify potential biomarkers for IBD.

In **Paper I,** we investigated the association between juvenile appendicitis, treated with appendectomy or conservatively treated without surgery, and adult risk of IBD. We found that childhood appendicitis with appendectomy was associated with lower risk of adult UC and CD, whereas conservative treatment was associated with lower risk of adult UC only.

In **Paper II,** we investigated the association between bariatric surgery and new onset of IBD. We found that individuals operated on with “gastric bypass” had an increased risk of later development of CD whereas individuals who underwent “gastric sleeve” had an increased risk of UC.

In **Paper III**, the aim was to analyse plasma concentrations of SCFA in relation to IBD and to evaluate SCFA as a potential biomarker for disease. We found that CD and UC were not associated with alterations in plasma SCFA concentration.

In **Paper IV**, we aimed to assess alterations of plasma BA profiles in association to CD. We found that the immune dysfunction in CD may be associated with altered bile acid composition in blood plasma.

Abstract

Inflammatory bowel disease (IBD) is a chronic and idiopathic disorder that causes inflammation in the gastrointestinal tract. Overall, it can be classified into two types: ulcerative colitis (UC) and Crohn's disease (CD). The causes of IBD have been extensively studied, with heredity, lifestyle, and environmental factors being identified as possible contributors. These factors can trigger an imbalance in the bacterial flora in the colon, which is increasingly thought to play a crucial role in the development of IBD. As dysbiosis in the gut microbiota has been frequently reported in inflammatory bowel disease, it has been proposed that both UC and CD may be caused by an auto-immune response to gut bacteria in genetically susceptible individuals. However, the exact aetiology of these diseases is still largely unknown. The aim of this thesis was to investigate epidemiological aspects of surgical abdominal procedures and possible biochemical markers associated with gut microbiota, in relation to IBD.

In **Paper I,** we investigated the association between juvenile appendicitis, treated with appendectomy or conservatively treated without surgery, and adult risk of IBD. This, nation-wide, population-based retrospective cohort study, based on Swedish national registers, included all individuals with a diagnosed appendicitis before the age of 16, during the time-period 1973-1996, and matched controls. The study population was followed until 2017 for any development of UC and CD. We found that childhood appendicitis with appendectomy was associated with lower risk of UC (aHR 0.30 95% CI 0.25-0.36) and CD (aHR 0.82 95% CI 0.68-0.97), whereas conservative treatment was associated with lower risk of adult UC (aHR 0.29 95% CI 0.12-0.69), only, compared to unexposed individuals. Our findings warrant further research of the appendix in relation to gut microbiota and IBD pathogenesis.

In **Paper II,** we investigated the association between bariatric surgery and new onset of IBD. This population-based retrospective cohort study included Swedish individuals registered in the Scandinavian Obesity Surgery Registry who underwent primary Roux-en-Y gastric bypass (RYGB) or sleeve gastrectomy (SG) during 2007 – 2018 and matched controls. The study population was followed up until 2019 to determine the development of CD and UC. We found that individuals operated on with RYGB had an increased risk of later development of CD (HR 1.8 95% CI 1.5 - 2.2) whereas individuals who underwent SG had an increased risk of UC (HR 1.8 95% CI 1.1-3.1). The findings should encourage further studies on surgical procedures for obesity and their effect on gut microbiota and development of IBD.

In **Paper III**, the aim was to analyse plasma concentrations of short chain fatty acids (SCFA) in relation to CD and UC and to evaluate SCFA as a potential biomarker for IBD. This cross-sectional study included 132 and 119 individuals with CD and UC respectively

and 205 controls. Although we found lower plasma concentrations of succinic acid among individuals with CD and UC in comparison to controls in univariate analysis, the difference did not remain after adjusting for sex, age and dietary factors. For all other SCFA, no differences could be found between the groups. In conclusion, CD and UC were not associated with alterations in plasma SCFA concentration.

In **Paper IV**, we aimed to assess alterations of plasma bile acid (BA) profiles in association to CD. This cross-sectional study included 88 individuals with CD and 88 controls. CD was found to be associated with lower concentrations of most secondary BA, particularly derivatives of deoxycholic acid and lithocholic acid. Moreover, plasma concentration of secondary BA among participants with active CD was lower in comparison to participants with CD in remission. We concluded that the immune dysfunction in CD may be associated with altered bile acid composition in blood plasma.

List of scientific papers

I. **Kiasat A**, Ekström LD, Marsk R, Löf Granström A, Gustafsson UO. Childhood appendicitis and future risk of inflammatory bowel disease - A nationwide cohort study in Sweden 1973-2017. *(Colorectal Dis. 2022 Aug;24(8):975-983)*

II. **Kiasat A**, Löf Granström A, Stenberg E, Gustafsson UO, Marsk R. The risk of inflammatory bowel disease after bariatric surgery. *(Surg Obes Relat Dis. 2022 Mar;18(3):343-350)*

III. **Kiasat A**, Rautiainen S, Prast-Nielsen S, Engstrand L, Schuppe-Koistinen I, Gustafsson UO, Löf Granström A.
Evaluation of plasma Short Chain Fatty Acid levels as markers for Inflammatory bowel disease.
(Submitted manuscript)

IV. **Kiasat A**, Prast-Nielsen S, Rautiainen S, Engstrand L, Andersson F, Lindberg J, Schuppe-Koistinen I, Löf Granström A, Gustafsson UO.
Plasma bile acids in association with Crohn's disease.
(Submitted manuscript)

Contents

1 Introduction 1
2 Literature review 3
2.1 Inflammatory bowel disease 3
2.1.1 Epidemiology 3
2.1.2 Aetiology 4
2.1.3 Treatment of IBD 4
2.2 Surgical procedures and risk for later development of IBD 5
2.2.1 Appendectomy 5
2.2.2 Bariatric surgery 7
2.3 Human microbiota and IBD 8
2.3.1 Human microbiota 8
2.3.2 Colonic microbiota 9
2.3.3 Colonic microbiota and IBD 11
2.3.4 Sequencing microbiota 11
2.4 Short chain fatty acids, microbiota and IBD 12
2.4.1 Short chain fatty acid synthesis and metabolism 12
2.4.2 SFCA in IBD 13
2.5 Bile acids, microbiota and IBD 13
2.5.1 Bile acid synthesis and metabolism 13
2.5.2 Bile acid function 14
2.5.3 Bile acid and IBD 14
2.6 Short chain fatty acids, bile acids, microbiota and IBD 15
3 Research aims 17
4 Materials and methods 19
4.1 Data sources 19
4.1.1 Swedish national patient register (NPR) 19
4.1.2 Cause of death registry (CDR) 19
4.1.3 Scandinavian obesity register (SOReg) 19
4.1.4 KOLBIBAKT 19
4.2 Studies 21
4.2.1 Paper I 22
4.2.2 Paper II 23
4.2.3 Paper III 23
4.2.4 Paper IV 24
4.3 Statistics and data analysis: 25
4.4 Ethical considerations 25
5 Main Results 27
5.1 Paper I 27

5.2 Paper II 31
5.3 Paper III 35
5.4 Paper IV 38
6 Discussion 43
6.1 Appendicitis and subsequent risk of IBD 43
6.2 Risk of IBD after bariatric surgery 44
6.3 Metabolites in blood, dependent on gut microbiota 45
6.4 Methodical considerations 48
6.4.1 Precision 48
6.4.2 Validity 48
6.4.3 Multiple hypothesis testing 50
7 Conclusions 53
8 Future perspectives 55
9 Acknowledgements 57
10 References 59
11 Appendix 69
11.1 KOLBIBAKT Questionnaire 69

List of abbreviations

AHEI	Alternate Healthy Eating Index
aHR	Adjusted Hazard ratio
BA	Bile acids
BAM	Bile acid malabsorption
BAR	Bile acid activated receptor
BMI	Body mass index
C4	7α-hydroxy-4-cholesten-3-one
CA	Cholic Acid
CC	Clean colon
CD	Crohn's disease
CDCA	Chenodeoxycholic Acid
CDR	Cause of death registry
CI	Confidence Interval
CRP	C-reactive protein
DCA	Deoxycholic Acid
FMT	Fecal microbiota transplantation
FXR	Farnesoid x-receptors
GCA	Glycocholic Acid
GCDCA	Glycochenodeoxycholic Acid
GDCA	Glycodeoxycholic Acid
GHCA	Glycohyocholic Acid
GI	Gastrointestinal
GLCA	Glycolithocholic Acid
GPCR	G-protein-coupled receptors
GUDCA	Glycoursodeoxycholic Acid
HDCA	Hyodeoxycholic Acid
HR	Hazard ratio
IBD	Inflammatory bowel disease
IBD-U	Inflammatory bowel disease – unclassified
RYGB	Roux-en-Y gastric bypass
IBS	Irritable bowel syndrom
ICD	International Classification of Diseases
IDCA	Iso-deoxycholic Acid
ILCA	iso-Lithocholic Acid
KOLBIBAKT	Koloskopi-Biopsi-Bakterier (name of a study project)
LC-MS	Liquid chromatography-mass spectrometry
LCA	Lithocholic Acid
LCA.3S	Lithocholic Acid 3-Sulfate

MCA	Muricholic acid
NBHW	National board of Health and Welfare
NPR	Swedish national patient register
SCFA	Short chain fatty acids
SD	Standard deviation
SE	Standard error
SES-CD	Simplified endoscopic activity score point system
SG	Sleeve gastrectomy
SOReg	Scandinavian obesity register
TaMCA	Tauro-α-Muricholic acid
TCA	Taurocholic Acid
TCDCA	Taurochenodeoxycholic Acid
TDCA	Taurodeoxycholic Acid
THCA	Taurohyocholic Acid
TLCA	Taurolithocholic Acid
TLCA.3S	Taurolithocholic Acid 3-Sulfate
TRG-5	Seven-transmembrane G-protein-coupled receptors
TUDCA	Tauroursodeoxycholic Acid
UC	Ulcerative colitis
UDCA	Ursodeoxycholic Acid
UPLC	Ultra Performance Liquid Chromatography

1 Introduction

Inflammatory bowel disease (IBD) is a chronic idiopathic disorder that causes inflammation in the gastrointestinal tract and is roughly divided into ulcerative colitis (UC) and Crohn's disease (CD) [1-3]. The prevalence of IBD in Sweden is 0.65% [4], and there has been an observed increase in incidence in industrialized countries during the second half of the 20th century [5]. Both heredity and lifestyle factors such as smoking habits, diet, antibiotic use and hygiene have been studied as in relation to both UC and CD. However, they cannot fully explain the majority of the disease burden. As a result, the primary causes of IBD remain unclear [5].

The human gut hosts approximately 1000 different bacterial species [6] of which approximately 70% of all bacteria are located in the colon. Studies have shown that in addition to aiding in the breakdown of starch, bacteria can also synthesize amino acids and vitamins, as well as metabolize drugs [7]. Recent evidence suggests that bacteria engage in a process known as "cross-talk" with cells from different organs through microbial metabolites, influencing the metabolic, immunological, and neurological systems [8]. As a result, intestinal bacteria may play a significant role in the causal chain of various diseases, which explains the growing global interest and research into the microbial environment in the colon, particularly in relation to bowel disease [9].

Several studies have observed changes in the gut microbial composition in individuals with IBD [10]. While clear evidence of causality is lacking, the most widely accepted hypothesis regarding the aetiology of UC and CD is that both diseases are caused by an autoimmune response to a subset of commensal gut bacteria in genetically susceptible hosts [11]. Heredity, lifestyle and environmental factors but also surgical removal of bodily organs have been studied as causes of IBD. These factors can disrupt the natural balance of bacteria in the colon, which is believed to play a crucial role in the development of the disease. However, the available evidence on this topic is limited, and further studies are necessary to advance our understanding of IBD.

The objective of this thesis is to explore possible epidemiological links between surgical procedures that may affect colonic bacterial diversity which could increase the risk of development of IBD. Additionally, the thesis aims to determine whether bacterial compositions specific to inflammation can be identified through variations in short-chain fatty acids and bile acids, as inflammatory biomarkers in plasma.

Timely treatment and close monitoring of IBD can improve patient outcomes, potentially reducing the need for major surgery. This approach could lead to significant gains in survival rates, lower healthcare costs, and alleviate suffering and morbidity for a large patient population.

2 Literature review

2.1 Inflammatory bowel disease

2.1.1 Epidemiology

Inflammatory bowel disease (IBD) is a chronic disorder of unknown origin that results in inflammation of the gastrointestinal (GI) tract. It is classified into two main types: ulcerative colitis (UC) and Crohn's disease (CD) [1-3]. In cases where there are indications of chronic colitis but the clinical, pathological and endoscopic features are insufficient to differentiate between UC and CD, the condition is referred to as inflammatory bowel disease-unclassified (IBD-U) [12]. Due to the complex nature of the disease, IBD patients may experience changes in symptoms and may transition between different disease entities as the condition progresses [13]. The prevalence of IBD overall, UC and CD in Sweden is 0.65%, 0.35% and 0.19% [4]. IBD is a severe chronic condition, which requires lifelong monitoring and symptomatic treatment for more than 2.5 million patients in Europe [5]. There was a rise in the incidence of IBD industrialized countries during the second half of 20th century [5]. Today, the incidence of IBD have stabilized, and even decreased [14] in regions with historically high incidence rates, such as North America and northern Europe. However, in newly industrialized areas and regions with previous low incidence rates such as South America, Asia, and Africa, the incidence of IBD continues to increase [1]. Thus, the rise in incidence and prevalence shown in newly industrialized countries mirrors the changes seen in the western world during the 20th century under rapid socioeconomic development [15].

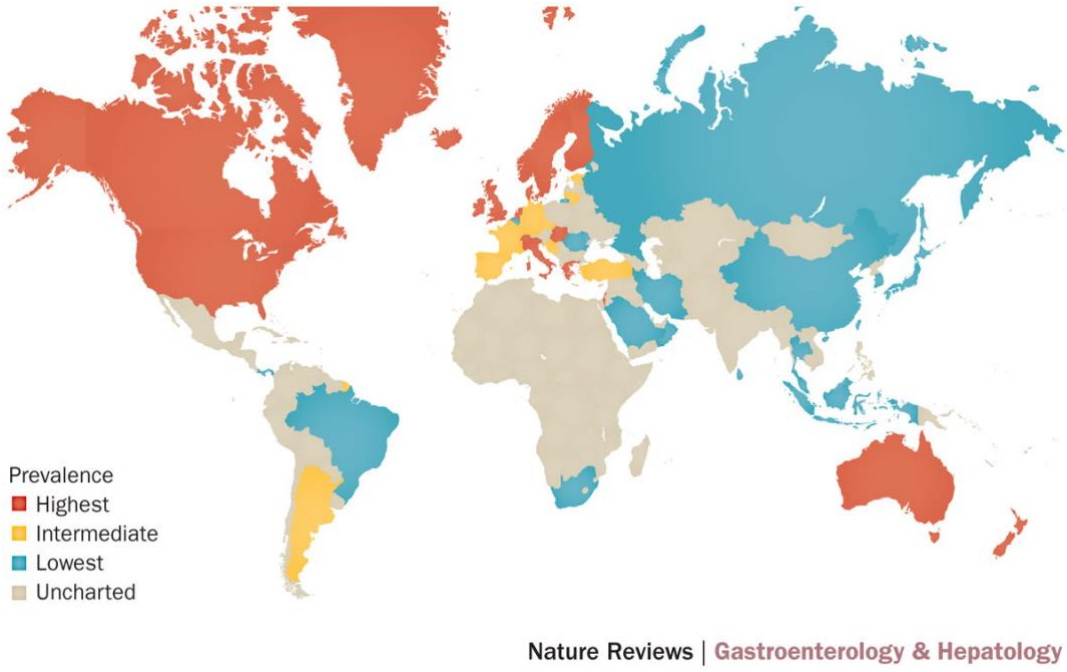

Figure 1: The global prevalence of IBD in 2015. Reproduced with permission from Springer Nature[5].

2.1.2 Aetiology

The aetiology of UC and CD is still largely unknown, but according to one widely accepted hypothesis, both diseases are caused by an autoimmune response to a subset of commensal gut bacteria in individuals who are genetically susceptible [11]. In addition, there are several environmental risk factors that have been associated with both CD and UC although a definitive causal relationship has not been established [16-19]. These factors include cigarette smoking [20, 21], appendectomy [22-25], vitamin-D deficiency [26], tonsillectomy [27, 28], antibiotic exposure [29, 30], oral contraceptive use [31-33], urban living, consumption of soft drinks, physical activity, breastfeeding, tea consumption, diet, stress, depression and Helicobacter pylori infection [17, 19].

Genetic factors are also widely studied, where 163 loci have been associated with CD and UC [34, 35]. Strongest associations are found in genes involved in the immune (NOD2) and inflammatory response (IL23R) to bacteria, including autophagy (ATG16L1) [34, 36, 37]. However, despite extensive genetic research indicating a genetic link, this has not been verified in twin studies and IBD patients seldom report family history of UC or CD [38].

All lifestyle, and environmental factors, including surgical removal of bodily organs, can cause a disturbance in the bacterial flora of the colon, which is increasingly believed to play a crucial role in the development of IBD. The inflammation appears to be a result of an inadequate immune response to the gut flora in individuals who are genetically predisposed to developing the disease, but the causal relationships are still unclear.

In summary the aetiology of IBD seems to be multifactorial where environmental factors, genetic factors and gut microbiota all contribute to the development of the disease.

2.1.3 Treatment of IBD

Both UC and CD can lead to strictures in the intestine, severely impaired intestinal function, and cancer. IBD can be treated with medications, surgery, or a combination of both. The goal of medical treatment is to reduce inflammation, alleviate symptoms, and prevent complications [39-42]. The specific treatment approach will depend on the type and severity of the disease. Medications are often the first line of treatment for IBD. Commonly used medications for IBD include: Aminosalicylates (5-ASAs), Corticosteroids, Immunomodulators, Biologics and Antibiotics[39, 40].

In cases where medications are not effective, surgery may be necessary. In both UC and CD, surgery can involve the removal of part or all of the colon and/or rectum. This can be curative for UC but not for CD. In CD, small bowel surgery is often needed. For IBD in general, surgery can also be used to treat complications, such as abscesses or strictures[41, 42]. However, there are major risks with surgery and there is hope that new modern drugs will reduce the percentage of patients who undergo surgery. Nevertheless, despite the rapid development of biological drugs in the past decade, the

10-year rate of surgery after CD and UC diagnosis is 47% and 16% respectively and often involves multiple procedures [43].

Early medical treatment and monitoring of treatment outcomes are important to reduce the risk of major surgery [44]. Targeted medical treatment is recommended for IBD, which means there is a great need to monitor the disease to ensure that the goals are being achieved. However, currently, there are only non-specific markers to track inflammation. C-reactive protein (CRP) is elevated in all types of inflammation and infections in the body, and Calprotectin in faeces (a substance from granulocyte cytoplasm) is not only elevated in IBD but also in other types of inflammation and cancer [44]. Therefore, there is a great need to find better markers to better optimize the treatment of IBD.

If the bacterial composition is found to be specific for IBD and if this can be detected through biomarkers in plasma, IBD can be detected earlier, and the disease can be better monitored during treatment. Early treatment and monitoring to optimize treatment can reduce the risk of the need for major surgery, which would mean significant survival gains, significantly reduce healthcare costs, and reduce suffering and morbidity for a large patient group.

2.2 Surgical procedures and risk for later development of IBD

Several surgical procedures have been associated with both new onset of IBD and severity of disease. Although the evidence is sparse, appendectomies [22-25], tonsillectomies [27, 28] and bariatric surgery [45, 46] have been associated with increased or decreased risk of IBD. Even if the reason for this association remains unclear, there is a compelling need to investigate whether the alteration of gut bacterial flora subsequent to these surgical procedures could be a risk factor for the development of IBD. By performing additional epidemiological studies to further map the associations between surgical procedures and subsequent IBD development, valuable insights can be obtained for the study of gut dysbiosis.

2.2.1 Appendectomy

The vermiform appendix has been suggested to play a role associated with IBD [22-25, 47, 48]. Since the first report in 1987 showing that fewer UC patients had a previous history of appendectomy, compared to non-UC controls [49], several studies have confirmed the negative association between appendectomy and UC [50]. Over the last two decades two large cohort studies both reported a negative association between appendectomy due to appendicitis or lymphadenitis, before the age of 20, and subsequent development of UC [22, 25].

With regards to CD, several smaller studies have shown inconsistent association with appendectomy, both positive [51, 52], negative [47] and no association at all [48]. However, two large registry-based cohort studies from Sweden and Denmark found an increased, transient [24] versus long-term [23], risk of CD after appendectomy. In addition, a meta-analysis investigating the relationship between appendectomy and the risk of developing CD found that the risk was increased for up to 5 years after surgery, but returned to baseline levels after that period [53]. The authors argue that the transient increased risk could reflect diagnostic difficulties in individuals with incipient CD [53].

Appendectomy has also been shown to affect the severity of UC [54]. Appendectomy for appendicitis before the age of 20 and before UC diagnosis is associated with milder severity of disease and lower risk of subsequent colectomy, whereas appendectomy for appendicitis after the age of 20 in established UC is associated with worse severity of disease and higher risk of subsequent colectomy [54]. Furthermore, undergoing an appendectomy prior to an IBD diagnosis has been linked to a delayed onset of both UC and CD when compared to controls [47].

Although attempts have been made to investigate the relationship between appendectomy and the risk of developing IBD, the outcomes of these studies have varied. Further studies are needed, particularly those that include individuals with appendicitis who have not undergone surgery, in order to better elucidate the associations.

2.2.1.1 Appendix vermiformis and bacteria

"Appendix vermiformis" has long been regarded as a vestigial organ that can be removed without negative medical consequences. However, new research indicates that the appendix has several important immunological functions [55] and also serves as a reservoir for gut bacteria that can restore the gut flora in the colon if it has been affected by, for example, antibiotic treatment [56]. Approximately 8% of the world's population, usually at a young age, undergoes removal of the appendix (appendectomy) due to appendicitis (inflammation of the appendix)[57]. If appendectomy is found to be associated with an increased risk of later disease development, patients with appendicitis should be treated conservatively without surgery to a greater extent.

Population-based cohort studies have recently shown a possible association between appendectomy and an increased risk of colorectal cancer [58], ischemic heart disease [59] including myocardial infarction [60] and gallbladder disease and gallstones [61] later in life. The prognosis for Clostridium difficile infection appears to be significantly worse in individuals who have undergone appendectomy [62].

The function of the appendix as an antibody producer and bacterial reservoir can be very important. Unlike the rest of the gastrointestinal tract, the appendix contains large

amounts of dense lymphatic tissue with B cells that continuously secrete IgA antibodies into the intestine [56]. Although both tonsils and so-called Peyer's patches in the small intestine also contain lymphoid tissue with some IgA production, the appendix accounts for the clear majority [56]. IgA antibodies bind with high affinity to pathogenic bacteria and viruses to neutralize them, but they also bind to "good" bacteria to regulate their amount and composition [63]

It is estimated that up to 75% of the bacteria in the intestine are coated with IgA antibodies [63]. IgA deficiency is the most common immunodeficiency in humans, and although many people with IgA deficiency are asymptomatic (likely due to compensatory mechanisms) [64], allergies, autoimmune diseases, and gastrointestinal disorders are more common in this group [65]. In animal studies where the appendix has been removed, low levels of serum and intestinal IgA have been noted, however this has not been investigated in humans [56].

The appendix's protected anatomical location and its unique environment for bacterial biofilms, which are extremely difficult to reach with antibiotics, provide a reservoir function for essential "good" bacteria that can be distributed in the intestine if the microbial balance is disrupted [66]. The few studies that so far have examined the types of bacteria in healthy appendices have shown a wide variation between individuals but are based on bacterial culture and not DNA sequencing techniques. With modern DNA sequencing techniques, we can map the bacterial diversity in the colon of individuals who have had an appendectomy versus those who have not, as well as the bacterial diversity in relation to IgA levels in the intestinal lumen, intestinal mucosa, and serum, and correlate this with the presence of an appendix and its bacterial flora.

2.2.2 Bariatric surgery

Bariatric surgery, such as Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) have increased during the last decades due to the overweight and obesity endemic [67]. It has been estimated that 20-40% of the IBD patients in western countries suffer from obesity [68].

Gastric restriction and gastrointestinal diversion cause weight loss after bariatric surgery [69], but other mechanisms may also be important, involving the central nervous system, the neuroendocrine system and the bile acid metabolism [70]. Changes in the gut microbiome may affect both the neuroendocrine system and the bile acid metabolism and has been suggested to play a role in weight reduction after bariatric surgery where a postoperative increase in Bacteroidetes and Proteobacteria and a decrease in Firmicutes have been reported in several studies [71, 72].

Since bariatric surgery may cause alterations in the gut microbiome, and development of IBD may be triggered by a changed bacterial diversity, associations between bariatric

surgery and onset of IBD has previously been studied, but mostly in small case series [73-78] and with conflicting results [45, 46].

A recent Danish cohort study showed increased risk of new-onset CD but not UC after bariatric surgery [45]. In contrast, a recent American study showed lower prevalence of de-novo CD and UC among individuals going through bariatric surgery compared to individuals with persistent obesity, defined as a BMI>30 kg/m² [46].

To gain a better understanding of the relationship between bariatric surgical procedures and the development of IBD, further studies are required, particularly those that investigate different types of surgeries and impact on gut microbiota.

2.2.2.1 The stomach and bacteria

Although the stomach is considered to be a hostile environment for bacteria due to its acidic pH, it has been found that some bacteria can still survive and thrive in the stomach. For example, *Helicobacter Pylori*, known as the major cause of peptic ulcers and gastric cancer, may also be linked to changes in other gut microbiota, potentially impacting overall health [79].

In this context, it is noteworthy that medical interventions for gastric diseases can significantly affect the microbial environment. For instance, the administration of proton pump inhibitors (PPI), which are commonly prescribed for conditions like acid reflux and ulcers, can modify the stomach microbiota. This modification can result in a reduction in the diversity of bacteria in the stomach and an increase in the prevalence of oral bacteria [80].

As medical interventions can impact the microbial environment, it is logical to consider that different types of surgical interventions on the stomach may also affect the microbiome. Alterations in the gut microbiome have the potential to influence both the neuroendocrine system and bile acid metabolism, which has been proposed to contribute to weight loss following bariatric surgery [70, 71].

2.3 Human microbiota and IBD

2.3.1 Human microbiota

The human microbiota consists of all the bacteria, fungi, and viruses present in the human body, with bacteria being the most extensively studied. According to estimates, an adult carries around $3.8 * 10^{13}$ bacteria, weighing approximately 0.2 kg, which exceeds the number of human cells ($3.0 * 10^{13}$) [81]. Most of these bacteria are located in the colon [81] as reflected in research that primarily focuses on the colonic microbiome.

In 2007, the Human Microbiome Project was the first project to define composition and function of the healthy human microbiome [82] and during the past two decades new DNA sequencing methods have made it possible to explore its interaction with the human cells [83].

2.3.2 Colonic microbiota

The human gut hosts approximately 1000 different bacterial species [6] of which approximately 70% of all bacteria are located in the colon. The predominant colonic bacterial phyla are Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteria [84], Figure 2.

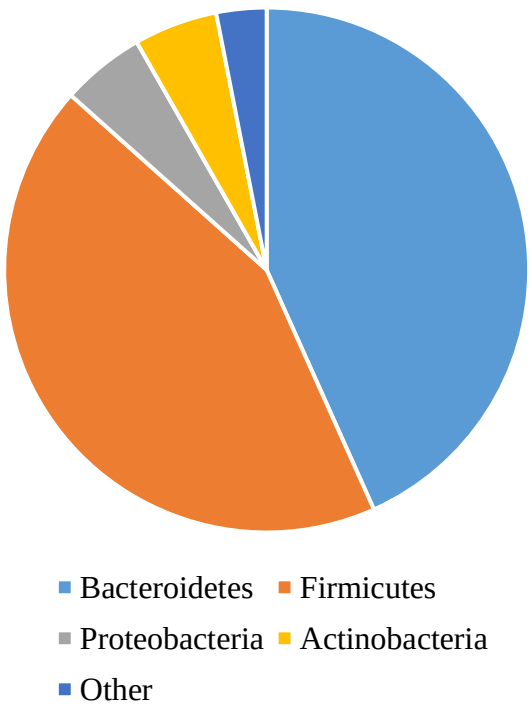

Figure 2: The dominant bacterial phyla in human colon.

The initial colonization of bacteria in the human body occurs during birth when the newborn is exposed to the mother's microbiota [85]. Subsequently, the gut microbiota rapidly develops and attains a structure similar to that of an adult by the age of 1-3 years [86]. During the early stages of life, environmental factors such as delivery mode and breastfeeding [85] as well as pet keeping and antibiotic use [87], have been shown to affect the development and composition of gut microbiota in childhood.

In contrast to infants, the gut microbiota in adults tends to be more stable, although the specific microbial species and their relative abundance can differ significantly between individuals [86]. Nevertheless, the functional capacity of the adult gut microbiota, which refers to the metabolic pathways present in a particular set of bacteria, remains relatively consistent across healthy individuals [6].

The intestinal microbiota is associated with various potential health benefits for humans, including the production of vitamins, absorption of essential ions such as calcium, magnesium, and iron, protection against harmful microorganisms and promotion of a healthy immune system [84]. One microbial function of considerable interest is the fermentation process of non-digestible food components, such as indigestible dietary fibres into short-chain fatty acids in the colon (See separate chapter 2.4).

The microbial environment of the colon in relation to intestinal disease has become the subject of increasingly intense research. Data suggests that the gut flora of healthy individuals is more diverse than, for example, patients with low-grade inflammation in the intestine. Therefore, the diversity of bacteria appears to be correlated with disease [88]. It is further believed that bacteria interact with cells in the intestinal system. Structural proteins (metabolites) in bacteria, such as flagellin, lipopolysaccharides, and specific antigens such as Polysaccharide A, are recognized by receptors in the intestinal mucosal cells, which in turn affect the immune system in terms of recruiting T cells and releasing cytokines. This may be significant for the onset of inflammation and later cancer development in the intestine [89]. Much indicates that this communication is specific to different disease areas [7] and therefore the bacterial population needs to be locally mapped by sampling from the current intestinal pathology to study these processes further. Local sampling is also advantageous as bacteria in the mucosa are likely to be less sensitive to laxatives than bacteria lying freely in the intestine [90].

As collecting samples from various segments of the gastrointestinal (GI) tract typically requires invasive procedures through colonoscopy, most studies on the microbiota of the GI tract in healthy individuals rely on faecal samples. However, such samples only provide information about the luminal microbiota and may not accurately reflect the microbiota that adheres to the host tissue, which is better assessed through mucosal biopsies [84]. This is crucial, as the luminal and mucosal microbiota harbour heterogenous microbial communities [91]. The mucosal tissue microbiota has lower alpha-diversity, indicating a lesser variation of microbes in a single sample (in terms of species richness and distribution), and lower abundance of most microbes with some exceptions; *Bacteroides*, *Subdoligranulum*, *Escherichia* and *Propionibacteriaceae* which are more prevalent on the mucosa than in the colonic lumen [91].

Certain species of *Bacteroides* (*B. caccae, B. fragilis and B. vulgatus*) as well as *Akkermansia mucinophila* are capable of degrading mucin and are primarily located in the mucin layer of the colon, where they derive nutrition from mucin [92-94]. The greater prevalence of *Propionibacterium* and *Escherichia* near the mucosa can be attributed to their ability withstand oxidative stress as the oxygen concentration near the mucosa is higher compared to in the lumen (known as the intraluminal oxygen gradient) [95]. In addition, the composition of the gut microbiota varies throughout the GI-tract with

increasing diversity and density. Therefore, a faecal sample cannot accurately represent the microbiota at a single location [96].

Dysbiosis i.e., a disruption of the gut microbiota homeostasis and changes in microbic metabolic activities and function [97] has been associated with numerous diseases such as inflammatory bowel disease, overweight, diabetes and autism spectrum disease [98].

2.3.3 Colonic microbiota and IBD

The role of dysbiosis in the GI-tract has been proposed as a significant factor in the pathogenesis of IBD [10]. However, due to the cross-sectional nature of most studies, it is challenging to determine whether the alterations in the microbiota are the root cause of IBD or a consequence of it. There are several publications comparing the colonic microbiota in IBD patients and healthy controls, most of them with small samples and inconsistent results. The lack of consistency may be due to various factors, such as differences in the assessment methods of the microbiome (e.g., 16S rRNA sequencing versus shotgun metagenomics, mucosa samples versus stool samples), the ways in which the data are pooled into subgroups (CD/UC, active disease/remission, treatment naïve/ treated) and differences in the control groups. It is also worth noting that the healthy gut microbiome has diverse composition across different parts of the world [99].

In a recent systematic review including 48 studies, a common finding was reduced alpha-diversity (total number of different species within a defined area) in CD patients compared to controls. In studies including both CD and UC patients, 9 out of 12 studies showed reduced alpha-diversity among CD patients in comparison to controls and 5 out of 17 studies showed reduced alpha-diversity among UC patients vs. controls [10].

The interpretation of results pertaining to the abundance and deficit of specific bacterial taxa is challenging due to inconsistencies in the various methodologies used for analysis. Nonetheless, some findings have been more frequently reported, such as the reduction of *Faecalibacterium prausnitzii* (CD and UC), *Christensenellaceae* (CD), *Akkermansia* (UC), *Eubacterium rectale* (UC) and Actinobacteria (UC and CD). Conversely, the increase of *Escherechia, Veillonella* (CD) and *Actonimyces* (CD) has also been reported [10].

Faecal microbiota transplantation (FMT), a treatment primarily used thus far in patients with *C. difficile* colitis, has also been suggested as a potential treatment for IBD patients. Despite some small studies showing positive results, there is currently limited evidence to support the use of FMT as an effective treatment for IBD [100, 101].

2.3.4 Sequencing microbiota

Over the past centuries, studies on gut microbes have primarily relied on culture-dependent methods. However, these methods have limitations as a significant

proportion of bacteria in the GI tract cannot be cultured. With the emergence of new culture-independent methods, based on DNA sequencing technologies, known as next-generation sequencing, new opportunities have arisen to analyse the gut microbiome and its correlation with different diseases. There are mainly two methods for microbiome profiling, "16S rRNA sequencing" and "metagenomic sequencing".

2.3.4.1 16S rRNA sequencing

The 16S rRNA gene, which is present in all bacteria is 1500 base pair long. The gene consist of 9 highly conserved and 9 hypervariable regions (V1-V9) that can be amplified by polymerase chain reaction and sequenced. To identify bacterial species in a sample, the sequences can be compared to a collection of gene sequences available in large databases [102, 103].

2.3.4.2 Metagenomic sequencing

In metagenomic sequencing all available DNA in a sample is sequenced non-selectively which enables higher taxonomic resolution and allows for identification of bacteria on a strain level. Since all genes in a sample are sequenced, metagenomic sequencing also gives information on what type of genes are present in a sample. Thus, a sample can also be analysed on a functional level [102, 103].

2.4 Short chain fatty acids, microbiota and IBD

2.4.1 Short chain fatty acid synthesis and metabolism

Short chain fatty acids (SCFA) are produced in the colon through fermentation of indigestible dietary fibres, proteins and peptides by the gut microbiota. SCFA are defined as fatty acids with fewer than six carbons, including formic acid, acetic acid, propionic acid, butyric acid and valeric acid where acetic acid, propionic acid and butyric acid account for more than 95% of all SCFA in the human intestine [104]. SCFA are subsequently absorbed by the colonocytes and enter the circulation via the portal vein affecting general metabolism, liver function, skeletal muscle and fatty tissue [105].

Some SCFA are key promoters of colonic heath and integrity. Butyrate for example, is the major and preferred metabolic substrate for colonocytes providing at least 60–70% of their energy requirements, required for proliferation and differentiation [106]. Apart from the function as a major energy source for colonocytes, SCFA in the gut exhibits various physiological functions with effect on colonic mobility, colonic blood flow, and gastrointestinal pH, which in turn can influence uptake and absorption of electrolytes and nutrients [107].

SCFA also plays an important role in regulation of intestinal immunity. For example, the SCFA-sensing G-protein-coupled receptors (GPCR) are expressed on immune cells [108] and regulate intestinal barrier integrity, as well as the activity and proliferation of immune cells (T-cells, CD-cells, macrophages, neutrophils, monocytes) [109].

The fermentation process responsible for the formation of SCFA is mediated by specific gut bacteria. Acetate, for example, is produced by several enteric bacteria, while Bacteroidetes and Firmicutes are the main producers of propionate. *Faecalibacterium prausnitzii, Eubacterium rectale, Eubacterium hallii* and *Ruminococcus bromii* are all producers of butyrate [109].

2.4.2 SFCA in IBD

Since SCFA are involved in both pro- and anti-inflammatory processes in the intestines, dysbiosis observed in IBD patients could affect the production of SCFA, thereby contributing to the development of IBD. Lower abundance of SCFA producing bacteria e.g., Firmicutes have been reported in IBD patients [110]. A recent meta-analysis has also shown a reduction of intraluminal acetate, propionate, butyrate and valerate as well as an increase in lactate in IBD patients compared to controls [111]. Furthermore, UC patients with active disease were found to have reduced butyrate levels compared to those in remission [111]. This may indicate functional differences of the microbiota in IBD patients compared to healthy subjects leading to alterations in the SCFA production and further damage on the intestinal barrier and increased inflammation.

2.5 Bile acids, microbiota and IBD

2.5.1 Bile acid synthesis and metabolism

Bile acids (BA) are synthesised in the liver from cholesterol by several enzymatic reactions. The synthesis requires 17 enzymes [112]. There are two pathways in which bile acids are synthesised, the classical pathway and the alternate pathway which accounts for 80% and 20% of the BA pool respectively [113]. These two pathways produce mainly two primary BA in the liver, cholic acid (CA) and chenodeoxycholic acid (CDCA). The primary BA are then conjugated with glycine (G) or taurine (T) giving rise to bile salts (GCA, TCA, GCDCA and TCDCA) [113].

Conjugated BA, i.e., bile salts, are stored in the gallbladder and secreted into the duodenum, mainly after meals, where they are transformed by bacterial enzymes to secondary BA e.g., lithocolic acid (LCA) and deoxylithocolic acid (DLCA). Thus, the gut microbiota highly affects the composition of BA.

After being secreted into the duodenum most BA are reabsorbed and transported back to the liver via the portal blood. This cycle is called the entero-hepatic circulation [114]. About 95% of the BA are reabsorbed through the portal blood minimizing loss which allows low rate of de novo synthesis. A small amount of BA can enter to the systemic circulation and are subsequently cleared in urine [113].

2.5.2 Bile acid function

The major physiological functions of BA are lipid digestion and cell signalling. BA form micelles in small intestine facilitating digestion and absorption of intestinal cholesterol, triglycerides, fatty acids and fat-soluble vitamins [115].

BA can also act as signalling molecules, interacting with cell membranes and nuclear receptors (bile acid activated receptors, (BAR)) which are expressed in the GI tract and immune cells. This enables communication between the intestinal microbiota and the host [114].

Each BA has distinct chemical properties and biochemical activities. Most studies on BAR are conducted on Farnesoid x-receptors (FXR) and seven-transmembrane G-protein-coupled receptors (TGR5). FXR is expressed at high levels in the GI tract, primarily in hepatocytes and ileal epithelial cells. It plays a key role in regulating the enterohepatic circulation and the synthesis of BA. FXR is also expressed in immune cells and is involved in various immunological functions, such as inflammatory response, maintaining the integrity and function of the intestinal barrier, and regulating the growth of the intestinal microbiota [116].

TRG5 is expressed in intestinal epithelial cells, ileal endocrine L-cells, biliary epithelial cells, gallbladder cells, and adipose tissue [114]. Activation of TRG 5 by secondary bile acids in enteroendocrine cells stimulates secretion of glucagon-like peptide, which in turn promotes insulin secretion. TRG signalling also promotes adipose tissue browning and energy metabolism to reduce weight [114]. In addition to regulating metabolic homeostasis, TGR5 is also expressed by immune system cells, which suggests a potential role for BA in immune cell homeostasis and function [114].

Examples of other BAR are Liver-X-Receptor, Constitutive Androstane Receptor, Vitamin D receptor, Pregnane-X-Receptor, Retinoid Related Orphan Receptor, Sphingosine-1-phosphate receptor 2 and Muscarinic receptor [114].

2.5.3 Bile acid and IBD

Patients with IBD often have an altered composition and dysbiosis of intestinal microbiota, as well as an inflamed intestinal epithelium. This inflamed epithelium is believed to decrease BA reabsorption resulting in more BA reaching the colon and being eliminated with faeces. Bile acid malabsorption (BAM) has been found in up to 50% of

adult patients with CD, which can lead to diarrhoea, steatorrhea with malabsorption of fat-soluble vitamins and formation of gallstones and kidney stones. BAM can also result from surgical ileal resection. Additionally, the dysbiosis associated with IBD leads to a reduction in the enzymatic capacity of the microbiota, affecting their ability to metabolize primary BA into secondary BA [114]. Given that BA are ligands for BAR, which are highly expressed in immunological cells in the GI tract, these changes could contribute to the immune dysfunction observed in IBD patients [114].

2.6 Short chain fatty acids, bile acids, microbiota and IBD

There are compelling reasons to further investigate the relationship between BA and SCFA with bacterial diversity and inflammation in the colon. It is important to determine whether inflammation-specific bacterial compositions can be identified through different compositions of BA, SCFA and inflammatory biomarkers in plasma. If it is possible to detect a bacterial composition that is specific to inflammatory bowel disease (IBD) through plasma biomarkers, the disease can be detected earlier and better monitored during treatment. Early intervention and monitoring to optimize treatment can decrease the likelihood of major surgery, leading to significant gains in survival, reduced healthcare costs, and less suffering and morbidity.

3 Research aims

The aim of this thesis is to investigate the relationship between appendectomy and bariatric surgery and later development of IBD, as well as to identify potential biomarkers for IBD.

The specific aims of the included papers were:

Paper I:

To investigate if juvenile appendicitis treated with appendectomy and conservatively treated appendicitis both are associated with a reduction of later onset of UC and CD.

Paper II:

To investigate if bariatric surgery is associated with increased risk of later development of IBD.

Paper III:

To compare plasma concentrations of SCFA, among patients with IBD, both with and without active inflammation, with healthy controls and to investigate these metabolites as potential markers for bowel inflammation.

Paper IV:

To identify alterations in the BA profile in blood plasma of CD patients in comparison to controls, and to investigate these metabolites as potential biomarkers for bowel inflammation.

4 Materials and methods

4.1 Data sources

4.1.1 Swedish national patient register (NPR)

The Swedish National Patient Register (NPR) is a register held by the National board of Health and Welfare (NBHW). It contains data on inpatient care such as discharge diagnoses according to the international classification (ICD) codes, surgical procedure codes and dates of hospital admission and discharge [117]. Data in the NPR and other national registers are linked to the Swedish personal identity number (PIN) that identifies every legal resident in Sweden [118].

The registry was established in 1968 and achieved national coverage in 1987. Since 2001, the registry has also been recording outpatient specialist care visits. Currently almost 100% of the inpatient and 80% of the outpatient specialist care visits are registered [119].

4.1.2 Cause of death registry (CDR)

The Swedish cause of death registry is held by the national board of health and welfare (NBHW). It is an almost complete registry containing information on all deaths in Sweden since 1952 including data on date, place, and cause of death according to ICD-codes [120]. The data is based on death certificates issued by physicians and the accuracy of the cause of death in the registry may vary based on age groups and diagnostic categories [121].

4.1.3 Scandinavian obesity register (SOReg)

SOReg is a national quality registry containing data on bariatric surgery in Sweden since 2007 [122]. The registry covered 97.4% of all bariatric surgical procedures performed in Sweden 2012-2018 [123] and includes baseline and perioperative data, as well as follow-up data [122]. The internal validity is considered high. The 2 and 5-year follow-up in the registry is 70% and 50% respectively [123].

4.1.4 KOLBIBAKT

An overview of the Koloskopi-Biopsi-Bakterier (KOLBIBAKT) cohort is shown in Figure 3. During the period, 1 November 2016 to 1 July 2019, 2,395 individuals (aged ≥ 18 years) who were referred for colonoscopy at Danderyd Hospital were asked for participation in the study. Of these, 1,136 (47.4%) denied participation and two were excluded due to interrupted colonoscopy. All patients included (N = 1,257) gave written informed consent before participating in the study. Prior to bowel preparation (Movprep®), patients were asked to submit a stool sample which was then stored in a standardized microbiological DNA stabilizing solution. This sample was saved for bacterial analysis.

Participants were also asked to complete a validated 13-page questionnaire with 277 questions about previous illnesses, diet, lifestyle habits (eg, smoking, alcohol, exercise, and sleep habits), bowel habits (including IBS scores, and the Bristol stool scale), previous colonoscopies and antibiotic treatment (Appendix).

Before colonoscopy, four blood samples were taken: Two for analysis of HbA1c, hemoglobin and CRP, and two for the hospital's biobank for later analysis of biomarkers (BA, SCFA, Olink-inflammation panel). During the colonoscopy, in addition to routine diagnostic tissue samples, two extra biopsies were taken from healthy mucosa, near the disease area and in the disease area, in all individuals. From each area, a biopsy was saved for DNA sequencing and one for culture. The severity of IBD disease was assessed and recorded by the examining endoscopist.

Of those included (645 men and 612 women) in the study, 1,247 (99.2%) filled out the lifestyle form and 1,165 (92.8%) submitted a stool sample. Blood samples were obtained from 1,255 (99.8%) individuals.

The colonoscopy findings showed that there were 595 (47.3%) cases of precancerous lesions, 403 (32.1%) cases of diverticulosis, 263 (20.9%) cases of IBD, 97 (7.7%) cases of former cancer and 14 (1.1%) cases of present cancer. 213 (16.9%) individuals had no pathological findings on colonoscopy, clean colon (CC), Table 1.

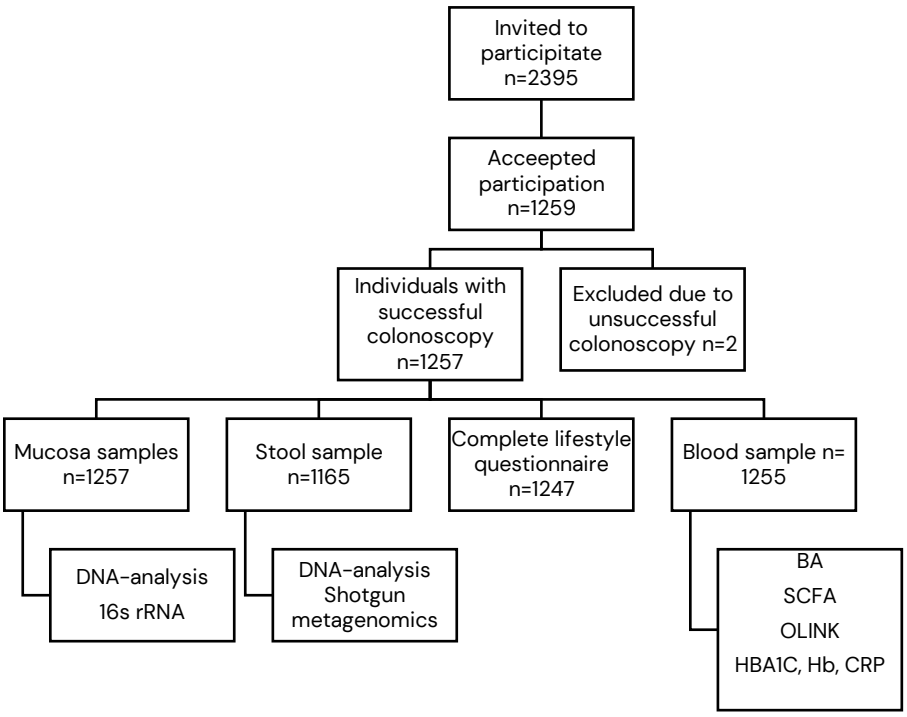

Figure 3: Overview of the KOLBIBAKT cohort.

Table 1: Colonoscopy findings in the KOLBIBAKT cohort.

	N	%	Age (SD)	Sex (M/F)
Precancerous lesions	595	47.3	65.6 (10.7)	314/281
Diverticulosis	403	32.1	66.2 (9.6)	213/190
IBD	263	20.9	58.5 (16.2)	153/210
Former cancer	97	7.7	68.3 (10.4)	56/41
Present cancer	14	1.1	67.1 (8.1)	9/5
Clean colon	213	16.9	56.8 (14.9)	93/120

SD = standard deviation. M/F = Male/female.

4.2 Studies

An overview of the study design of all papers included in the thesis are presented in Table 2.

Table 2: Overview of included papers in the thesis.

Paper	Study design	Exposure	Outcome	Statistical analysis
I	Retrospective cohort	Appendicitis before the age of 16 and matched controls 1:1	IBD (CD and UC)	T-test. Wilcoxon rank-sum test. Cox proportional hazard ratio models
II	Retrospective cohort	Bariatric surgery (RYGB and SG) and matched controls 1:10	IBD (CD, UC and IBD-U)	T-test. Pearson chi2 test. Cox proportional hazard ratio models
III	Cross-sectional	IBD (CD and UC) and controls	SCFA in plasma	ANOVA. Pearson chi2-test. Linear regression
IV	Cross-sectional	CD and aged matched controls 1:1	BA in plasma	T-test. Pearson chi2-test. Linear regression

4.2.1 Paper I

Design:
Population-based retrospective cohort study.

Participants:
The study population included all individuals (n = 52,435) with a discharge diagnosis of appendicitis before the age of 16 years in Sweden, during the period of 1 January 1973 – 31 December 1996. Each exposed individual was matched to individuals without history of appendicitis according to age, sex and region of residence. Unexposed individuals who were diagnosed with appendicitis after inclusion but before the age of 16 were excluded as well as well as individuals with UC or CD before or within one year after inclusion. The final cohort consisted of 52,391 individuals with juvenile appendicitis (appendicitis with appendectomy N = 50,421, appendicitis without appendectomy N = 1970) and 51,415 unexposed individuals.

Exposure:
The exposure variable was appendicitis before age 16 ('juvenile appendicitis') and the diagnosis was retrieved from the NPR using relevant ICD-codes. Individuals with juvenile appendicitis were further stratified into either appendectomy or conservative treatment without appendectomy using ICD-codes for appendectomy from the NPR, Table 3.

Outcome:
The primary outcomes were obtained from the NPR using relevant ICD-codes, Table 3. Diagnosis of outcome diagnosis (either CD or UC) was based on the most recent diagnosis in NPR, and the onset of disease was defined as the date of the first UC or CD diagnosis regardless of the final outcome diagnosis. The cohort was followed until 31 December 2017

Table 3: Exposure and outcome based on following ICD codes in the National Patient Register.

Exposure	**ICD-8**	**ICD-9**	**ICD-10**
Appendicitis	540, 541, 542	540, 541, 542	K35X K36X, K37X
Appendectomy	4510, 4511	4510, 4511	JEA00, JEA01, JEA10
Outcome			
Ulcerative colitis	563.10	556X	K51
Crohn's disease	563.00	555A-X	K50

4.2.2 Paper II

Design:
Population-based retrospective cohort study.

Participants and exposure:
The study population consisted of all individuals who underwent primary bariatric surgery (RYGB or SG) and were registered in SOReg, during the period of 1 January 2007 – 31 December 2018. Controls were matched in a 1:10 ratio based on sex, age and area of residence. Individuals who had been diagnosed with IBD either before or within one year after the inclusion period were excluded, as were controls of any excluded cases. The final cohort consisted of 64,188 individuals with bariatric surgery (RYGB n=54,465, SG n= 9723) and 634,530 controls.

Outcome:
The primary outcomes were obtained from the NPR using ICD-codes, Table 4. Outcome diagnosis (CD, UC or IBD-U) was determined by the most recent diagnosis in NPR and onset of disease was defined as the date of the first UC, CD or IBD-U diagnosis, regardless of final outcome diagnosis. The cohort was followed until 31 December 2019.

Table 4: Outcome based on following ICD codes in the National Patient Register

Outcome	**ICD-10**
Crohn's disease	K.50
Ulcerative colitis	K.51
IBD-unclassified	K52.3

4.2.3 Paper III

Design:
Cross-sectional study.

Participants:
The study included all individuals from the KOLBIBAKT cohort (4.1.4) who had undergone colonoscopy and had been diagnosed with either CD, UC, or showed no pathological findings on colonoscopy (CC). After primary inclusion, Individuals with unsuccessful SCFA analysis were excluded (n=20). The final cohort consisted of 132, 119 and 205 individuals with CD, UC and CC, respectively.

Exposure:
Severity of disease in CD and UC individuals was assessed by the examining

endoscopist and graded in accordance with Simplified endoscopic activity score point system for CD (SES-CD) [124] and Mayo endoscopic sub score for UC [125]. Individuals with no pathological findings (CC) were included as controls.

Outcome:
SCFA (formic-, acetic-, propionic-, butyric-, isobutyric-, isovaleric-, and valeric acid) as well as caprionic- and succinic- acids, were analyzed plasma by liquid chromatography-mass spectrometry (LC-MS). Results were reported in the unit μM.

Covariates:
Data on nutritional and dietary variables were collected from the questionnaire. Average daily intakes of specific nutrients were estimated according to the Alternate Healthy Eating Index (AHEI) score where a higher score indicates a healthier diet [126].

4.2.4 Paper IV

Design:
Cross-sectional study.

Participants:
This study included individuals with CD as well as individuals without pathological findings on colonoscopy or history of colorectal cancer or precancerous lesions (CC) from the KOLBIBAKT cohort (4.1.4). From the total of 141 individuals with CD and 213 individuals with CC, 88 age-matched pairs, who had not received antibiotic treatment within three months prior to inclusion were selected for analysis.

Exposure:
The exposure was CD. Severity of disease was assessed by the examining endoscopist and graded in accordance with Simplified endoscopic activity score point system for CD (SES-CD) [124].

Outcome:
Plasma bile acid levels were measured using Ultra Performance Liquid Chromatography (UPLC). The outcome data was analysed according to semi-quantitative measures.

Covariates:
Diet:(see chapter 4.2.3)

4.3 Statistics and data analysis:

Paper I and II:
Students t-test, Wilcoxon rank-sum test, and Pearson chi2-test were used for crude group comparison of continuous and categorical variables.

The hazard ratio of developing each outcome in relation to exposure status were estimated by fitting Cox proportional-hazards regression models for each exposure (juvenile appendicitis, appendectomy and conservative treatment in paper I and bariatric surgery, RYGB and SG in paper II) with each outcome (IBD, UC and CD in paper I and IBD, UC, CD, and IBD-U in paper II). Hazard ratios were calculated with (adjusted) and without (unadjusted) covariates (sex, age, educational level, disposable income in paper I and age, sex and preoperative BMI in paper II). Kaplan-Meier incidence plots comparing exposed and unexposed individuals for each outcome during follow-up were constructed to illustrate outcome incidence during follow-up. A p-value <0.05 was considered statistically significant and all analyses were performed in Stata 15.1 and 16 (StataCorp.)

Paper III and IV
ANOVA-test and Students t-test were used for crude group comparisons of continuous variables and chi2-test was used for comparisons of categorical variables. For associations a univariate linear regression analysis was performed for each exposure (CD, UC, CC in paper III and CD, CC in paper IV) and outcome (SCFA in paper III and BA in paper IV). In paper III, we also used a multivariate linear regression model including sex age and AHEI. Results were presented as mean ± standard error for each exposure and outcome and in paper IV, also as ratios between exposed and unexposed (CD/CC). A p-value <0.05 was considered statistically significant in paper III. In paper IV, after Bonferroni correction [127] a p-value<0.002 was considered significant (further discussed in chapter 6.4.3).

In paper IV, seven machine learning algorithms were trained and their ability to separate CD from CC were tested.

All analyses were performed using STATA 16.1 (Stata Corp.), except the machine learning algorithms in paper IV where R version 4.1.2 was used.

4.4 Ethical considerations

Paper I and II are retrospective cohort studies based on Swedish registers. All data were de-identified by the registries before being provided to us. Thus, the risk of integrity infringement was minimal, and the participants were not exposed to any medical risks. As register-based studies, there is generally no requirement for informed consent.

In paper III and IV there were some ethical concerns since mucosal biopsies (2-6 extra biopsies) increases the risk of bowel perforation during the colonoscopy investigation. However, the risk of perforation due to biopsy, 1/6000, is considered small and can be treated with antibiotic in the vast majority cases. Overall, in the entire KOLBIBAKT project, no patient experienced any complications before, during or after their colonoscopy. All participants gave informed consent prior to the colonoscopy. Collected samples and other data were registered and stored according to current legislation. The collected data will be destroyed 25 years after inclusion. Personal identification numbers were replaced by anonymous key numbers. A code key was kept on a secure server in Danderyd hospital.

5 Main Results

5.1 Paper I

Population characteristics

The study population characteristics of the cohort are shown in Table 5. The cohort consisted of 52,391 exposed and 51,415 unexposed individuals. Of the individuals exposed to juvenile appendicitis, 50,421 were treated with appendectomy while 1,970 were treated without appendectomy (conservative treatment). All covariates were evenly balanced between groups, except for sex. The male sex was predominant in all groups, except for the conservative treatment group where the sexes were equally balanced.

Follow-up

Table 6 presents the events that occurred during the follow-up period, the corresponding person-years at risk, and the adjusted Cox proportional-hazards ratio estimates. In total 619 individuals developed UC and 529 individuals developed CD during a follow-up time of 3.3 million person-years.

Out of the 52,391 individuals with juvenile appendicitis, 145 (0.28%) developed UC (140 (0.28%) treated with appendectomy and 5 (0.25%) treated conservatively), during 1.7 million person-years at risk. Additionally, 226 (0.43%) developed CD (215 (0.43%) treated with appendectomy and 11 (0.56%) treated conservatively) during the same follow-up period. These figures compared to corresponding figures of 474 (0.92%) and 303 (0.59%) respectively, among the 51,415 unexposed who were followed for 1.6 million person-years at risk.

Individuals with juvenile appendicitis had decreased risk of later development of both UC (aHR 0.30 95% CI 0.25 - 0.36) and CD (aHR 0.82 95% CI 0.69 - 0.99).

The analyses which were stratified by the method of appendicitis treatment (i.e., appendectomy and conservative treatment), showed similar reduction in risk of UC and CD, except for conservatively treated juvenile appendicitis with CD. Individuals treated with appendectomy had decreased risk of both UC (aHR 0.30 95% CI 0.25 - 0.36) and CD (aHR 0.82 95% CI 0.68 - 0.97) while individuals treated conservatively had decreased risk of UC (aHR 0.29 95% CI 0.12 - 0.69) but not CD (aHR 1.12 95% CI 0.61 – 2.06).

Table 5: Population characteristics.

	No juvenile appendicitis (unexposed)	Juvenile appendicitis (exposed)	Appendectomy	Conservative treatment
	n=51,415	**n=52,391**	**n=50,421**	**n=1,970**
Age[1], years (SD)	11.26 (2.86)	10.94 (2.84)	10.95 (2.81)	10.50(3.39)
Sex				
Male, n (%)	27,737 (53.95%)	28,255 (53.93%)	27,263 (54.07%)	992 (50.36%)
Female, n (%)	23,678 (46.05%)	24,136 (46.07%)	23,158 (45.93%)	978 (49.64%)
Highest Educational Level [2]				
Low, n (%)	4,161 (8.10%)	4237 (8.08%)	4,084 (8.10%)	153 (7.76 %)
Intermediate, n (%)	24,071 (46.82%)	23,944 (45.70%)	23,016 (45.65%)	928 (47.10%)
High, n (%)	23,183 (45.09%)	24,210 (46.21%)	23,321 (46.26%)	889 (45.13%)
Household disposable income [3]				
Mean, SEK(median)	6,105 (5391)	6,029 (5,391)	6,037 (5,391)	5,834 (5,391)
Individual disposable income [3]				
Mean, SEK(median)	3,473 (3,022)	3,471 (3,022)	3,475 (3,022)	3,368 (3,022)

[1] Age at appendicitis (exposed) or age at appendicitis for corresponding exposed patient (unexposed) .
[2] Highest educational level achieved during follow up. Low ≤9 years, intermediate 10–12 years and high ≥13 years of schooling.
[3] Monthly disposable income in Swedish Krona (SEK).

Table 6: Association between juvenile appendicitis with ulcerative colitis and Crohn's disease stratified by treatment method.

Disease outcome	Number of patients	Events/Person years	aHR[1] (95% CI)
Ulcerative colitis	103,806	619 / 3,321,105	
No juvenile appendicitis	51,415	474/ 1,643,120	1.00 (ref.)
Juvenile appendicitis	52,391	145 / 1,677,985	0.30 (0.25 - 0.36)
Appendectomy	50,421	140 / 1,618,040	0.30 (0.25 - 0.36)
Conservative treatment	1,970	5 / 59,945	0.29 (0.12 - 0.69)
Crohn's disease	103,806	529 / 3,321,186	
No juvenile appendicitis	51,415	303 / 1,644,677	1.00 (ref.)
Juvenile appendicitis	52,391	226 / 1,676,509	0.82 (0.69 - 0.99)
Appendectomy	50,421	215 / 1,616,662	0.82 (0.68 - 0.97)
Conservative treatment	1,970	11 / 59,847	1.12 (0.61 – 2.06)

[1] *Adjusted Hazard ratio models accounted for sex, age at the time of exposure, educational level, disposable income as well as for appendicitis with appendectomy, appendicitis without appendectomy and appendectomy without appendicitis among unexposed individuals.*

Kaplan–Meier incidence plots comparing 'no juvenile appendicitis' versus 'juvenile appendicitis' for each outcome, UC and CD during follow-up is shown in Figure 4.

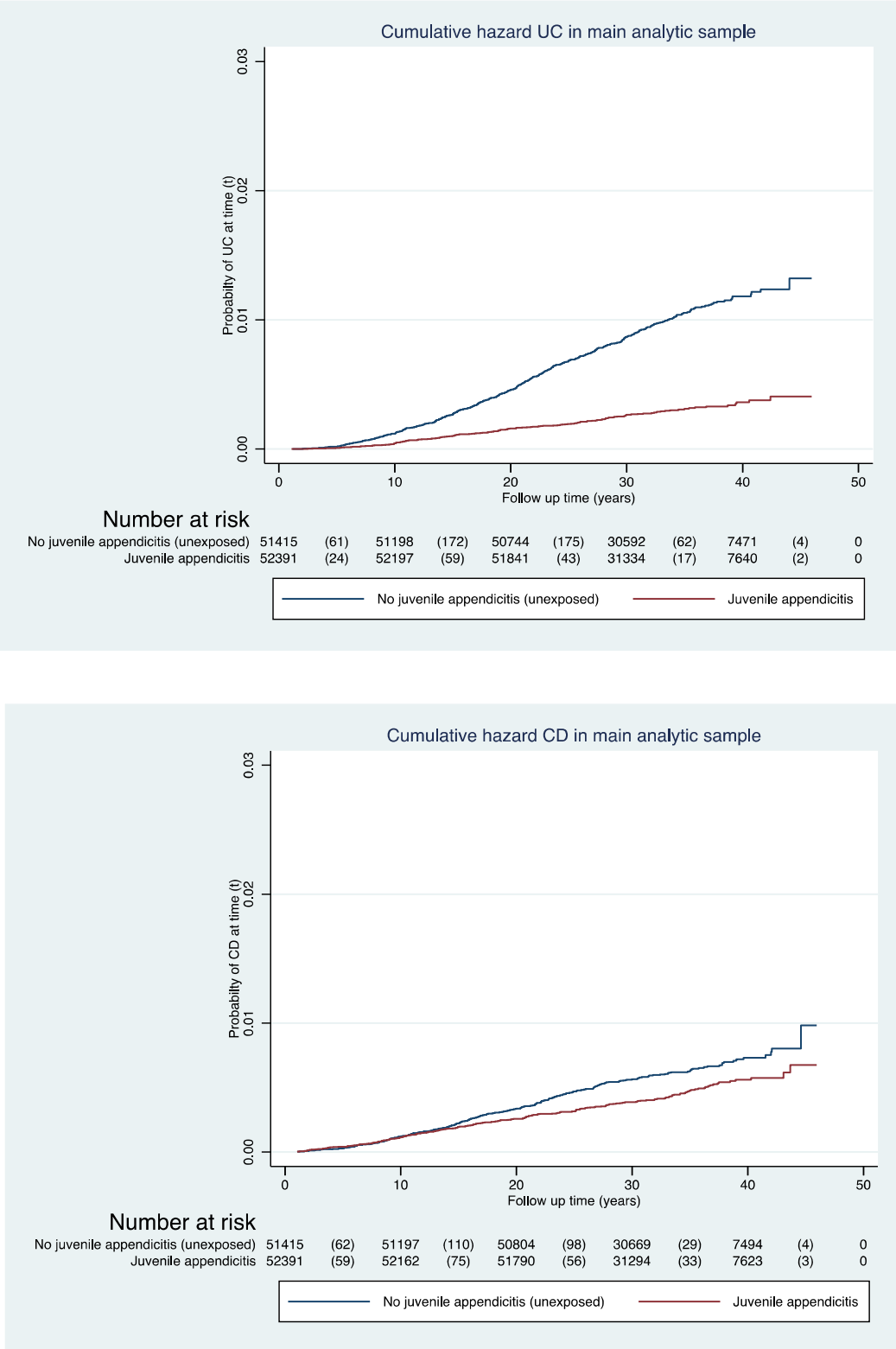

Figure 4: Kaplan–Meier incidence plots comparing 'no juvenile appendicitis' versus 'juvenile appendicitis' for each outcome, UC and CD during follow-up.

5.2 Paper II

Population characteristics

Population characteristics are shown in Table 7. The final analytic sample consisted of 64,188 individuals with bariatric surgery (RYGB n=54,465, SG n=9,723) and 634,530 controls. There was a clear predominance of females (76.4%) vs males (23.6%) in the entire cohort including individuals operated with RYGB (75.6% vs. 24.4%) and SG (80.6% vs. 19.4%) respectively. Of those who underwent bariatric surgery, 41.5% had a preoperative BMI of less than 40 kg/m², 50.7% had a BMI between 40-50 kg/m² and 7.8% had a BMI greater than 50 kg/m². The proportion of individuals with a lower BMI was significantly higher in those who underwent SG compared to those who underwent RYGB ($p<0.001$).

Except for weight, for which no data was available for the control group, all other covariates were evenly distributed between the surgery group and the control group.

Table 7: Population characteristics in analytic sample and sub-samples

	No Bariatric surgery n=634,530	Bariatric Surgery n=64,188	p-value	RYGB n=54,465	SG n=9,723	p-value
Sex						
Male, n (%)	149,904 (23.6%)	15,163 (23.6%)		13,274 (24.4%)	1,881 (19.4%)	
Female, n (%)	484,626 (76.4%)	49,025(76.4%)	0.993 π	41,191 (75,6%)	7,834 (80.6%)	<0.001 π
Age[1], years (SD)	41.4 (11.2)	41.4 (11.2)	0.410 β	41.4 (11.2)	41,6 (10.9)	0.043 β
Preoperative BMI[2], mean (SD)		41.8 (5.5)		42.3 (5.4)	39.5 (5.6)	<0.001 β
Preoperative BMI[2] category, n (%)						
BMI <40		26,627 (41.5%)		20,834 (38.3%)	5,793 (59.6%)	
BMI 40-50		32,525 (50.7%)		29,005 (53.3%)	3,520 (36.2%)	
BMI >50		5,035 (7.8%)		4,626 (8.5%)	409 (4.2%)	

[1] Age at surgery (case patients) or age at surgery for corresponding case patient (control patients).
[2] Body mass index kg/m2
β=Two-sample t-test. π= CHI2-test. SD=standard deviation

Follow-up

Events during follow-up and person years at risk are shown in Table 8.

Over a follow-up period of 3.4 million person-years in the control group, 701 individuals (0.1%) developed CD, 1,116 (0.2%) developed UC and 189 (0.03%) developed IBD-U. In the surgery group, 123 (0.2%), 111 (0.2%) and 51 (0.1%) developed CD, UC and IBD-U respectively during a follow-up time 347 thousand person-years.

Table 8: Events during follow up.

	No Bariatric surgery	**Bariatric Surgery**	**RYGB[1]**	**SG[2]**
	N=634,530	**N=64,188**	**N=54,465**	**N=9,723**
Follow up time, person years	3,444,186	346,860	323,886	22,974
Crohn's disease, n (%)	701 (0.1%)	123 (0.2%)	119 (0.2%)	4 (0.04%)
Ulcerative colitis, n (%)	1,116 (0.2%)	111 (0.2%)	97 (0.2%)	14 (0.1%)
IBD-U[3], n (%)	189 (0.03%)	51 (0.1%)	48 (0.1%)	3 (0.03%)
IBD[4], n (%)	2006 (0.3%)	285 (0.4%)	264 (0.5%)	21 (0.2%)

[1] Roux-en-Y gastric bypass, [2]Sleeve gastrectomy, [3]Inflammatory bowel disease- unclassified, [4]Inflammatory bowel disease

Incidence rates and Cox proportional-hazard ratio estimates for each exposure and outcome are shown in Table 9.

Individuals treated with bariatric surgery had increased risk of CD (HR 1.7 95% CI 1.4-2.1) and IBD-U (HR 2.7 95% CI 2.0–3.7) but not UC (HR 1.0 95% CI 0.8-1.2) compared to controls.

Among individuals who underwent RYGB, the risk of CD (HR 1.8 95% CI 1.5-2.2) and IBD-U (HR 2.7 95% CI 2.0–3.7) was increased compared to controls, but there was no significant difference in risk of UC (HR 0.9 95% CI 0.8–1.1). In individuals who underwent SG, there was an increased risk of UC (HR 1.8 95% CI 1.1-3.1) compared to controls, but no differences in risk of CD (HR 0.8 95% CI 0.3–2.1) or IBD-U (HR 2.5 95% CI 0.8–7.8) could be detected.

Table 9: Association between bariatric surgery and inflammatory bowel disease, unstratified and stratified by treatment method.

	No Bariatric Surgery	**Bariatric Surgery**	**RYGB[1]**	**SG[2]**
Number of patients	634,530	64,188	54,465	9,723
IR[1] IBD[2] (event/1000 person years)	0.6	0.8	0.8	0.9
Hazard ratios (95% CI)				
Crohn's disease	1 (ref)	1.7 (1.4- 2.1)	1.8 (1.5- 2.2)	0.8 (0.3 – 2.1)
Ulcerative colitis	1 (ref)	1.0 (0.8-1.2)	0.9 (0.8-1.1)	1.8 (1.1-3.1)
IBD-U[4]	1 (ref)	2.7 (2.0-3.7)	2.7 (2.0-3.7)	2.5 (0.8-7.8)
IBD[5]	1 (ref)	1.4 (1.2-1.6)	1.4 (1.2-1.6)	1.5 (1.0-2.3)

[1]Roux-en-Y gastric bypass, [2]Sleeve gastrectomy, [3] Incidence rate, [4]Inflammatory bowel disease, [5]Inflammatory bowel disease – unclassified.

Weight and IBD

Higher preoperative BMI was associated with lower risk of IBD. Individuals with preoperative BMI >50 (regardless type of surgical procedure) had a lower risk of developing IBD (HR 0.5 95% CI 0.3-0.9) compared to individuals with preoperative BMI <40. Although the decreased risk of IBD in preoperative BMI 40-50 (HR 0.8 95% CI 0.6-1.0) compared to BMI<40 was not significant, the results indicate a linear relation between higher BMI and lower risk of IBD, Figure 5.

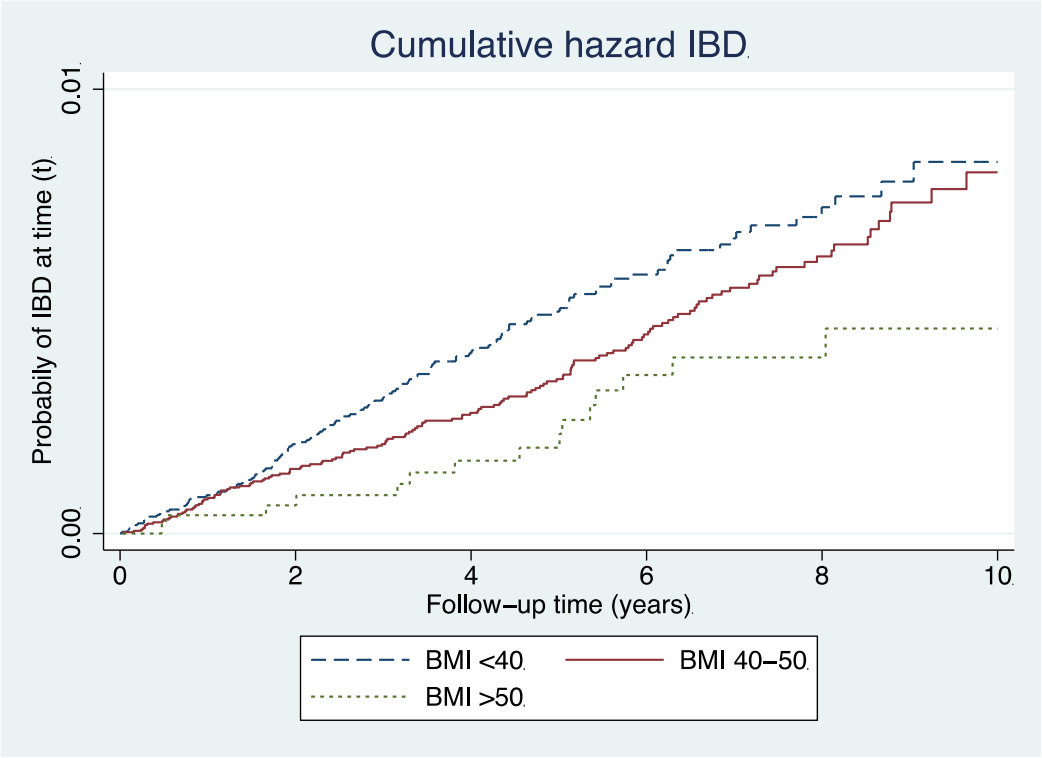

Figure 5: Kaplan–Meier incidence plots comparing body mass index <40 kg/m², body mass index 40–50 kg/m², and body mass index >50 kg/m² for inflammatory bowel disease during follow-up.

5.3 Paper III

Population characteristics:

The study population characteristics are shown in Table 10. The final cohort consisted of 132 individuals with CD, 119 with UC and 205 with CC. The sex distribution differed between CD and UC in comparison to CC with more males with CD and UC, (p=0.056, p<0.001).

Individuals with CD and UC were younger, 44.8 years and 51.5 years compared to individuals with CC, 56.7 years (p<0.001, p=0.002).

The severity of CD, assessed by SES-CD, was as follows: 77 individuals (58.3%) were in remission, while 36 (27.3%), 18 (13.6%) and 1 (0.8%) had mild, moderate, and severe disease, respectively. In UC, assessed by MES, 88 individuals (73.9%) were in remission, while 8 (6.7%), 12 (10.1%) and 11 (9.2%) had mild, moderate, and severe disease respectively, at the time for colonoscopy.

In analysis of dietary components, AHEI score, was significantly lower in UC compared to CC (36.01 vs.39.64, p=0.010), but not in CD compared to CC (37.64 vs 39.64, p=0.14).

Table 10: Study population characteristics of men and women with Crohn's disease (CD) and Ulcerative Colitis (UC) as compared to clean colon (CC)

		CD n=132	p-value	UC n=119	p-value	CC n=205
Sex	Male	72 (54.5%)	0.056 π	75 (63.0%)	<0.001 π	90 (43.9%)
	Female	60 (45.5%)		44 (37.0%)		115 (56.1%)
Age	years	44.8 (16.8)	<0.001 β	51.5 (14.6)	0.002 β	56.7 (15.0)
Antibiotics	yes/no	21 (15.9%)	0.47 π	13 (10.9%)	0.056 π	39 (19.0%)
SES-CD	remission	77 (58.3%)				
	mild	36 (27.3%)				
	moderate	18 (13.6%)				
	severe	1 (0.8%)				
MES	remission			88 (73.9%)		
	mild			8 (6.7%)		
	moderate			12 (10.1%)		
	severe			11 (9.2%)		
AHEI		37.64 (11.93)	0.14 β	36.01 (12.32)	0.010 β	39.64 (11.86)

Data are presented as mean (SD) for continuous measures. and n (%) for categorical measures. β=ANOVA test. π= Pearson's chi2 test. Antibiotics = Any use of antibiotics within 3 months before colonoscopy. SES-CD = Simplified endoscopic activity score point system for Crohn's disease. MES = Mayo endoscopic sub score. AHEI = Alternate healthy eating index.

Levels of SCFA:

Mean levels of SCFA for CD, UC and CC are shown in Table 11. Succinic acid was lower in CD 3.00μM (SE 0.10) and UC 3.13μM (SE 0.10) in comparison to CC 3.41μM (SE 0.08), p<0.05, however not significant when adjusting for sex, age and AHEI. No other significant differences were observed between groups.

Subgroup analysis for active disease versus remission is shown in Table 12. All subgroups had lower mean Succinic acid levels than individuals with CC, but there was no statistically significant difference between individuals with UC remission and individuals with CC. In addition, lower concentration of valeric acid was shown in CD active 0.07μM (SE 0.01) in comparison to CC 0.10SDμM (SE 0,01), p<0.05. However, after adjusting for sex, age, and AHEI, there were no differences in mean SCFA levels.

Table 11: Plasma concentration of SCFA according to CD and UC diagnosis.

	Formicacid	Aceticacid	Propionicacid	Butyricacid	Isobutyricacid	Succinicacid	Valericacid	Isovalericacid	Caprionicacid
CD	214.33 (13.35)	111.10 (10.38)	0.47 (0.03)	0.22 (0.02)	0.22 (0.01)	3.00 (0.10) *	0.09 (0.01)	0.20 (0.01)	0.29 (0.02)
UC	199.53 (14.06)	103.24 (10.89)	0.46 (0.03)	0.27 (0.02)	0.21 (0.01)	3.13 (0.10) *	0.09 (0.01)	0.20 (0.02)	0.28 (0.02)
CC (ref)	207.93 (10.71)	105.72 (8.30)	0.45 (0.03)	0.24 (0.02)	0.21 (0.01)	3.41 (0.08)	0.10 (0.01)	0.20 (0.01)	0.33 (0.02)

*Data are presented as mean and standard error in parathesis in μM. CC=Clean Colon. CD= Crohn's disease. UC=Ulcerative colitis. * p<0.05.*

Table 12: Plasma concentration of SCFA according to CD and UC active or in remission diagnosis.

	Formicacid	Aceticacid	Propionicacid	Butyricacid	Isobutyricacid	Succinicacid	Valericacid	Isovalericacid	Caprionicacid
CD remission	228.21 (17.48)	127.78 (13.54)	0.50 (0.04)	0.20 (0.02)	0.22 (0.01)	3.02 (0.13)*	0.10 (0.01)	0.22 (0.02)	0.28 (0.03)
CD active	194.89 (20.68)	88.06 (15.92)	0.43 (0.05)	0.25 (0.03)	0.21 (0.02)	2.97 (0.15)*	0.07 (0.01)*	0.18 (0.02)	0.30 (0.03)
UC remission	192.48 (16.35)	90.03 (12.58)	0.45 (0.04)	0.26 (0.02)	0.20 (0.01)	3.22 (0.12)	0.09 (0.01)	0.18 (0.02)	0.28 (0.03)
UC active	219.56 (27.54)	140.71 (21.20)	0.50 (0.07)	0.27 (0.04)	0.23 (0.02)	2.86 (0.20)*	0.07 (0.02)	0.23 (0.03)	0.28 (0.05)
CC(ref)	207.93 (10.71)	105.72 (8.24)	0.45 (0.03)	0.24 (0.02)	0.21 (0.01)	3.41 (0.08)	0.10 (0.01)	0.20 (0.01)	0.33 (0.02)

*Data are presented as mean and standard error in parathesis in μM. CC=Clean Colon. CD= Crohn's disease. UC=Ulcerative colitis. * p<0.05.*

5.4 Paper IV

Population characteristics:
Population characteristics are shown in Table 13. The cohort consisted of 88 age matched pairs. Age, sex and BMI were evenly distributed across the cohort and there was no significant difference in AHEI.

Of the individuals with CD, 53 (60%) were in remission, while 21 (24%), 14 (16%) and 0 (0%) had mild, moderate, and severe disease respectively. Of these individuals, 66 (75%) did not have history of bowel resection, while 19 (22%) had previously undergone ileocolic resection. Among individuals with CC, 81 (92%) did not have history of bowel resection.

Table 13: Population characteristics

		CD n=88	CC n=88	p-value
Sex	Male	47 (53%)	39 (44%)	0.23 π
	Female	41 (47%)	49 (56%)	
Age		44.92 (16.22)	48.69 (14.94)	0.11 β
BMI		25.74 (4.97)	25.97 (6.22)	0.79 β
SES-CD	remission	53 (60%)		
	mild	21 (24%)		
	moderate	14 (16%)		
	severe	0 (0%)		
Previous Surgery	None	66 (75%)	81 (92%)	<0.001 π
	Ileocolic resection	19 (22%)	2 (2%)	
	Colon or rectum resection	2 (2%)	3 (3%)	
	Colectomy	1 (1%)	0 (0%)	
	RYGB	0 (0%)	2 (2%)	
AHEI		37.94 (11.74)	39.55 (12.15)	0.38 β

Data are presented as mean (SD) for continuous measures and n (%) for categorical measures. β=two sample t-test test. π= Pearson's chi2 test. SES-CD = Simplified endoscopic activity score point system for Crohn's disease. AHEI = Alternate healthy eating index.

Bile acids levels:
Levels of primary and secondary bile acids in blood plasma for individuals with CD and CC and the CD/CC ratio is shown in Table 14.

Primary bile acids: Levels of CA was similar between groups, CD/CC ratio = 1.00 (SE 0.18). Point estimates of CD/CC ratio for all the other primary BA was 1.12-2.73, however not significant. Thus, no statistically significant differences in levels of primary BA were shown.

Secondary bile acids: The levels of all deoxycholic acids were lower in CD compared to CC, with a CD/CC ratio of 0.20 - 0.73. The differences were statistically significant for DCA, CD/CC ratio 0.47 (SE 0.11), $p<0.001$ and HDCA CD/CC ratio 0.20 (SE 0.07), $p<0.001$. Additionally, the levels of all lithocholic acids were significantly lower in CD, CD/CC ratio 0.40-0.52, $p<0.001$.

There were no statistically significant differences in the levels of hyocholic acids and ursodeoxycholic acids, except TUDCA, with a CD/CC ratio of 0.28 (SE 0.21), $p<0.001$ and there were no differences in levels of MCA, TaMCA and C4. The total amount of secondary BA was lower in CD, with a CD/CC ratio of 0.60 (SE 0.12) $p=0.001$.

The relation between total amount of secondary BA and primary BA (SecondaryBA/PrimaryBA) for CD and CC was 6.50 (SE 0.51) and 12.05 (SE 1.50) respectively.

Subgroup analysis of the statistically significant alterations in secondary BA levels in the main analysis is shown in Table 15. Significantly lower levels of HDCA, LCA, ILCA and TLCA-3S were shown in CD in remission vs. CC and significantly lower levels of DCA, HDCA, LCA, LCA.3S ILCA, GLCA, TLCA and TLCA.3S were shown in CD with active disease vs. CC.

Moreover, plasma concentration for secondary BA among individuals with CD with active disease was significantly lower compared to those with CD in remission, CD active / CD remission ratio 0.65 (SE 0.11), $p<0.002$ (not shown in table).

Table 14: Plasma concentration of BA.

	CD n=88	CC n=88	CD/CC	p-value*
Primary BA				
Cholic Acids				
Cholic Acid(CA)	25.34 (3.18)	25.42 (3.16)	1.00 (0.18)	0.985
Glycocholic Acid(GCA)	12.02 (4.92)	6.84 (1.99)	1.76 (0.82)	0.359
Taurocholic Acid (TCA)	7.63 (5.03)	4.04 (1.47)	1.88 (1.44)	0.537
Chenodeoxycolic Acids				
Chenodeoxycholic Acid (CDCA)	33.09 (8.85)	12.11 (1.66)	2.73 (0.86)	0.044
Glycochenodeoxycholic Acid (GCDCA)	18.50 (4.72)	13.20 (3.00)	1.40 (0.46)	0.385
Taurochenodeoxycholic Acid (TCDCA)	7.51 (2.32)	6.68 (1.42)	1.12 (0.40)	0.758
Total	104.09 (20.74)	68.30 (8.36)	1.52 (0.33)	0.112
Secondary BA				
Deoxycholic acids				
Deoxycholic Acid (DCA)	14.80 (2.22)	31.34 (5.42)	0.47 (0.11)	<0.001
Glycodeoxycholic Acid (GDCA)	26.38 (4.71)	49.50 (15.00)	0.53 (0.18)	0.011
Iso-deoxycholic Acid (IDCA)	45.83 (4.97)	62.74 (7.66)	0.73 (0.11)	0.015
Taurodeoxycholic Acid (TDCA)	24.87 (6.12)	44.77 (12.05)	0.56 (0.19)	0.020
Hyodeoxycholic Acid (HDCA)	7.39 (1.43)	36.78 (9.09)	0.20 (0.07)	<0.001
Litocholic Acids				
Lithocholic Acid (LCA)	15.53 (1.48)	38.85 (7.40)	0.40 (0.08)	<0.001
Lithocholic Acid 3-Sulfate (LCA.3S)	28.97 (4.89)	48.82 (6.50)	0.59 (0.12)	0.001

iso-Lithocholic Acid (ILCA)	19.49 (1.51)	37.38 (5.27)	0.52 (0.07)	<0.001
Glycolithocholic Acid (GLCA)	16.18 (2.99)	36.99 (9.82)	0.44 (0.15)	<0.001
Taurolithocholic Acid (TLCA)	29.14 (4.00)	55.81 (12.35)	0.52 (0.15)	0.001
Taurolithocholic Acid 3-Sulfate (TLCA.3S)	31.86 (3.90)	63.27 (7.95)	0.50 (0.08)	<0.001
Hyocholic acids				
Glycohyocholic Acid (GHCA)	22.83 (6.89)	13.87 (2.42)	1.65 (0.58)	0.262
Taurohyocholic Acid (THCA)	4.94 (2.21)	3.33 (1.04)	1.48 (0.87)	0.581
Ursodeoxycholic acids				
Ursodeoxycholic Acid (UDCA)	5.10 (2.99)	1.70 (0.48)	3.00 (1.97)	0.310
Glycoursodeoxycholic Acid (GUDCA)	1.62 (0.27)	2.73 (1.32)	0.59 (0.28)	0.142
Tauroursodeoxycholic Acid (TUDCA)	0.45 (0.12)	1.62 (1.17)	0.28 (0.21)	<0.001
Other bile acids				
Muricholic acid (MCA)	24.50 (5.79)	18.31 (2.80)	1.34 (0.39)	0.390
Tauro-α-Muricholic acid (TaMCA)	21.71 (5.84)	19.83 (5.75)	1.09 (0.43)	0.826
7α-hydroxy-4-cholesten-3-one (C4)	90.24 (3.29)	90.29 (2.94)	1.00 (0.05)	0.992
Total	341.61 (35.42)	567.65 (72.16)	0.60 (0.12)	0.001
Secondary BA/ primaryBA	6.50 (0.51)	12.05 (1.50)	0.54 (0.086)	<0.001

*Data are presented as mean of an arbitrary unit, standard error in parenthesis, and the ratio between CD and CC (CD/CC ratio). CC =clean colon. CD = Crohn's disease. * Wald test. Level of significance p<0.002.*

Table 15: Subgroup analysis. Plasma concentration of significant secondary BA.

	CDrem n=53	CDrem/CC ratio	p-value *	CDact n=35	CDact/CC ratio	p-value*	CC n=88
Deoxycholic Acid (DCA)	17.50 (3.61)	0.56 (0.15)	0.004	10.72 (2.72)	0.34 (0.11)	<0.001	31.34 (4.86)
Hyodeoxycholic Acid(HDCA)	8.46 (2.03)	0.23 (0.08)	<0.001	5.78 (2.01)	0.16 (0.06)	<0.001	36.78 (8.66)
Lithocholic Acid(LCA)	15.83 (2.11)	0.41 (0.08)	<0.001	15.09 (2.19)	0.39 (0.91)	<0.001	38.85 (7.15)
Lithocholic Acid 3-Sulfate(LCA.3S)	36.15 (8.54)	0.74 (0.19)	0.180	18.10 (3.45)	0.37 (0.09)	<0.001	48.83 (6.95)
iso-Lithocholic Acid(ILCA)	20.08 (2.06)	0.54 (0.09)	<0.001	18.60 (2.71)	0.50 (0.09)	<0.001	37.38 (4.90)
Glycolithocholic Acid(GLCA)	19.59 (4.16)	0.53 (0.19)	0.012	11.01 (1.96)	0.30 (0.10)	<0.001	36.99 (10.11)
Taurolithocholic Acid(TLCA)	32.81 (5.50)	0.59 (0.16)	0.011	23.60 (4.74)	0.43 (0.14)	<0.001	55.82 (12.74)
Taurolithocholic Acid 3-Sulfate(TLCA.3S)	39.60 (5.51)	0.63 (0.12)	0.002	20.14 (3.48)	0.32 (0.07)	<0.001	63.27 (7.43)
Total	190.03 (24.71)	0.54 (0.10)	<0.001	123.03 (17.72)	0.35 (0.07)	<0.001	349.25 (44.00)

*Data are presented as mean of an arbitrary unit, standard error in parenthesis, and the ratio between CD and CC (CD/CC ratio). CDrem=CDremission, CDact=CD active disease. CC=clean colon * = Wald test. Level of significance p<0.002.*

6 Discussion

6.1 Appendicitis and subsequent risk of IBD

In Paper I, we discovered an association between childhood appendicitis that was treated with appendectomy and a reduced likelihood of developing ulcerative colitis (UC) and Crohn's disease (CD) later on. A negative association was also found between childhood appendicitis treated without appendectomy (conservatively treated) and UC but not CD.

There have been reports indicating an association between the appendix and various immunological functions in humans, suggesting that it could play a crucial role in maintaining intestinal health. Specifically, the appendix contains significant amounts of lymphatic tissue that produces an IgA biofilm, which is transferred to the colon [128]. This mechanism could play and important role in in preserving the equilibrium between the microorganisms present in the colon and the immune system. Another theory suggests that the appendix may function as a reservoir that enables the rapid re-establishment of gut bacteria in the event they are eradicated from the colon, for instance due to antibiotics or infection [129].

Previous studies have shown that childhood appendectomy is linked to various diseases, such as colorectal cancer [58], kidney disease [130], myocardial infarction [60] and mood and anxiety disorders [131], thereby suggesting that the appendix may have a crucial role in maintaining our overall health. In this context, evaluation of appendicitis in relation to risk for development of inflammatory bowel disease is highly relevant.

Although the function of the appendix in maintaining overall health is contradicted by our data suggesting that surgical removal of the appendix reduces the risk of developing IBD, this finding can be explained by the following mechanisms: Firstly, histological studies of inflamed appendixes from patients with IBD have showed features consistent with the colonic mucosa [132, 133], which is different from the changes seen in acute appendicitis in non-UC patients. In UC patients, there is an excess of neutrophilic infiltration in the appendices, which suggests a skip lesion [134] or a priming site for UC [132]. Therefore, the appendix may serve as a source of origin for IBD. Secondly: While the appendix may serve as a 'safe house' for important commensal bacteria in the gut, it may also function as a reservoir for gut bacteria involved in the pathogenesis of IBD in some individuals [135]. Finally: the appendix contains substantial amount of IgA-producing B-cells and NK cells and constitutes a significant part of the gut-associated lymphatic tissue (GALT) system [55]. Consequently, an imbalance in this system could trigger the development of IBD.

Our results confirm the results from two previous large population-based cohort studies [22, 25] showing an inverse association between appendectomy before the age of 20 and later development of UC. With regards to CD, and in contrast to our results, two large registry-based cohort studies from Sweden and Denmark found an increased transient [24] versus long-term [23] risk of developing CD after appendectomy. The heterogeneity of these results could be due to methodical differences regarding exposure (appendicitis vs. appendectomy) and study population (pediatric population vs all ages). None of the previous studies, investigated the association between appendicitis per se, without appendectomy, and IBD. Our study revealed a correlation between conservatively treated appendicitis and a reduced risk for UC, but not CD. This observation may suggest that the mechanisms that cause appendicitis also have a protective effect against UC, or that inflammation alters or destroys the appendix's function as a reservoir for commensal or pathogenic bacteria. Nonetheless, patients who undergo conservatively treated appendicitis could serve as a useful comparison group, as significant appendiceal functions may be preserved, potentially influencing the later development of disease.

Strengths and limitations

Paper I has several strengths, including a large sample size and the long follow-up period. The study is based on high-quality registries that cover all Swedish residents and the NPR has been specifically validated for use in IBD research [136]. However, there are some potential weaknesses to consider. For example, the study had relatively small number of individuals (N = 1970) with conservatively treated appendicitis, which makes it difficult to draw conclusions about the relationship with IBD. Therefore, any, differences in outcome of conservatively treated appendicitis in the study may be speculative. In addition, the ICD-codes used in the NPR, for appendicitis and appendectomy have not been validated, as discussed further in chapter 6.4.2.

6.2 Risk of IBD after bariatric surgery

In Paper II we found an association between bariatric surgery (RYGB and SG) and an increased risk of CD and IBD-U. Upon subdividing bariatric surgery for analysis, RYGB was associated with increased risk of CD and IBD-U while SG was associated with increased risk of UC later in life. We also found an association between lower preoperative BMI and increased risk of IBD in the surgery group.

Our results confirm a recent study from Denmark [45] which also showed an increased risk of developing new onset CD after bariatric surgery. However, the Danish study, unlike our study did not analyse the two treatment methods separately. In contrast to our results an American study showed lower prevalence of de-novo CD and UC among individuals operated on with bariatric surgery compared to individuals with persistent

obesity [46]. The inconsistent results could be due to methodical differences such as lack of data on follow-up time and a control group with consistent obesity.

One possible explanatory mechanism for the association between bariatric surgery and IBD may be changes in the gut microbiome resulted by the altered anatomical environment in the gut. After RYGB surgery, a decrease of Firmicutes and an increase of Bacteroidetes and Proteobacteria [71] in addition to increased microbial diversity and richness [71, 137, 138] have been observed. An increase in Bacteroidetes and Proteobacteria and a decrease of Firmicutes are also common findings after SG [71]. Similar findings, i.e., decrease of Firmicutes and Bacteroidetes and increase in Proteobacteria are also frequently reported in IBD patients [71, 72]. The common gut microbial findings in IBD patients and in patients operated with bariatric surgery may suggest a link between gut microbiota, bariatric surgery and development of IBD.

Strengths and limitations

The strength of **Paper II** is its large sample size and the utilization of high-quality national registers. SOReg includes almost all patients going though bariatric surgery in Sweden [123] and NPR provides extensive coverage of both inpatient and outpatient specialist care visits. Furthermore, NPR has been validated for use of cases of IBD [136]. In contrast to previous studies, we had access to information regarding the specific surgical methods used, namely RYGB and SG.

One potential weakness of our study is the lack of information on weight among controls. The control group was drawn from the general population, so their weight distribution reflects that of the general population. Both over and underweight, has been identified as risk factors for CD, but not for UC [139, 140]. Some researchers have proposed that the relation between BMI and CD is U-shaped rather than linear [141, 142]. Therefore, weight could be a confounding factor in our findings. However, we observed that high BMI was associated with a lower risk of inflammatory bowel disease in the bariatric surgery group. Additionally, since SG has gained popularity more recently, we had relatively few individuals and shorter follow-up in the SG group.

6.3 Metabolites in blood, dependent on gut microbiota

Dysbiosis of the gut microbiota is frequently reported in UC and CD patients and it has been suggested to play an important role in the development and pathogenesis of IBD [10]. While some studies have reported a microbial composition of the gut microbiota in IBD patients with reduced diversity, reduced Firmicutes and Bacteroides, and an increase of Proteobacteria [72], there is a considerable heterogenicity in reported results. This heterogenicity may be due methodological differences, as well as various known and unknown factors that can affect the gut microbiota, such as diet, medication and ethnicity. Furthermore, it should be noted that bacteria from the same species might not have the same functional capacity, as some genes may be more active in certain

bacterial strains. By studying small molecules, produced or transformed by commensal bacteria within cells, biofluids or tissues, we can gain a better understanding of microbiota host cross-talk [143] and the functional capacity of the microbiota. This approach can also uncover new biomarkers and insights into the pathogenesis of diseases [144].

In Paper III and Paper IV, we investigated plasma concentrations of two groups of metabolites (SCFA and BA) which are highly influenced by the gut microbiota. SCFA are produced by the gut microbiota through fermentation of indigestible dietary fibres, while BA are transformed by the gut microbiota from primary BA to secondary BA. The aim was to evaluate if a "fingerprint" of the gut microbiota could be identified in plasma.

In **Paper III**, we compared the levels of SCFA in plasma among individuals with IBD (both CD and UC) and those with a clean colon. Our univariate analysis showed lower plasma concentrations of succinic acid among individuals with IBD, but the results were not significant in multivariate analysis after adjusting for age, sex and diet. We obtained similar findings when we analysed the IBD group separately in terms of active disease and remission. Therefore, we concluded that SFCA levels in plasma may not be a reliable biomarker for IBD.

The association between IBD and SCFA in plasma has previously only been evaluated in one small study including 15 individuals [145]. SCFA levels in faeces in IBD patients are on the other hand more widely studied. A meta-analysis reported that intraluminal concentrations of lactate were lower, while concentrations of propionate, butyrate and valerate were higher among IBD patients in comparison to controls. Furthermore, there was an inverse relation between disease activity and intraluminal levels of butyrate [111]. However, our study was unable to confirm these results as the modified plasma concentrations of SCFA may not necessarily reflect faeces concentrations. This is because a significant amount of SCFA is metabolized by the enterocytes and the liver, and hence, does not enter the systemic blood circulation.

In **Paper IV** we discovered that the distribution of secondary and primary BA in plasma differed between CD and controls. Specifically, in comparison to controls, individuals with CD had lower plasma concentrations of most secondary BA including DCA, HDCA, LCA, LCA.3S ILCA, GLCA, TLCA and TLCA.3S. Additionally, we observed that plasma concentrations of selected secondary BA were significantly lower in active CD disease compared to CD in remission.

Previous studies investigating BA levels in faeces in relation to IBD have demonstrated lower levels of the secondary BA such as LCA, TLCA in both CD and UC [146]. Additionally, lower levels of the secondary BA, specifically LCA and DCA, have been observed in individuals with CD [147]. A small cross-sectional study on both faeces and plasma that included 42 patients with IBD (12 CD and 30 UC) and 29 healthy controls showed a lower

proportion of secondary BA in plasma and faeces and a higher proportion of conjugated and sulfated BA in faeces among IBD patients in comparison to healthy controls [148]. A study evaluating plasma concentrations only, showed decreased levels of LCA but increased levels of HDCA, both secondary BA in CD and UC respectively [149].

Our study has confirmed the results from previous research which revealed lower levels of LCA and DCA in individuals with CD as well as lower levels of secondary BA in general. In addition, we showed that the reduction of certain BA was more significant in individuals with active CD compared to those in remission.

The findings of our study can be explained by several mechanisms. The primary site for BA reabsorption is the terminal ileum, and bile acid malabsorption (BAM) is a common feature of CD, especially in cases with ileitis or ileal resection [150]. However, BAM has also been observed in individuals with normal histology [151]. The decrease in secondary BA and the increase in primary BA, although not statistically significant, could be explained by BAM, reduced entero-hepatic circulation and compensatory increased de-novo BA synthesis. The gut microbiota is responsible for metabolizing primary BA into secondary BA and thus, dysbiosis associated with IBD could also explain the altered BA composition in individuals with IBD.

The first step in the formation of LCA and DCA is conducted by the bacterial enzymes, bile salt hydroxylase (BSH) and 7-α-hydroxylase. While BSH is found in all major bacterial phyla of the gut microbiota, 7-α-hydroxylase is expressed by single bacteria such as *Clostridium* and *Eubacterium* [114]. Decreased abundance of the Firmicutes phyla, including e.g. *Clostridium* and *Eubacterium rectale* have been reported in CD [114]. This indicates a possible link between dysbiosis, CD and altered bile acid composition and illustrates the importance of the gut bacteria in this context.

Both SCFA and BA have multiple functions that are closely linked to the immune system. For example, SCFA are involved in neutrophil migration to inflammatory sites, maintaining the integrity of the intestinal barrier, promoting the differentiation of immune cells and inhibiting proinflammatory cytokines [109]. Bile acid activated receptors (BAR), which are primarily expressed along the GI tract and on immune cells such as Faensoid x-receptors (FXR) and Pregnane x-receptor (PXR) play a critical role in immunological functions including regulating the inflammatory response and maintaining the intestinal barrier[114] .Therefore, a disruption in the SCFA and BA composition could be a contributing factor to the immune dysfunction observed in IBD patients [109, 114].

Strengths and limitations

One of the major strengths of **Paper III** and **Paper IV** is that the data were obtained from a large population-based cohort (KOLBIKAKT) with a high level of compliance to a comprehensive lifestyle questionnaire. This questionnaire contained detailed information on the participants' dietary and lifestyle habits, bowel habits, and other

clinically important data, such as previous surgery and endoscopic disease activity, all of which were objectively collected.

The use of this cohort allowed us to account for differences in antibiotic treatment or diet. In **Paper IV** we only included individuals who had not received antibiotic treatment in the three months prior to inclusion. Antibiotics can significantly impact the gut microbiota [152, 153], and previous or ongoing treatment could potentially skew the results. This approach also enabled us to rule out any possible differences in dietary intake between the groups.

However, the studies have some potential limitations. Blood samples were taken before colonoscopy, after participants had undergone bowel preparation and were fasting which could potentially impact our results or mask any possible differences in outcome between groups. Furthermore, the IBD patients in our cohort were not treatment-naïve, and the effects of medications used to treat IBD on the gut microbiome are still unknown.

6.4 Methodical considerations

The accuracy of a study is dependent on its precision and validity. Precision mainly depends on random errors and sample size. It is most often expressed by confidence intervals (CI) and p-values. A wider CI means poorer precision. The p-value refers to the probability to find results at least as extreme as the results observed under the assumption that the null hypothesis is true. Validity is divided into external and internal validity. While external validity refers to generalizability and whether the results of the study can be used in other populations the internal validity refers to whether the study measures what it is intended to do. Internal validity is dependent on different type of bias (systematic errors).

6.4.1 Precision

Paper I and II are studies based on Swedish National registers with large sample sizes. However, subgroups in both studies were relatively small (conservatively treated appendectomies in paper I and Sleeve gastrectomy in paper II) resulting in varying precision in both studies. In paper III and IV the results are based on a smaller cohort, but at the same time, they represent an intervention study that is much larger compared to previously published cohorts.

6.4.2 Validity

External validity

Paper I and II are population-based studies based on a Swedish national register

covering almost all Swedish residents, which means that results may be generalizable to other populations with a similar demography. The generalizability of the results presented in Paper III and IV poses some challenges. A significant number of individuals, both with and without confirmed intestinal disease, declined to participate in the study. Although we did map the reasons for non-participation, we cannot dismiss the possibility that these individuals may have shared a common biology that could have potentially influenced the study results. In addition, all participants were referred to Danderyd hospital, which is a tertiary centre for colonoscopy. Therefore, our sample might not be representative for the entire population.

Internal validity

Misclassification (information bias) refers to the assignment of study participants to the wrong category and can occur at any stage of a study. It is categorized as either non-differential or differential misclassification. Non-differential misclassification are random errors that typically lead to an underestimation of the true effect size, resulting in bias towards the null hypothesis. In contrast, differential misclassification is non-random and differs between groups which could lead to false associations.

Paper I and II included diagnostic data from the NPR. While data from NPR is generally considered to have high validity [119], it is important to note that not all disease groups have been separately validated. In Paper I, there were two groups under observation: patients treated for appendicitis with appendectomy, defined by ICD codes for both appendicitis and appendectomy, and patients treated conservatively for appendicitis, defined by ICD codes for appendicitis and the absence of any codes for appendectomy in the NPR. Since our study utilized data from 1973-1997, a time period when preoperative radiologic diagnostics and postoperative pathological examinations were not routinely conducted, it is possible that some individuals in the appendicitis group were actually experiencing abdominal pain due to causes other than appendicitis.

In Paper I and II the outcome diagnoses (UC, CD and IBD-U), are based on ICD-codes registered in the NPR. The accuracy of these diagnoses has previously been validated [136] and are considered to have high validity. However, changes between subtypes can occur, particularly in the early stages of a disease, which can potentially result in misclassification. Nevertheless, in our studies, the changes from the original classifications to different subtypes appear to be similar between all groups [13]. Furthermore, we utilized the most recent classification of IBD diagnosis in the NPR to distinguish between subtypes, as we believe the that the most recent diagnose is likely to be more correct. Nevertheless, we believe that the misclassification of IBD outcome diagnoses is in the current studies is most likely non-differential.

In Paper II we observed a higher risk of IBD following bariatric surgery. However, it is important to note that individuals who have undergone bariatric surgery often suffers

from abdominal pain, which may result in a higher likelihood of referral for colonoscopy. As a result, individuals with asymptomatic IBD may be more likely to be identified in the bariatric surgery group. Therefore, we cannot rule out that our results may be influenced by referral bias.

Selection bias arises when the process of sample selection fails to accurately represent the target population. In Paper III and IV there were one major potential source of selection bias. The control group comprising individuals without confirmed intestinal disease or pathological findings was referred for colonoscopy for some reason. Consequently, they cannot be considered a genuinely healthy control group, which poses challenges to the generalizability of the study findings.

Confounding is a variable associated with both the outcome and exposure but is not a direct link between them. It is important to note that this variable may be unevenly distributed between exposure groups, which can lead to alternative explanations for the observed association between exposure and outcome. However, there are various methods to handle confounding, such as restriction, randomization, matching, regression analysis and stratification. Despite controlling for confounding, residual confounding may still be present in observational studies, which can lead to distortion in the results.

In Paper I, the exposed and the unexposed group were matched according to sex age and region of residence. The covariates were evenly balanced between the groups except for the sex distribution when the exposed groups were subdivided into appendectomy and conservative treatment. This difference was adjusted for in the regression analysis.

In Paper II, all covariates except weight were evenly balanced between groups. There was no available information on weight among unexposed individuals, which is a potential source of bias (previously discussed 6.2). In a subgroup analysis, the sex distribution differed between RYGB and SG. This difference was adjusted for in the regression analysis.

6.4.3 Multiple hypothesis testing.

Statistical hypothesis testing involves two types of errors, type I and type II errors [154]. Type I error occurs when a null hypothesis that is true is rejected, while a type II error occurs when a false null hypothesis is not rejected. The p-value represents the probability of obtaining results at least as extreme as the ones observed, assuming that the null hypothesis is true. In medical research, the critical level of significance is typically set at $p<0.05$, which means that the probability of making a type I error is in a single hypothesis test is less than 5%.

When multiple hypothesis tests are conducted, the probability of obtaining at least one significant test increases. Specifically, the probability of obtaining at least one statistically significant result when performing multiple tests is calculated as $1-0.95^n$, where n represents the number of statistical tests performed [155].

In Paper IV we tested for difference in mean levels of 25 BA between CD and CC. To keep the overall critical level of significance at 0.05, the critical level of significance for each test was adjusted by Bonferroni correction [127] . In paper IV, 0.05 was divided by the number of tests, i.e., 25. Thus a p-value <0.002 for each specific test was considered statistically significant.

Although Bonferroni's correction is the most widely used methods to control for type 1 error, it has its downsides. When reducing the critical level of significance, the probability of making a type II error increase.

In Paper I-III, a p-value of 0.05 was considered significant since the numbers of test were few.

7 Conclusions

Paper I:

Appendicitis, treated with appendectomy before the age of 16 is associated with decreased risk of inflammatory bowel disease, both ulcerative colitis and Crohn's disease, later in life, whereas conservatively treated appendicitis is associated with decreased risk of ulcerative colitis only.

Paper II:

Roux-en-Y gastric bypass is associated with increased risk of development of Crohn's disease and inflammatory bowel disease-unclassified, but not ulcerative colitis. Sleeve gastrectomy is associated with increased risk of development of ulcerative colitis but not Crohn's disease and inflammatory bowel disease-unclassified.

Paper III:

Inflammatory bowel disease does not have an impact on the levels of short-chain fatty acids in the systemic circulation, as measured in plasma.

Paper IV:

Plasma concentrations of secondary bile acids are lower in individuals with inflammatory bowel disease compared to controls. The findings are more prominent in active disease indicating an association between disease severity and secondary bile acid concentrations.

8 Future perspectives

Over the last few decades, significant progress has been made in the field of gut microbiota and its relation to inflammatory bowel disease. This progress is evident from the exponential rise in publications that have explored the gut microbiota's role in IBD. However, despite these efforts, a well-described causal relationship is yet to be established, since most studies conducted are observational in nature. In order to advance from a correlation to a causality framework, experimental studies need to be conducted. Hypothesis-generating studies must be followed by hypothesis- testing studies.

This thesis involved a significant amount of effort towards the collection of tissue samples and the creation of the "KOLBIBAKT" database. However, even after the completion of two studies included in this thesis, a major portion of the collected material still needs to be analysed.

The next step in the current research involves analysing the microbiota in detail and its association with biomarkers and IBD. Until now, the possibilities for analysing the bacterial composition in the gut have been limited, resulting in small, published studies. However, new analytical methods based on powerful DNA sequencing techniques provide us with greater opportunities to map the bacterial composition in the gut in relation to various diseases.

In this future study, our samples will be analysed with respect to the bacterial composition in relation to potential biomarkers in plasma, as well as data in the lifestyle questionnaire in relation to IBD. To achieve this, we will carry out DNA sequencing, genetic investigations on the intestinal mucosa (metagenomics), and analysis of plasma samples in collaboration with the Centre for Translational Microbiome Research at Karolinska Institutet. The aim is to determine if specific bacterial compositions related to inflammation can be identified through different combinations of biomarkers in plasma. If the bacterial composition is found to be specific to IBD and can be detected through plasma biomarkers, then it may be possible to detect IBD at an earlier stage and better monitor the disease during treatment. Early detection and monitoring can optimize treatment and reduce the risk of requiring major surgery.

The project's interdisciplinary approach, combining important clinical questions with advanced microbiological analyses, will provide unique information. This project serves as a good example of how translational research could be implemented in clinical practice and contribute to the advancement of the surgical research field.

9 Acknowledgements

Det har varit många personer inblandade i den här avhandlingen och jag känner en stor tacksamhet till er alla. Ett särskilt tack vill jag ge till:

Ulf O Gustafsson, huvudhandledare och sektionschef på kolorektalsektionen på KS, för att du växelvis dragit och knuffat mig framåt i avhandlingsarbetet samt även för råd och hjälp med det som inte berör forskning och kirurgi. Du är en förebild både på och utanför planen.

Anna Löf Granström, för det stora ansvar du tagit som bihandledare och för att du också ringde 1200 patienter. Ditt genuina intresse för forskning och din effektivitet har varit en förutsättning för att jag ska komma i mål.

Richard Marsk, bihandledare, för alla kloka synpunkter och för att du får det komplicerade att bli lätt både inom forskning och det kliniska arbetet.

Kollegorna på CTMR, **Ina Schuppe-Koistinen, Stefanie Prast-Nielsen** och **Lars Engstrand** för samarbetet kring KOLBIBAKT och **Susanne Rautiainen** för analys av kostdata.

Klas Pekkari, sektionschef på DS, för du som handledare under ST och senare chef hjälpt mig att komma dit jag är nu.

Fredrik Hjern för att du som chef på DS och KS gett mig de utmaningar och förutsättningar jag behövt för att utvecklas.

Henrik Iversen, mentor och kollega på KS**,** för goda råd och den kirurgiska noggrannhet och de kunskaper som du förmedlar.

Kollegorna på kolorektalsektionen på KS, **Anders Hansson Elliot, Annika Sjövall, Christian Buchli, Gabriella Jansson Palmer, Mirna Abraham Nordling, Naseer Baloch, Patrik Lundström, Kristina Stenstedt, Pelle Nilsson, Petri Rantanen, Björn Bolmstrand, Caroline Nordenvall, Mattias Soop, Monica Egenvall, Ulrik Lindfors, Stefan Carlens och Anna Martling** för det varma välkomnande jag fått på KS och för den kunskap ni delar med er av. Jag är otroligt stolt över att vara en del av er grupp.

Kollegorna på kolorektalsektionen på DS, **Karin Westberg, Mahmood Wael Mahmood, Daniel Asklid, Mats Bragmark, Roger Gerjy, Mikael Sundin och Ylva Falkén och Claes Anderin** för all kirurgi ni lärt mig genom åren.

Erik Näslund, professor, för att du gjort det möjligt för mig att kombinera klinik och forskning och slutföra den här avhandlingen.

Håkan Wallén, professor, studierektor på KI DS och **Nina Ringart** handläggare på KI DS för all hjälp från doktorandregistrering till disputation.

Christina Edberg, administratör i forskargruppen på KS. Tack för all hjälp med avhandlingen.

Mina vänner från åren på DS, **Emma Öistämö** och **Emma Rosander** för fina samtal och svar på frågor rörande disputation.

Vännerna från läkarprogrammet i Uppsala, **Jennie Engstrand, Karin Kempe, Jonna Karlén och Sanam Zoghi Scott, Sami Bui-Quy Abu Hamdeh, Robert Lindberg, John Möller, Erik Ljungars, Micael Taavo, Oskar Ljunquist, Patrik Dofs och Karl Sörelius** för vägledning inom yrket och genom livet, och för bra häng.

Mina svärföräldrar, **Tara och Ali** för att ni med glädje alltid engagerar er i barnen och ställer upp som barnvakter.

Mina syskon **Bahar** och **Rouzbeh** samt min svåger **Farshad** med deras familjer, för den nära relationen vi har där vi alltid ställer upp för varandra.

Mina föräldrar, **Simin och Ebrahim** för all kärlek och stöd genom åren och för att ni gett mig möjligheten att leva ett liv i frihet.

Ava och Darya för all glädje ni sprider och för allt ni har lärt mig. Jag älskar er.

Mithra, mitt livs kärlek, för att du finns. Tack för allt.

10 References

1. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet 2017;390:2769-2778.
2. Baumgart DC, Sandborn WJ. Crohn's disease. Lancet 2012;380:1590-605.
3. Ordás I, Eckmann L, Talamini M, et al. Ulcerative colitis. Lancet 2012;380:1606-19.
4. Busch K, Ludvigsson JF, Ekstrom-Smedby K, et al. Nationwide prevalence of inflammatory bowel disease in Sweden: a population-based register study. Aliment Pharmacol Ther 2014;39:57-68.
5. Kaplan GG. The global burden of IBD: from 2015 to 2025. Nat Rev Gastroenterol Hepatol 2015;12:720-7.
6. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207-14.
7. Li D, Wang P, Wang P, et al. The gut microbiota: A treasure for human health. Biotechnol Adv 2016;34:1210-1224.
8. Sommer F, Bäckhed F. Know your neighbor: Microbiota and host epithelial cells interact locally to control intestinal function and physiology. Bioessays 2016;38:455-64.
9. Marchesi JR, Adams DH, Fava F, et al. The gut microbiota and host health: a new clinical frontier. Gut 2016;65:330-9.
10. Pittayanon R, Lau JT, Leontiadis GI, et al. Differences in Gut Microbiota in Patients With vs Without Inflammatory Bowel Diseases: A Systematic Review. Gastroenterology 2020;158:930-946.e1.
11. Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol 2006;3:390-407.
12. Thurgate LE, Lemberg DA, Day AS, et al. An Overview of Inflammatory Bowel Disease Unclassified in Children. Inflamm Intest Dis 2019;4:97-103.
13. Everhov Å H, Sachs MC, Malmborg P, et al. Changes in inflammatory bowel disease subtype during follow-up and over time in 44,302 patients. Scand J Gastroenterol 2019;54:55-63.
14. Forss A, Ludvigsson JF, Olén O. The Burden of IBD: Comparing Denmark and Sweden. Gastroenterology 2022.
15. Kaplan GG, Ng SC. Globalisation of inflammatory bowel disease: perspectives from the evolution of inflammatory bowel disease in the UK and China. Lancet Gastroenterol Hepatol 2016;1:307-316.
16. Cosnes J, Gower-Rousseau C, Seksik P, et al. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology 2011;140:1785-94.
17. Ananthakrishnan AN. Environmental risk factors for inflammatory bowel diseases: a review. Dig Dis Sci 2015;60:290-8.

18. Ananthakrishnan AN. Epidemiology and risk factors for IBD. Nat Rev Gastroenterol Hepatol 2015;12:205-17.

19. Piovani D, Danese S, Peyrin-Biroulet L, et al. Environmental Risk Factors for Inflammatory Bowel Diseases: An Umbrella Review of Meta-analyses. Gastroenterology 2019;157:647-659.e4.

20. Mahid SS, Minor KS, Soto RE, et al. Smoking and inflammatory bowel disease: a meta-analysis. Mayo Clin Proc 2006;81:1462-71.

21. Benjamin JL, Hedin CR, Koutsoumpas A, et al. Smokers with active Crohn's disease have a clinically relevant dysbiosis of the gastrointestinal microbiota. Inflamm Bowel Dis 2012;18:1092-100.

22. Andersson RE, Olaison G, Tysk C, et al. Appendectomy and protection against ulcerative colitis. N Engl J Med 2001;344:808-14.

23. Andersson RE, Olaison G, Tysk C, et al. Appendectomy is followed by increased risk of Crohn's disease. Gastroenterology 2003;124:40-6.

24. Kaplan GG, Pedersen BV, Andersson RE, et al. The risk of developing Crohn's disease after an appendectomy: a population-based cohort study in Sweden and Denmark. Gut 2007;56:1387-92.

25. Frisch M, Pedersen BV, Andersson RE. Appendicitis, mesenteric lymphadenitis, and subsequent risk of ulcerative colitis: cohort studies in Sweden and Denmark. Bmj 2009;338:b716.

26. Fletcher J, Cooper SC, Ghosh S, et al. The Role of Vitamin D in Inflammatory Bowel Disease: Mechanism to Management. Nutrients 2019;11.

27. Bager P, Gørtz S, Feenstra B, et al. Increased Risk of Inflammatory Bowel Disease in Families with Tonsillectomy: A Danish National Cohort Study. Epidemiology 2019;30:256-262.

28. Sun W, Han X, Wu S, et al. Tonsillectomy and the risk of inflammatory bowel disease: A systematic review and meta-analysis. J Gastroenterol Hepatol 2016;31:1085-94.

29. Nguyen LH, Örtqvist AK, Cao Y, et al. Antibiotic use and the development of inflammatory bowel disease: a national case-control study in Sweden. Lancet Gastroenterol Hepatol 2020;5:986-995.

30. Shaw SY, Blanchard JF, Bernstein CN. Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. Am J Gastroenterol 2010;105:2687-92.

31. Khalili H, Granath F, Smedby KE, et al. Association Between Long-term Oral Contraceptive Use and Risk of Crohn's Disease Complications in a Nationwide Study. Gastroenterology 2016;150:1561-1567.e1.

32. Khalili H. Risk of Inflammatory Bowel Disease with Oral Contraceptives and Menopausal Hormone Therapy: Current Evidence and Future Directions. Drug Saf 2016;39:193-7.

33. Khalili H, Neovius M, Ekbom A, et al. Oral Contraceptive Use and Risk of Ulcerative Colitis Progression: A Nationwide Study. Am J Gastroenterol 2016;111:1614-1620.

34. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature 2012;491:119-24.

35. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature 2011;474:307-17.

36. Bianco AM, Girardelli M, Tommasini A. Genetics of inflammatory bowel disease from multifactorial to monogenic forms. World J Gastroenterol 2015;21:12296-310.

37. Loddo I, Romano C. Inflammatory Bowel Disease: Genetics, Epigenetics, and Pathogenesis. Front Immunol 2015;6:551.

38. Halme L, Paavola-Sakki P, Turunen U, et al. Family and twin studies in inflammatory bowel disease. World J Gastroenterol 2006;12:3668-72.

39. Raine T, Bonovas S, Burisch J, et al. ECCO Guidelines on Therapeutics in Ulcerative Colitis: Medical Treatment. J Crohns Colitis 2022;16:2-17.

40. Torres J, Bonovas S, Doherty G, et al. ECCO Guidelines on Therapeutics in Crohn's Disease: Medical Treatment. J Crohns Colitis 2020;14:4-22.

41. Adamina M, Bonovas S, Raine T, et al. ECCO Guidelines on Therapeutics in Crohn's Disease: Surgical Treatment. J Crohns Colitis 2020;14:155-168.

42. Spinelli A, Bonovas S, Burisch J, et al. ECCO Guidelines on Therapeutics in Ulcerative Colitis: Surgical Treatment. J Crohns Colitis 2022;16:179-189.

43. Frolkis AD, Dykeman J, Negrón ME, et al. Risk of surgery for inflammatory bowel diseases has decreased over time: a systematic review and meta-analysis of population-based studies. Gastroenterology 2013;145:996-1006.

44. Turvill J, O'Connell S, Brooks A, et al. Evaluation of a faecal calprotectin care pathway for use in primary care. Prim Health Care Res Dev 2016;17:428-36.

45. Allin KH, Jacobsen RK, Ungaro RC, et al. Bariatric surgery and risk of new-onset inflammatory bowel disease: A nationwide cohort study. J Crohns Colitis 2021.

46. Kochhar GS, Desai A, Syed A, et al. Risk of de-novo inflammatory bowel disease among obese patients treated with bariatric surgery or weight loss medications. Aliment Pharmacol Ther 2020;51:1067-1075.

47. Radford-Smith GL, Edwards JE, Purdie DM, et al. Protective role of appendicectomy on onset and severity of ulcerative colitis and Crohn's disease. Gut 2002;51:808-13.

48. Russel MG, Dorant E, Brummer RJ, et al. Appendectomy and the risk of developing ulcerative colitis or Crohn's disease: results of a large case-control study. South Limburg Inflammatory Bowel Disease Study Group. Gastroenterology 1997;113:377-82.

49. Gilat T, Hacohen D, Lilos P, et al. Childhood factors in ulcerative colitis and Crohn's disease. An international cooperative study. Scand J Gastroenterol 1987;22:1009-24.

50. Koutroubakis IE, Vlachonikolis IG, Kouroumalis EA. Role of appendicitis and appendectomy in the pathogenesis of ulcerative colitis: a critical review. Inflamm Bowel Dis 2002;8:277-86.

51. Koutroubakis IE, Vlachonikolis IG, Kapsoritakis A, et al. Appendectomy, tonsillectomy, and risk of inflammatory bowel disease: case-controlled study in Crete. Dis Colon Rectum 1999;42:225-30.

52. Kurina LM, Goldacre MJ, Yeates D, et al. Appendicectomy, tonsillectomy, and inflammatory bowel disease: a case-control record linkage study. J Epidemiol Community Health 2002;56:551-4.

53. Kaplan GG, Jackson T, Sands BE, et al. The risk of developing Crohn's disease after an appendectomy: a meta-analysis. Am J Gastroenterol 2008;103:2925-31.

54. Myrelid P, Landerholm K, Nordenvall C, et al. Appendectomy and the Risk of Colectomy in Ulcerative Colitis: A National Cohort Study. Am J Gastroenterol 2017;112:1311-1319.

55. Sahami S, Kooij IA, Meijer SL, et al. The Link between the Appendix and Ulcerative Colitis: Clinical Relevance and Potential Immunological Mechanisms. Am J Gastroenterol 2016;111:163-9.

56. Girard-Madoux MJH, Gomez de Agüero M, Ganal-Vonarburg SC, et al. The immunological functions of the Appendix: An example of redundancy? Seminars in Immunology 2018;36:31-44.

57. Humes DJ, Simpson J. Acute appendicitis. Bmj 2006;333:530-4.

58. Wu SC, Chen WT, Muo CH, et al. Association between appendectomy and subsequent colorectal cancer development: an Asian population study. PLoS One 2015;10:e0118411.

59. Chen CH, Tsai MC, Lin HC, et al. Appendectomy increased the risk of ischemic heart disease. J Surg Res 2015;199:435-40.

60. Janszky I, Mukamal KJ, Dalman C, et al. Childhood appendectomy, tonsillectomy, and risk for premature acute myocardial infarction—a nationwide population-based cohort study. European Heart Journal 2011;32:2290-2296.

61. Chung SD, Huang CC, Lin HC, et al. Increased Risk of Clinically Significant Gallstones following an Appendectomy: A Five-Year Follow-Up Study. PLoS One 2016;11:e0165829.

62. Clanton J, Subichin M, Drolshagen K, et al. Fulminant Clostridium difficile infection: An association with prior appendectomy? World J Gastrointest Surg 2013;5:233-8.

63. van der Waaij LA, Limburg PC, Mesander G, et al. In vivo IgA coating of anaerobic bacteria in human faeces. Gut 1996;38:348-54.

64. Brandtzaeg P, Karlsson G, Hansson G, et al. Immunohistochemical study of nasal mucosa in patients with selective IgA deficiency. Int Arch Allergy Appl Immunol 1987;82:483-4.

65. Odineal DD, Gershwin ME. The Epidemiology and Clinical Manifestations of Autoimmunity in Selective IgA Deficiency. Clin Rev Allergy Immunol 2020;58:107-133.

66. Tytgat HLP, Nobrega FL, van der Oost J, et al. Bowel Biofilms: Tipping Points between a Healthy and Compromised Gut? Trends Microbiol 2019;27:17-25.

67. Frühbeck G. Bariatric and metabolic surgery: a shift in eligibility and success criteria. Nat Rev Endocrinol 2015;11:465-77.

68. Singh S, Dulai PS, Zarrinpar A, et al. Obesity in IBD: epidemiology, pathogenesis, disease course and treatment outcomes. Nat Rev Gastroenterol Hepatol 2017;14:110-121.

69. Kral JG, Näslund E. Surgical treatment of obesity. Nat Clin Pract Endocrinol Metab 2007;3:574-83.

70. Sandoval DA. Mechanisms for the metabolic success of bariatric surgery. J Neuroendocrinol 2019;31:e12708.

71. Luijten J, Vugts G, Nieuwenhuijzen GAP, et al. The Importance of the Microbiome in Bariatric Surgery: a Systematic Review. Obes Surg 2019;29:2338-2349.

72. Lane ER, Zisman TL, Suskind DL. The microbiota in inflammatory bowel disease: current and therapeutic insights. J Inflamm Res 2017;10:63-73.

73. Ahn LB, Huang CS, Forse RA, et al. Crohn's disease after gastric bypass surgery for morbid obesity: is there an association? Inflamm Bowel Dis 2005;11:622-4.

74. Bernstein GR, Pickett-Blakely O. De Novo Inflammatory Bowel Disease After Bariatric Surgery: A Case Series and Literature Review. Dig Dis Sci 2017;62:817-820.

75. Braga Neto MB, Gregory M, Ramos GP, et al. De-novo Inflammatory Bowel Disease After Bariatric Surgery: A Large Case Series. J Crohns Colitis 2018;12:452-457.

76. Janczewska I, Nekzada Q, Kapraali M. Crohn's disease after gastric bypass surgery. BMJ Case Rep 2011;2011.

77. Korelitz BI, Sonpal N, Schneider J, et al. Obesity/Bariatric Surgery and Crohn's Disease. J Clin Gastroenterol 2018;52:50-54.

78. Kotze PG, Bremer-Nones R, Kotze LM. Is there any relation between gastric bypass for morbid obesity and the development of Crohn's disease? J Crohns Colitis 2014;8:712-3.

79. Bik EM, Eckburg PB, Gill SR, et al. Molecular analysis of the bacterial microbiota in the human stomach. Proc Natl Acad Sci U S A 2006;103:732-7.

80. Imhann F, Bonder MJ, Vich Vila A, et al. Proton pump inhibitors affect the gut microbiome. Gut 2016;65:740-8.

81. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol 2016;14:e1002533.

82. Turnbaugh PJ, Ley RE, Hamady M, et al. The human microbiome project. Nature 2007;449:804-10.

83. Arnold JW, Roach J, Azcarate-Peril MA. Emerging Technologies for Gut Microbiome Research. Trends Microbiol 2016;24:887-901.

84. Hillman ET, Lu H, Yao T, et al. Microbial Ecology along the Gastrointestinal Tract. Microbes Environ 2017;32:300-313.

85. Bäckhed F, Roswall J, Peng Y, et al. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. Cell Host Microbe 2015;17:690-703.

86. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. Nature 2012;486:222-7.

87. Kim H, Sitarik AR, Woodcroft K, et al. Birth Mode, Breastfeeding, Pet Exposure, and Antibiotic Use: Associations With the Gut Microbiome and Sensitization in Children. Curr Allergy Asthma Rep 2019;19:22.

88. Cotillard A, Kennedy SP, Kong LC, et al. Dietary intervention impact on gut microbial gene richness. Nature 2013;500:585-8.

89. Ni J, Wu GD, Albenberg L, et al. Gut microbiota and IBD: causation or correlation? Nat Rev Gastroenterol Hepatol 2017;14:573-584.

90. Gorkiewicz G, Thallinger GG, Trajanoski S, et al. Alterations in the colonic microbiota in response to osmotic diarrhea. PLoS One 2013;8:e55817.

91. Sun S, Zhu X, Huang X, et al. On the robustness of inference of association with the gut microbiota in stool, rectal swab and mucosal tissue samples. Sci Rep 2021;11:14828.

92. Collado MC, Derrien M, Isolauri E, et al. Intestinal integrity and Akkermansia muciniphila, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. Appl Environ Microbiol 2007;73:7767-70.

93. Derrien M, Vaughan EE, Plugge CM, et al. Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. Int J Syst Evol Microbiol 2004;54:1469-1476.

94. Martens EC, Chiang HC, Gordon JI. Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. Cell Host Microbe 2008;4:447-57.

95. Albenberg L, Esipova TV, Judge CP, et al. Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota. Gastroenterology 2014;147:1055-63.e8.

96. Aldars-García L, Chaparro M, Gisbert JP. Systematic Review: The Gut Microbiome and Its Potential Clinical Application in Inflammatory Bowel Disease. Microorganisms 2021;9.

97. Bäckhed F, Fraser CM, Ringel Y, et al. Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. Cell Host Microbe 2012;12:611-22.

98. Lynch SV, Pedersen O. The Human Intestinal Microbiome in Health and Disease. N Engl J Med 2016;375:2369-2379.

99. Mobeen F, Sharma V, Tulika P. Enterotype Variations of the Healthy Human Gut Microbiome in Different Geographical Regions. Bioinformation 2018;14:560-573.

100. Imdad A, Nicholson MR, Tanner-Smith EE, et al. Fecal transplantation for treatment of inflammatory bowel disease. Cochrane Database Syst Rev 2018;11:Cd012774.

101. Paramsothy S, Paramsothy R, Rubin DT, et al. Faecal Microbiota Transplantation for Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. J Crohns Colitis 2017;11:1180-1199.

102. Davidson RM, Epperson LE. Microbiome Sequencing Methods for Studying Human Diseases. Methods Mol Biol 2018;1706:77-90.

103. Boers SA, Jansen R, Hays JP. Understanding and overcoming the pitfalls and biases of next-generation sequencing (NGS) methods for use in the routine clinical microbiological diagnostic laboratory. Eur J Clin Microbiol Infect Dis 2019;38:1059-1070.

104. Cummings JH, Pomare EW, Branch WJ, et al. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 1987;28:1221-7.

105. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. Nat Rev Endocrinol 2015;11:577-91.

106. Suzuki T, Yoshida S, Hara H. Physiological concentrations of short-chain fatty acids immediately suppress colonic epithelial permeability. Br J Nutr 2008;100:297-305.

107. Tazoe H, Otomo Y, Kaji I, et al. Roles of short-chain fatty acids receptors, GPR41 and GPR43 on colonic functions. J Physiol Pharmacol 2008;59 Suppl 2:251-62.

108. Brown AJ, Goldsworthy SM, Barnes AA, et al. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J Biol Chem 2003;278:11312-9.

109. Sun M, Wu W, Liu Z, et al. Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. J Gastroenterol 2017;52:1-8.

110. Gonçalves P, Araújo JR, Di Santo JP. A Cross-Talk Between Microbiota-Derived Short-Chain Fatty Acids and the Host Mucosal Immune System Regulates Intestinal Homeostasis and Inflammatory Bowel Disease. Inflamm Bowel Dis 2018;24:558-572.

111. Zhuang X, Li T, Li M, et al. Systematic Review and Meta-analysis: Short-Chain Fatty Acid Characterization in Patients With Inflammatory Bowel Disease. Inflamm Bowel Dis 2019;25:1751-1763.

112. Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. Annu Rev Biochem 2003;72:137-74.

113. Chiang JY. Bile acids: regulation of synthesis. J Lipid Res 2009;50:1955-66.

114. Fiorucci S, Carino A, Baldoni M, et al. Bile Acid Signaling in Inflammatory Bowel Diseases. Dig Dis Sci 2021;66:674-693.

115. Hofmann AF, Borgstroem B. The intraluminal phase of fat digestion in man: The lipid content of the micellar and oil phases of the intestinal conten obtained during fat digestion and absorbtion. J Clin Invest 1964;43:247-57.

116. Makishima M, Okamoto AY, Repa JJ, et al. Identification of a nuclear receptor for bile acids. Science 1999;284:1362-5.

118. Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, et al. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. Eur J Epidemiol 2009;24:659-67.

119. Ludvigsson JF, Andersson E, Ekbom A, et al. External review and validation of the Swedish national inpatient register. BMC Public Health 2011;11:450.

120. Brooke HL, Talbäck M, Hörnblad J, et al. The Swedish cause of death register. Eur J Epidemiol 2017;32:765-773.

121. Johansson LA, Björkenstam C, Westerling R. Unexplained differences between hospital and mortality data indicated mistakes in death certification: an investigation of 1,094 deaths in Sweden during 1995. J Clin Epidemiol 2009;62:1202-9.

122. Hedenbro JL, Näslund E, Boman L, et al. Formation of the Scandinavian Obesity Surgery Registry, SOReg. Obes Surg 2015;25:1893-900.

123. Sundbom M, Näslund I, Näslund E, et al. High acquisition rate and internal validity in the Scandinavian Obesity Surgery Registry. Surg Obes Relat Dis 2021;17:606-614.

124. Daperno M, D'Haens G, Van Assche G, et al. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. Gastrointest Endosc 2004;60:505-12.

125. Mohammed Vashist N, Samaan M, Mosli MH, et al. Endoscopic scoring indices for evaluation of disease activity in ulcerative colitis. Cochrane Database Syst Rev 2018;1:Cd011450.

126. Chiuve SE, Fung TT, Rimm EB, et al. Alternative dietary indices both strongly predict risk of chronic disease. J Nutr 2012;142:1009-18.

127. Sedgwick P. Multiple hypothesis testing and Bonferroni's correction. Bmj 2014;349:g6284.

128. Randal Bollinger R, Barbas AS, Bush EL, et al. Biofilms in the large bowel suggest an apparent function of the human vermiform appendix. Journal of Theoretical Biology 2007;249:826-831.

129. Kooij IA, Sahami S, Meijer SL, et al. The immunology of the vermiform appendix: a review of the literature. Clin Exp Immunol 2016;186:1-9.

130. Chang CH, Kor CT, Wu CL, et al. Increased chronic kidney disease development and progression in diabetic patients after appendectomy: a population-based cohort study. PeerJ 2018;6:e5019.

131. Ekström LD, Ekström H, Dal H, et al. Childhood appendectomy and adult mental disorders: A population-based cohort study. Depress Anxiety 2020;37:1108-1117.

132. Matsushita M, Uchida K, Okazaki K. Role of the appendix in the pathogenesis of ulcerative colitis. Inflammopharmacology 2007;15:154-7.

133. Scott IS, Sheaff M, Coumbe A, et al. Appendiceal inflammation in ulcerative colitis. Histopathology 1998;33:168-73.

134. Park SH, Loftus EV, Jr., Yang SK. Appendiceal skip inflammation and ulcerative colitis. Dig Dis Sci 2014;59:2050-7.

135. Rath HC, Ikeda JS, Linde HJ, et al. Varying cecal bacterial loads influences colitis and gastritis in HLA-B27 transgenic rats. Gastroenterology 1999;116:310-9.

136. Jakobsson GL, Sternegård E, Olén O, et al. Validating inflammatory bowel disease (IBD) in the Swedish National Patient Register and the Swedish Quality Register for IBD (SWIBREG). Scandinavian Journal of Gastroenterology 2017;52:216-221.

137. Cortez RV, Petry T, Caravatto P, et al. Shifts in intestinal microbiota after duodenal exclusion favor glycemic control and weight loss: a randomized controlled trial. Surg Obes Relat Dis 2018;14:1748-1754.

138. Lee CJ, Florea L, Sears CL, et al. Changes in Gut Microbiome after Bariatric Surgery Versus Medical Weight Loss in a Pilot Randomized Trial. Obes Surg 2019;29:3239-3245.

139. Harpsøe MC, Basit S, Andersson M, et al. Body mass index and risk of autoimmune diseases: a study within the Danish National Birth Cohort. Int J Epidemiol 2014;43:843-55.

140. Khalili H, Ananthakrishnan AN, Konijeti GG, et al. Measures of obesity and risk of Crohn's disease and ulcerative colitis. Inflamm Bowel Dis 2015;21:361-8.

141. Mendall M, Harpsøe MC, Kumar D, et al. Relation of body mass index to risk of developing inflammatory bowel disease amongst women in the Danish National Birth Cohort. PLoS One 2018;13:e0190600.

142. Mendall MA, Jensen CB, Sørensen TIA, et al. Body mass index in young men and risk of inflammatory bowel disease through adult life: A population-based Danish cohort study. Sci Rep 2019;9:6360.

143. Chen MX, Wang SY, Kuo CH, et al. Metabolome analysis for investigating host-gut microbiota interactions. J Formos Med Assoc 2019;118 Suppl 1:S10-s22.

144. Agus A, Clément K, Sokol H. Gut microbiota-derived metabolites as central regulators in metabolic disorders. Gut 2021;70:1174-1182.

145. Jaworska K, Konop M, Bielinska K, et al. Inflammatory bowel disease is associated with increased gut-to-blood penetration of short-chain fatty acids: A new, non-invasive marker of a functional intestinal lesion. Exp Physiol 2019;104:1226-1236.

146. Weng YJ, Gan HY, Li X, et al. Correlation of diet, microbiota and metabolite networks in inflammatory bowel disease. J Dig Dis 2019;20:447-459.

147. Franzosa EA, Sirota-Madi A, Avila-Pacheco J, et al. Gut microbiome structure and metabolic activity in inflammatory bowel disease. Nat Microbiol 2019;4:293-305.

148. Duboc H, Rajca S, Rainteau D, et al. Connecting dysbiosis, bile-acid dysmetabolism and gut inflammation in inflammatory bowel diseases. Gut 2013;62:531-9.

149. Gnewuch C, Liebisch G, Langmann T, et al. Serum bile acid profiling reflects enterohepatic detoxification state and intestinal barrier function in inflammatory bowel disease. World J Gastroenterol 2009;15:3134-41.

150. Vítek L. Bile acid malabsorption in inflammatory bowel disease. Inflamm Bowel Dis 2015;21:476-83.

151. Davie RJ, Hosie KB, Grobler SP, et al. Ileal bile acid malabsorption in colonic Crohn's disease. Br J Surg 1994;81:289-90.

152. Maier L, Goemans CV, Wirbel J, et al. Unravelling the collateral damage of antibiotics on gut bacteria. Nature 2021;599:120-124.

153. Palleja A, Mikkelsen KH, Forslund SK, et al. Recovery of gut microbiota of healthy adults following antibiotic exposure. Nat Microbiol 2018;3:1255-1265.

154. Sedgwick P. Pitfalls of statistical hypothesis testing: type I and type II errors. Bmj 2014;349:g4287.

155. Sedgwick P. Pitfalls of statistical hypothesis testing: multiple testing. Bmj 2014;349:g5310.