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GASTROESOPHAGEAL REFLUX:

ETIOLOGICAL FACTORS

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"The most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!', but rather 'hmm... that's funny'..."

Isaac Asimov

ABSTRACT

Gastroesophageal reflux disease (GERD) is one of the most common health problems in the Western world today, affecting up to 20% of the adult population weekly and 50% monthly, generating substantial suffering among patients as well as significant costs to both patients and also to society in general. GERD can result in serious complications such as esophageal strictures, Barrett's esophagus, and esophageal adenocarcinoma. Several risk factors for developing GERD have been identified, but there are still large bits of information on the etiology of GERD and its consequences missing. The purpose of this thesis is therefore to bring to light more knowledge in the etiology of GERD.

In the first paper, the association between several respiratory symptoms and gastroesophageal reflux symptoms is evaluated based on data from a large population-based health survey from Norway, comprising more than 40,000 participants. In study participants with respiratory symptoms, a two- to threefold increased risk for reflux symptoms was seen.

The second paper uses a nested case-control study, also based on the large Norwegian population-based health survey, to investigate the relation between *Helicobacter pylori* infection, gastric atrophy, and gastroesophageal reflux symptoms. A total of 944 study participants were included. Infection with *Helicobacter pylori* did not influence the risk for reflux symptoms after adjustments for confounding.

The third paper employs a monozygotic co-twin control design, within the Swedish Twin Registry, to determine the effect of lifestyle factors on the risk of GERD. The study shows a dose-response association between increasing body mass and GERD in women, but not in men. When genetic and early environmental factors were taken into account an increased risk for reflux symptoms was seen among both women and men. Smoking, physical activity at work and a low educational level were also associated with an increased risk of reflux symptoms, while recreational physical activity seemed to decrease this risk. No association with reflux was seen for the potential risk factors alcohol consumption, or dietary items.

The fourth paper is also based on the Swedish Twin Registry. In monozygotic co-twin control analyses and in prospective nested case-control analyses, the potential effect of female sex hormones, in postmenopausal hormone therapy and oral contraceptives, on reflux symptoms was evaluated. In women who had ever used estrogen postmenopausal therapy there was a 60% increased risk of reflux symptoms. This association was seen in normal-weight women, and was slightly further increased in overweight and obese women. There were indications of an association between use of both progestin postmenopausal hormone therapy and oral contraceptives on the risk of reflux symptoms, but this did not remain in the prospective nested case-control analyses.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals (I-IV).

I. Helena Nordenstedt, Magnus Nilsson, Saga Johansson, Mari-Ann Wallander, Roar Johnsen, Kristian Hveem, Jesper Lagergren.
The relation between gastroesophageal reflux and respiratory symptoms in a population-based study: The Nord-Trondelag health survey.
Chest 2006;129:1051-8.

II. Helena Nordenstedt, Magnus Nilsson, Roar Johnsen, Jesper Lagergren, Kristian Hveem.
Helicobacter pylori infection and gastroesophageal reflux in a population-based study: (The HUNT Study).
Helicobacter 2007;12:16-22.

III. Zongli Zheng, Helena Nordenstedt, Nancy L Pedersen, Jesper Lagergren, Weimin Ye.
Lifestyle factors and risk for symptomatic gastroesophageal reflux in monozygotic twins.
Gastroenterology. 2007;132:87-95.

IV. Helena Nordenstedt, Zongli Zheng, Alan J Cameron, Weimin Ye, Nancy L Pedersen, Jesper Lagergren.
Use of postmenopausal hormone therapy and oral contraceptives in relation to gastroesophageal reflux in a population-based twin study.
Manuscript submitted.

CONTENTS

LIST OF ABBREVIATIONS

BMI	Body mass index
cagA	Cytotoxin-associated gene A
CI	Confidence interval
DZ	Dizygotic
GERD	Gastroesophageal reflux disease
H. pylori	*Helicobacter pylori*
HT	Postmenopausal hormone therapy
HUNT	Helseundersøkelsen i Nord-Trøndelag (The Nord-Trøndelag Health Survey)
LES	Lower esophageal sphincter
MZ	Monozygotic
OR	Odds ratio
S-PGI	Serum pepsinogen I

INTRODUCTION

Gastroesophageal reflux disease (GERD) is one of the most common health problems in the Western world today, affecting up to 20% of the adult population at least weekly,[1, 2] generating a great amount of suffering among patients. Less than half of the affected will see their primary care physician with their symptoms and less than 20% will undergo endoscopy.[3] Nonetheless there are estimates that patients presenting with GERD symptoms may account for up to 5% of the primary care workload in Europe, and that this rate is increasing.[4] Moreover, up to 80% of patients with frequent GERD symptoms experience difficulty sleeping, working, and eating which contributes to the impaired quality of life seen in several studies.[5-7] GERD produces substantial costs to patients as well as society in general. Considerable amounts are spent on antireflux medication with estimates that over $10 billion are spent annually for the care of GERD in the United States, of which $6 billion are spent on antireflux medication.[8] However, even higher costs may be produced by loss in work productivity.[9] It is also increasingly recognized that GERD can contribute to a number of extraesophageal symptoms such as sleep apnea, laryngitis, chronic hoarseness, non-cardiac chest-pain and respiratory disorders.[10, 11] GERD can further result in complications such as esophagitis, esophageal stricture, esophageal hemorrhage, Barrett's esophagus and esophageal adenocarcinoma,[12] a cancer with poor prognosis and which incidence is rapidly increasing in the Western world.[13] Despite all the facts stated above, large portions of information on the etiology of GERD and its consequences remain missing.

This thesis, based on four original papers, attempts to unearth more pieces of GERD's etiological puzzle. By increasing the knowledge basis of GERD, including identifying risk factors, we can hopefully make our contribution to find a more effective treatment of GERD, improve quality of life in GERD patients, and most importantly, help to prevent GERD from developing in the first place.

BACKGROUND

HISTORICAL PERSPECTIVE

Esophagitis (inflammation of the esophagus) one of the complications of GERD, was first described by Galen in the second century. Even though he did not recognize the association with acid reflux, he did note that the esophagus, when inflamed, could hinder swallowing due to pain.[14] The first to suggest that gastric acid was associated with esophageal disease was Rokitansky in the 19th century. Some confusion as to the nature and causes of esophagitis remained during the first half of the 20th century. In 1935 Winkelstein defined peptic esophagitis clinically by recognizing "an esophagitis resulting from the irritant action on the mucosa of free hydrochloric acid and pepsin".[15] However, in 1946 Allison equated the presence of esophageal ulcer with that of a hiatal hernia.[16] This last notion resulted in hiatal hernia being more or less synonymous with reflux esophagitis well into the 1960s when Palmer reported that many patients in a prospective study with hiatal hernias neither had reflux symptoms nor esophagitis, and vice versa, that many other patients had esophagitis in the absence of hiatal hernia.[17] In 1950, Norman Barrett of Guy's Hospital in London introduced the term reflux esophagitis.[18] Since then, the interest in GERD has increased notably and within the last decade there has been an explosion in the number of publications relating to GERD.[19]

DEFINITIONS

Gastroesophageal Reflux Disease

A crucial issue when investigating GERD is its definition. There is still no universally accepted definition and classification of GERD. This results in difficulties comparing studies since the definition of GERD might vary from study to study. It is not like a pregnancy – either it is there or not – where researchers actually can agree on what the condition stands for. When it comes to GERD, there have been many different definitions throughout the years based on symptoms, endoscopic or histological findings, extraesophageal manifestations, and complications. The definition of GERD as endoscopically or histologically visible changes in the esophageal mucosa was challenged early on when it was shown that many patients with symptoms do not have such mucosal damage.[20] Moreover, the definition solely based on symptoms, has also recently been challenged when it was shown that as many as 37% of individuals with endoscopically verified esophagitis did not have classic reflux symptoms.[21] This uncertainty has led to an over- as well as under-diagnosis of the GERD, throughout the years.

The aforementioned confusion is well recognized and many are the researchers who have tried to find a generally accepted definition. Until recently, these ambitions were futile. However, in 2006 a consensus document was finally agreed upon.[22] This document, called the Montreal Definition and Classification of GERD, was developed

by an International Consensus Group consisting of 44 experts from around the world (18 countries). The consensus was reached by using a modified so-called Delphi process, based on repeated iterative voting. The hope is that this document, coupling evidence-based medicine with modern consensus development techniques, can provide a basis for a universally accepted terminology and thus simplify management of GERD and improve comparative research and generalizations. However, it still awaits to be widely accepted by clinicians and researchers throughout the world.

According to the Montréal definition, GERD is "a condition which develops when the reflux of the stomach contents causes troublesome symptoms and/or complications". These symptoms are further classified as troublesome "when they adversely affect an individual's general well-being" and thus symptoms that are not troublesome "should not be diagnosed as GERD". Furthermore, the characteristic symptoms of GERD are defined as heartburn and regurgitation. It is stated that "in population-based studies, mild symptoms occurring 2 or more days a week, or moderate/severe symptoms occurring more than 1 day a week, are often considered troublesome by patients.[22]

In this thesis, due to the nature of the reflux questionnaires used, and referring to the Montréal definition, I will use the term reflux symptoms for papers I and II and the term GERD for papers III and IV.

Esophagitis

The diagnosis of esophagitis, inflammation of the esophageal mucosa, is defined endoscopically by visible breaks of the distal esophageal mucosa.[22] The classification system currently most generally accepted to grade the severity of the disease is the Los Angeles classification (A to D denoting increasing severity of inflammation).[23]

Barrett's Esophagus

Barrett's esophagus is generally defined as a partial replacement of esophageal squamous epithelium with metaplastic columnar epithelium, from the gastroesophageal junction and proximally. However, there is some confusion about this term, since the way to diagnose the metaplasia varies throughout the world, from solely endoscopy-based diagnoses to histologically-verified diagnoses,[22] although the latter definition is gaining more support.

EPIDEMIOLOGY

Prevalence of GERD Symptoms

Prevalence estimates of GERD vary considerably from study to study. This is not surprising, considering the previous lack of a consistent definition of GERD. However, from the studies that have been performed, it can be concluded that the prevalence of GERD seems to be increasing. In a study among veterans in the United States, hospitalizations due to GERD have increased four- to sevenfold from the 1970s to the 1990s,[24] and in a recently published systematic review evaluating 17 cross-sectional population-based studies of GERD symptoms there was a significant increasing trend

in the prevalence from 1982-2005, and it was suggested that this trend might even gradually be increasing.[25] In the Western world it is estimated that as much as 20% of the adult population currently suffer from one or more reflux episodes per week.[2, 26-29] The prevalence of GERD varies considerably from country to country. Studies from South America and Asia, predominantly China, indicate that the prevalence is considerably lower than in Europe and North America with a prevalence of 5-10% of the population reporting at least one reflux episode weekly.[30-34] The high prevalence in the Western part of the world is further mirrored by the increasing use of acid suppression medications and the rapidly rising incidence of esophageal adenocarcinoma – the most feared complication of GERD.[12, 13, 35]

The reasons for the increase of GERD are not established, but the rising prevalence of GERD has been paralleled by both an increase in obesity and a more sedentary behavior of populations in modern times, which both have been suggested to play a role in the etiology of GERD. Furthermore, changes in smoking habits, diet, medication use, and the diminishing prevalence of *Helicobacter pylori (H. pylori)* have been proposed to contribute to the increase in GERD.

Progress of GERD

The natural history of GERD is still not clear. Some researchers suggest that the different manifestations of GERD is a continuous spectrum[36] with disease progression (from reflux symptoms to esophagitis to Barrett's esophagus) in some individuals, while others believe that the different manifestations represent a group of separate, albeit related conditions, and that it is unusual for patients to change from one group to another.[37, 38] In a systematic review on the progression of GERD it was concluded that there is progression in a small proportion of patients, but also that in some patients regression is observed.[39] More studies are needed to fully evaluate this issue.

Prevalence of Esophagitis

In a recent population-based study of upper endoscopies from northern Sweden, the prevalence of erosive esophagitis was 15.5%, based on endoscopic findings.[40] More than one third of those with erosive esophagitis did not report any GERD symptoms. On the other hand, only 25% of patients diagnosed with GERD had signs of esophagitis on endoscopy.[41]

Prevalence of Barrett's Esophagus

The prevalence of Barrett's esophagus in the community is unknown, and most cases are thought to be unrecognized. Researchers even talk about "the tip of the iceberg".[42] In an autopsy study from USA a frequency of 0.4% was found, while in a population-based study in Sweden the prevalence was found to be as high as 2%.[43, 44] Studies on individuals with chronic GERD symptoms show a prevalence of Barrett's esophagus in the range of 3% to 12%.[45, 46]

PATHOPHYSIOLOGY OF GASTROESOPHAGEAL REFLUX

Gastroesophageal reflux basically occurs when the pressure in the lower esophageal sphincter (LES) is lower than the intra-abdominal pressure. The LES is formed by a 2.5-4.5 cm segment of circular smooth muscle fibers in the distal esophagus. Reflux episodes are very common in healthy individuals, occurring up to 50 times a day, usually during meals, in the postprandial state or due to gravitational influence such as bending forward,[47] but only in a minority of individuals do these episodes cause symptoms or mucosal damage. To understand why reflux episodes become symptomatic in some people but not in others, it is important to have a good understanding of the pathophysiology underlying GERD.

Antireflux Barrier

The physiological antireflux mechanism has three main components and it is only when this defense is overcome that reflux-induced damage ensues.[48] The first component is persistent high LES pressure. A normal resting pressure is between 15 and 35 mm Hg.[49, 50] Even pressures of 5 to 10 mmHg seem to prevent reflux episodes efficiently and the normal pressure range should therefore present a generous pressure reserve. This mechanism mainly prevents reflux episodes at rest, why it therefore sometimes is referred to as the static antireflux barrier.[49] The second component is the crural diaphragm which can be said to form an external sphincter, exerting pressure on the outside of the LES. The esophagus runs through the crural diaphragm in the so-called diaphragmatic hiatus and thereafter enters the abdomen. The diaphragmatic hiatus itself is approximately 2 cm in length. The size of the hiatus is not fixed, but it contracts whenever the intra-abdominal pressure increases, such as when coughing or during exercise. This mechanism can therefore be called the dynamic antireflux barrier. The third component of the antireflux barrier is thought to be the flap valve mechanism formed by the sharp angle between the cardia of the stomach and the distal esophagus, commonly called the angle of His.[51]

Mechanisms

Throughout the last century, there have been many different beliefs as to the mechanism of GERD. For instance, during much of the first part of the 20th century even the existence of a sphincter in the lower part of the esophagus (LES) in humans was questioned or as Ingelfinger put it: “The sphincter that is a sphinx”.[52] A popular belief in the history of GERD has been that episodes of reflux can be ascribed to a permanent low resting tone in the LES. A study in asymptomatic healthy volunteers challenged this belief by showing that the episodes of reflux were rather due to transient relaxations of the LES.[53] The same research group then showed in a classic study of patients with esophagitis and healthy controls,[50] that reflux episodes could occur by three different mechanisms:

(i) transient relaxations of the LES
(ii) a transient increase in intra-abdominal pressure
(iii) spontaneous free reflux associated with a low resting pressure of the LES

In healthy controls it was by far most common to register reflux episodes due to transient relaxations of the LES, while in the GERD patients the mechanisms varied and more than one mechanism was often seen at the same episode of reflux.

The current views on reflux mechanisms have not changed substantially compared to Dodds' results in the study described above. Transient relaxation of the LES is still believed to be the main mechanism for physiological as well as most pathological reflux.

Once a reflux episode has occurred, the degree of mucosal damage varies, depending on a number of variables. These include the ability of the esophagus to get rid of the refluxate, the contents of the reflux material, and the degree of mucosal resistance, which is perceived to be maintained by the stratified squamous epithelial barrier. Additional protection is afforded by the swallowing of saliva, which both neutralizes the acid and helps in removing it.[54] There is some correlation between the exposure of the esophagus to acid and the severity of GERD. Individuals with longer periods and more frequent episodes tend to suffer from more severe disease and their esophageal pH remains below 4 for significantly longer periods of time than in individuals with milder forms of disease.[55] This is a qualified truth though, since there is other data pointing in the opposite direction.[56] One study shows that elderly patients, despite more severe esophagitis, have less intense heartburn symptoms than younger patients.[57] There is also some evidence that patients with Barrett's esophagus have less frequent or less severe symptoms due to the development of a more acid resistant columnar mucosa.[58]

Gastric Emptying

Delayed gastric emptying, on the basis of retention at 4 hours, has been shown to occur in 26% of GERD patients.[59] It is hypothesized that delayed emptying could lead to GERD, due to increased gastric content that could increase the frequency of transient relaxations of the LES and gastric acid secretion via a gastric distention mechanism.[60] However, as most patients do not have delayed gastric emptying this is probably only an enhancing cofactor in some patients.

Hiatal Hernia

It is generally perceived that people with a hiatal hernia have more reflux.[61] There have been different suggestions as to why this is the case. Mechanistically, the gastro-esophageal junction must protect against reflux during both static and dynamic conditions. During sudden increases in intra-abdominal pressure, the crural diaphragm normally serves as a "second sphincter". In individuals with a hiatal hernia, this mechanism is substantially impaired since the diaphragmatic sphincter is anatomically distanced from the gastroesophageal junction and therefore loses its ability to function as an antireflux mechanism.[62] Large hernias can also impair the process of esophageal emptying, thereby prolonging acid clearance time following a reflux event.[61, 63]

ETIOLOGY OF GASTROESOPHAGEAL REFLUX

Heredity

It has since long been accepted that there must be a substantial genetic component in GERD. Familial aggregation has been observed, not only for GERD, but for Barrett's esophagus as well.[64, 65] However, it has been unclear to what extent heredity accounts for GERD. There are to this day two large twin studies that confirm these earlier observations. Our group showed in a large, population-based study from the Swedish Twin Registry, that heredity accounts for 31% of the liability to GERD.[66] In another study, Mohammed et al (2003) found that 43% of the variance in liability to GERD is due to additive genetic factors.[67] The finding that as much as one third of GERD can be explained by genetic factors does not only emphasize the importance of taking heredity into account when evaluating risk factors for GERD - it also stresses that environmental factors play a key role in the etiology of this disease.

Life Style Factors

Obesity

During the last decades there has been such a rapid increase in the prevalence of overweight and obesity, not only in the Western world, but in lower middle-income countries such as India and China as well, that it is called a new global epidemic.[68-71] High body mass index (BMI) has in a number of recent studies, including meta-analyses, convincingly been shown to be a risk factor for GERD.[72-74] There are several proposed mechanisms for this association, most associated with a disruption of the gastroesophageal junction integrity. A high BMI has been linked with an increased abdominal pressure,[75] but it is unlikely that this alone can cause reflux symptoms.[76] Obesity has further been linked with impaired gastric emptying and an increased frequency of transient relaxations of the LES.[77, 78] Another proposed mechanism is via increased occurrence of hiatal hernia, which also disrupts the esophageal junction, but even though the majority of studies point in this direction data is not conclusive as to whether there is an association between a high BMI and hiatal hernia.[79-81]

Tobacco smoking

There is data, based on studies using esophageal pH monitoring and/or esophageal manometry, indicating that smoking can induce reflux episodes by lowering the LES resting pressure.[82-85] However, only a few studies point to that long-time smoking is a risk factor for GERD,[86, 87] and these studies did not take heredity into account.

Alcohol

Intake of alcohol can lower the LES pressure and trigger reflux episodes.[29, 88, 89] However, when it comes to long-term effects of alcohol consumption on the risk of GERD the existing data is conflicting, with some studies showing an increased risk of GERD and others showing no influence at all. [86, 87, 90, 91]

Dietary Factors

Certain foods including coffee, chocolate and peppermints have been found to precipitate reflux episodes.[92] In physiological studies, ingestion of dietary fat and mints

have been shown to decrease LES pressure and increase esophageal acid exposure.[93, 94] Whether dietary items can increase the long-term risk of GERD, i.e. be etiological factors, is another question though. In the studies undertaken to evaluate the influence of high dietary fat results have been conflicting, but there are some evidence that intake of dietary fiber can lower the risk of GERD.[86, 92, 95] In a population-based study, using the same health survey database as in papers I and II of this thesis, it was shown that intake of dietary fiber in bread decreased the risk of GERD while a high salt intake increased the risk of GERD.[87] The intake of coffee and tea did not affect the risk of GERD in this study. Intake of fruit was in one smaller study not associated with GERD,[95] but in another population-based study fruit consumption was shown to reduce the risk of reflux.[91] Bias due to confounding and reversed causality might have hampered these studies. To summarize, more data is needed before the effect of dietary items can be properly estimated.

Physical Exercise

Reflux symptoms are common among athletes which is most likely to be due to an increased esophageal acid exposure during exercise.[96] However, data on the general population show conflicting results regarding the possible association between GERD and physical exercise. The five studies that have evaluated this relation used definitions of physical activity ranging from less than 3 hours of watching TV a day to physical exercise more than 3 times a week.[86, 87, 91, 92, 95] All studies but one[95] demonstrated that physical exercise exert a protective effect on GERD. In a cohort study both recreational and professional exercise decreased the risk hospitalization for reflux-related diagnoses,[86] consistent with the results from a population-based study from Germany where individuals with reflux symptoms were less physically active than those without symptoms.[91] In a large population-based study based on The Nord-Trøndelag Health Survey (HUNT), the same register used in papers I and II in this thesis, physical exercise was associated with a decreased risk of reflux.[87]

Lifestyle Modifications as Treatment of GERD

As described above, the evidence that several life style factors play an important role in the etiology of GERD is rather solid. As a result, primary care physicians often suggest lifestyle modifications as a first line therapy in GERD. There is support that weight loss decreases the prevalence of GERD,[72] but according to a recently published systematic review, the evidence for other lifestyle measures improving GERD symptoms was rather weak.[97]

Education

The association between education level and GERD is largely unknown. Two smaller have evaluated this relation, but with inconclusive results.[92, 95] To clarify the potential association, more research is needed.

Female Sex Hormones

In two previous studies from our group it has been suggested that the association between obesity and GERD in women might be mediated by estrogen.[72, 98] In the first study, there was a statistically significant association between obesity and esophagitis in women, an association that was further strengthened by the use of estrogen

replacement therapy in postmenopausal women.[98] The second study was based on the same public health surveys as paper I and II in this thesis, HUNT. In that study it was found that obese women had an increased risk of reflux symptoms compared with obese men, and that the risk was highest in premenopausal women and postmenopausal women on estrogen therapy.[72] However, in another study the obesity-related risk for GERD was not affected by sex.[79]

The mechanism hypothesized to account for the relation between estrogen and reflux symptoms is nitric oxide.[72] Estrogen has been shown to increase nitric oxide synthesis.[99] Nitric oxide, in turn, is the predominant relaxing transmitter substance of the gastrointestinal tract in general and the LES in particular.[100, 101] Furthermore, in support of our hypothesis, there is one study showing that postmenopausal women on postmenopausal hormone therapy (HT) with estrogen have an increased nitric oxide synthesis.[102]

In this thesis, the term HT is replacing the outdated terminology "hormone replacement therapy" (HRT).

Psychological Factors

It has been suggested that a reason for the observed difference in prevalence of GERD between countries is not only a result of variation in exposure to risk factors, but also from how cultural diversity makes people in different parts of the world perceive symptoms in different ways.[103] This assumption builds on the notion that GERD is linked to psychological factors. In one study, up to 60% of patients with GERD reported worsening of their symptoms in times of stress.[104] Further, a number of psychiatric disorders can appear as comorbidities with gastrointestinal diseases,[41, 105] but to establish whether there is any true causal association or not more research is warranted.

Helicobacter pylori

H. pylori infection is now widely accepted to be an important risk factor for a number of gastric disorders. For instance, it is established that this bacterial infection through the development of gastric atrophy is the major risk factor for non-cardia gastric cancer.[106] During the last decade, interest in *H. pylori* has extended from its role in the etiology of diseases of the stomach to its possible role in the etiology of diseases of the esophagus. Focus has been on the possible protective role for *H. pylori* in the development of GERD, Barrett's esophagus and esophageal adenocarcinoma.[107] When it comes to the association between *H. pylori* infection and GERD, studies have been showing conflicting results. Especially in studies from the East, *H. pylori* infection seems to play a protective role in GERD,[108] while results from the West have been more ambiguous.[109] This difference might be due to different patterns of gastritis induced by *H. pylori.* In the West, *H. pylori* infection is often associated by a predominantly antral infection, and a high acid secretion, which may make GERD more serious. In contrast, in the East, *H. pylori* infection tends to be associated with a high incidence of atrophic gastritis resulting in a low acid secretion, seemingly protecting against GERD.[110, 111]

Respiratory Symptoms

The relation between respiratory disorders and reflux symptoms has been debated at least since the beginning of the 20th century. Parallel to the increasing insight into the pathophysiology of GERD the interest in this association has increased. The majority of studies have focused on the relation with asthma and chronic cough, but predominantly the studies have been performed in selected patient populations, seeking health care for their respiratory symptoms, and also lacking a control population for comparison. It has been estimated that GERD, alone or in combination with other conditions, is one of the most common causes of chronic cough in adults in the world, with estimates of prevalence of GERD in chronic cough patients ranging from 21-41%.[112-114] The reported prevalence of GERD in asthmatics is even higher, ranging from 32-82%.[115-118] In a large case-control study on military veterans in USA, it was shown that patients with erosive esophagitis are at an increased risk of a spectrum of laryngeal and pulmonary diseases, including sinusitis, laryngitis, bronchial asthma and pneumonia.[119]

The mechanism whereby GERD can cause respiratory disorders such as asthma or chronic cough is not fully established. Indeed not even the causal direction is yet clear. However, it is pertinent to believe that the causal pathway might go both ways, even though the majority of explanations suggest that GERD causes respiratory symptoms and not vice versa. One of the exceptions is the notion that asthma can cause or aggravate reflux symptoms by increasing the negative pleural pressure through airway obstruction and thereby increasing the thoraco-abdominal pressure gradient over the diaphragm.[120] Furthermore, it has been suggested that bronchodilator medication might predispose to gastroesophageal reflux by having a relaxing effect on the LES.[121] Several of the proposed mechanisms whereby GERD can cause respiratory symptoms are similar in asthma and chronic cough and include a vagally mediated reflex, heightened bronchial sensitivity and microaspiration.[122] Avidan et al. investigated the temporal association between coughing or wheezing and reflux episodes in asthmatics and concluded that even though occasional coughing can lead to reflux, the opposite is far more common.[123] In that study almost half of all occasions of coughs and wheezes were associated with reflux.

Due to the commonness of GERD as well as asthma, chronic cough and other respiratory disorders in the society, it is important to establish the relation on a population basis to improve current therapy strategies.

AIMS

The general aim of this thesis is to advance our understanding about the etiology of GERD.

Specific aims are:

- To determine the association between gastroesophageal reflux symptoms and respiratory disorders on the population level.
- To clarify the relation triangle between *H. pylori* infection, gastric atrophy and gastroesophageal reflux symptoms.
- To determine whether lifestyle factors such as tobacco smoking, weight, alcohol consumption, coffee drinking, dietary factors, education, professional and recreational physical activity influence the risk of GERD and if any potential effect is affected by genetic factors.
- To clarify whether exposure to female sex hormones, in postmenopausal hormone therapy and oral contraceptives, is a risk factor for GERD in women, including evaluation of confounding by heredity.

MATERIALS AND METHODS

This thesis uses data from two large epidemiological materials. Papers I and II are based on the large Norwegian health survey HUNT 2 and papers III and IV are based on the nationwide Swedish Twin Registry. An overview of the materials and methods used can be seen in Table 1.

Table 1. *Overview of the four studies included in this thesis.*

	Study I and II	**Study III and IV**
Register / Data source	**HUNT 2**	**Swedish Twin Registry**
Source population	Adult population above 20 yrs in the Norwegian county of Nord-Trøndelag	All twins born in Sweden in 1958 or earlier
Design	Study I: Cross-sectional, population-based case-control study Study II: Nested, population-based case-control study	Study III: Population-based co-twin control study Study IV: Population-based, co-twin control study **and** nested case-control study
Study population	Study I: Those in the source population who participated in HUNT 2. Study II: A randomly selected sample of 944 study participants of the population in study I.	Study III: All same sexed twin pairs born in Sweden in 1958 or earlier, who in 1967/1970 or 1973 **and** 1998-2002 responded to questionnaires. Study IV: All female twins born in Sweden in 1958 or earlier who responded to questionnaires in 1998-2002.
Outcomes	Severe reflux symptoms (heartburn and or acid regurgitations) as assessed in 1995-97.	GERD, determined by frequent reflux symptoms assessed by a validated questionnaire in 1998-2002.
Adjustments	Study I: Age, sex, BMI, smoking, asthma medication Study II: Age, sex, BMI, smoking, education	Study III: Age, BMI, smoking, coffee, physical activity, education Study IV: Smoking, coffee, education, physical activity, BMI
Statistical analyses	Unconditional logistic regression	Conditional logistic regression, generalized estimating equations, Wald statistic

THE HUNT PUBLIC HEALTH SURVEYS

Papers I and II are based on data from HUNT, two consecutive public health surveys conducted in the Norwegian county of Nord-Trøndelag. The first survey, HUNT 1, was carried out in 1984-86 and did not include any information on gastroesophageal reflux symptoms. This thesis only uses data from HUNT 2, which was conducted from 1995-97. Out of 94,197 eligible adult inhabitants of the Nord-Trøndelag county, 65,363 (71%) participated in HUNT 2. At local research centers, temporary set up for the purposes of HUNT, participants were asked to fill in extensive questionnaires covering a wide range of questions about education, lifestyle, working situation, health related issues and use of medications. Furthermore, all participants underwent a physical examination with measurements of body weight and height. Finally, blood samples were taken from all participants for immediate analysis of some parameters and for future storage in a biobank.

Measurement of Reflux Symptoms

In the HUNT 2 questionnaire there was one question regarding reflux symptoms: "To what degree have you had heartburn/regurgitation of acid during the last 12 months?" The response alternatives were "none", "minor" or "major"". Out of the 65,363 participants in HUNT 2, 58,596 individuals (90%) answered this question. Among the responders, 40,210 (69%) had not experienced any heartburn or acid regurgitation, 15,233 (26%) had experienced minor such symptoms and 3,153 (5%) reported severe symptoms.

Validation Study of Reflux Symptoms

The question regarding reflux symptoms in HUNT 2 included the two cardinal symptoms of GERD.[22, 124] However, information on frequency and duration of symptoms, use of antireflux medications, effect on symptoms of antireflux medications and nightly symptoms was not included in the questionnaire. To be able to account for this lack of information a separate validation study of the question used in HUNT 2 was performed. In the validation study, the HUNT 2 question was compared to questions in a previously used, validated questionnaire,[12] including more detailed questions regarding the nature of reflux symptoms. A total of 1,102 individuals participated in the validation study. The study was carried out among outpatients at the Karolinska Hospital in Stockholm (n = 731) and among outpatients visiting primary care physicians or outpatients at the community hospital in Levanger in Nord-Trøndelag (n = 371). The results from Nord-Trøndelag and Stockholm were similar, showing that 95% of the participants reporting severe reflux symptoms in the HUNT 2 questionnaire suffered from reflux symptoms at least once weekly according the more extensive questionnaire. This resulted in a specificity of 99.5% for reflux symptoms occurring at least once weekly in that group and a corresponding sensitivity of 58%. Out of those reporting minor symptoms, 75% had symptoms less than once weekly. Patients who reported taking antireflux medications regularly were counted as having reflux at least daily.

Identification of Cases and Controls

The participants of HUNT 2 who reported severe reflux symptoms during the last 12 months were selected as case group, resulting in 3,153 cases. Those reporting no reflux

symptoms for the last 12 months, 40,210 individuals, were selected as control group. The intermediate group, i.e. those reporting minor symptoms, was excluded from the analyses since the results from the validation study (see above) showed this group to be heterogeneous with a higher risk of misclassification of the outcome. Such misclassification would decrease the specificity of reflux disease.

THE SWEDISH TWIN REGISTRY

One of the purposes of twin studies is to study the separate importance of genetic and environmental influences for behavioral characteristics and diseases, and make it possible to account for genetic factors.

Papers III and IV are based on data from the Swedish Twin Registry. The Swedish Twin Registry[125, 126] was established in late 1950s by professors Lars Friberg and Rune Cederlöf who wanted to investigate how smoking affected health. They were among the first to show that smokers more often than non-smokers developed lung cancer.[125] Today the Swedish Twin Registry is the largest population-based register of twin births in the world and includes more than 170,000 twins out of which 61,000 twin pairs are both still alive. It covers, with few exceptions, all twins born in Sweden since 1886. The register is divided into three age cohorts: the older cohort, the middle cohort, and the younger cohort. In a review article in the journal Science, the Swedish Twin Registry was referred to as part of "Sweden's population goldmine".[127]

Papers III and IV in this thesis are based on data from twins of the first cohort (born from 1886 through 1925) and the middle cohort (born from 1926 through 1958). The data collection for the papers of this thesis was performed in three sessions. First, in 1967 and 1970, all surviving same-sexed twin pairs from the first cohort were mailed a questionnaire, covering information on zygosity, certain diseases and symptoms, alcohol, smoking, diet, stress and occupation. Second, in 1973, a similar questionnaire, but with additional questions about education and selected medications, including oral contraceptives, were sent to all same-sexed twin pairs in the second cohort. Out of approximately 36,000 eligible twins, responses were received from 30,000 individuals, including 14,000 twin pairs. Third, within the frames of the Screening Across the Lifespan Study, a full scale screening of all surviving twins, regardless of sex, born in 1958 or earlier, was initiated. From 1998 through 2002, a total of 45,809 twins (response rate 74%) participated in a structured computer-assisted telephone interview, conducted by trained professional interviewers. The questions covered lifestyle habits, socioeconomic situation, health-related items including symptoms of GERD, use of prescribed and over-the-counter medications as well as more basic questions regarding zygosity and body length and height. In all, 28,486 twins both filled in the questionnaire in 1967/1973 and participated in the interviews in 1998 through 2002.

Zygosity Diagnoses

The zygosity assignment in the Swedish Twin Registry is mainly based on responses to the validated question: ”During childhood, were you and your twin partner as like as ‘two peas in a pod’ or not more alike than siblings in general?” that has been included

in all questionnaires since 1967 (in the Swedish version the twins are actually asked if they "are similar as two berries"). If both twins in a pair responded "alike as two peas in a pod" they were classified as monozygotic (MZ) and if both responded "not alike" they were considered to be dizygotic (DZ). If the twins did not agree, or if only one member of the pair responded to the question, the zygosity was considered to be "not determined". This method of zygosity determination has been proven to correctly diagnose 98% of the twins, when compared to analyses with 13 DNA markers.[128]

Measurement of Reflux Symptoms

In the Screening Across the Lifespan Study telephone interview questionnaire, there was one section regarding reflux symptoms, including 10 questions. This specific reflux symptoms questionnaire has been used and described in detail previously[12, 66] and is based on earlier questionnaires.[12, 26] In summary, all twins were first asked whether they had regularly suffered from heartburn, pain behind the breastbone*, or regurgitation of sour fluid into the mouth. If the response to any of these three key questions was positive, the participants were further asked about the frequency and duration of such symptoms, as well as about radiation of discomfort toward the neck, night waking, antacid relief, and use of proton-pump inhibitors, histamine-receptor antagonists or other antireflux medications. Presence of GERD was defined, a priori, as the occurrence at least weekly of either retrosternal pain with antacid relief, retrosternal burning with antacid relief, retrosternal burning with radiation toward the neck or regurgitation of bitter or sour fluid. Twins reporting these symptoms before present treatment with acid-suppressant medications were also considered as having GERD. Participants reporting no symptoms or symptoms less than once weekly were classified as not having GERD. Finally, all twins with reflux symptoms were asked to report their age when their reflux symptoms first started.

Statistical methods in twin studies

Twin studies can be used to investigate the importance of a putative risk factor allowing controlling for genetic and early environmental effects. The statistical method used for this purpose is called the co-twin control method and it employs the fact that MZ and DZ twins share different degrees of genetic relatedness. This approach can be used for disease discordant twins as well as exposure discordant twins.

When studying disease discordant twins, two control groups are usually used: external (not related) controls and internal (co-twin) controls. The analyses may be performed in three steps.[126] In the first step external control analysis is performed, where the association between exposure and outcome is evaluated using a classical case-control study approach. Twins classified as cases are compared to external controls (other twins, regardless of relatedness) to evaluate the risk for disease given an exposure. The results from external control analysis can be compared with those from ordinary non-twin case-control studies. The second step is controlling for confounding from environmental factors early in life, where the healthy co-twin (in both MZ and DZ twin pairs) is used as control. This design provides an effective way to control for

* This question was modified after the start of the study to distinguish it from cardiac pain. However, the modification had little effect and the data from both versions of the questions were pooled.

differences in early environment since twins share the same intrauterine environment and in addition, most often grow up together. If analyses with external controls (the first step) show an association between exposure and disease and the relative risk is weakened or disappears in the second step, this implies that environmental factors early in life, for instance maternal smoking, nutrition and socioeconomic background, are responsible for the initially observed finding. The second step only needs to be undertaken when there is a need to separate the effect of hereditary factors and early environmental factors. In the third step, the healthy MZ co-twin is set as control and matched with its diseased twin, which means that only disease discordant MZ pairs are included in the analyses. This method is used to control for potential confounding from genetic factors as the cases and controls are genetically identical. As a result, if an association is observed in the second step, among disease discordant DZ pairs, but the association disappears in the third step, among MZ pairs, the results were likely to have been confounded by genetic factors. Since there was no need to separate genetic influence from early environmental influence, the second step described above was omitted in papers III and IV.

PAPER I

Design

Using the HUNT 2 public health survey we performed a population-based cross-sectional case-control study among the adult inhabitants of 20 years and above in the county of Nord-Trøndelag in Norway during 1995-1997.

Statistical Analyses

Occurrence of severe reflux symptoms, as described above, was defined as the outcome variable. The following respiratory disorders, experienced during the past 12 months, were defined as exposures: 1) occurrence of asthma; 2) breathlessness, with three alternative answers: no symptoms, minor symptoms or major symptoms; 3) attacks of heavy breathing or wheezing; 4) daily coughs and, in the case of a positive answer, for how many months and whether the cough was productive; 5) use of asthma medication. Except suffering from breathlessness, all respiratory variables were encoded as dichotomous, indicating presence or absence of the symptom or condition. Use of asthma medication was in the same way encoded dichotomously. Odds ratios (OR) and their 95% confidence intervals (CI), derived from unconditional multivariable logistic regression analysis, were used to assess the relation between the respiratory variables under study and the risk of reflux symptoms.

Potential confounding effects of age (in 10-year intervals), sex, tobacco smoking (years of daily smoking divided into three categories), alcohol consumption (number of occasions of drinking alcoholic beverages in four categories), BMI (body weight in kilograms divided by the square of body height in meters, in four categories), occurrence of asthma (yes or no), and use of asthma medication (yes or no) were tested by introducing them one by one into a multivariable model. The data for all these variables was cross-sectional, except for smoking, for which we used data representing lifetime exposure. The GENMOD procedure of the statistical software SAS (SAS 8e;

SAS Institute; Cary, NC) was used for the multivariable unconditional logistic regression analysis to calculate ORs and CIs. The covariates asthma and alcohol consumption did not show any confounding effect in the multivariable analysis and were therefore omitted from the final model.

PAPER II

Design

A population-based nested case-control study comprising a study base of all adult inhabitants of 20 years and above in the county of Nord-Trøndelag in Norway during 1995-1997.

Assessment of *H. pylori* and cagA

H. pylori infection status was assessed in 10,000 randomly selected cohort members. Among these, 472 individuals were found to have severe reflux symptoms and thereby constituted our case group. The same number of individuals was randomly selected from those without reflux symptoms, and these constituted the control group. Serum samples from the study participants were stored for research purposes (1.5 ml) at -70˚C. Sera were screened with a commercial immunoblot assay (HelicoBlot 2.1; Genelabs Diagnostic, Singapore) for *H. pylori* IgG antibodies and anti-cagA (cytotoxin-associated gene A) antibodies. Ambiguous test strips were repeated before classification. This assay has been reported to denote a sensitivity and specificity for assessment of *H. pylori* of 96% and 93% respectively,[129] and for detection of anti-cagA antibodies 97% and 88%, respectively[130]. Gastric atrophy was assessed by serum pepsinogen I levels (S-PGI), measured with the help of a sandwich enzyme immunoassay (Pepsinogen I ELISA Kit 96, Biohit Oy, Helsinki). The pre-defined cut-off point 25 µg/l for S-PGI was used to denote gastric atrophy. This cut-off point has been reported to stand for a sensitivity and specificity of 88% and 94%, respectively, for detecting severe corpus-predominated gastric atrophy.[131] The interpretation of the results of the immunoblot strips was performed blindly to the case/control status of the study participants.

Statistical Analyses

ORs and their 95% CIs, derived from logistic regression analysis were used to estimate relative risks. The occurrences of *H. pylori* infection, cagA-positive *H. pylori* infection, and gastric atrophy were dichotomized into yes or no. The effect of all covariates that were considered as potential confounders was evaluated in a multivariable model. The following potential confounders were tested: age (in 10-year intervals), sex, BMI (in four categories), smoking (years of daily smoking in 10-year intervals), alcohol consumption (occasions of drinking alcoholic beverages in the last two weeks categorized into four classes), dietary intake of fiber in bread (in four classes), intake of salt (two different exposures: intake of salted fish or meats, in five categories, as well as average use of extra salt on regular meals categorized into four classes), intake of coffee and tea (four and three classes, respectively) and educational level (in five classes). S-PGI levels were categorized into the median values and lower and higher

quartile values. Each of these potential confounders was introduced one by one into the multivariable logistic regression model. Since intake of the covariates salt, tea, coffee, fiber and alcohol consumption were found to have no or only a limited confounding effect, they were left out of the final model. For all statistical calculations, SAS computer software (SAS Institute; Cary, NC) release 8.02 was used and for the logistic regression model, PROC GENMOD was used.

PAPER III

Design

A population-based twin study using the Swedish Twin Registry including all twins in Sweden born in 1958 or earlier who were alive at the time of data collection.

Assessment of lifestyle factors

The questionnaires, sent out in 1967 and 1973, included questions about age, sex, education, height, weight, tobacco smoking, consumption of alcohol and coffee, and physical exercise.[126] BMI was categorized into <20 (lean), 20–22.4 (defined as normal, used as reference), 22.5–24.9 (upper normal weight), 25–29.9 (overweight), and >30 (obese). Tobacco smoking was assessed by consumption of cigarettes, cigars or pipe tobacco. Smoking status was categorized into ever smokers and never smokers. A cigarette equivalent index was created to assess the amount of smoking based on nicotine content (1 cigarette = 1 cigarette if inhaled in lungs or 0.25 if not, 1 cigar = 4 cigarettes if inhaled in lungs or 1 if not, 1 gram of pipe tobacco = 1.43 cigarettes if inhaled in lungs or 0.36 if not). Total alcohol consumption was evaluated by summing total amount of ethanol (beer, spirits and wine) consumed per month in grams. Physical exercise was assessed separately at work (divided into four categories) and at leisure time (classified into four categories). Moreover, combined physical exercise was calculated as a mean of physical activity at work and at leisure time, and was categorized into almost no physical exercise (mean level 1) or at least light physical exercise (mean level 2-4). Education level was dichotomized into elementary school level and above elementary school level. Specific dietary items, including vegetables, fruits, fish, meat, rice, flour-based foods, milk, sandwiches, grilled or fried food, were all rated on a scale from 1 to 5 (<1 time/month, occasionally/month, several times/month or once/week, several times/week, and daily).

Statistical Analyses

Two different methods of comparison of controls were used in the analyses. First, in the external control analysis, we compared twins with GERD (n = 4,083) with unrelated twins without reflux symptoms (n = 21,383), not related to the index probands. This is essentially a classic case-control study. Second, in the MZ co-twin control analysis, twins with reflux symptoms were compared with their MZ co-twins who did not report any reflux symptoms (n = 869 pairs). In the external control analysis, in view of the dependency between the twins in a pair, ORs and their corresponding 95% CIs were obtained by Generalized Estimating Equations model,[132] by applying the generalized linear model (GENMOD) procedure of SAS software (SAS Institute; Cary, NC). In the

MZ co-twin control analysis, ORs and 95% CIs were obtained by conditional logistic regression using the PHREG procedure. All analyses were stratified by sex, since previous studies have indicated sex differences. In basic models, year of birth (five categories) was included. In multivariable analyses, BMI (five categories), smoking (ever or never), coffee (four categories), physical activity at work (four categories) and at leisure time (four categories), and education (two categories) were further included in the models. Dose-response associations between the tested factor and risk of reflux symptoms were examined using a test for trend in the logistic regression, based on ordinal categories of that factor. To test the difference between the ORs derived from the external control and MZ co-twin control analyses, we compared the regression coefficients derived from these two models by the Wald statistic.[133] The differences between the ORs derived from women and men were likewise tested both in the external control and MZ

PAPER IV

Design

A population-based twin study and a nested case-control study using the nationwide Swedish Twin Registry including all female twins in Sweden born in 1958 or earlier who were alive at the time of data collection.

Assessment of Female Sex Hormones

In the questionnaire in 1973, the use of oral contraceptives and the duration of any such use were assessed, thus being of prospective nature. Previous or current use of postmenopausal hormone therapy (HT) and oral contraceptives was determined from brand names reported by the study participants during the Screening Across the Lifespan Study interviews in 1998 through 2002. Furthermore, these participants were asked at what age they started and stopped using the HT and the total duration of the use in years. Reported preparations, used either as HT or for contraception, included oral estrogens and progestins, as well as combined estrogen and progestin preparations.

Statistical Methods

In this study, two different designs were used: co-twin control and nested case-control designs.

Co-twin Control Design
The main design employed was the co-twin control method (see above for a more detailed description) where the exposure (HT and oral contraceptives) and the outcome (GERD) were assessed simultaneously, i.e., cross-sectionally. First external control analyses were carried out, where twin individuals with GERD were compared with unrelated twin individuals without GERD, comparable to a classic case-control design. Second, MZ co-twin control analyses were performed to evaluate the potential confounding effect by heredity and early environmental factors. In this approach, MZ twin pairs who were discordant, i.e. one twin had GERD and the other did not, were considered as matched pairs and then compared to eachother.[134]

Nested Case-Control Design

To allow for a prospective estimation of the associations, a nested case-control design with density sampling was used. Information about the time of onset of reflux symptoms and start and end of exposure made this latter design possible. Eligible as controls were participants without reflux symptoms at the time when reflux symptoms were first experienced by the index cases. The exposure was defined as reporting ever use of HT at this point. Five control participants were selected for each case of GERD. Since data regarding oral contraceptives was already available in 1973, we did not conduct any nested case-control analysis for this exposure.

Statistical Analyses

In the analyses the HT use of estrogen and combined estrogen and progestin were first categorized into two groups: ever and never use. Estrogen therapy was further classified into use of estrogen only. Progestin therapy was dichotomized into never use and use of progestin only. The different HT exposed groups were then categorized according to duration of the use into three groups: never used, >0-4 years, or >4 years. Use of contraceptive pills was dichotomized into never and ever use. This exposure was analyzed separately for data collected in the 1973 questionnaire and in the Screening Across the Lifespan Study questionnaire in 1998-2002. Finally, the analyses were stratified according to BMI. ORs with 95% CIs, obtained from multivariable logistic regression using the Generalized Estimating Equations model,[132] estimated the relative risk. A basic model included adjustment for age alone as a continuous variable. In a multivariable model, we further evaluated all covariates that were considered plausible confounders. These included birth year (five categories, adjusted for age and calendar period effects), education (four categories), tobacco smoking (ever or never), coffee use (four categories), physical activity (four categories), and BMI (six categories). In the stratified analyses, BMI was categorized in to three classes (<25; 25-30; or >30 kg/m^2), based on the World Health Organization definitions of overweight and obesity.[135] Possible dose-response associations were examined using a trend test in the logistic regression, based on ordinal categories of the covariate tested. For the logistic regression models the GENMOD procedure of SAS software release 9.13 (SAS Institute; Cary, NC) was used. In the MZ co-twin control analyses, relative risks were estimated in the form of ORs with 95% CIs, derived from conditional logistic regression in the PHREG procedure of SAS software. The potential interaction effect between BMI and HT was tested by introducing the cross product of the two variables into the model.

RESULTS

PAPER I

Analyses in paper I were based on the 40,210 (68.6%) asymptomatic controls and the 3,153 (5.4%) individuals who reported suffering from severe reflux symptoms. Demographics and some characteristics of the study participants in HUNT are shown in Table 2.

Table 2. *Basic characteristics of study participants in paper I.*

	Cases		Controls		Total	
	No.	**(%)**	**No.**	**(%)**	**No.**	**(%)**
Sex						
Men	1,555	(49.3)	18,814	(46.8)	20,369	(47.0)
Women	1,598	(50.7)	21,396	(53.2)	22,994	(53.0)
Total	3,153	(100.0)	40,210	(100.0)	43,363	(100.0)
Age						
Median	51.0		46.0		46.0	

Respiratory Symptoms

The risk of reflux symptoms was increased for all respiratory symptoms under study, (Table 3). Persons with heavy and wheezy breathing, daily cough, daily productive cough, or chronic cough (daily productive cough for more than three months during the past two years) showed a statistically significantly two- to threefold increase in the risk of reflux symptoms (Table 3). Furthermore, breathlessness showed a significant dose-response association with reflux symptoms (p for trend <0.0001). The OR of reflux symptoms was 12.0 (95% CI 9.5-15.2) among persons with severe breathlessness compared to those without such symptoms. Individuals with asthma suffered from reflux symptoms twice as much as those without asthma (OR 2.0; 95% CI 1.8-2.2) in the basic model, an association that was only slightly attenuated in the multivariable analysis (OR 1.6; 95% CI 1.4-1.9). The risk estimates in the basic model, adjusted for age and sex, were slightly higher than the risk estimates obtained in the multivariable model indicating a lack of strong confounding by the covariates studied.

Table 3. *Respiratory disorders and risk of reflux symptoms.*

	No. of		Basic model*		Multivariable model†	
	Cases	**Controls**	**OR**	**95 % CI**	**OR**	**95 % CI**
Breathlessness						
No	1,947	37,607	1.0	(reference)	1.0	(reference)
Minor symptoms	402	1,971	3.9	(3.5-4.4)	3.5	(3.1-4.0)
Severe symptoms	138	196	13.3	(10.6-16.6)	12.0	(9.5-15.2)
P value for trend				<0.0001		<0.0001
Chronic cough‡						
No	712	6,259	1.0	(reference)	1.0	(reference)
Yes	242	970	2.1	(1.8-2.5)	2.0	(1.7-2.4)
Asthma						
No	2,733	37,270	1.0	(reference)	1.0	(reference)
Yes	420	2,940	2.0	(1.8-2.2)	1.6	(1.4-1.9)
All respiratory symptoms incl. asthma						
No	1,724	31,416	1.0	(reference)	1.0	(reference)
Yes	1,429	8,794	2.9	(2.7-3.2)	2.7	(2.5-3.0)
Months of asthma medication for the last 12 months						
0	2,948	38,974	1.0	(reference)	1.0	(reference)
1-6	71	481	2.0	(1.5-2.5)	1.7	(1.3-2.2)
7-12	134	755	2.2	(1.8-2.7)	2.0	(1.7-2.4)
P value for trend				<0.0001		<0.0001

* Adjusted for age and sex.

† In the multivariable logistic regression model, adjustments were made for age, sex, body mass index, tobacco smoking, and use of asthma medication where appropriate. The influence of the occurrence of asthma and alcohol use was also tested, but they were omitted from the final model since they had no significant confounding effect.

‡ Daily productive cough for more than 3 months during the past 2 years.

PAPER II

A total of 944 study participants were included in study 2; 472 were included as cases and 472 as controls. Their demographics and some clinical characteristics are presented in Table 4.

Table 4. *Characteristics of study participants in paper II.*

	Cases		Controls	
	No.	**(%)**	**No.**	**(%)**
Sex				
Total	472	(100.0)	472	(100.0)
Men	226	(48)	198	(42)
Women	246	(52)	274	(58)
Age (years)				
Median	51		47	
BMI (kg/m^2)				
Median	28.2		26.0	
***Helicobacter pylori* infection**				
Serology IgG-positive	193	(41)	157	(33)
cagA-positive	159	(34)	136	(29)
Gastric atrophy*	3	(1)	16	(3)

* Defined as S-PGI levels <25µg/l.

H. pylori Infection and Reflux Symptoms

In total, *H. pylori* infection was present in 37% of the participants. The frequency of infection was higher in the case group (41%) than in the control group (33%). Antibodies against cagA were detected in 84% of the *H. pylori*-positive subjects, with similar proportions in the case and control groups (Table 4). Results of the analyses of the relation between *H. pylori* infection and reflux symptoms can be seen in Table 5. In the crude model, *H. pylori* infection was associated with an increased risk of reflux symptoms (OR 1.4; 95% CI 1.1-1.8). However, after adjusting for potential confounders in the basic and multivariable models, no association remained (OR 1.1, 95% CI 0.8-1.6). Among *H. pylori*-positive persons with cagA-positive strains, i.e. with a more virulent *H. pylori* infection, the risk of reflux symptoms was not significantly lower (OR 1.1, 95% CI 0.8-1.5) than among those with cagA-negative strains (OR 1.5, 95% CI 0.8-2.9). Exclusion of participants with gastric atrophy did not substantially change any of these results. The covariates age, BMI, sex, smoking, and level of education were found to have weak confounding effects, since adjustment for each of them slightly decreased the crude risk estimates.

Table 5. *H. pylori infection, cagA status and risk of reflux symptoms.*

	No. of		Basic model*		Multivariable model†	
	Cases	Controls	OR	95% CI	OR	95% CI
H. pylori						
No	279	315	1.0	(reference)	1.0	(reference)
Yes	193	157	1.2	(0.9-1.6)	1.1	(0.8-1.6)
cagA-positive	159	136	1.1	(0.8-1.6)	1.1	(0.8-1.5)
cagA-negative	34	21	1.5	(0.8-2.6)	1.5	(0.8-2.9)

* Adjusted for age and sex.
† Adjusted for age, sex, body mass index, tobacco smoking and educational level.

Gastric Atrophy and Reflux Symptoms

Gastric atrophy, predefined as an S-PGI level below 25 µg/l, was detected in 16 control persons and in 3 case persons. This resulted in an overall prevalence of gastric atrophy of 2% in the study population. Gastric atrophy was associated with an 80% reduction in the risk of reflux symptoms (OR 0.2, 95% CI 0.0-0.6). There was seemingly a dose-response relation between decreasing S-PGI levels and reduced risk of reflux symptoms, as shown in Table 6.

Table 6. *Gastric atrophy and risk of reflux symptoms.*

	No. of		Basic model†		Multivariable model‡	
	Cases	Controls	OR	95% CI	OR	95% CI
Gastric atrophy*						
No	469	455	1.0	(ref.)	1.0	(ref.)
Yes	3	16	0.1	(0.0-0.5)	0.2	(0.0-0.6)
Serum pepsinogen I levels						
>113 µg/l (4^{th} quartile)	143	88	1.0	(ref.)	1.0	(ref.)
86-113 µg/l (3^{rd} quartile)	122	118	0.7	(0.5-1.0)	0.7	(0.5-1.1)
67-85 µg/l (2^{nd} quartile)	110	120	0.6	(0.4-0.9)	0.7	(0.5-1.0)
<67 µg/l (1^{st} quartile)	97	146	0.5	(0.3-0.7)	0.5	(0.3-0.8)

* Defined as serum levels of pepsinogen I <25µg/l.
† Adjusted for age and sex.
‡ Adjusted for age, sex, body mass index, tobacco smoking and educational level.

H. pylori Infection and Gastric Atrophy

Participants infected with *H. pylori* showed a significant, almost nine-fold increase in the risk of gastric atrophy compared to non-infected persons (OR 8.9, 95% CI 2.0-39.9). All participants with *H. pylori* infection and gastric atrophy had cagA-positive strains, which meant that the estimated risk of gastric atrophy was even higher in those infected with a cagA-positive strain of the bacterium (OR 12.7, 95% CI 2.9-56.4).

PAPER III

Some basic characteristics of the study participants in study 3 are shown in Table 7. The analyses are based on 15,014 female and 12,703 male twins. Among these there were 523 female MZ twin pairs and 346 MZ male twin pairs discordant for GERD. The prevalence of GERD was 15.5% in women and 13.8% in men. The results from the multivariable analyses evaluating the association between potential risk factors and the risk of reflux symptoms can be seen in Table 8 and Table 9 for women and men, respectively.

Table 7. *Basic characteristics of study population in paper III.*

	Women	Men
Non-GERD participants (No.)	12,684	10,950
GERD participants (No.; %)	2,330 (15.5)	1,753 (13.8)
Monozygotic twin pairs discordant for GERD (No.)	523	346
Age at assessment of GERD, median (range)	58 (42-104)	57 (42-99)
Age at onset of GERD, median (range)	46 (17-83)	45 (18-83)

Obesity and Overweight

Among women, a dose-response association between increasing BMI and GERD was observed in the external control analysis. Compared with normal weight women, underweight women had 19% decreased risk, whereas upper normal weight, overweight, and obese women had approximately 25%, 46%, and 59% increased risk for GERD, respectively. In the MZ co-twin control analysis, these risk estimates were of similar strength. In men, no association between BMI and GERD was observed in the external control analysis. However, in the MZ co-twin control analysis, there was a clear trend with 28% decrease and 44%, 187%, and 277% increase in the risk for lean, upper normal weight, overweight, and obese men, respectively, compared with normal weight men. The difference between the estimates obtained from the external control and MZ control analyses was statistically significant ($P = 0.0203$).

Tobacco Smoking

There was a dose-response pattern in the association between tobacco smoking and GERD in women as well as in men. In the external control analysis, ever tobacco smoking and smoking more than 20 cigarettes per day was associated with 18% and 37% increased risk, respectively, among women when compared with never smoking. These estimates were slightly attenuated in the MZ co-twin control analysis, but the attenuation did not reach a significant level. In men, the corresponding estimates were 36% and 53%, respectively, in the external control analysis and were of similar strength in the MZ co-twin control analysis.

Table 8. *Associations between lifestyle factors and GERD in women*

	External control comparison			MZ control comparison†	
	Cases No.	Controls No.	Adjusted* OR (95%CI)	Adjusted* OR (95%CI)	*P*‡ value
BMI					
<20	638	3,432	0.78 (0.69-0.88)	0.76 (0.46-1.26)	
20.0-22.4	754	3,644	1.0 (reference)	1.0 (reference)	
22.5-24.9	412	1,939	1.25 (1.06-1.46)	1.38 (0.75-2.54)	
25-25.9	249	1,058	1.46 (1.19-1.80)	1.89 (0.72-5.01)	
≥30	33	154	1.59 (1.00-2.54)	1.71 (0.20-14.6)	0.9863
P value for trend			<0.0001	0.0825	0.5718
Ever smoking					
No	1,014	5,671	1.0 (reference)	1.0 (reference)	
Yes	1,063	4,578	1.18 (1.05-1.32)	1.08 (0.66-1.74)	0.7166
Cigarette equivalent /day					
1-9	386	1,900	1.12 (0.97-1.30)	0.82 (0.45-1.48)	
10-19	478	1,968	1.16 (1.01-1.34)	1.21 (0.71-2.44)	
≥20	178	621	1.41 (1.12-1.68)	1.10 (0.49-2.45)	0.5234
P value for trend			0.0027	0.6390	0.7896
Education					
Above elementary	992	5,187	1.0 (reference)	1.0 (reference)	
Elementary	1,072	4,962	1.21 (1.08-1.35)	1.87 (1.07-3.28)	0.1347

* Adjusted for year of birth, education, smoking, coffee, BMI as continuous variable, and physical exercise (almost no / at least light) wherever appropriate.
† The *No.* of twin pairs included in analysis for each factor ranged from 416 (physical activity at work) to 522 (alcohol) in women due to missing data.
‡ *P* value of the Wald test of comparing ORs derived from the external and MZ control analyses.

Alcohol

Alcohol consumption was not associated with risk of GERD in the external control analysis in women, but in the MZ co-twin control analysis an inverse dose-response association was observed. When consumption of beer, wine, and spirits was analyzed separately by dichotomous (yes/no) categories or by amount (quartiles), none of them was associated with GERD in the MZ control analysis. In men, alcohol consumption was associated with a modestly decreased risk of GERD but of borderline statistical significance and without dose-response pattern in the external control analysis. Similar results were observed in the MZ co-twin analysis.

Dietary Variables

None of the studied dietary items, i.e., vegetables, fruits, fish, meat, rice, flour-based foods, milk, sandwiches, potatoes, or grilled and fried food, was associated with risk of GERD in either the external control or the MZ co-twin analysis.

Table 9. *Associations between lifestyle factors and GERD in men*

	External control comparison			MZ control comparison†	
	Cases No.	Controls No.	Adjusted* OR (95 %CI)	Adjusted* OR (95%CI)	*P*‡ value
BMI					
<20	240	1,278	1.00 (0.83-1.20)	0.72 (0.35-1.49)	
20.0-22.4	548	2,884	1.0 (reference)	1.0 (reference)	
22.5-24.9	430	2,858	0.85 (0.73-1.00)	1.44 (0.77-2.68)	
25-25.9	295	1,585	1.03 (0.85-1.24)	2.87 (1.08-7.59)	
≥30	20	100	1.01 (0.56-1.83)	3.77 (0.40-35.9)	0.2230
P value for trend			0.6533	0.0232	0.0203
Ever smoking					
No	495	3,383	1.0 (reference)	1.0 (reference)	
Yes	989	5,138	1.36 (1.18-1.55)	1.37 (0.70-2.66)	0.9801
Cigarette equivalent /day					
1-9	178	1,196	1.22 (0.98-1.52)	1.21 (0.44-3.27)	
10-19	327	1,735	1.30 (1.09-1.54)	1.05 (0.47-2.37)	
≥20	460	2,003	1.53 (1.30-1.79)	1.82 (0.86-3.83)	0.7324
P value for trend			<0.0001	0.1034	0.6413
Education					
Above elementary	845	4,719	1.0 (reference)	1.0 (reference)	
Elementary	692	3,992	0.95 (0.84-1.08)	0.86 (0.44-1.71)	0.7806

* Adjusted for year of birth, education, smoking, coffee, BMI as continuous variable, and physical exercise (almost no / at least light) wherever appropriate.
† The number of twin pairs included in analysis for each factor ranged from 297 (cigarettes equivalent / day) to 346 (alcohol) due to missing data.
‡ *P* value of the Wald test of comparing ORs derived from the external and MZ control analyses.

Coffee

In the external analysis, coffee use was dose-dependently associated with an increased risk of GERD in women, with an approximately 45% increased risk among heavy users (>7 cups per day) compared with nonusers in the basic model, but no such association was observed in the multivariable analysis or in the MZ co-twin analysis. In men, an inversely dose-dependent association was observed for heavy users in the multivariable analysis. The sex difference in the associations was statistically significant (P for trend = 0.0127). The MZ co-twin analysis revealed similar estimates.

Physical Activity

In both sexes, physical activity at work was associated with an increased occurrence of GERD, whereas physical activity at leisure time in contrast rendered a decreased risk in the external control analyses. A 40% increased risk for GERD was observed in women with physically strenuous work compared with primarily sedentary work, but the risk was attenuated in the multivariable analysis. This change occurred specifically when introducing the variables education and BMI into the model. In men, the corresponding figures were 27% and 23% in the basic and multivariable analyses, respectively. In contrast, those who exercised “much” (the highest of 4 categories) at leisure time had approximately 40% decreased risk of GERD compared with those who did “almost no”

exercise, in both sexes. The MZ co-twin control analyses showed no significantly different results for both sexes.

Education

Lower education rendered a 21% increased risk for the development of GERD in women, but not in men, with a statistically significant sex difference (P = 0.0057) in the external control analysis. The association did not significantly change in the MZ co-twin analyses.

PAPER IV

A total of 24,040 female twin individuals were included in the cross-sectional study cohort presented under Methods. Of these, 4,365 twins were defined as cases, i.e. as having reflux symptoms, and 17,321 as controls, without reflux symptoms. Information on reflux symptoms status was missing in 2,354 participants (9.8%). Among all participating twins, 597 were discordant MZ twin pairs regarding reflux symptoms. Some characteristics of the study participants are presented in Table 10. The overall prevalence of reflux symptoms was 20.1%. In the nested case-control design, 2,678 (61.4%) case twins and 13,390 control twins were included. The general characteristics of these participants were similar to those of the cross-sectional study cohort.

Table 10. *Characteristics of study population in paper IV.*

	Control participants		**Case participants**		**Total**	
	No.	**(%)**	**No.**	**(%)**	**No.**	**(%)**
Total	17,321	(79.9)	4,365	(20.1)	21,686	(100.0)
Age (years)						
Median (Range)	58	(42-104)	56	(42-96)	57	(42-104)
Body mass index (kg/m^2)						
< 25	10,171	(58.7)	2,192	(50.2)	12,363	(57.0)
25-30	4,645	(26.8)	1,511	(34.6)	6,156	(28.4)
>30	1,144	(6.6)	529	(12.1)	1,673	(7.7)
Missing	1,361	(7.9)	133	(3.0)	1,494	(6.9)
Postmenopausal estrogen therapy						
Never	10,670	(61.6)	2,347	(53.8)	13,017	(60.0)
Ever	3,757	(21.7)	1,335	(30.6)	5,092	(23.5)
Missing	2,894	(16.7)	683	(15.6)	3,577	(16.5)

Postmenopausal Hormone Therapy with Estrogen

The risk of reflux symptoms was 59% higher in women who had ever used estrogen HT compared to women who had never used such therapy (OR 1.59; 95% CI 1.43-1.76). This estimate did not change when analyzing women who had used estrogen-only HT. After stratification for BMI the risk estimates for reflux symptoms slightly increased with a higher BMI, but the association also remained statistically significantly increased among women of normal weight. An increased duration of estrogen use did not further increase the risk of reflux. The point estimates did not differ materially in the MZ co-twin control analyses, suggesting that genetic factors play a limited role in the observed associations. (Table 11).

In the analyses of the nested case-control design an increased risk of reflux symptoms was observed among women exposed to HT with estrogen (OR 1.44; 95% CI 1.15-1.80) compared to those who had never used estrogen HT.

Postmenopausal Hormone Therapy with Progestin

Women who had ever used progestin alone (no estrogen) showed a 60% increase in the risk of reflux symptoms compared to those who had not used it (OR 1.60; 95% CI 1.15-2.22). The risk of reflux symptoms increased only slightly with higher BMI. There was no statistically significant trend with regard to the duration of progestin use. In the MZ co-twin control analysis the point estimate of ever users was decreased, but the number of cases was too small to get reliable results (OR 1.14; 95% CI 0.41-3.15). (Table 11).

In the nested case-control analysis, the observed increased risk of reflux symptoms disappeared among ever users of progestin (OR 0.96; 95% CI 0.42-2.20).

Postmenopausal Hormone Therapy with Combined Estrogen and Progestin

The risk of reflux symptoms in women who had ever used combined HT was statistically significantly increased (OR 1.19; 95% CI 1.04-1.36). The risk of reflux increased with increasing BMI, but in the MZ co-twin control analysis the association disappeared.

The association observed between combined HT and reflux symptoms was stronger in the nested case-control analysis (OR 1.44; 95% CI 1.07-1.93) than in the cross-sectional analysis.

Table 11. *Postmenopausal HT and risk of GERD among female twins.*

	Cross-sectional: External control comparison			Cross-sectional: MZ control comparison	
	Controls (No.)	Cases (No.)	Adjusted* OR (95% CI)	Discordant pairs (No.)	Univariate OR (95% CI)
Ever used estrogen					
No	10,670	2,347	1.00 (reference)	423	1.00 (reference)
Yes	3,757	1,335	1.59 (1.43-1.76)		1.28 (0.87-1.89)
Never-user†			1.00 (reference)		1.00 (reference)
BMI <25	2,420	674	1.49 (1.29-1.72)	178	1.21 (0.66-2.22)
BMI 25-30	1,031	472	1.68 (1.42-1.99)	56	1.40 (0.44-4.41)
BMI >30	243	159	1.81 (1.33-2.46)	25	4.00 (0.45-35.8)
Ever used progestin alone					
No	14,238	3,603	1.00 (reference)	423	1.00 (reference)
Yes	189	79	1.60 (1.15-2.22)		1.14 (0.41-3.15)
Never-user†			1.00 (reference)		1.00 (reference)
BMI <25	129	37	1.40 (0.88-2.24)	178	2.00 (0.37-10.9)
BMI 25-30	44	26	1.49 (0.83-2.66)	56	2.00 (0.18-22.1)
BMI >30	14	15	3.52 (1.43-8.68)	25	-

* In the multivariable analyses, the covariates tobacco smoking, coffee, educational level, physical activity, and BMI from the SALT study were included. BMI was excluded from the model in the BMI-stratified analyses.
† The reference category for each analyzed BMI stratum was based on hormone therapy never-users in the same stratum.

Oral Contraceptives

In total, 12,669 female twins who responded to the questionnaire from 1973 also participated in the Screening Across the Lifespan Study data collection. Out of these, 8,663 were defined as controls and 1,993 as cases. Use of contraceptive pills, as assessed in 1973, resulted in a tendency toward an increased risk of reflux symptoms (OR 1.14; 95% CI 0.99-1.30), and the point risk estimate was higher in the MZ co-twin control analysis (OR 1.33; 95% CI 0.91-1.96). Having ever used contraceptive pills, as assessed in 1998-2002 entailed a statistically non-significantly 10% increased risk of reflux symptoms (OR 1.09; 95% CI 0.94-1.26). This estimate was slightly increased in the MZ co-twin control analysis (OR 1.34; 95% CI 0.83-2.17). The effect on reflux symptoms by contraceptive pills was not affected by BMI in the stratified analyses in the external case-control design, but in the MZ co-twin control analyses there was a tendency toward a higher risk for reflux symptoms with higher BMI.

No interaction was seen between BMI and the use of HT. Generally, the results from those in the basic model, adjusted only for birth year, did not differ materially from those of the multivariable model.

Table 12. Use of oral contraceptives and risk of GERD among female twins.

	Cross-sectional: External control comparison			Cross-sectional: MZ control comparison	
	Controls (No.)	Cases (No.)	Adjusted* OR (95% CI)	Discordant pairs (No.)	Univariate OR (95% CI)
Ever contraception pill use in 1973					
No	3,769	911	1.00 (reference)	425	1.00 (reference)
Yes	3,725	1,082	1.14 (0.99-1.30)		1.33 (0.91-1.96)
Ever contraception pill use in 1998-2002					
No	12,678	3,177	1.00 (reference)	423	1.00 (reference)
Yes	1,749	505	1.09 (0.94-1.26)		1.34 (0.83-2.17)

* In the multivariable analyses, the covariates tobacco smoking, coffee, educational level, physical activity, and BMI from the SALT study were included. BMI was excluded from the model in the BMI-stratified analyses.
† The reference category for each analyzed BMI stratum was based on hormone therapy never-users in the same stratum.

DISCUSSION

METHODOLOGICAL CONSIDERATIONS

In this thesis, epidemiological approaches are used to address risk factors for GERD. But what does epidemiological mean? Epidemiology used to be defined as the study of occurrence of illness,[136] but during recent decades the definition has evolved and according to one of the most well known epidemiologists today, Professor K Rothman, epidemiology is "the distribution and determinants of disease in human populations".[137]

There are two main types of epidemiological studies: experimental and observational. There is a general perception in today's evidence-based medicine that experimental studies, more specifically, randomized control trials in humans yield the most reliable results regarding disease causation. However, carrying out experimental studies is not always feasible or possible since the exposure might be harmful to humans, making the study unethical or it can just be too impractical or costly to perform in comparison to the expected research value. In response to the most hardheaded advocates of randomized controlled trials a new approach has been proposed, called the Parachute approach,[138] suggesting that more common sense is needed to determine when a randomized controlled trial is warranted and when not!

Study Design

There are two main types of epidemiological observational studies: cohort studies and case-control studies. Whereas the cohort study normally deals with frequency of disease in exposed and non-exposed individuals, the case-control study on the other hand is concerned with the frequency and amount of exposure in individuals with or without a specific disease.

Cohort Studies

Cohort studies have been called "the archetype for all epidemiological studies".[137] In such studies cohort members who are at risk for the disease under study (i.e. disease-free at the onset of the study) are followed for a certain time, and in the end of the follow-up period the occurrence of disease is measured. The main advantages of the cohort study are that temporal relations can be taken into account, the risk of selection bias is reduced, and the prospective assessment of exposure eliminates the risk for recall bias. Disadvantages are mainly the impracticality and high costs, brought on by the amount of time needed.

Case-control Studies

All four papers in this thesis are to some extent case-control studies. In a case-control study, individuals with the disease or condition under study (referred to as cases) are identified and then compared to disease-free individuals (controls). The history of the cases and of the controls regarding exposure and other characteristics, prior to onset of the disease, is recorded through interviews and sometimes by means of records. The

exposure information can be of either prospective or retrospective nature. The purpose of the control group is to provide an estimate of the frequency of exposure in disease-free individuals from the same source population as the cases. This brings on the most important feature when carrying out a case-control study: the selection of controls. The controls should come from the same source population as the cases and be sampled independently of exposure status. The advantages of case-control studies compared to cohort studies are that they are usually quicker and cheaper to perform and moreover, it is the only feasible design for rare disorders or those with long lag between exposure and outcome. Drawbacks include reliance on recall or records to determine previous exposure status, selection of controls might be difficult, and the effect of confounding has to be taken into account. However, when properly carried out, case-control studies provide the same information as cohort studies, but usually faster and at a lower cost.[137]

Paper I is a cross-sectional, population-based case-control study, i.e. cross-sectional in the meaning that the exposures (respiratory symptoms) and the outcome (reflux symptoms) were assessed at the same point in time.

Paper II is a nested, population-based, case-control study. Even though the main exposure (*H. pylori* infection) and the outcome (reflux symptoms) were assessed at the same point in time, the data cannot be considered to be entirely cross-sectional. Since *H. pylori* infection is contracted before the age of five in the vast majority of infected individuals,[139] and the onset of reflux symptoms is usually in middle age,[140] it is unlikely that reflux symptoms would precede contraction of *H. pylori*.

Papers III and IV employ to some extent the case-control design as part of the co-twin control method, which will be further discussed under this topic.

Population-based Design

All four papers in this thesis can be said to be of population-based design. This requires full coverage of cases occurring in the population being studied. Either all ascertained cases during a given time period, or a random sample of them (i.e. nested), were included in the studies. The population-based design allays concern of selection bias, since there is theoretically no selection if the participation frequency is 100%. However, such complete participation is as a rule not possible in reality. The conditions in Sweden and Norway are favorable for population-based studies since there is a tradition in the Scandinavian countries to keep large and complete registers of different aspects of their populations. Furthermore, since the middle of the last century, every resident in Sweden and Norway has been assigned a unique identification number at birth, making the establishment of the large population-based registers used in this thesis possible. Paper I uses data from the HUNT 2 survey, where 71% of the adult inhabitants of one Norwegian county participated. Out of those who participated, 90% answered the question about reflux symptoms, which means that in total 64% of the adult population of the county of Nord-Trøndelag were included in the study. This participation frequency is good, but opens for a risk of some level of selection bias. Paper II employs HUNT2 as source population, but a randomly selected sample of this source population was used in the analyses, thereby making this study a

nested case-control study. When the sampling is done randomly, the study is still considered population-based. Papers III and IV are both based on the Swedish Twin Registry, which is a nationwide twin register, compiled by in principle all twins born in Sweden since 1886 and thus of population-based nature as well. A total number of 30,000 individuals, out of which there were approximately 14,000 same-sexed pairs, responded to the questionnaire in 1973 resulting in a response rate of 83%. In the data collection in 1998 through 2002, 45,809 twins participated, corresponding to a response rate of 74%. The participation rate is good, albeit not excellent, and the risk of selection bias cannot be ruled out.

Co-twin Control Analyses
In papers III and IV co-twin control analyses were used. These analyses were performed in two steps: first as external control analyses (classic case-control studies not taking zygosity into account) and second as MZ co-twin control analyses. By comparing the results from these two analyses, it is possible to separate genetic and shared early environmental effects from other effects. There are several advantages to the co-twin control method over other matched pair designs. First, twin pairs are genetically matched - that is, MZ twins share 100% of their genes and DZ twins share on average 50%. Second, twins are matched closely for a large number of known and unknown environmental factors since they share prenatal environment and usually early life environment such as location, socioeconomic background, possible toxic exposures, and nutrition as well. Third, MZ twins are of the exact same age and the same sex. In a co-twin control study twin pairs discordant for a trait or disease are thus used as matched pairs.

Validity

There are two main types of error in the kind of studies that are included in this thesis: systematic error (often called bias) and random error. Internal validity in a study, i.e. the absence of systematic error, refers to the extent to which results actually measure what the study is aimed to measure. Internal validity is also a prerequisite for external validity, i.e. the generalizability of the findings to other populations. Random error on the other hand affects the precision in a study.

Internal validity is commonly classified into three categories: selection bias, information bias, and confounding.

Selection Bias
If the selection of cases or controls is influenced by the occurrence of the disease or exposure under study, this will lead to selection bias, which might result in distorted risk estimates. The population-based design used in all studies in this thesis and the relatively high participation rates reduce the risk of selection bias. However, whenever there is any degree of non-participation there is always a risk for selection bias, (see under Population-based Design for a more thorough discussion).

Information Bias
Information bias, also known as misclassification, refers to errors in the information collected about the study variables. Misclassification regarding either exposure or

outcome can be differential or non-differential. If the misclassification is different between cases and controls because of the disease or the exposure under study, respectively, it is called differential and can result in severely distorted risk estimates in either direction. In contrast, non-differential misclassification is independent of the exposure and disease under study, and only leads to dilution of the effects observed. Hence, the risk estimates go towards null, making it less severe than differential misclassification. There are some specific risks of misclassification of the outcome in the studies of this thesis. Case classification in papers I and II was based on self-reported symptoms of heartburn and acid regurgitation during the past 12 months. Those reporting severe symptoms were defined as cases while those with minor symptoms were excluded from the analyses. In our validation study, those with severe symptoms corresponded to having symptoms at least daily with a specificity of 99.5%. Although there is no perfect definition of GERD, it has been shown that frequent reflux symptoms have a high specificity for true GERD.[141, 142] Thus, by defining those with severe symptoms as case group and those reporting no symptoms as control group, the risk of misclassification of the outcome should be limited. In papers III and IV the use of a validated questionnaire with more comprehensive questions regarding reflux symptoms should result in an even lower risk for misclassification of the outcome.

When determining *H. pylori* infection status, including cagA status, and the presence of gastric atrophy, the serological methods used might result in some degree of misclassification. Any such misclassification should in the case of *H. pylori* infection be limited, according to the high sensitivity and specificity of the applied test.[129] Using S-PGI as a biomarker for gastric atrophy is disputed. There are studies reporting a reasonably good accuracy for the test we used,[131] but other investigators have found a substantially lower sensitivity for detecting gastric atrophy.[143] By using a low cut-off point when determining gastric atrophy, we achieved a high specificity, but at the cost of a low sensitivity. Thus, the cases that we did capture with this cut-off point were likely to have had a significant gastric atrophy, but some level of misclassification of atrophy is likely, which might partly explain the low number of individuals with gastric atrophy detected in our study.

Recall bias is a common type of information bias in retrospective studies, where information on exposures is collected after the occurrence of the disease or condition under study. The concern of recall bias in the studies of this thesis was reduced by the fact the questions about reflux symptoms were part of extensive questionnaires covering a wide range of information. It is therefore unlikely that our cases would report information on exposures differently from the controls and therefore any exposure misclassification stemming from difficulty to remember would rather be non-differential and thus only lead to a dilution of risk estimates.

Confounding

In observational studies, there is an inherent risk of confounding. Confounding can be described as the confusion of effects between the exposure under study and other factors. A confounder is a variable that is associated both with the outcome (disease under study) and the studied exposure, which is not part of the causal pathway between the exposure and the outcome.[137] In non-experimental research, there are three main methods to control for confounding: multivariable regression analyses, stratification,

and matching. In the four papers of this thesis, these three methods were employed to some extent. Adjustments by multivariable regression modeling were used in all four papers to control for the potential confounding effect of covariates, e.g. age, sex, BMI etc. Stratification for sex was used in paper III since the effect of lifestyle factors on reflux symptoms has been seen in previous studies to differ between sexes.[144] Stratification for BMI was further used in paper IV, since results from our previous studies on the effect of female sex hormones on the risk of reflux symptoms was seen to be strengthened by being overweight or obese. A special case of matching was used in the MZ co-twin control analyses in papers III and IV, where disease discordant monozygotic pairs were compared. By definition, MZ twins are matched for age, sex, genetic factors, and early environmental factors. In summary, even though confounding was rather well accounted for in the studies of this thesis, residual confounding from other variables, known or unknown, can never be ruled out.

Generalizability

Generalizability is the external validity of the conclusions in a study, i.e. how well the findings can be transferred to other populations. The internal validity of a study carries markedly more importance when evaluating the quality of a study, and the general rule is to be cautious when extrapolating results from single studies to other populations. However, there are some remarks to be made about the generalizability of the studies in this thesis. The source population used in papers I and II, i.e. the adult population of the Norwegian county of Nord-Trøndelag, is essentially representative of the Norwegian population as a whole, but the average income and level of education are slightly lower than the national.[145] Furthermore, the population is homogenous, with 97% of the residents being of White Caucasian origin. In papers III and IV, the question is rather if twins differ from singletons. Studies investigating this issue have found that prevalence and incidence for several diseases and symptoms in twins are equal to those yielded in singleton studies.[146, 147] However, one thing to keep in mind when comparing the results yielded in co-twin control studies with those obtained from ordinary case-control studies is that when the co-twin control method is used factors in the biological pathway between exposure and disease might be concealed, which in turn may result in an underestimation of the association studied. In summary, the generalizability of the studies of this thesis should be satisfactory due to the population-based nature of all studies.

Precision

Precision, the influence of random error or chance, is mirrored statistically by the size of the CIs and P values. The sheer size of our studies with large numbers of cases and controls provides a good basis to avoid random errors and thus results in a good precision. However, in the MZ co-twin control analyses in papers III and IV, the number of individuals studied was notably lower and consequently the confidence intervals yielded were wider than in the external control analyses.

FINDINGS AND IMPLEMENTATIONS

Respiratory Symptoms

In paper I we found evidence for a link between several respiratory disorders and symptoms, including asthma, and reflux symptoms. The strengths of the observed associations ranged from ORs of 1.6 (asthma) to 12.0 (severe breathlessness). Individuals with breathlessness showed a particularly strong and dose-response association with reflux symptoms. Moreover, we found that use of asthma medication did not affect the observed associations.

Our results are consistent with the findings in most earlier large-scale studies, although a lack of association between self-reported asthma and reflux symptoms has been reported.[1] In a study by El-Serag et al,[119] investigating the co-morbid occurrence of laryngeal and pulmonary disease with esophagitis, in more than 100,000 military veterans in the United States, the ORs ranged between 1.2 and 1.5 for the respiratory diseases investigated. The reason for these risk estimates being lower than the ones observed in our study could be due to the difference in study populations analyzed. In HUNT 2 the whole population of a county was invited to participate, thereby healthy individuals as well as individuals with comorbid conditions were included. In the study of the military veterans, the controls were selected from other inpatients, which is likely to result in a higher prevalence of respiratory disorders over all than in healthy controls, and thereby the observed ORs are likely to be rather conservative. In another study, hospitalization due to hiatal hernia or reflux esophagitis increased the risk of future respiratory disease hospitalization to the same extent as our results.[148]

Our findings may be viewed in a clinical context. The associations reported point to the need to consider reflux symptoms as a cause or contributory factor of respiratory disorders, particularly those respiratory symptoms that do not respond well to conventional treatment. In these individuals, anti-reflux therapy might be an alternative treatment. Indeed several studies have focused on this issue and in the majority of them improvements in asthma symptoms, but not in objective measures of pulmonary function were noted after anti-reflux therapy. [115, 149, 150] In a Cochrane review on the use of antireflux medication in chronic cough, it was concluded that there is not enough evidence to support treatment with proton-pump inhibitors in GERD-associated chronic cough, but future research might change this conclusion.[151]

Since patients with GERD, as well as most respiratory conditions, in the majority of cases are treated by primary health care physicians, it is important that these be aware of this association.

H. pylori, Gastric Atrophy and the Risk of Reflux Symptoms

In paper II, our hypothesis, i.e. that *H. pylori* infection might reduce the risk of reflux symptoms and that the mechanism behind such association would be the development of gastric atrophy, was refuted. No reduction in the risk of gastroesophageal reflux symptoms was observed among infected persons. In the crude prevalence results, there was a difference in the occurrence of *H. pylori* infection between cases and controls (33% vs. 41%), but this disparity disappeared in the multivariable analysis.

Age and sex adjustment were the strongest contributors to this effect, but some impact was also seen by smoking, BMI and education level. However, two other distinct associations were revealed. Firstly, gastric atrophy (as denoted by a serum level of S-PGI of less than 25 µg/l) was associated with a considerably decreased risk of reflux symptoms, as expected.[111] This was further supported by the observed dose-response relation between S-PGI levels and decreased risk of reflux symptoms. Secondly, *H. pylori* infection, especially the more virulent type (cagA-positive strains), was strongly associated with an increased risk of gastric atrophy, as confirmed by other studies.[152]

Although *H. pylori* infection clearly increased the risk of gastric atrophy, which in turn protected against reflux symptoms, there were too few individuals with gastric atrophy for this relation to have any impact on the overall association in the population-based setting. Nonetheless, atrophy may be a mediator that could contribute to explain the inverse association between *H. pylori* and GERD found in some non-population-based, studies.[94, 153, 154] Moreover, in other parts of the world, where the prevalence of gastric atrophy has been reported to be considerably higher, the impact of this association might play a more important role.

The possible relation between *H. pylori* and GERD has been debated intensely, since there are some important clinical implications. If *H. pylori* infection is protective against GERD, medical eradication of the infection should lead to or aggravate the condition. On the other hand, if there is no association, eradication of *H. pylori* should not give rise to increased risk of GERD. Previous studies regarding eradication of *H. pylori* and the risk of reflux are inconclusive. Some authors have found that eradication might make it more difficult to control GERD.[155] Other studies have shown a lack of aggravation of GERD following of eradication, a finding that is consistent with the presented results.[156, 157]

In the absence of support for the hypothesis that *H. pylori* has a protective effect against reflux symptoms, the evidence to refrain from eradicating *H. pylori in Western societies,* when such treatment is indicated, in persons with reflux symptoms is not convincing.

Lifestyles

Paper III demonstrates a clear role for lifestyle factors in the etiology of GERD, independently of genetic and early environmental factors. Heritability in GERD can explain about one third of the cases,[66, 67] which implies that genetic factors should account for a considerable part of the variation in liability to GERD. Thus, it is important to be able to control for heredity when investigating risk factors for GERD.

Overweight and Obesity

We found a clear and dose-dependent association between a high BMI and increased risk for reflux symptoms. This finding is consistent with previous studies; among others, two meta-analyses and a large cohort study within the Nurses' Health Study.[72-74, 158] The latter also showed that only a moderate weight gain can cause or

exacerbate reflux symptoms. Furthermore, we found that the association between BMI and GERD seemed to be negatively influenced by genetic factors in men.

Smoking
The finding of a dose-dependent association between smoking and GERD, regardless of sex, is consistent with previous studies investigating this relation.[87, 90] Together with previous literature, our results, which have taken heredity in to account, provide evidence that smoking is a true risk factor for GERD.

Alcohol
Alcohol consumption was not found to be associated with the GERD. Moreover, none of the specific types of alcoholic beverages, i.e., beer, wine, or spirits, was associated with GERD. This is consistent with previous population-based epidemiologic studies.[87, 91]

Dietary Factors
No associations between any of the studied dietary factors and the development of GERD were found, and these findings were not influenced by BMI. These results are consistent with earlier literature.[92, 159]

Coffee
Coffee consumption might be a protective factor for GERD in men but not in women. This finding is consistent with an earlier large population-based study from our group.[87] The prospective collection of dietary exposures in our study should act against reversed causality, i.e., that patients with GERD avoided coffee use in order to avoid reflux symptoms, which has been a concern in previous studies of cross-sectional design.[87, 91] However, the mechanism behind this association is unclear and more research is certainly needed before causality can be considered.

Physical Activity
We found that physical activity at work was a risk factor for GERD, while recreational physical activity seemed to reduce the risk of GERD. One explanation for this is that physical activity at work might be linked with postprandial exercise, which has been found to be a risk factor for development of GER symptoms.[160] Physical exercise at leisure, on the other hand, is most likely not to be performed directly after meals. Speculatively, yet another reason might be that physical activity is usually performed under more stress than physical activity in leisure time, which can evoke reflux symptoms.[104] One explanation for the conflicting results in previous studies regarding effects of physical activity might be due to inability to separate these two kinds of physical activities.[92, 95] The present result is consistent, in terms of both direction and strength of association, with our previous large population-based study, in which physical exercise was defined as leisure time exercise, such as cross-country skiing, and jogging.[87]

Education
We found an increased risk of GERD in women with lower education, but no such association was found in men. Previous studies on the association between education and GERD yield inconsistent results and more research is warranted.[92,95] Moreover,

we have no good explanation for a sex difference. Our finding might thus be an effect of chance error.

In conclusion, this large MZ co-twin study provides evidence that BMI, tobacco smoking, and physical activity at work contribute to the development of GERD, whereas physical activity at leisure time appears to be a protective factor. The association between BMI and GERD among men may be attenuated by genetic factors. In addition, heavy coffee intake may be a protective factor of GERD in men and lower education may be a potential risk factor in women. However, whether appropriate lifestyle measures can reduce the risk of GERD is still not determined,[97] except in the case of weight loss.[72]

Female Sex Hormones

In paper IV it is demonstrated that use of postmenopausal estrogen therapy is associated with an increased risk of GERD of 59%, independently of heredity, body mass, tobacco smoking or other plausible confounding factors. The risk of GERD among estrogen therapy users increased slightly with increasing BMI. The risk estimates remained in the nested case-control analysis, rendering a prospective evaluation of the association and supporting our finding. There was an increased risk for reflux symptoms of 14% among women who had used oral contraceptives in the 1970s when the estrogen content was five times higher than today, but this association did not reach statistical significance. In the case of postmenopausal progestin therapy, a 60% increased risk of reflux symptoms was observed in the external control analysis, but this risk disappeared in the nested case-control analysis, indicating that a true relation is uncertain.

In the literature there are only two studies investigating the possible role of female sex hormones in GERD, both emanating from our research group.[72, 98] Our finding of a moderately increased risk of reflux symptoms among women using HT is consistent with the findings from these two studies. Moreover, the weak association between oral contraceptives and GERD finds some support in a study from the 1970s, where it was shown that women using oral contraceptives showed decreased lower esophageal sphincter (LES) pressures.[161] Furthermore, reflux symptoms are common among pregnant women, often starting in the first trimester before there is any extra pressure on the LES from the enlarged uterus.[162-164] Taken together, these studies suggest that during pregnancy, the increased occurrence of reflux symptoms might be explained by raised levels of female sex hormones.[165-167]

If our results are confirmed in future research, the clinical implications of our findings might be considered. The postmenopausal woman considered for HT should be informed that reflux symptoms is a possible side effect and if a woman who uses HT develops reflux symptoms, a treatment alternative might be an attempt to stop using HT, particularly among obese women.

CONCLUSIONS

- There is a strong link between a number of respiratory disorders, i.e. asthma, breathlessness, chronic cough, productive cough and heavy breathing with wheezing, and gastroesophageal reflux symptoms.

- *H. pylori* infection seems not to be associated with risk of reflux on a population basis. Although *H. pylori* is a strong risk factor for atrophic gastritis and atrophic gastritis, in turn, protects against reflux, the limited frequency of persons with atrophic gastritis in the Western population studied does not have much influence on the overall risk of reflux.

- Obesity, tobacco smoking, and physical activity at work appear to be risk factors for GERD, whereas physical activity at leisure time appears to be a protective factor. These associations seem to be at least partly confounded by genetic factors. Lower education may increase the risk of reflux symptoms in women, while coffee use may decrease the risk in men. Alcohol and studied dietary factors do not seem to influence the risk of reflux symptoms.

- The use of estrogen as HT seems to be an independent risk factor for reflux symptoms, thus giving support for the hypothesis that sex hormones can contribute to the etiology of reflux. Use of oral contraceptives or progestin HT does not influence the risk of GERD.

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FUTURE STUDIES

When looking at the huge number of studies that have investigated the prevalence of GERD, there are surprisingly few studies designed to evaluate the incidence of GERD. During 2006-2008 a third data collection within the HUNT public health survey series is taking place. In HUNT 3, the questions regarding GERD are the same as in HUNT 2 and comparing the participants reporting reflux symptoms in HUNT 2 with those in HUNT 3 will make it possible to measure incident GERD on a population basis.

Since the data regarding an association between GERD and respiratory disease is rather convincing, it would be of interest to carry this research further. GERD is a known risk factor for esophageal adenocarcinoma, a cancer which incidence has increased rapidly during the recent decades. Parallel to this, the incidence of adenocarcinoma of the lung has also increased. Taking the anatomical relation into consideration, it could therefore be hypothesized that GERD might be a risk factor for adenocarcinoma of the lung as well. A study to evaluate this hypothetical association could be performed using the linking the Swedish Inpatient Register to the Swedish Cancer Register, using patients with other histological types of lung cancer than adenocarcinoma as controls to account for the plausible confounding effect of smoking.

The Swedish Twin Registry is a unique resource to determine potential risk factors for GERD, for several reasons. First, it is possible to evaluate the effect of potential risk factors without the risk of confounding from genetic and early environmental factors. Second, the occurrence of reflux symptoms is based on a validated questionnaire. Third, the size of the register provides good precision. Fourth, the register is population-based thus minimizing the risk of selection bias. Fifth, there is information on most biologically plausible confounders. Possible future research includes investigating the role of psychosocial and socioeconomic factors in the etiology of GERD. Further, it would be of interest to evaluate the relation between psychiatric disorders (such as depression, anxiety, and mania) and GERD. For the studies suggested, there is some evidence pointing to a relation between the disorders stated and GERD, but they need to be confirmed in a large population-based material with heredity taken into account. Finally, a relation between sleep disorders and GERD has been hypothesized based on studies conducted in sleep laboratories. It would be of great interest to see if this hypothesis would be rejected or accepted in a study based on the Swedish Twin Registry.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Gastroesofageal refluxsjukdom (nedan kallad refluxsjukdom eller reflux) innebär att magsäcksinnehåll når upp i matstrupen och orsakar störande halsbränna eller sura uppstötningar. Ordet reflux kommer från latinet och betyder just ”återflöde”. I västvärlden är reflux en folksjukdom: nästan varannan person har symtom någon gång i månaden och var femte person har symtom minst en gång i veckan. Under de senaste årtiondena har refluxsjukdom dessutom blivit allt vanligare. Refluxsjukdomen medför ökad risk för allvarliga följdsjukdomar såsom matstrupscancer, genererar höga behandlingskostnader, och leder till nedsatt livskvalitet hos de drabbade. Med dessa fakta i åtanke är det därför av stor betydelse att vi kartlägger alla aspekter av refluxsjukdomen noggrant. Av särskild vikt är att hitta sätt att förebygga refluxsjukdomen på, vilket kan ske genom att identifiera riskfaktorer. Syftet med denna avhandling är att undersöka förhållandet mellan ett antal olika faktorer och refluxsjukdom, för att se om de har någon effekt på sjukdomsrisken.

Det är känt att refluxsjukdom är vanligare hos personer med luftvägssymtom (såsom astma, andfåddhet och hosta) än hos friska personer. Vilka luftvägssymtom detta gäller och hur vanligt det är i befolkningen att man är drabbad av både reflux och luftvägssymtom har dock inte varit känt. I **studie 1** undersökte vi därför sambandet mellan ett flertal luftvägssymtom och risken för refluxsjukdom. Vi använde oss av data från HUNT 2, en stor norsk folkhälsoundersökning som inkluderar ca 70 % av alla vuxna i ett norskt fylke. Sammanlagt svarade nästan 60 000 personer som deltog i HUNT 2 (90 %) på frågan om de led av refluxsymtom. Av dessa angav 5 % att de hade svåra refluxbesvär, 26 % att de hade måttliga besvär och 69 % att de var besvärsfria. I de statistiska analyserna fann vi att risken för refluxsjukdom var mellan två- och tredubblad hos personer med de olika luftvägssymtom som vi undersökte. **Hos en del av de drabbade, där vanlig medicinering mot luftvägssymtom inte hjälper, skulle man därför kunna överväga att pröva med läkemedel mot reflux i syfte att förbättra luftvägsproblemen.**

En av de vanligaste infektionerna i världen är infektion med bakterien *Helicobacter pylori* (*HP*) i magsäcken, en infektion som oftast fås under barnaåren och sedan kvarstår livet ut. I västvärlden har förekomsten av *HP*-infektion minskat stadigt under 1900-talet i takt med minskad trångboddhet och ökad antibiotikaanvändning. *HP* orsakar en inflammation i magslemhinnan som i sin tur kan leda till att det produceras mindre magsyra. Forskare har därför föreslagit att minskad syraproduktion på grund av *HP*-infektion skulle kunna motverka refluxsjukdom. Den minskade förekomsten av *HP*-infektion skulle därmed kunna förklara den ökade förekomsten av refluxsjukdom. Eftersom *HP*-infektion också kan leda till magsår och magsäckscancer har idén om att *HP*-infektion ska behandlas på bred front lanserats. Innan en sådan behandling införs måste dock eventuella effekter på refluxsjukdomens förekomst analyseras. Genom att med antibiotika avlägsna *HP* minskas risken för magsäckscancer drastiskt, men om *HP*-infektion visar sig skydda mot refluxsjukdom skulle en antibiotikabehandling innebära att förekomsten av matstrupscancer istället skulle kunna öka. Det är därför av stor vikt att ta reda på alla effekter som kan förväntas om hela befolkningar behandlas

för *HP*-infektion. I **studie 2** använde vi oss av data från samma norska folkhälsoundersökning som i studie 1, HUNT 2, för att klarlägga sambandet mellan *HP*-infektion och refluxsjukdom. Sammanlagt valdes slumpvis ur denna befolkning 500 personer med refluxsymtom och 500 personer utan refluxsymtom. Genom analyser av blodprover kunde graden av tillbakabildande av magsäcksslemhinnan samt förekomsten av *HP*-infektion bestämmas. I vår studie fann vi inga belägg för att *HP*-infektion skulle påverka risken för refluxsjukdom. **Resultaten från denna studie indikerar därmed att det inte finns någon anledning att avstå från att behandla *HP*-infektion på grund av oro för att refluxsjukdom skulle kunna tillstöta.**

Individer som är drabbade av reflux får ofta rådet av sin läkare att ändra sin livsstil, t.ex. att gå ner i vikt och att undvika vissa typer av mat. Det finns dock bara ett begränsat antal vetenskapliga studier om hur olika så kallade livsstilsfaktorer inverkar på risken för refluxsjukdom. För att närmare utreda sådana samband, och dessutom kunna ta hänsyn till om det är ärftlighet eller uppväxtmiljö som spelar in, genomförde vi **studie 3**. Vi använde oss av data från det Svenska Tvillingregistret. Detta register har uppgifter om alla tvillingar födda i Sverige från 1886 och framåt. Sammanlagt fann vi knappt 28 000 tvillingar som i början på 1970-talet tillfrågats om olika livsstilsfaktorer och sedan ca 30 år senare fått rapportera sin förekomst av refluxsymtom. Ungefär 4 000 personer (15 %) uppgav att de led av refluxsymtom. För att kunna se hur ärftlighet spelar in jämförde vi vidare förekomsten av reflux hos 869 enäggstvillingpar där den ena tvillingen led av refluxsymtom och den andra inte hade sådana symtom. Alla analyser gjordes separat för män och kvinnor. Vi fann att övervikt, rökning och hög fysisk aktivitet på arbetet ökar risken för refluxsymtom, medan hög fysisk aktivitet på fritiden skyddar mot refluxsymtom. De observerade sambanden var endast i måttlig grad påverkade av ärftlighet. **Resultaten från studie 3 visar att livsstilsfaktorer spelar stor egen roll för att förklara förekomsten av refluxsjukdomen.**

I en tidigare studie från vår forskningsgrupp etablerades ett samband mellan övervikt och refluxsymtom. Detta samband befanns vara särskilt starkt för kvinnor, framför allt före klimakteriet. Dessutom verkade behandling med det kvinnliga könshormonet östrogen efter klimakteriet stärka sambandet mellan övervikt och refluxsymtom. För att närmare undersöka hur kvinnliga könshormoner inverkar på risken för refluxsjukdom, och dessutom kunna utvärdera eventuell ärftlig påverkan, genomförde vi **studie 4** med data från samma register som i studie 3, det Svenska Tvillingregistret. Sammanlagt deltog ungefär 4 000 kvinnor med refluxsymtom och drygt 17 000 kvinnor utan refluxsymtom i studien. Hos de kvinnor som använde östrogenbehandling mot klimakteriebesvär var risken för refluxsymtom 60 % högre än för de kvinnor som inte använde östrogen. Detta samband kunde observeras både hos normalviktiga och överviktiga kvinnor, även om sambandet var något starkare för överviktiga kvinnor. Ärftlighet påverkade inte detta samband. Vi undersökte även sambandet mellan användning av det kvinnliga könshormonet progesteron och p-piller å ena sidan och risken för refluxsymtom å andra sidan. Risken för refluxsymtom tycktes öka vid användning av även dessa hormoninnehållande preparat, men sambandet var inte lika starkt och verkade dessutom delvis kunna förklaras av ärftliga faktorer. **Sammanfattningsvis pekar våra resultat i studie 4 på att kvinnor som behandlas med östrogen och har refluxsymtom skulle kunna överväga att på försök göra uppehåll i östrogenmedicineringen för att minska sina refluxsymtom.**

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