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ETIOLOGY OF GASTROESOPHAGEAL REFLUX

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ABSTRACT

Although gastroesophageal reflux disease is among the most common disorders at all in Western populations, the etiology is largely unknown. The objective of this thesis is to increase the knowledge of the etiology of reflux disease by studying a number of potential risk factors, using population-based methods.

The first paper of the thesis estimates the prevalence of reflux symptoms, both totally and as a function of age and sex, in a public health survey of the adult population of Nord-Trøndelag, Norway. Among the 58,596 persons included in this study 15,233 (26%) had experienced minor symptoms of heartburn or regurgitation while 3,154 (5.4%) had severe symptoms of heartburn or regurgitation. Among women there was a gradual increase in the prevalence of reflux symptoms with age, while among men the prevalence of reflux symptoms peaked in the 51 – 70 age groups, only to decline among the oldest men.

In the second paper, which was based on a case-control study including 184 cases of esophagitis and 184 control subjects, matched to the cases with regard to age, sex and area of residence, there was a dose-dependent association between increasing body-mass and endoscopically verified esophagitis among women, while no association was found among men. The association between body-mass and esophagitis among women was strengthened by the use of postmenopausal hormone replacement therapy.

The third paper, a cross-sectional case-control study nested within the public health survey from Nord-Trøndelag, including 3,113 case subjects with severe reflux symptoms and 39,872 asymptomatic control subjects (pregnant women were excluded), assessed the influence of body-mass and female sex-hormones on the risk of reflux symptoms. Dose-dependent associations between increasing body-mass and reflux symptoms were seen both among men and women, although 2-fold stronger among women. The association among women was stronger premenopausally than postmenopausally, and was significantly augmented by postmenopausal hormone replacement therapy (effect-modification), especially by estrogen-only hormone therapy. Weight loss during the ten year period between the two data collections of the health survey was associated to a significant 40% decrease in the risk of reflux symptoms.

The last paper, a case-control study investigating the effect of life-style exposures on the risk of symptomatic reflux, was nested within the above-mentioned Norwegian public health survey, and included 3,153 case subjects with severe reflux symptoms and 40,210 asymptomatic control subjects. Tobacco smoking, alcohol use, tea and coffee drinking, table salt use, dietary fibers in bread and physical exercise were assessed. The study demonstrated dose-dependent positive associations between tobacco smoking as well as table salt intake and the risk of reflux symptoms. Negative associations were seen between dietary fibers in bread and reflux symptoms and between physical exercise and reflux symptoms. No associations were seen between alcohol intake and reflux or tea drinking and reflux. A negative association was observed between coffee drinking and reflux, although this finding may be explained by reversed causality.

LIST OF ORIGINAL PAPERS

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

- I. Magnus Nilsson, Roar Johnsen, Weimin Ye, Kristian Hveem, Jesper Lagergren.
The prevalence of gastro-oesophageal reflux symptoms and the influence of age and sex. *Manuscript submitted*.

- II. Magnus Nilsson, Göran Lundegårdh, Lasse Carling, Weimin Ye, Jesper Lagergren.
Body mass and reflux oesophagitis: an oestrogen-dependent association?
Scand J Gastroenterol 2002;37(6):626-30.

- III. Magnus Nilsson, Roar Johnsen, Weimin Ye, Kristian Hveem, Jesper Lagergren.
Obesity and estrogen as risk factors for gastroesophageal reflux symptoms.
JAMA 2003;290(1):66-72.

- IV. Magnus Nilsson, Roar Johnsen, Weimin Ye, Kristian Hveem, Jesper Lagergren.
Life-style related risk factors in the aetiology of gastro-oesophageal reflux.
Manuscript submitted.

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LIST OF ABBREVIATIONS

GERD	Gastroesophageal reflux disease
LES	Lower esophageal sphincter
TLESR	Transient lower esophageal sphincter relaxations
HCl	Hydrochloric acid
BMI	Body mass index
NO	Nitric oxide
IBS	Irritable bowel syndrome
HRT	Hormone replacement therapy
HUNT	Helseundersøkelsen i Nord-Trøndelag
WHO	World Health Organization
OR	Odds ratio
CI	Confidence interval
SHBG	Sex hormone binding globulin
NSAIDs	Non-steroid anti inflammatory drugs
H. pylori	Helicobacter pylori
CagA	Cytotoxin-associated gene

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a significant public health problem in the industrialized countries of Europe, North America, Australia and New Zealand, while it seems to be less prevalent in the developing countries of Asia and Africa. Previous studies from Western populations suggest that between 27 and 59% of adults experience reflux symptoms [1-8], and between 9 and 25% do so at least weekly [1-5, 9]. Frequent reflux symptoms have a strong negative effect on the quality of life of affected persons [10, 11]. The treatment of reflux, given the high prevalence of reflux symptoms and the effective symptom relief provided by proton pump inhibitors, is very costly on a societal level [12-15]. Furthermore, symptomatic gastroesophageal reflux is the strongest known risk factor for adenocarcinoma of the esophagus [16, 17], a cancer with poor prognosis [18], and rapidly increasing incidence [19-23].

Knowledge about the etiology of GERD is limited. A majority of the research work published about reflux concerns either pathogenesis, i.e. the mechanisms of reflux or *how* reflux happens, or it concerns treatment of reflux. Pathogenesis is mainly studied by applying physiological laboratory methods on patients, volunteers or laboratory animals. Etiology, on the other hand, deals with the question *what* causes reflux, and is appropriately studied using epidemiological methods to identify risk factors, and subsequently evaluating causality. There is a scarcity of valid and precise studies concerning risk factors for GERD.

This thesis, based on four original papers, attempts to increase the knowledge about the internal and external factors that influence the occurrence of pathological reflux. By identifying valid risk factors we can provide a scientific basis for effective primary prevention, to the benefit of reflux patients in terms of quality of life and lower treatment costs, the health care system in terms of lower costs and more efficient resource utilization, and possibly also halt the rising incidence of esophageal adenocarcinoma. Moreover, increased knowledge about risk factors for GERD may provide important clues as to still unknown aspects of the pathogenesis of reflux.

BACKGROUND

DEFINITIONS OF GASTROESOPHAGEAL REFLUX

Gastroesophageal reflux is the passing of acid or other stomach contents, from the stomach into the esophagus, and often further up into the pharynx and oral cavity. From 24-hour ambulatory pH monitoring it is known that daily brief reflux episodes are normal and occur in virtually all healthy, asymptomatic individuals, especially postprandially [24-27]. Most reflux episodes are thus asymptomatic, and are neither associated with any morphologic changes in the esophageal mucosa, nor with symptoms [26, 27]. This may be called physiological gastroesophageal reflux.

In some individuals however, the exposure of the esophageal mucosa to refluxate is severe enough to cause symptoms in the form of heartburn, a burning sensation retrosternally. In still some individuals the refluxate is voluminous enough to pass into the oral cavity, called acid regurgitation. Frequent episodes of heartburn and/or acid regurgitation are highly specific for true gastroesophageal reflux, as measured by 24-hour ambulatory pH monitoring [28], and are considered the cardinal symptoms of reflux [28-31]. As many as 59% of adults, in some Western populations, experience heartburn or acid regurgitation occasionally [2]. When then are reflux symptoms considered pathological? When do they warrant the diagnosis gastroesophageal reflux *disease*? In epidemiological studies GERD classification has most commonly been based on symptom frequency, and the arbitrary cut-off point has been symptoms at least once weekly [9, 32, 33]. Another proposed definition of pathological reflux symptoms is based on when affected persons subjectively experience significant impairment of health related quality of life, due to reflux symptoms [25]. Moreover, there are a number of validated questionnaires to assess and quantify reflux symptoms [17, 29, 34-36].

Table 1. Ways of defining *pathological* gastroesophageal reflux (i.e. GERD)

Method	Criteria
Symptoms	Heartburn or acid regurgitation at least once weekly over a prolonged period of time. Alternatively, significantly impaired health related quality of life, due to reflux.
24 hour pH monitoring	Total percentage* of time with a distal esophageal pH < 4 and correlation between acid episodes and symptoms
Morphological signs	Esophagitis, esophageal ulcer, esophageal stricture, Barrett's esofagus

* Cut-off percentage varies between different laboratories, >3-4 % is generally considered pathological.

Another basis for classification of reflux as pathological depends on 24-hour pH monitoring, where the total percentage of time with a distal esophageal pH < 4, and correlation between acid episodes and symptoms, are the main determinants. This method has by some been called a gold standard for GERD diagnosis, an opinion challenged by others [25, 37], as false negative results are common due to natural day to day variation in reflux episode frequency, impairing the sensitivity of the method.

Finally, reflux induced morphological changes in the esophageal mucosa, such as esophagitis, esophageal ulcers, strictures and Barrett's esophagus [38] are sound bases for the diagnosis of GERD (Table 1), as specificity is high, although sensitivity for GERD is low.

PREVALENCE OF GASTROESOPHAGEAL REFLUX

Symptoms

The prevalence of heartburn and/or acid regurgitation, occurring at any frequency, seems to vary between 27 and 59% [1-6] (Table 1), while symptoms occurring at least weekly are reported by between 9 and 25% [1-5, 9] (Table 2), in studies performed among adults in Western populations.

Prevalence of reflux symptoms seems to be considerably lower in the developing countries of Asia and Africa. Studies from Asian populations demonstrate prevalence figures clearly lower than those from Europe and North America, with at least weekly reflux symptoms occurring in 2 – 3% [39-41] (Table 2). Similarly, a recent publication concerning the prevalence of reflux disease in Sub-Saharan Africa concluded that the prevalence of GERD and its complications is low [42].

It is generally assumed that the prevalence of reflux symptoms increases more or less linearly with age. The scientific basis for this assumption is weak, as only one study shows a significant age related increase [1], and others do not [2, 3, 43].

Most previous studies of the prevalence of symptoms of reflux are of small to moderate sample size, either not permitting combined age- and sex-stratification, or else compromising the precision of such analyses. Neither of two studies presenting age- and sex-stratified prevalence data revealed any significant difference between sexes in age-stratified prevalence [2, 43]. The need for such analyses with sufficient sample size to allow good precision, is further stressed by the strong (7:1) and unexplained male predominance among patients with esophageal adenocarcinoma [21].

24-hour pH monitoring-based prevalence

A few studies have attempted to quantify the prevalence of reflux in the normal population using ambulatory 24-hour pH monitoring [44-47]. In these studies it is clearly difficult to choose the correct cut-off point between normal and pathological, as there is substantial overlap in measured reflux between asymptomatic, endoscopy-negative individuals and symptomatic, endoscopy-positive ones [48]. Moreover, the invasive nature of pH-monitoring negatively affects the positive response rates of inclusion, potentially introducing severe selection bias.

Table 2. Previous population-based studies of the prevalence of gastroesophageal reflux symptoms

Author & reference	Publication year	Analyzed sample size	Response rate	Population origin	Prevalence	
					Heartburn and/or acid regurgitation ≥1/week	Of any frequency
Murray (4)	2003	4,902	38%	UK	13%*	46%*†
Wong (41)	2003	2,209	61%	Hong Kong	2.5%	30%
Khoshbaten (40)	2003	4,207		Iran	2.7%*‡	
Louis (3)	2002	2,000		Belgium	12%*	28%*
Haque (5)	2000	817	82%	New Zealand	16%	30%§
Kennedy (43)	2000	3,179	72%	UK		29%
Frank (7)	2000	2,056	20%	USA & Canada		20%*†
Talley (9)	2000	1,643	74%	USA	25%*	
		730	64%	Australia	15%*	
		401	80%	Germany	9%*	
		1,139	75%	Sweden	17%*	
Ho (39)	1998	696	93%	Singapore		1.6%§
Locke (2)	1997	1,511	73%	USA	20%	59%
Isolauri (1)	1995	1,700	68%	Finland	15%*	27%*¶
Agréus (8)	1994	1,156	90%	Sweden		19%†
Kay (6)	1994	3,608	79%	Denmark		45%¶**

* Heartburn only.

† ≥1 symptom episode during last 3 months.

‡ ≥3 symptom episodes during last 2 weeks.

§ ≥1 symptom episode during last month.

|| ≥6 symptom episodes during last 6 months.

¶ ≥1 symptom episode during last 12 months.

Esophagitis

As the diagnosis of reflux esophagitis depends on endoscopy, most studies of esophagitis prevalence are based on patients referred for endoscopy [49-51], reflux symptomatic patients recruited for clinical trials [35, 52, 53], or on healthy volunteers [54], all recruitment methods with potential problems of selection bias. In a study from Finland 167 of 1,128 patients (14.8%) referred for upper gastrointestinal endoscopy had esophagitis, esophageal ulcers or strictures [49]. Pooling of data based on primary care patients included in clinical trials of reflux symptoms demonstrated that 38% of study subjects with troublesome heartburn or acid regurgitation had endoscopic signs of esophagitis [25]. In a study of 355 healthy volunteers, Akdamar et al. reported that 8.5% had erosive esophagitis [54]. As of yet, there are no accurate data available on the true prevalence of reflux esophagitis in a population.

An early report suggested that esophagitis is more prevalent among elderly patients [55], and in an endoscopy series from a defined catchment area the severity of esophagitis increased significantly with age [56]. Other studies with up to 22 years follow-up of esophagitis patients do not support this notion [57, 58], and there is no firm evidence in favor of prevalence increasing with age.

Several endoscopy series show a male predominance among diagnosed cases of esophagitis [49, 51, 56], although care should be taken in the interpretation of these data, given the substantial risk of selection bias in this study design.

Is the prevalence of GERD increasing?

It's clear that the incidence of adenocarcinoma of the esophagus has increased over the last decades [19-23], but is reflux, its strongest known risk factor, also increasing? Because of the varying methods of assessing reflux, it is generally not possible to accurately compare older prevalence studies with recent ones. There is however, some evidence of increasing prevalence of GERD [59]. In a study from American veterans' hospitals hospitalization rates for erosive esophagitis increased 4-fold over the last three decades [59].

PATHOGENESIS OF GASTROESOPHAGEAL REFLUX

Limited postprandial reflux occurs every day in all individuals. Heartburn and morphologic mucosal changes, the indicators of tissue damage, however, occur in a minority of individuals. Four factors influence their occurrence: 1. The frequency, duration and extent of reflux. 2. Esophageal clearance of refluxate. 3. The composition of the refluxate. 4. Mucosal resistance to injury.

The anti-reflux barrier

A healthy individual can stand upside down after a heavy meal without any reflux of food into the esophagus or oral cavity. Evidently, there must be some type of valve-mechanism at the esophagogastric junction. This valve mechanism has three main components: 1. The resting pressure of the so-called high pressure zone in the distal esophagus. This resting pressure is maintained by the circular smooth muscle fibers in this 4-5 cm long segment, often referred to as the lower esophageal sphincter (LES) (Figure 1), and amounts to between 10 and 35 mm Hg [24, 60, 61]. This mechanism mainly prevents reflux at rest [24, 61] and may be called the static anti-reflux barrier. 2.

The striated muscle fibers of the crural diaphragm surround the esophagus at the level of the LES (Figure 1), augmenting the high pressure zone, especially increasing the pressure drastically during exercise, coughing and Valsalva maneuver, when abdominal pressure may reach very high levels [24, 61]. The rapid changes in abdominal pressure caused by physical activity are thus met by an almost simultaneous contraction in the crural diaphragm, effectively preventing exercise-associated reflux [24, 61]. This may be called the dynamic anti-reflux barrier. 3. The flap valve mechanism of the angle of His, by which the pressure in the gastric fundus creates a flap that presses against the intraabdominal portion of the esophagus, reinforcing the high pressure zone [24, 62]. This mechanism is active both at rest and during rapid fluctuations in abdominal pressure.

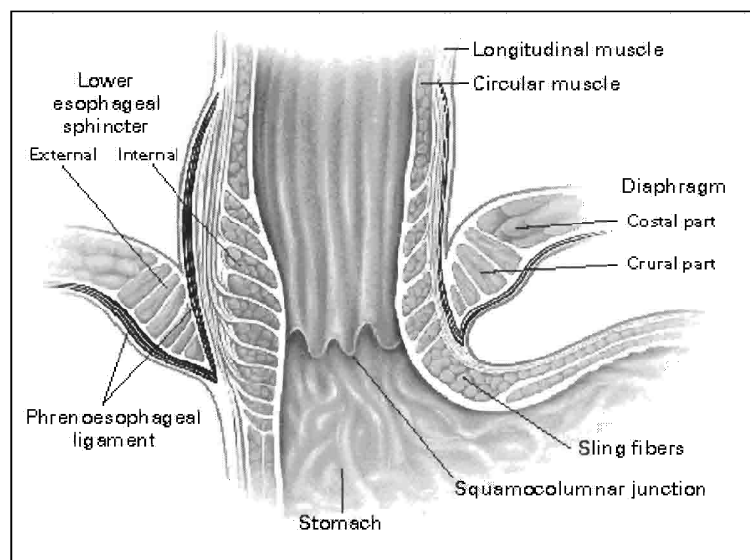


Figure 1. Anatomy of the esophagogastric junction. The lower esophageal sphincter and crural diaphragm are anatomically superimposed and constitute the internal static and external dynamic anti-reflux barriers, respectively. Adopted from Mittal et al. (24) with permission from the New England Journal of Medicine (copyright © 1997).

Reflux mechanisms

Transient lower esophageal sphincter relaxations (TLESR)

The mechanism behind physiological, as well as most pathological reflux, is transient lower esophageal sphincter relaxations [24, 61, 63]. These are periods of simultaneous relaxation in the smooth muscle of the LES and in the striated muscle of the crural diaphragm, resulting in a total loss of pressure in the esophagogastric junction [24, 61, 63]. These transient relaxations are evoked by distension of the gastric fundus, most commonly by the ingestion of food, physiologically facilitating the expulsion of swallowed air [64, 65], but normally also resulting in a short asymptomatic reflux episode [24, 66, 67]. Food with a high content of fat has been observed to evoke a higher frequency of TLESRs than food with low fat content, at the same level of fundal distension [63]. Interestingly, transient relaxations are most

easily evoked in the upright position, and do normally not take place when lying down flat on the back [24, 61, 63]. The distension of the gastric fundus results in the activation of receptors in the gastric wall and transmission of impulses via the vagus nerve to the brain stem where efferent signals are evoked, and simultaneously relax both the LES, (via the vagus nerve, nitric oxide being the postganglionic transmitter substance), and the striated muscle fibers of the crural diaphragm (via the phrenic nerves, acetylcholine being the postganglionic transmitter). These TLESRs, occurring postprandially in all individuals, normally last for 10 to 60 seconds [24, 61, 63, 68]. In some persons the postprandial frequency of TLESRs increase, resulting in more severe reflux, which may lead to mucosal damage, symptoms, and if persistent to GERD. The factors influencing the increase in TLESR frequency in some individuals, discriminating physiological from pathological reflux, are unknown.

Reduced LES pressure

Until the mid 1980s low resting pressure in the LES was thought to be the sole mechanism behind gastroesophageal reflux. At this time however, esophageal manometry studies revealed that most reflux patients had normal or even high LES resting pressures [68, 69]. It was recognized that in a majority of reflux patients, TLESRs was the mechanism of reflux. A subgroup of patients however, often with severe esophagitis, was noted to have reduced LES resting pressure [24, 68, 70].

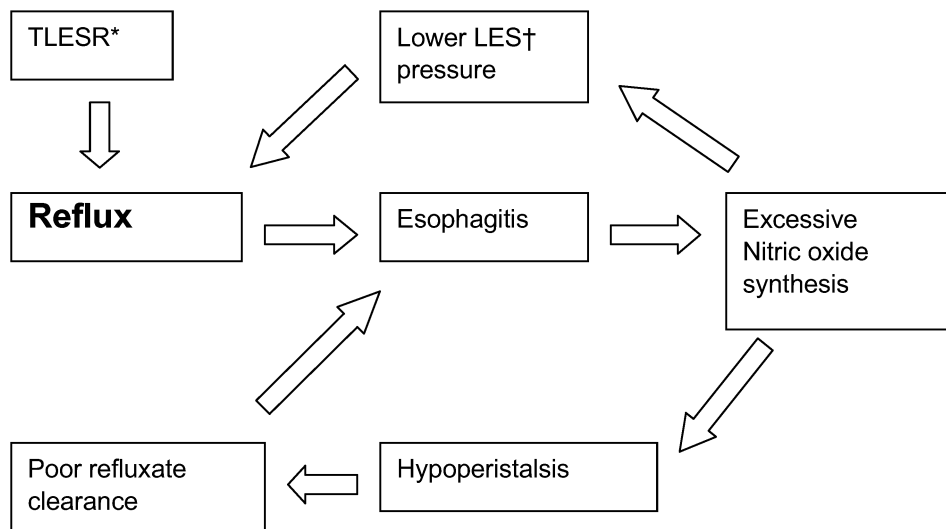


Figure 2. Hypothesis for the pathogenesis of GERD.

*TLESR (transient lower esophageal sphincter relaxation)
 † LES (lower esophageal sphincter)

It was further observed that patients with esophagitis often had low peristaltic amplitude in the distal esophagus, and thus poor clearance of refluxate [24, 66]. A plausible hypothesis to explain this chain of events, supported by data from an experimental model of feline esophagitis [71], would be that esophagitis induces mucosal synthesis of high concentrations of nitric oxide, the main relaxing transmitter substance of the esophagus, which then both lowers the resting pressure of the LES, and causes loss of esophageal peristaltic amplitude, resulting in both increased reflux and in poor clearance of refluxate (Figure 2).

Hiatal hernia

A large proportion of patients with moderate to severe reflux have hiatal hernias [24]. Hiatal hernias promote reflux by intervening with all three above mentioned anti-reflux barrier mechanisms: 1. The negative intrathoracic pressure may annul the LES pressure, facilitating reflux [72]. 2. Hiatal hernias are associated with a widening of the hiatus, making the anti-reflux effect of the crural diaphragm substantially less effective [72] 3. The flap-valve mechanism of the angle of His is abolished [73].

How then does hiatal hernia fit into the pathogenesis of GERD? There is some evidence that hiatal hernias may be caused by persistent reflux. Animal experimental studies have demonstrated that sliding hiatal hernias can be induced by means of experimental esophagitis, causing fibrosis and shortening of the longitudinal esophageal muscle layer, and subsequently hiatal hernia [74] (Figure 3). Alternatively, hiatal hernias may be caused by other etiologic factors suggested to be associated to reflux, such as genetic factors, obesity, strenuous exercise or lack of physical exercise.

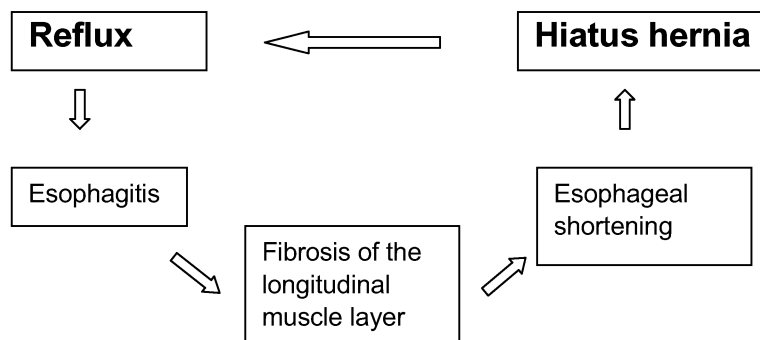


Figure 3. Hypothesis of hiatus hernia induction by esophagitis. Adopted from Paterson et al (74).

Refluxate composition

The noxious effects of refluxate are mainly caused by hydrochloric acid (HCl) and pepsin, which is activated from its inactive pro-form pepsinogen at pH < 3.5. Other potentially noxious components of the refluxate are deconjugated bile salts and pancreatic enzymes, both inactivated by an acidic pH, and thus unlikely to play a role in reflux disease [27]. Further, conjugated bile salts, although active at acidic pH, are unlikely to be of major importance in reflux-related esophageal mucosal injury, since 1. they are present in low, non-cytotoxic concentrations in the refluxate [75]; 2. typical signs of acid-bile injury are lacking in reflux injured esophageal mucosa [76-78].

Mechanisms of mucosal resistance to injury

As the esophageal mucosa in all individuals is exposed to refluxate from physiological reflux, in most persons without any mucosal damage, it is evident that there is a mucosal defense against injury. This defense has three levels [27]: 1. Pre-epithelially there is a thin but significant mucous layer, capable of sustaining a lumen-to-cellsurface pH gradient of 2:3 [27]. 2. The epithelial defense consists of an intercellular junctional complex, serving as a diffusion barrier [79], and of intercellular and cytosolic buffers (bicarbonate, phosphate, proteins), for acid neutralization [80]. 3. Post-epithelially the mucosa receives vital substrates such as oxygen, nutrients and additional bicarbonate, and removes potentially noxious agents such as hydrogen ions [27], which is vital to resistance, and repair of injury.

ETIOLOGY OF GASTROESOPHAGEAL REFLUX

Due to a scarcity of well designed, sufficiently powered epidemiological studies, knowledge about the causes of GERD is to a large extent lacking. Several etiological factors have been proposed, and in the following section I will discuss the most prominent of them, one by one.

Genetic factors

Genetic factors have for many years been suspected to be important in the etiology of GERD. In several studies familial aggregation of GERD has been observed [81, 82]. Although this aggregation may be explained by genetic factors, the sharing of a common environment can not be ruled out as the cause. Moreover, studies demonstrating familial aggregation of Barrett's esophagus suggest that in a minority of individuals with severe reflux, the liability to reflux disease is inherited in an autosomal-dominant manner [83, 84]. Furthermore, severe reflux with childhood onset has been shown to be inherited in an autosomal-dominant way [85], and seems to be associated with a gene of undetermined function mapped to chromosome 13q14 [86]. Carre et al. [85] were able to show that in a large five generation family that they studied, the childhood onset reflux was mediated by congenital hiatal hernia, with the same autosomal-dominant inheritance pattern.

For the majority of GERD patients, however, the influence of genetic factors until recently has been less clear. In 2002 Cameron et al. [87], in a large, population-based twin study, with reflux symptoms at least weekly as outcome measure, demonstrated that genetic factors are important in the etiology of GERD, and account for approximately 31 % of the liability to reflux disease in the studied population. This

finding has later been confirmed in another twin study, which estimated heritability to account for approximately 43% of the propensity for GERD [88] (Table 3).

Obesity

During the last decades the prevalence of obesity has increased in an almost epidemic manner in Western populations [89-92]. Among physicians there is by tradition a widespread belief that obesity causes reflux, and reflux patients are often recommended to lose weight in order to treat GERD. The scientific basis for this belief has during recent years gradually been strengthened, although some uncertainty still exists. Of five previous population-based studies estimating the risk of reflux symptoms associated with high body mass index (BMI), four, of which one cohort study [93] and three cross-sectional studies [1, 4, 32], concluded that obesity does increase the risk for reflux [1, 4, 32, 93], while in one study there was no association [33] (Table 3). No previous studies have examined whether obesity affects the propensity for reflux differently between the sexes.

If obesity causes GERD, what then could the mechanism be? A commonly suggested pathogenetic pathway is via increased abdominal pressure. This is unlikely to be the principal mechanism, as obesity only causes a moderate increase in abdominal pressure, and since high abdominal pressure alone does not cause reflux in experimental models [94, 95]. Another proposed mechanism is by obesity causing hiatal hernia, here possibly at least in part through increased abdominal pressure. This notion is supported by data from two studies; one a hospital-based case-control study that showed a strong and dose-dependent association between increasing BMI and esophagitis, which was substantially weakened upon adjustment for concomitant hiatal hernia [96], the other an endoscopy case-series assessing BMI in relation to esophagitis and hiatal hernia in which obesity was strongly associated with combined esophagitis and hiatal hernia [51].

Female sex-hormones

Female sex-hormones are known to be the predominant cause of gastroesophageal reflux during pregnancy, although there is some contribution by the increased abdominal pressure during the late stage of pregnancy [94, 95, 97, 98]. It has further been shown that the use of sequential oral contraceptives lowers the resting pressure of the LES [99]. There are no data available concerning whether it is estrogens, gestagens or both, that cause reflux during pregnancy, and lowering of the LES resting pressure during treatment with oral contraceptives. The effect of female sex-hormones has not been studied with respect to GERD in any previous population-based study.

Tobacco smoking

Several studies using esophageal pH-monitoring and/or esophageal manometry have presented convincing data indicating that tobacco smoking induces reflux episodes and among some individuals also corresponding reflux symptoms, mainly by lowering the LES resting pressure [67, 100-103]. It is generally believed, and supported by some evidence, that this effect is mediated by nicotine [103]. It is however, a completely different matter if long-term smoking causes GERD. Data from a population-based cohort study by Ruhl et al. demonstrated a non-significant but interesting positive dose-response trend between tobacco smoking and hospitalization for reflux disease, where the relatively low values of the point estimates may well be explained by severe

Table 3. Previous population-based studies concerning risk factors for gastroesophageal reflux

Author & reference	Publication year	Analyzed sample size	Response rate	Design	Exposures	
					Analyzed	Association (+, - or 0)§
Murray (4)	2003	4,902	38%	Cross-sectional	BMI*	+
Mohammed (88)	2003	3,920	56%	Twin study	Heritability†	+ (43%)
Cameron (87)	2002	16,802	76%	Twin study	Heritability†	+ (31%)
Haque (5)	2000	817	82%	Cross-sectional	Smoking Alcohol Aspirin	+ 0 0
Lagergren (33)	2000	820	73%	Cross-sectional	BMI	0
Locke (32)	1999	1,524	72%	Cross-sectional	PSCS‡ BMI Smoking Alcohol Coffee Aspirin	+ + + + 0 0
Ruhl (93)	1999	12,349		Cohort study	BMI Dietary fat Smoking Alcohol Coffee/tea Aspirin Physical exercise	+ 0 0 (+?) 0 0 0 -
Isolauri (1)	1995	1,700	68%	Cross-sectional	BMI Smoking	+ +

* Body mass index (BMI) is calculated as body weight in kilograms divided by the square of the body height in meters.

† Heritability here estimates the proportion of the variance in the liability to symptomatic reflux disease due to additive genetic factors.

‡ The psychosomatic symptom checklist score (PSCS) is in instrument designed to detect and quantify psychosomatic behavior.

§ Positive (+), negative (-) or no (0) association.

dilution of the association from major non-differential misclassification of the reflux outcome [93]. The other three previous population-based studies demonstrate clear associations between smoking and reflux symptoms [1, 5, 32], in support of the hypothesis that smoking causes GERD. Problems with these studies include relatively small sample sizes, with risk of chance findings, lack of dose-response analysis and cross-sectional design with risk of reversed causality (Table 3). In spite of these drawbacks, the consistency of the results between these studies, as well as the fact that reversed causality here means that reflux symptoms would cause smoking, which is highly unlikely, together with the point estimate trend seen in the cohort study mentioned above [93], points in the direction of a causal relation, although further studies are necessary before this conclusion can be drawn.

Alcohol

In resemblance to tobacco smoking, the intake of alcoholic beverages has been shown to evoke reflux episodes detected by esophageal pH-monitoring [104, 105], to relax the LES [106], and to induce reflux symptoms [104-108], immediately after ingestion. In conflict with studies of the immediate effects of alcohol, a study among chronic alcoholics demonstrated relative hypertension of the LES in this group [109]. Furthermore, available population-based data are conflicting, with one study suggesting a weak association [32], and two observing no association [5, 93] (Table 3). Further large population-based studies are necessary to elucidate the role of long-term alcohol exposure on the risk of GERD.

Dietary factors

Dietary fat

Several studies have demonstrated that meals with a high content in fat increase the frequency of postprandial TLESRs, and can induce reflux symptoms [24, 63]. Concerning the long term effects of high dietary fat intake, data is only available from one study [93]. In a cohort study by Ruhl et al. 12,349 persons were followed for a median of 18.5 years and followed-up for GERD associated hospitalization. Hospitalization for GERD was not associated to a diet high in fat, although it was associated to overweight and obesity [93] (Table 3). More studies are needed to elucidate the role of dietary fat in the etiology of GERD.

Dietary fibers

Dietary fibers, especially those of cereal origin, have been demonstrated to be associated with a reduced risk of esophageal and gastric cardia adenocarcinoma [110, 111], cancers for which GERD is the main risk factor. Could this protective effect be mediated by a reduction in the occurrence of reflux, i.e. dietary fibers have a protective effect against GERD? Another suggested mechanism by which dietary fibers may protect against esophageal and cardia cancers may be mediated by cereal fibers scavenging carcinogenic nitrosamines [110, 112, 113].

A possible biological mechanism by which dietary fibers may reduce the occurrence of reflux could be linked to nitric oxide production. In the acidic environment of the stomach large amounts of nitric oxide are produced non-enzymatically from nitrites in the diet [114-116]. Nitric oxide has a potent relaxing effect on the LES [117-122], which could promote reflux. Dietary fibers are well known to scavenge nitrites in the stomach [110, 112], thereby decreasing the availability of the substrate for non-

enzymatic nitric oxide synthesis. This might reduce nitric oxide concentration in the gastroesophageal junction, and thus prevent reflux.

There are no data from epidemiological studies available concerning the effect of dietary fibers on the risk of GERD.

Coffee and tea

Coffee drinking is widely believed to trigger reflux symptoms [123-125], but only one [126] out of three studies addressing the issue conclusively observed that, while the other two did not [107, 127]. In data from three epidemiological studies, one population-based cross-sectional [32], a population-based cohort-study [93] and an endoscopy series of persons going through endoscopy as a part of a general health check-up [50], coffee drinking was neither associated to reflux symptoms nor esophagitis, respectively. Although most of the available evidence points against that coffee drinking is associated to GERD, more studies are needed in order to determine the relation.

Tea drinking has been studied sparsely in relation to GERD. Only in one study of reflux episode triggering [127], in the above mentioned cohort study [93] and in an endoscopy series [50], has it been evaluated, and neither of these three reports demonstrate any association. Although an association seems unlikely, the available evidence from previous studies is inadequate to definitely rule out the possibility of tea playing a role in GERD etiology.

Helicobacter pylori

H. pylori infection of the stomach is a global phenomenon, affecting 25 – 50% of the population in the industrialized countries of Europe and North America, and 70 – 90% of the population in the developing countries of Asia, Africa and South America [128-130]. Historically it is likely that a vast majority of the population in all parts of the world have been infected [130]. During the last century, probably mainly influenced by socio-economic development, the prevalence of *H. pylori* has decreased substantially in Western populations, while it has remained high in developing countries [130]. In parallel to this development, the incidence of *H. pylori* associated disorders such as peptic ulcer disease and distal gastric cancer have decreased, while the incidence of adenocarcinoma of the esophagus and the gastric cardia have increased in Western populations. This transition has not taken place in the developing countries where the prevalence of *H. pylori* infection is still very high [128, 130, 131]. Moreover, the prevalence of GERD seems to be substantially higher in populations with low prevalence of *H. pylori* infection, than in populations where *H. pylori* prevalence is still very high [1-5, 9, 39, 40, 130]. These circumstances have prompted the hypothesis that *H. pylori* infection may protect against GERD, which has good support in the findings of several epidemiological studies, mainly from Asia. In these studies the risk of GERD has been demonstrated to be lower among persons infected with *H. pylori*, especially in infection with CagA-positive strains [132-139], indicating more virulent infection.

The effects of *H. pylori* infection on acid secretion are complex, and depend on both host factors and characteristics among different strains of *H. pylori* [140, 141]. Infection of the gastric antrum, which is associated with duodenal ulcer disease, causes increased gastrin secretion and subsequent *hypersecretion* of HCl [140-142], which would be likely to actually increase the risk of GERD. Infection also of the corpus and

fundus areas of the stomach (so-called pangastritis), in addition to causing increased gastrin secretion causes atrophy of the acid-producing parietal cells with severe decrease in acid secretion, causing a gastric pH above 4 (comparable to treatment with proton pump inhibitors) in most affected individuals [134, 141, 143-146]. Pangastritis is very common in the developing countries where *H. pylori* prevalence is high, and is associated to CagA-positive infection [140, 143]. This loss of acid secretion is completely reversible upon eradication of the *H. pylori* infection [141, 147]. To determine the net effect of *H. pylori* infection on GERD, population-based studies that can differentiate between pangastritis and antral infections, would be of great value.

Pharmaceutical drugs

Several pharmaceutical drugs such as nitroglycerines, β -receptor agonists, aminophyllines, anticholinergic drugs and benzodiazepines have been reported to relax the smooth muscle of the LES, [148-160]. These agents have been suggested both to precipitate reflux episodes, and through increased occurrence of reflux, cause adenocarcinoma of the esophagus [161]. Data from a population-based case-control study demonstrated that long-term use of the five groups of drugs mentioned above, both combined and each separately, were associated to adenocarcinoma of the esophagus, an effect which diminished on adjustment for reflux symptoms, suggesting that reflux contributed substantially to the effect [162]. Furthermore, in a recent cohort study of 92,986 adult patients hospitalized for asthma with an average follow-up of 8.5 years there was an overall 40 % increase in risk of esophageal adenocarcinoma [163]. This increase in risk may be explained by that exposure to β -agonists and aminophyllines cause reflux and subsequently also cancer, but may also be explained by reversed causality, i.e. that reflux, in addition to causing the cancer, also may have caused the asthma that gave rise to the hospitalization.

Aspirin (acetylsalicylic acid) and non-steroid anti-inflammatory drugs (NSAIDs) have been proposed to cause GERD by damaging the esophageal mucosa [93, 164] and thereby making it more vulnerable to the influence of physiological reflux. Three population-based studies have addressed this issue without finding any support for such an association [5, 32, 93], making it very likely that Aspirin/NSAIDs do not cause GERD (Table 3).

Physical exercise

In the cohort-study by Ruhl et al. [93], which is the only published study addressing this issue, both recreational and professional physical exercise decreased the risk of hospitalization for reflux-related diagnoses. A reasonable hypothesis to explain the mechanism for such an association could be that frequent and regular exercise could strengthen the crural diaphragm, thereby reinforcing the dynamic, striated muscle-part of the anti-reflux barrier (Table 3).

Psychosomatic and psychological factors

A cross-sectional population-based study [32] reported a moderate positive association between obtaining a high score in the psychosomatic symptom checklist score, a validated measure of somatization [165], and reflux symptoms. This finding can either be interpreted as suggesting a role for psychological factors, such as somatization, in the etiology of GERD, or it may be explained as a sign of bias from misclassification of somatization as true reflux, in studies using reflux symptoms as outcome. In some contradiction to the latter interpretation, are the findings of Agréus et al., who in a

longitudinal follow-up study of a population-based sample of individuals, reported that subjects with symptomatic GERD were a distinct and separate group from individuals with irritable bowel disease (IBS), and upper dyspepsia, and that very few individuals with GERD changed over time to any of the other groups [166]. Moreover, Johnston et al. compared psychological profiles of reflux symptomatic patients with and without endoscopic or pH-monitoring evidence of GERD, without finding any significant difference [167, 168].

Many reflux patients report worsening of symptoms under the influence of psychological stress in the daily life [169]. Several studies in laboratory settings have evaluated the effect of physiological and cognitive stress on the occurrence of reflux episodes, mainly yielding negative results [169, 170]. Data concerning the long-term effect of stress on the risk of GERD are not available [169].

Other factors

An association between cholecystectomy and GERD has been reported [93, 171, 172] and some investigators have proposed that cholecystectomy may cause changes in the motor function of the stomach and esophagus leading to GERD [171]. Others have suggested that this association is caused by a clustering of upper gastrointestinal disorders, and not by the cholecystectomy itself [173]. This proposed association needs further investigation for its potential role in the etiology of GERD to become clear.

Duodenal ulcer disease has been proposed to be positively associated to GERD [174-176]. This association is likely to be a result of antral *H. pylori* infection, resulting in acid hypersecretion, leading both to duodenal ulcer disease and GERD [140-142, 146].

AIMS

The aims of this thesis are:

- To estimate the prevalence of gastroesophageal reflux symptoms in an adult Western population, with the means of a large population-based study.
- To estimate the prevalence of gastroesophageal reflux symptoms among men and women of different ages, i.e. to determine to what extent age and sex influence the occurrence of reflux symptoms, and how these relations may be affected by potential risk factors.
- To investigate whether obesity is a risk factor for GERD.
- To determine whether there are any differences between the sexes in the effect of body-mass on the risk for GERD.
- To explore whether, female sex-hormones, most specifically in the form of post-menopausal hormone therapy (HRT), influence the risk of GERD, or the relation between obesity and GERD.
- To elucidate whether the potential effect of HRT on the risk of GERD, and on the influence of obesity on GERD, may be mediated by estrogens, gestagens or both.
- To investigate whether weight reduction may reduce the risk of GERD.
- To explore whether life-style related habits such as tobacco smoking, alcohol use, coffee use, tea use, table salt use, intake of dietary fibers in bread and physical exercise, influence the risk for GERD.

SUBJECTS AND METHODS

THE 'HUNT' PUBLIC HEALTH SURVEYS

Papers I, II and IV are based on data from Helseundersøkelsen i Nord-Trøndelag (HUNT), two consecutive public health surveys conducted in the Norwegian county of Nord-Trøndelag. The first survey, HUNT 1, was performed in 1984 – 1986 and included 74,599 individuals, representing 88.1% of the population from the year when population members turned 20 and older. Data for the second survey, HUNT 2, was collected in 1995 – 1997 and included 65,363 individuals, representing 71.2% of the population of age 20 and older. A total of 47,556 individuals, 72.8% of all HUNT 2 participants, participated in both surveys.

At local temporary research centers throughout the county of Nord-Trøndelag, all the participants of the two health surveys, completed extensive written questionnaires covering a wide variety of information about life-style, social relations, education and employment, diseases and symptoms etc. Moreover, all participants underwent a limited physical examination including measurements of height, weight and repeated assessment of blood pressure. A majority of individuals also underwent blood sampling, both for immediate analysis of routine parameters such as hemoglobin, glucose, lipids, liver enzymes, kreatinine etc, and for storage in a biobank for future research purposes. A total of 813 variables were collected and are included in the combined HUNT 1 and 2 databases.

Measurement of reflux symptoms

Reflux symptoms were assessed in the HUNT 2 survey only. In the HUNT 2 questionnaire study participants were asked whether they had experienced heartburn or acid regurgitation during the past 12 months, and if so, if the symptoms were minor or severe. Of 65,363 participants in HUNT 2, 58,596 (90%) responded to the question about reflux symptoms. Among the responders 40,210 (69%) had had no heartburn or acid regurgitation during the past 12 months, 15,233 (26%) reported minor symptoms, while 3,153 (5%) had experienced severe symptoms.

VALIDATION STUDY OF REFLUX SYMPTOMS

Heartburn and acid regurgitation are considered the cardinal symptoms of gastroesophageal reflux and have, when experienced frequently, been shown to have high specificity for true gastroesophageal reflux [28-31]. To evaluate the reflux assessment in HUNT 2, a validation study was performed, comparing the exact question in HUNT 2 concerning heartburn or acid regurgitation during the past 12 months, with an established questionnaire [17] assessing frequency of heartburn or acid regurgitation, duration of symptoms, effect on daily life, effect of anti-reflux medications, regular or on demand use of anti-reflux medication and nightly symptoms (See Appendix on page 54).

The validation study, including 1,102 subjects, was conducted among outpatients at general practitioners and the community hospital of Levanger in Nord-Trøndelag (N=371) and among outpatients at the Karolinska Hospital in Stockholm (N=731).

Table 4. Frequency and duration of symptoms in the validation study

Frequency of heartburn or acid regurgitation among persons reporting none, minor or severe symptoms during the last 12 months

	< Weekly N (%)	Weekly to < daily N (%)	≥ Daily N (%)	Total N (%)
None	719 (100)	0 (0)	0 (0)	719 (100)
Minor	236 (84)	37 (13)	7 (3)	280 (100)
Severe	7 (7)	46 (45)	50 (49)	103 (100)

Frequency of heartburn or acid regurgitation among persons reporting none, minor or severe symptoms during the last 12 months, including regular anti-reflux medication*

	< Weekly N (%)	Weekly to < daily N (%)	≥ Daily* N (%)	Total N (%)
None	719 (100)	0 (0)	0 (0)	719 (100)
Minor	210 (75)	27 (10)	43 (15)	280 (100)
Severe	5 (5)	24 (23)	74 (72)	103 (100)

Frequency of nightly symptoms among persons reporting none, minor or severe symptoms during the last 12 months†

	< Monthly N (%)	Monthly to < weekly N (%)	≥ Weekly N (%)	Total N (%)
None	719 (100)	0 (0)	0 (0)	719 (100)
Minor	221 (80)	45 (16)	10 (4)	276 (100)
Severe	25 (25)	29 (29)	45 (45)	99 (100)

Duration of reflux symptoms‡

	< 5 years N (%)	5 – 10 years N (%)	> 10 years N (%)	Total N (%)
Minor	151 (55)	41 (15)	85 (31)	277 (100)
Severe	59 (57)	18 (17)	26 (25)	103 (100)

* Persons taking regular anti-reflux medication are analyzed in the ≥ daily symptom group

† Missing data: 8.

‡ Missing data: 3.

The results were similar in Nord-Trøndelag and Stockholm. Out of the 1,102 participants, 103 (10%) persons reported severe symptoms of heartburn or regurgitation during the past 12 months. In this group with severe symptoms, 72% (68% in Nord-Trøndelag and 74% in Stockholm) reported heartburn or regurgitation occurring at least daily or were on regular anti-reflux medication, 23% (26% in Nord-Trøndelag and 22% in Stockholm) had heartburn or regurgitation one or several times per week, while 5% (5.9% in Nord-Trøndelag and 4.3% in Stockholm) had symptoms less frequently than once weekly (Table 4). Hence, 95% (94% in Nord-Trøndelag and 96% in Stockholm) of the validation study participants corresponding to the case group in papers III and IV had experienced reflux symptoms at least once per week during the preceding 12-month period. The specificity for symptoms occurring at least once per week among persons with severe symptoms during the last 12 months was 99.5% (99.4% in Nord-Trøndelag and 99.6% in Stockholm) and the corresponding sensitivity was 58% (78% in Nord-Trøndelag and 52% in Stockholm).

Among the 280 (25%) subjects who reported minor reflux symptoms in the validation study, 15% (9% in Nord-Trøndelag and 16% in Stockholm) had daily symptoms or used anti-reflux medication on a regular basis, 10% (17% in Nord-Trøndelag and 9% in Stockholm) had symptoms at least once per week, and 75% (74% in Nord-Trøndelag and 75% in Stockholm) had symptoms less frequently than once a week. Hence, the majority of validation study participants reporting minor symptoms, and thus corresponding to the group excluded from the main analyses in papers III and IV, had reflux symptoms occurring less frequently than once per week (Table 4).

Among study participants reporting severe symptoms 45% had nightly symptoms at least weekly, 29% at least monthly and only 25% less than monthly, while in the group reporting minor symptoms the corresponding percentages were 4%, 16% and 80%, respectively (Table 4).

Concerning the duration of reflux symptoms, 57% of those who reported severe symptoms had less than five years duration of symptoms, 17% had experienced their symptoms for between five and ten years, while 25% had had symptoms for more than ten years. Among those participants reporting minor symptoms, 55% had experienced symptoms for less than five years, 15% for between five and ten years and 31% for more than ten years (Table 4).

PAPER I

Design

Using the HUNT 2 public health survey we performed a population-based study of the prevalence of reflux symptoms, specifically heartburn or acid regurgitation, during the last 12 months.

Statistical analyses

The prevalence of any, minor and severe heartburn or acid regurgitation during the last 12 months, was calculated as the frequency of study subjects corresponding to these groups among all persons who answered the reflux question in the survey. Furthermore, the study participants were stratified by sex and age. Age was categorized in 10-year intervals. P-values from Chi-square tests were calculated to evaluate the differences in prevalence between sexes in the various age categories.

The HUNT 2 survey did not specifically assess symptoms occurring at least weekly, which is a commonly used measure of reflux disease in epidemiological studies. To facilitate comparisons between our results and previous prevalence studies, we calculated at least weekly symptom frequencies by extrapolating the reflux symptom frequency data found in our validation study. We multiplied the number of persons with severe symptoms in the survey with 0.95, i.e. the proportion of persons with severe symptoms in the validation study that had symptoms at least weekly. Thereafter, we multiplied the number of persons with minor symptoms in the survey with 0.25, i.e. the proportion of persons reporting minor symptoms in the validation study that had symptoms at least weekly. Finally, we added these two categories and divided the sum by the total number of study persons. Hence, for this calculation we used the formula: *Prevalence at least once weekly = (number of persons with severe symptoms in the survey x 0.95 + number of persons with minor symptoms in the survey x 0.25) divided by all participants answering the reflux question in the survey.* We then applied the same formula to all age and sex strata described above.

To determine whether any differences found in prevalence between men and women in different age strata could be explained by confounding from suspected risk factors, i.e. body-mass index (BMI), tobacco smoking, alcohol use, coffee drinking, intake of table salt, intake of dietary fibers, or physical exercise, we performed multivariate analyses using unconditional logistic regression. All suspected confounding variables were categorized to facilitate logistic regression analysis. We categorized BMI into four categories. Tobacco smoking was categorized by lifetime total number of cigarettes smoked and grouped into six categories. Alcohol consumption, defined as the number of occasions on which wine, liquor or non-low-alcohol beer had been consumed during the preceding two weeks using data from HUNT 1, i.e. prospectively collected, was grouped into four levels. Coffee drinking was assessed by reported average daily coffee use and categorized into four groups. Table salt use (prospective data collected in HUNT 1), was assessed by the average frequency of meals of salted fish or meat and categorized into five exposure levels, and by the average use of extra salt on regular meals categorized into four groups. Dietary fiber content in bread, expressed as the dry weight percentage of the type of bread predominantly consumed, was categorized into four levels. Regular physical exercise of at least 30 minutes duration (HUNT 1, prospectively collected data) was categorized into four groups corresponding to increasing frequency of exercise. The potential confounding effect of each variable was tested by introducing them one by one into the multivariate model. Possible interaction between age and sex was tested by introducing a cross-product term into the logistic regression model.

PAPER II

Design

This study is a population-based case-control study, matched for age, sex and area of residence, attempting to test the hypothesis that obesity is associated with reflux esophagitis.

Data collection

Patients with endoscopically diagnosed reflux esophagitis were recruited at 17 county and community hospitals in 11 out of a total of 20 Swedish counties, as part of a clinical trial of a proton-pump inhibitor. Since residents in the 11 counties under study

receive medical care at these county and community hospitals, and very few exceptions to this occur, our sampling of esophagitis patients can be considered to be population-based. All patients older than 16 years, presenting with esophagitis at endoscopy, at all the participating centers, where eligible as cases in the present study, during the study period of October 1996 to November 1997. A total of 224 patients with esophagitis, grade I to IV in the modified Savary-Miller classification [177, 178], were invited to participate in the study and all patients (100%) accepted participation. The patients completed a written questionnaire at the time of endoscopy.

The control subjects were randomly selected by Statistics Sweden from the computerized and continuously updated national population register. They were matched for age, sex and area of residence. One control subject was selected for each case. The sampled 224 controls received, by mail, the same questionnaire as the case subjects. Of the sampled controls 184 (82%) completed and returned the questionnaire. Of the 40 control subjects that after an additional letter was sent to them, still did not respond, 36 were men and 4 women. Among the remaining 184 case-control pairs, information about height or weight was missing for three case patients and two control subjects, thus leaving a remainder of 179 case-control pairs for further analysis. The questionnaire covered the following exposure data: age, sex, height (in meters), current weight (in kilograms) [179, 180], current tobacco smoking habits, previous cholecystectomy and previous and present pharmaceutical drug use. For all study subjects BMI, which is well validated as a measure of body mass independent of height [181], was calculated as body weight in kilograms divided by the square of body height in meters (kg/m^2).

Statistical analyses

The cut-off points for BMI were based on the World Health Organisation (WHO) classification of overweight and obesity [182]. A BMI value between 25 and 30 represents overweight and BMI higher than 30 was defined as obesity. Thus, a BMI value of less than 25 was regarded as normal, and this cut-off level was used as the reference category in comparisons between BMI levels. Odds ratios (OR) and 95% confidence intervals (CI) derived from conditional logistic regression were used to assess the association between body mass and risk of reflux esophagitis [183]. Linear trend of the association was tested by treating categorical variables as continuous in the model. Potential confounding effects by smoking and pharmaceutical drug use were controlled by introducing these variables into the model. The potential interaction-effect between overweight/obesity and estrogen use was tested by introducing a cross-product term, representing the interaction between the two variables, into the model.

PAPER III

Design

A population-based, cross-sectional, case-control study performed among participants of the HUNT public health surveys. The objectives of the study were to study the relation between reflux symptoms and obesity, to test the hypothesis that there is a difference between the sexes concerning this relation, and to evaluate whether female sex hormones, particularly HRT, influence the relation. Furthermore, this study evaluates whether changes in BMI during the decade between HUNT 1 and 2 influence the risk of reflux symptoms, especially if weight-loss is associated to a corresponding decrease in the risk of reflux symptoms.

Among the 58,596 persons answering the reflux question in HUNT 2, the 3,153 reporting severe heartburn or acid regurgitation were categorized as cases and the 40,210 without reflux symptoms were classified as control subjects in the study. The 15,233 persons who reported minor symptoms of heartburn or regurgitation were excluded from the analyses as the symptoms in this group may be less specific for true reflux, with an increased risk of misclassification of the reflux outcome. To eliminate the risk of confounding from pregnancy to influence relations between BMI, female sex hormones and reflux, the 378 pregnant women in the study were excluded, leaving 3113 cases and 39,872 control subjects for analysis.

Statistical analyses

In the main analyses the BMI data were from the HUNT 2 survey. The cut-off points for BMI were pre-determined and based on the WHO classification of overweight and obesity [182]. A BMI value between 25 and 30 represents overweight and BMI higher than 30 is defined as obesity. We further defined a BMI higher than 35 as representing severe obesity. A BMI value lower than 25 is regarded as normal, and all persons with a BMI value lower than this cut-off level constituted the reference group in comparisons between BMI levels. ORs and their 95% CIs, derived from unconditional logistic regression, were used to assess the association between BMI and the risk of reflux [183]. Linear trends of the associations were tested in a multivariate model by treating categorical variables as continuous.

Potential confounding effects of age (in ten year intervals), tobacco smoking (years of daily smoking), alcohol drinking (number of alcoholic beverages consumed during the two weeks preceding data collection), and asthma medication (months of daily use during the last 12 months) were controlled by introducing these variables, one by one, into the model. For all potential confounders except asthma medication, for which only data from HUNT 2 was available, there were data from both surveys. Whenever possible, HUNT 1 data, or HUNT 2 data of lifetime exposure, were used rather than cross-sectional HUNT 2 data, in order to lessen the risk of reversed causality, i.e. that the level of exposure is affected by the occurrence of reflux symptoms.

The potential interaction effect (i.e. effect modification) between body mass and HRT was tested by introducing a cross-product term, representing the interaction between the two variables, into the model. To investigate differences in the risk of reflux related to BMI between pre- and postmenopausal women, female study subjects were stratified according to menstrual status and then entered into the multivariate model. The effect of weight loss and weight gain were assessed by entering the net change in BMI between the HUNT 1 and HUNT 2 surveys into the model, with adjustment for baseline BMI (i.e. the BMI measured in the HUNT 1 survey).

PAPER IV

Design

A population-based, case-control study performed among participants of the HUNT surveys, aiming to evaluate the relation between habits related to life-style and symptomatic gastroesophageal reflux. The life-style related exposures studied were tobacco smoking, the intake of alcohol, coffee, tea, table salt and dietary fibers in bread, and finally the effect of physical training. Smoking, alcohol, coffee and tea have all been previously proposed to be risk factors for reflux. Dietary fibers and physical exercise have been proposed to be protective against reflux. The intake of table salt has

not previously been discussed as a potential risk factor for reflux, and was analyzed only because of the unique salt exposure data available, due to the common habit of eating salted food, in the population of Nord-Trøndelag.

Among the participants of the HUNT 2 survey, the 3,153 reporting severe heartburn or acid regurgitation were categorized as cases and the 40,210 without reflux symptoms were classified as control subjects in the study. The 15,233 persons who reported minor symptoms of heartburn or regurgitation were excluded from the study as the symptoms in this group may be less specific for true reflux, compared to the group reporting severe symptoms.

Statistical analyses

All variables under study were categorized to facilitate unconditional logistic regression analysis. The categories for the number of years of daily tobacco smoking (cigarette, pipe or cigar) were < 1 year (reference), 1 – 5 years, 6 – 10 years, 11 – 20 years, and > 20 years. The lifetime total number of cigarettes smoked was grouped into < 100 cigarettes (reference), 101 – 25,000, 25,001 – 50,000, 50,001 – 100,000, 100,001 – 200,000, and finally > 200,000 cigarettes. The frequency of alcohol consumption, defined as the number of occasions on which wine, liquor or non-low-alcohol beer had been consumed during the preceding two weeks (HUNT 1, prospectively collected data), were grouped into: none (reference), 1 – 4 occasions, 5 – 10 occasions, and > 10 occasions. Estimated average daily coffee use (HUNT 2, cross-sectional data) was categorized into < 1 cup (reference), 1 – 3 cups, 4 – 7 cups, and > 7 cups. Daily average tea use (HUNT 2, cross-sectional data) was categorized into < 1 cup (reference), 1 – 3 cups and > 3 cups. Table salt use (HUNT 1, prospectively collected data) was assessed by two variables. First, the average frequency of meals of salted fish or meat was categorized into never (reference), < 3 / month, once / week, twice / week and > twice / week. Second, the average use of extra salt on regular meals was grouped into never (reference), sometimes, often and always. Dietary fiber content (HUNT 2, cross-sectional data), expressed as the dry weight percentage of the type of bread predominantly consumed, was categorized into plain white bread: 1 – 2 % (reference), soft medium fiber bread: 4 – 7 %, soft high fiber content bread: 6 – 10 %, and finally hard bread: 14 – 16 %. Regular physical exercise of at least 30 minutes duration (HUNT 1, prospectively collected data) was categorized into never (reference), < 1 / week, 1 – 3 / week, and > 3 / week.

ORs and their 95% CIs, derived from unconditional logistic regression, were used to assess the association between the potential risk factors under study and the risk of reflux symptoms [183]. Linear trends of the associations were tested by treating categorical variables as continuous in the multivariate model. Potential confounding effects of age, sex, body mass index, asthma medication as well as all the exposure variables under study, were tested by introducing them one by one into the model.

RESULTS

PAPER I

Prevalence of any, minor and severe reflux symptoms

Among the 58,596 study subjects, 40,210 (68.6%) reported no reflux symptoms, 15,233 (26.0%) reported minor symptoms, and 3,153 (5.4%) had experienced severe symptoms of reflux. The total prevalence of reflux during the last 12 months, combining study participants with minor and severe symptoms, was 33.1% for men, 29.8% for women and 31.4 % for all study participants together (Table 5).

The prevalence of reflux symptoms by age and sex is listed in Table 5. The prevalence of study participants who experienced minor reflux symptoms during the last 12 months was 27.6% among men, and 24.5% among women. Among women, the prevalence of minor reflux symptoms increased linearly with age from 18.4% in the 19 – 30 age group until the age of 60, after which it leveled-off at a prevalence of 29.7%. Among men, the prevalence of minor reflux symptoms increased from 22.1% in the 19 – 30 age group to peak at 29.9% in the age group 51 – 60 years, after which it gradually declined in the older age strata.

The prevalence of severe reflux symptoms was 5.5% among men and 5.3% among women. Among women, the prevalence increased linearly with age, and no leveling-off was found. The prevalence of severe symptoms among women was lowest at 3.6% in the youngest age category (19 - 30 years), and highest at 7.9% among women older than 70. Among men, the age stratified prevalence pattern of severe symptoms bears resemblance to that observed concerning minor symptoms. The prevalence of severe heartburn or acid regurgitation among men was lowest at 3.7% in the youngest age stratum to gradually increase to a peak in prevalence at 6.3% in the 61 - 70 age group, after which it declined to 5.2% among men older than 70.

Table 5. Prevalence of minor, severe and any symptoms of heartburn or regurgitation during the last 12 months, in different age groups.

Age groups	Minor symptoms			Severe symptoms			Any symptoms					
	Men %	Women %	P-value* %	All %	Men %	Women %	P-value* %	All %	Men %	Women %	P-value* %	All %
19-30	22.1	18.4	<0.001	20.1	3.7	3.6	0.628	3.7	25.8	22.1	<0.001	23.8
>30-40	27.5	21.9	<0.001	24.5	5.7	4.0	<0.001	4.8	33.2	25.9	<0.001	29.3
>40-50	28.9	23.6	<0.001	26.2	6.0	4.3	<0.001	5.1	34.9	27.9	<0.001	31.3
>50-60	29.9	27.0	<0.001	28.5	6.0	5.8	0.282	5.9	36.0	32.8	<0.001	34.3
>60-70	28.2	29.7	0.048	28.9	6.3	7.7	0.005	7.0	34.4	37.4	0.006	35.9
>70	28.6	29.7	0.070	29.2	5.2	7.9	<0.001	6.6	33.8	37.5	<0.001	35.8
All ages	27.6	24.5	<0.001	26.0	5.5	5.3	0.020	5.4	33.1	29.8	<0.001	31.4

* Chi square test

Prevalence of reflux symptoms occurring at least once weekly

The prevalence of reflux symptoms occurring at least once weekly during the last 12 months, extrapolated from the validation study as described in the methods section, was 11.6%, without any major difference between sexes (data not shown). The pattern by age groups was similar to that found among persons with severe reflux symptoms (data not shown).

Sex-stratified risk of severe reflux symptoms

Overall, the multivariate analysis revealed no statistically significant difference in the risk of reflux symptoms between men and women, although the overall multivariately adjusted relative risk estimate was 20% higher among men (OR 1.2, 95% CI 0.8-1.9). The risk of severe reflux symptoms was significantly 60% higher among men than among women in the age groups 19 – 30 and 31 – 40, using univariate logistic regression (Table 6), but after adjustment for the potential confounding variables listed in the methods section, this difference in risk between sexes diminished (Table 6). The variables mainly explaining this positive confounding effect were physical exercise and dietary intake of table salt (data not shown). Using univariate analysis, the risk of severe reflux was significantly 20% lower among women than among men in the 61 - 70 age group and 40% lower in the >70 age group. These point estimates did not change when multivariate analysis was used, indicating lack of significant confounding from any of the potential confounding factors tested in the model (Table 6). The P-value for the interaction term between age and sex was <0.0001, indicating significant effect modification.

Table 6. The influence of sex on the risk of severe symptoms of gastroesophageal reflux in different age groups*

Age	Univariate		Multivariate*					
	OR†	95 % CI‡	OR†	95 % CI‡	OR†	95 % CI‡	OR†	95 % CI‡
	Women		Men		Women		Men	
19-30	1.0	(reference)	1.1	(0.8-1.3)	1.0	(reference)	1.1	(0.9-1.4)
31-40	1.0	(reference)	1.6	(1.3-1.9)	1.0	(reference)	1.2	(0.7-1.9)
41-50	1.0	(reference)	1.6	(1.3-1.8)	1.0	(reference)	1.2	(0.9-1.7)
51-60	1.0	(reference)	1.1	(0.9-1.3)	1.0	(reference)	1.0	(0.7-1.3)
61-70	1.0	(reference)	0.8	(0.6-0.9)	1.0	(reference)	0.8	(0.6-1.1)
>70	1.0	(reference)	0.6	(0.5-0.8)	1.0	(reference)	0.6	(0.4-0.8)
All ages	1.0	(reference)	1.1	(1.0-1.2)	1.0	(reference)	1.2	(0.8-1.9)

*Body mass index, tobacco smoking, alcohol and coffee drinking, table salt intake, dietary fibers in bread and physical exercise were included in the logistic regression model.

† Odds ratio.

‡ Confidence interval.

P-value for interaction term between age and sex <0.0001.

PAPER II

BMI and risk of esophagitis

Of the 179 matched case-control pairs analyzed, 71 pairs were comprised of women and 108 of men. Among men, no association between overweight and/or obesity and the risk of reflux esophagitis was found (Table 7). Further control for smoking and pharmaceutical drug use did not change the results significantly (data not shown). Among women, on the other hand, there was a strong and dose-dependent association between increasing BMI and the risk of reflux esophagitis (p -value for trend = 0.0007), after controlling for the use of asthma medication. Among overweight women (BMI 25-30) the OR for reflux esophagitis was 2.9 (95% CI: 1.1-7.6), compared to women of normal body mass (BMI <25). Among obese women (BMI >30), the risk of reflux esophagitis increased almost 15-fold compared to persons of normal weight (OR 14.6; 95% CI: 2.6-80.9). Further control for smoking and HRT did not substantially alter the results of the study (Table 7).

Table 7. Number of cases and controls and risk of reflux esophagitis in different BMI categories among men and women.*

	Men		Women	
	Number of Cases/controls	OR† (95 % CI‡)	Number of Cases/controls	OR (95 % CI)
BMI§ < 25	30 / 34	1.0 (reference)	19 / 42	1.0 (reference)
BMI 25 - 30	69 / 62	1.2 (0.7 - 2.2)	33 / 26	2.9 (1.1 - 7.6)
BMI > 30	9 / 12	0.8 (0.3 - 2.3)	19 / 3	14.6 (2.6 - 80.9)
P-value for linear trend		0.91		0.0007

* The use of asthma medication was included in the model. Smoking and HRT were also tested, but excluded from the final model as they did not contribute significantly to the explained variance

† Odds ratio.

‡ Confidence interval.

§ Body mass index (BMI) was calculated as body weight in kilograms divided by the square of the height in meters.

Hormone replacement therapy, BMI and risk of esophagitis

Among women there seemed to be an interaction-effect between overweight/obesity (BMI > 25) and HRT (p -value = 0.08), and HRT tended to increase the association between overweight/obesity and esophagitis. The OR for the risk of reflux esophagitis among non HRT-treated women with a BMI > 25 was 3.4 (95% CI: 1.3-8.8) and the corresponding OR for estrogen medicating females was 9.7 (95% CI: 1.7-55.6) (Table 8).

Table 8. Joint effects of postmenopausal hormone replacement therapy (HRT) and BMI on risk of reflux esophagitis among women*

	HRT	Number of cases / controls	OR† (95% CI‡)
BMI§ < 25	No	18 / 34	1.0 (reference)
BMI < 25	Yes	1 / 8	0.4 (0.04 - 3.5)
BMI ≥ 25	No	40 / 27	3.4 (1.3 - 8.8)
BMI ≥ 25	Yes	12 / 2	9.7 (1.7 - 55.6)

* The use of asthma drugs was also included in the model.

† Odds Ratio.

‡ Confidence interval.

§ Body mass index (BMI) was calculated as body weight in kilograms divided by the square of body height in meters.

PAPER III

The relation between BMI and reflux symptoms

After exclusion of pregnant women, 3,113 case subjects with severe reflux symptoms and 39,872 asymptomatic control subjects remained for analysis. Among men, a moderate and dose-dependent association between increasing BMI and reflux symptoms was observed in the multivariate analysis (P for trend < 0.0001) (Table 9). Severely obese men (BMI > 35) demonstrated a more than 3-fold increase in risk of reflux symptoms (OR 3.3; 95% CI: 2.4 – 4.7), compared to men of normal weight (BMI < 25). The corresponding analysis among women revealed a similarly dose-dependent, but clearly stronger association, compared to the one observed in men (P<0.0001) (Table 9). There was a more than 6-fold increase in risk of reflux symptoms (OR 6.3; 95% CI: 4.9 – 8.0) in severely obese women (BMI > 35), compared to women of normal weight (BMI < 25). Adjustment for age only, revealed no major differences compared to the multivariate analyses, indicating lack of significant confounding by the variables that were tested (data not shown).

Menopause, BMI and reflux symptoms

The association between BMI and reflux symptoms was significantly more pronounced in premenopausal than in postmenopausal women (P<0.0001) (Table 10). There was an almost 7-fold increased risk of reflux symptoms in severely obese (BMI > 35) premenopausal women (OR 6.8; 95% CI: 4.7 – 9.7), while after menopause the risk was 4-fold increased (OR 4.2; 95 % CI: 3.2 – 5.5) compared to women of normal weight with the same menstrual status.

Table 9. Association between obesity and the risk of reflux symptoms in men and women* (pregnant women excluded from analysis)

	Men				Women			
	Number of		OR	(95% CI)	Number of		OR	(95% CI)
Cases	Controls	Cases			Controls			
BMI† < 25	317	7,378	1.0	(reference)	401	10,558	1.0	(reference)
BMI 25 - 30	916	9,151	2.2	(2.0 - 2.6)	612	7,402	2.0	(1.7 - 2.4)
BMI 30 - 35	271	1,926	3.1	(2.6 - 3.6)	365	2,245	3.9	(3.3 - 4.7)
BMI > 35	46	289	3.3	(2.4 - 4.7)	159	687	6.3	(4.9 - 8.0)
P-value for linear trend	< 0.0001				< 0.0001			

* In the multivariate logistic regression model adjustments were made for age, tobacco smoking, asthma medication and postmenopausal hormone replacement therapy (HRT) in women. Alcohol use was tested in the model but omitted in this presentation since it did not have any significant confounding effect.

†Body mass index (BMI) was calculated as body weight in kilograms divided by the square of the body height in meters using HUNT 2 data.

P-value for interaction term between BMI and sex <0.0001.

Missing BMI values: 280.

Table 10. Association between obesity and the risk of reflux symptoms among pre- and postmenopausal women* (pregnant women excluded from analysis)

	Premenopausal				Postmenopausal			
	Number of		OR	(95% CI)	Number of		OR	(95% CI)
Cases	Controls	Cases			Controls			
BMI† < 25	211	6909	1.0	(reference)	167	3020	1.0	(reference)
BMI 25 - 30	219	3579	2.0	(1.6 - 2.5)	354	3335	1.9	(1.6 - 2.3)
BMI 30 - 35	105	878	3.9	(3.0 - 5.1)	230	1234	3.2	(2.6 - 4.0)
BMI > 35	57	232	6.8	(4.7 - 9.7)	96	405	4.2	(3.2 - 5.5)
P-value for linear trend	<0.0001				< 0.0001			

* In the multivariate logistic regression model adjustments were made for age, tobacco smoking asthma medication and postmenopausal hormone replacement therapy (HRT). Alcohol use was tested in the model but omitted in this presentation since it did not have any significant confounding effect.

†Body mass index (BMI) was calculated as body weight in kilograms divided by the square of the body height in meters using HUNT 2 data.

P-value for interaction term between BMI and menopause status <0.0001.

Missing menopause status data: 1,687 (7.3 %).

Table 11. Risk of reflux symptoms among women in different BMI* categories including status of hormone replacement therapy (HRT)†

	BMI < 25	BMI 25 - 30	BMI 30 - 35	BMI > 35	All BMI categories
Never HRT					
Cases‡	253	347	200	92	892
Controls‡	7,613	4,941	1,458	441	14,453
OR (95% CI)	1.0 (ref.§)	2.0 (1.7 - 2.4)	3.8 (3.1 - 4.6)	5.5 (4.2 - 7.2)	1.0 (ref.**)
Ever HRT					
Cases‡	45	101	58	32	236
Controls‡	967	826	198	55	2,046
OR (95%CI)	1.3 (0.9 - 1.8)	3.3 (2.5 - 4.2)	7.9 (5.7 - 11.0)	16.0 (10.0 - 25.6)	1.7 (1.5 - 2.0)
Present HRT					
Cases‡	32	57	34	21	144
Controls‡	681	570	128	41	1420
OR (95%CI)	1.3 (0.9 - 1.9)	2.7 (1.9 - 3.6)	7.4 (4.9 - 11.1)	14.4 (8.2 - 25.1)	1.5 (1.3 - 1.9)
Previous HRT					
Cases‡	13	44	24	11	92
Controls‡	286	256	70	14	626
OR (95%CI)	1.3 (0.7 - 2.3)	4.5 (3.2 - 6.5)	8.7 (5.3 - 14.3)	20.2 (8.9 - 46.1)	2.1 (1.7 - 2.7)
Ever HRT, post hysterectomy††					
Cases‡	8	15	10	8	41
Controls‡	103	117	38	7	265
OR (95%CI)	2.3 (1.1 - 4.8)	3.2 (1.8 - 5.6)	6.8 (3.3 - 14.0)	33.3 (11.8 - 94.4)	2.1 (1.4 - 2.9)

*BMI was calculated as body weight in kilograms divided by the square of the body height in meters, HUNT 2 data.

†In the multivariate logistic regression model adjustments were made for age, tobacco smoking and asthma medication. Alcohol use was tested in the model but omitted in this presentation since it did not have any significant confounding effect. The far right column was, in addition to the adjustments mentioned above, also adjusted to BMI.

‡Number of subjects.

§Reference for all odds ratios presented in the table except for last column (concerning all BMI-categories).

**Reference for odds ratios presented in the last column.

†† Post hysterectomy status indicates HRT with estrogens only (no gestagens).

P-value for interaction term between BMI and HRT never-ever status <0.0001.

Hormone replacement therapy, BMI and reflux symptoms

Among women who had ever (presently or previously) been treated with HRT, there was a strong and dose-dependent increase in the risk of reflux symptoms, most pronounced in the highest BMI intervals, representing mainly an effect-modification ($P < 0.0001$) of the BMI-effect on risk of reflux symptoms (Table 11). The separate effect (independent of obesity) of HRT was tested among women of normal weight ($BMI < 25$), and a tendency towards increased risk of reflux among women ever on HRT was found, but it did not reach statistical significance (OR 1.3; 95% CI: 0.9 –

1.8). In the group of women treated with HRT post-hysterectomy, i.e. those who did not have any endometrium to protect and hence received estrogens only (without gestagens), the risk of reflux in normal weight women was significantly and more than 2-fold increased (OR 2.3; 95% CI: 1.1 – 4.8) (Table 10). The risk of reflux symptoms was 14-fold increased among presently HRT-treated women with BMI > 35 (OR 14.4; 95% CI: 8.2 – 25.1), and 20-fold increased among women with previous HRT in the same BMI category (OR 20.2; 95% CI: 8.9 – 46.1). The highest risk of reflux symptoms observed in our study was among women treated with estrogen-only HRT, i.e. previously hysterectomized, with BMI > 35. In this group, the risk of reflux symptoms was 33-fold increased compared to women of normal weight (BMI < 25) never treated with HRT (OR 33.3; 95% CI: 11.8 – 94.4) (Table 11). Generally, the age-adjusted estimates did not importantly differ from the multivariately adjusted data (data not shown).

BMI data from the HUNT 1 survey

For the 72.8% of the individuals who participated in both surveys, all analyses were also made utilizing BMI from the first survey, i.e. approximately a decade previous to the assessment of reflux symptoms. A weak association between increasing body mass and reflux symptoms was found among moderately obese men (BMI 30 – 35), with a 40% increase in risk of reflux symptoms (OR 1.4; 95% CI: 1.1 – 1.9) compared to men of normal weight (BMI < 25). Among severely obese men (BMI > 35), the association did not reach significance, in spite of a higher point estimate (OR 1.8; 95% CI: 0.9 – 3.5). In women, the association was stronger, with a significant increase in the risk of reflux symptoms in all three categories of overweight, when compared to those with BMI < 25. Among overweight women (BMI 25 – 30), there was a 2-fold increased risk of reflux symptoms compared to subjects with BMI < 25 (OR 2.1; 95% CI: 1.7 – 2.4). In women with a BMI of 30 – 35, the risk of reflux symptoms was 3-fold increased compared to women of normal weight (OR 3.2; 95% CI: 2.5 – 4.1), and the corresponding risk was 2.5-fold increased in severely obese women (BMI > 35), (OR 2.5; 95% CI: 1.7 – 4.0). The analyses concerning exposure to HRT based on data from HUNT 1 resemble the results from HUNT 2, although the strengths of the associations are somewhat diluted. Both analyses show strong and dose-dependent increases in risk of reflux symptoms among HRT-users. Similar to the results from HUNT 2, the strongest association observed was among severely obese women (BMI > 35) taking estrogen-only HRT (OR 17.4; 95% CI: 3.9 – 79.1).

Influence of weight changes

The persons participating in both surveys were evaluated for the effects of weight changes, identified during the time interval between the two surveys, on the risk of reflux (Table 12). The risk of reflux was dose-dependently higher with increasing net BMI gain. In the group who gained >3.5 kg/m² (BMI units), OR was 2.7 (95% CI: 2.3 – 3.2) compared to persons with stable BMI. The risk of reflux symptoms was significantly 40% lower among persons who lost net BMI >3.5 kg/m² (OR 0.6; 95% CI: 0.4 – 0.9).

Analyses using subjects with minor reflux symptoms as cases

Sex-stratified analyses of the relation between BMI and reflux symptoms, and analyses of the influence of HRT, were also performed using the 15,233 study subjects who reported minor reflux symptoms (discarded in the main analyses because of suspected heterogeneity) as the case group, instead of persons reporting

severe symptoms. These analyses gave similar results, only with lower point estimates and weaker trends, compared to the analyses using severe reflux outcome for case classification (data not shown).

Table 12. Effect of weight loss (net BMI* decrease) and weight gain (net BMI increase), between the 1984 -1986 and 1995-1997 surveys, on risk of gastroesophageal reflux symptoms†.

	Cases	Controls	OR	95% CI
No weight change	290	4628	1.0	(reference)
Weight loss, BMI Units*				
0.5 – 1.5	98	1684	0.8	(0.7 - 1.1)
>1.5 to 3.5	81	1097	0.9	(0.7 - 1.2)
>3.5	69	928	0.6	(0.4 - 0.9)
Weight gain, BMI Units*				
0.5 to 1.5	399	5792	1.2	(1.0 - 1.4)
>1.5 to 3.5	805	9287	1.6	(1.4 - 1.8)
>3.5	636	4394	2.7	(2.3 - 3.2)

*Body mass index (BMI) was calculated as body weight in kilograms divided by the square of the body height in meters.

†In the multivariate logistic regression model adjustments were made for baseline (1984-1986) BMI, age and sex.

PAPER IV

Tobacco smoking and alcohol consumption

Analyses were based on 3,153 case subjects with severe reflux symptoms and 40,210 asymptomatic control subjects. There was a moderately strong and dose-dependent association between increasing duration of daily tobacco smoking of cigarettes, pipes or cigars and risk of reflux symptoms (P-value for linear trend <0.0001) (Table 13). The multivariate analysis revealed that among individuals who had smoked daily for more than 20 years the risk of reflux was significantly increased by 70%, compared with those who had smoked daily for less than a year (OR 1.7; 95% CI: 1.5 – 1.9). Likewise, there was a dose-dependent association between the lifetime total number of cigarettes smoked and reflux symptoms (P-value for linear trend < 0.0001) (Table 13). Individuals who had smoked more than 50 000 cigarettes had a significant 60% increased risk of symptomatic reflux compared with those who had smoked less than 100 cigarettes in the multivariate analysis (OR 1.6; 95% CI: 1.4 – 1.8). Alcohol consumption, assessed in HUNT 1, and thus prospectively collected, was not associated with any change in the risk of reflux, independent of the level of consumption (Table 13). For both tobacco smoking and alcohol consumption, the multivariately adjusted odds ratios were not markedly different from the unadjusted estimates, indicating lack of strong confounding effects by the variables included in the model (listed in the methods section).

Table 13. Tobacco smoking, alcohol use and risk of symptomatic gastroesophageal reflux

	No. of		Univariate		Multivariate*	
	Cases	Controls	OR	95% CI	OR	95% CI
Tobacco smoking§						
Years of daily smoking						
< 1 year	1253	20414	1.0	(reference)	1.0	(reference)
1 - 5 years	133	2204	1.0	(0.8 - 1.2)	1.2	(0.9 - 1.6)
6-10 years	251	3000	1.4	(1.2 - 1.6)	1.5	(1.2 - 1.8)
10-20 years	540	5583	1.6	(1.4 - 1.8)	1.7	(1.4 - 1.9)
> 20 years	976	9009	1.8	(1.6 - 1.9)	1.7	(1.5 - 1.9)
P-value for linear trend						< 0.0001
Lifetime number of cigarettes smoked (in thousands)						
< 0.1	1391	21681	1.0	(reference)	1.0	(reference)
0.1 - 25	239	3744	1.0	(0.9 - 1.1)	1.1	(0.9 - 1.4)
>25 - 50	296	3345	1.4	(1.2 - 1.6)	1.5	(1.3 - 1.8)
>50 - 100	484	4968	1.5	(1.4 - 1.7)	1.6	(1.4 - 1.8)
>100 - 200	520	4816	1.7	(1.5 - 1.9)	1.6	(1.4 - 1.9)
>200	223	1656	2.1	(1.8 - 2.4)	1.6	(1.3 - 2.0)
P-value for linear trend						< 0.0001
Alcohol use§						
Occasions of liquor, wine or beer (non-low alcohol) consumption during last two weeks						
None	1066	11960	1.0	(reference)	1.0	(reference)
1 - 4	776	10029	0.9	(0.8 - 1.0)	0.9	(0.8 - 1.0)
5 - 10	58	695	0.9	(0.7 - 1.2)	0.9	(0.7 - 1.2)
> 10	67	655	1.1	(0.9 - 1.5)	1.0	(0.8 - 1.3)
P-value for linear trend						0.54

*In the multivariate logistic regression model adjustments were made for age, sex, body mass index, tobacco smoking, alcohol use, coffee use and asthma medication. Tea drinking, table salt intake, dietary fibers in bread and physical exercise were tested in the model, but omitted from the final model since they had no significant confounding effect.

§ Tobacco smoking and alcohol use variables represent exposure previous to reflux outcome assessment

Dietary factors

Data on the use of coffee and tea were collected from the HUNT 2 survey only, and are therefore of cross-sectional nature. In the multivariate analysis there was a negative association between coffee intake and reflux symptoms with an approximate 40% decrease in risk among persons who drank more than seven cups of coffee per day, compared with those who drank less than one cup (OR 0.6; 95% CI: 0.4 – 0.7). This finding differed markedly from the univariate analysis of coffee exposure, which demonstrated a slight increase in risk of reflux when comparing the same groups as

Table 14. Dietary factors and risk of symptomatic gastroesophageal reflux

	No. of		Univariate		Multivariate*	
	Cases	Controls	OR	95% CI	OR	95% CI
Coffee use						
Cups per day						
< 1	299	3,955	1.0	(reference)	1.0	(reference)
1 - 3	860	10,729	1.1	(0.9 - 1.2)	0.7	(0.6 - 0.9)
4 - 7	1,304	18,106	1.0	(0.8 - 1.1)	0.5	(0.4 - 0.6)
> 7	593	6,368	1.2	(1.1 - 1.4)	0.6	(0.4 - 0.7)
P-value for linear trend						< 0.0001
Tea use						
Cups per day						
< 1	812	10,527	1.0	(reference)	1.0	(reference)
1 - 3	880	11,750	1.0	(0.9 - 1.1)	1.0	(0.8 - 1.1)
> 3	139	1,365	1.3	(1.1 - 1.6)	1.1	(0.9 - 1.5)
P-value for linear trend						0.20
Table salt use[§]						
Meals of salted fish or meat						
Never	308	4,057	1.0	(reference)	1.0	(reference)
< 3 / month	593	7,746	1.0	(0.9 - 1.2)	1.0	(0.9 - 1.2)
Once / week	574	6,839	1.1	(1.0 - 1.3)	1.1	(1.0 - 1.3)
Twice / week	359	3,480	1.4	(1.2 - 1.6)	1.3	(1.1 - 1.5)
> twice / week	168	1,367	1.6	(1.3 - 2.0)	1.5	(1.2 - 1.8)
P-value for linear trend						0.0007
Extra salt on regular meals						
Never	905	11,154	1.0	(reference)	1.0	(reference)
Sometimes	717	8,828	1.0	(0.9 - 1.1)	1.1	(1.0 - 1.2)
Often	232	2,339	1.2	(1.1 - 1.4)	1.4	(1.2 - 1.6)
Always	146	1,241	1.4	(1.2 - 1.7)	1.7	(1.4 - 2.0)
P-value for linear trend						< 0.0001
Dietary fibers in bread						
Dry weight % dietary fibers in bread predominantly consumed						
1-2 (white, low fiber)	177	1,318	1.0	(reference)	1.0	(reference)
4 - 7 (medium fiber)	1,055	12,059	0.7	(0.6 - 0.8)	0.7	(0.6 - 0.9)
6 - 10 (high fiber)	921	13,747	0.5	(0.5 - 0.7)	0.5	(0.4 - 0.7)
14 - 16 (hard bread)	520	6,742	0.6	(0.4 - 0.6)	0.5	(0.4 - 0.7)
P-value for linear trend						< 0.0001

*In the multivariate logistic regression model adjustments were made for age, sex, body mass index, tobacco smoking, coffee use, tea use, table salt intake and dietary fibers in bread. Alcohol use, physical exercise and asthma medication were tested in the model, but omitted from the final model since they had no significant confounding effect.

§ Table salt data were prospectively collected and represent exposure previous to reflux outcome assessment.

|| Data on exposure to coffee, tea and dietary fibers in bread are of cross-sectional nature.

above (OR 1.2; 95% CI 1.1 – 1.4). The analyses revealed that the increased risk of reflux identified in the univariate data could be entirely explained by confounding from tobacco smoking. Tea drinking was not associated with any effect on the risk of reflux symptoms, independent of the adjustment for potential confounding variables (Table 14).

The use of table salt, based on data from HUNT 1, and thus collected prospectively, was estimated from the frequency of meals of salted fish or meat and from how often the person added extra salt to regular meals. A moderate and dose-dependent association between increasing frequency of meals of salted fish or meat and symptomatic reflux was observed (P-value for linear trend = 0.0007). The risk of reflux symptoms among persons who ate salted food three times per week or more was significantly increased by 50% compared with those who never ate salted food (OR 1.5; 95% CI: 1.2 – 1.8). Similarly, the increasing use of extra table salt on regular meals was associated with an increased risk of reflux in a dose-dependent manner (P-value for linear trend < 0.0001). The risk of reflux was 70% increased among persons who always added extra salt compared with those who never did so (OR: 1.7; 95% CI: 1.4 – 2.0) (Table 14). With increasing dietary fiber content in the predominantly consumed bread type (HUNT 2; cross-sectional data), the risk of reflux decreased significantly (P-value for linear trend < 0.0001) (Table 14). Persons who predominantly ate bread with 7 % dry weight of dietary fibers or more, had an approximately halved risk of symptomatic reflux compared with those who predominantly ate white, low fiber-content (1 – 2%) bread (OR 0.5; 95% CI: 0.4 – 0.7). No strong confounding effects were identified in the analyses of dietary salt or fibers (Table 14).

Table 15. Physical exercise and risk of symptomatic gastroesophageal reflux

	No. of		Univariate		Multivariate	
	Cases	Controls	OR	95% CI	OR	95% CI
Physical exercise§						
of > 30 minutes duration						
Never	275	2,231	1.0	(reference)	1.0	(reference)
< 1 / week	116	1,020	0.9	(0.7 - 1.2)	1.0	(0.8 - 1.2)
1 / week	233	3,970	0.5	(0.4 - 0.6)	0.5	(0.4 - 0.7)
1 - 3 / week	226	3,775	0.5	(0.4 - 0.6)	0.6	(0.5 - 0.7)
> 3 / week	116	1,432	0.7	(0.5 - 0.8)	0.7	(0.6 - 0.9)
P-value for linear trend					< 0.0001	

*In the multivariate logistic regression models adjustments were made for age, sex, body mass index, tobacco smoking, coffee use, table salt intake and dietary fibers in bread. Alcohol use, tea drinking and asthma medication were tested in the model, but omitted from the final model since they had no significant confounding effect.

§ Data on physical exercise represent exposure previous to reflux outcome assessment

Physical exercise

Increasing frequency of physical exercise sessions (HUNT 1 data; prospectively collected) lasting at least 30 minutes, exemplified by jogging, cross-country skiing and exercise-swimming, correlated with a decrease in risk of reflux symptoms (P-value for linear trend < 0.0001). Physical exercise once a week was associated with a significant, 50% decreased risk of reflux when compared with individuals who never did any organized physical exercise of at least 30 minutes duration (OR 0.5; 95% CI: 0.4 – 0.7) (Table 15). Univariate data did not markedly differ from the multivariate results, indicating limited influence of confounding factors.

DISCUSSION

METHODOLOGICAL ASPECTS

Study design

Case-control studies

Three out of the four papers that this thesis is based on are case-control studies. Papers II and III are cross-sectional case-control studies, where measurements of both reflux outcome and BMI, the main exposure under study, were performed at the same point in time. Paper IV is a case-control study of mixed longitudinal and cross-sectional design, as some exposure variables such as tobacco smoking, alcohol use, table salt use and physical exercise refer to exposure well in advance of the outcome assessment (longitudinal), while others such as the use of coffee, tea and dietary fibers in bread refer to exposure at the same time as outcome assessment (cross-sectional). The outcome measures of these three studies are for study II prevalent esophagitis and for studies III and IV prevalent reflux symptoms, as opposed to incident reflux disease. For paper IV this introduces the possibility that even the longitudinal exposures mentioned above, may not precede the onset of reflux disease, since we do not know how many of the reflux cases in the HUNT 2 survey that had reflux a decade earlier during the HUNT 1 data collection. Thus, inference from these studies is complicated by the risk of reversed causality, which has to be carefully considered for each exposure separately. The long-standing nature and significant variations over time in GERD makes prevalent reflux much easier to define and measure compared to incident reflux disease, for which reason very few studies have been published using incident GERD as the measure of outcome.

The main alternative to case-control design in analytic epidemiological research is the cohort study, which has the advantage of providing the incidence rate of the disease in the cohort and of having fewer pitfalls in obtaining a high internal validity compared to case-control studies, as the risk of selection bias is lower. Since the registration of incident disease outcome, including the time of each onset of disease, is necessary in conducting a cohort study, it is not generally a feasible design in the study of the etiology of GERD. The advantage with case-control studies is first of all the time- and resource-efficiency. Moreover, case-control studies allow the simultaneous assessment of many different potential risk factors, a significant advantage in the study of diseases which may have several unknown causes.

Population-based design

All four papers included in this thesis are of population-based design. Paper I, addressing the prevalence of reflux symptoms in the Norwegian county of Nord-Trøndelag, included the 90% of the participants of the HUNT 2 survey who answered the reflux symptom question in the survey. Since 71% of adults in the entire population of the county were included in the survey, this prevalence study is based on a majority (64%) of the population rather than a mere sample from it, which offers reassurance against selection bias and makes it well representative of the population at large. For the case-control studies (papers II – IV) this means that the cases and controls come from the same precisely defined and identified source populations.

In paper II the cases were drawn from patients at the endoscopy units of 11 county and community hospitals in Sweden, each with geographically defined patient catchment areas, corresponding to the populations living there. Since the Swedish healthcare system, especially at the time of data collection in 1996 to 1997 and in the 11 catchment areas which do not include any of the major cities of Sweden, has a strict geographical structure in which residents in a certain geographical area are provided for by a specific hospital, and very few exceptions to this occur, the case-sampling can be considered truly population-based. Controls were sampled randomly from the national population register and matched to the cases with respect to age, sex and area of residence, the latter making the controls representative of the same geographical area-defined populations as the cases. Hence, adjustment for any confounding by these variables was inherent in the design.

Papers III and IV are case-control studies nested within the HUNT public health surveys. The well defined nature of the participants of these surveys, provide excellent reassurance against selection bias occurring by cases and controls being sampled from different source populations.

Validity

There are two main sources of error in any quantitative research enterprise: 1. systematic error; often referred to as bias, affecting the validity of the results, and 2. random error, affecting the precision of the results. The validity of research findings is termed internal with respect to its bearing on the study subjects or source population in question, and external with respect to its generalizability. The main sources of bias in epidemiological studies are selection bias, information bias, confounding and reversed causality.

Selection bias

Selection bias is a systematic error emanating from the procedure of selecting study subjects, i.e. from the factors that influence study participation. In a prevalence study selection bias can occur either if selection of participants is non-random, or if the participation rate is low and the decision to participate or not, is in any way influenced by the condition being studied. In the prevalence study of reflux symptoms presented in paper I in this thesis, the influence of selection bias is likely to be small. Participation in the HUNT 2 survey was 71%. Due to the several hundred variables collected in the questionnaire, it is highly unlikely that the decision not to participate in the survey, to any large extent, was specifically related to the questions on reflux symptoms. Of the participants in the survey, 90% answered the reflux symptom questions. However, the selection bias potentially introduced by these 10% non-responders is very limited.

In case-control studies selection bias occurs mainly by two mechanisms: 1. by cases and controls being sampled from different source populations, 2. by any association between case- or control-selection and exposure status [184]. The population-based design of papers II, III and IV, especially the nested design in papers III and IV, provide good reassurance against selection bias by the mechanism of cases and controls being selected from different source populations. Moreover, participants were at the time of data collection not in any way more focused on reflux symptoms than on any of the hundreds of other topics in the questionnaire, making any associations between reported reflux symptom status and the exposure variables studied, very unlikely.

Information bias

Information bias is any systematic error in a study caused by misclassification of exposure variables or disease outcome. Misclassification of subjects for either exposure or disease may be differential or nondifferential. Exposure misclassification is called nondifferential if it is unrelated to the disease classification and differential if it is different for those with and without disease. Disease outcome misclassification is similarly differential if it is different for those exposed and unexposed to the variables under study [184]. Differential misclassification may lead to severe error, either exaggerating or underestimating the associations studied. Nondifferential misclassification on the other hand, has a much more predictable effect, as it tends to dilute any association studied; thus less seriously damaging the validity of a study [184].

In paper II misclassification of the main exposure variable BMI is possible, although self-reported height and weight have been shown to have high validity and reliability [179, 180]. It is, furthermore, not likely that any such misclassification should be differential with respect to case-control status. Significant misclassification of esophagitis outcome is unlikely as endoscopic esophagitis diagnosis is considered very reliable and specific for GERD [38].

In paper III significant misclassification of the main exposure variable BMI is unlikely as height and weight were objectively measured by survey personnel. Moreover, any possible misclassification of BMI is bound to be nondifferential as both study participants and survey personnel were unaware of reflux symptoms as a specific outcome. The same reasoning is applicable concerning possible misclassification of the exposure variables in paper IV: any misclassification is likely to be nondifferential, and would thus dilute (i.e. underestimate) the identified associations.

In papers III and IV, case classification was based on symptoms of heartburn or acid regurgitation during the last 12 months. Those with minor reflux symptoms were excluded from the main analyses and only those reporting that they had been severely troubled by reflux symptoms were assigned as cases. In our validation study of the HUNT 2 reflux question, the “severe symptoms” category mainly corresponded to frequent reflux symptoms (specificity for at least weekly symptoms 99.5%, sensitivity 58%). Frequent reflux symptoms have been demonstrated to have high specificity for true reflux disease [28-31]. Intentionally we selected a case group with a high specificity (rather than high sensitivity) for reflux symptoms and a control group only consisting of persons reporting absence of reflux symptoms. If there, in spite of this prudence in case and control classification should be significant misclassification, it is very likely to be nondifferential, with the same reasoning as for misclassification of exposure above.

Confounding

Confounding may be described as the confusion, or mixing of effects, between the exposure under study and other factors. To confound an association a so called confounding factor must both be associated to the disease outcome and to the exposure variable being studied. The main alternatives in controlling for confounding in non-experimental research are: 1. stratification 2. adjustment by multivariate regression modeling 3. matching. If several potential confounding factors are present and available for analysis, multivariate regression modeling is generally the method of preference. In

the studies included in this thesis multivariate logistic regression modeling using a wide array of potential confounding factors was used to control for confounding in papers I, III and IV. In paper II confounding from age, sex and residential location was controlled by case-control matching, and tobacco smoking, previous cholecystectomy, and the use of asthma medication and other pharmaceutical drugs were tested and when necessary adjusted for in a logistic regression model. All in all, confounding was well controlled for in all four studies of the thesis, although residual confounding from the tested variables or other unknown factors can not entirely be ruled out.

Reversed causality

Reversed causality is a systematic error that may particularly affect cross-sectional studies. It occurs when the studied disease outcome in any way affects an exposure under study. An example of possible reversed causality in this thesis is the effect of coffee drinking on reflux symptoms in paper IV. The risk of symptomatic reflux is 40% lower among those who drink more than seven cups per day than among those who do not drink coffee at all. This could either be explained as a protective effect of coffee drinking on reflux disease, or more likely since coffee has been described to trigger reflux symptoms, that people with reflux symptoms to a certain extent abstain from drinking coffee, i.e. reversed causality.

Concerning the exposure variables based on data from HUNT 1, as well as smoking, which is based on recalled life-time exposure reported in HUNT 2, together called longitudinal variables, there is on theoretical grounds a certain risk of reversed causality. This is because of the prevalent nature of the reflux symptom disease outcome, which implies that a certain proportion of the prevalent reflux cases had reflux symptoms already when HUNT 1 data were collected, thus making the exposures cross-sectional for this proportion. However, this concern is to a certain extent alleviated by the findings in the validation study for reflux symptoms which showed that only 25% of participants that reported severe reflux symptoms, corresponding to the case group in papers III and IV, had experienced reflux symptoms for more than ten years, and 57% had so for less than five years (Table 4).

For all cross-sectional variables, and to some extent also for the longitudinal variables, since prevalent reflux disease is the outcome, the possibility of reversed causality has to be carefully considered. For most of the studied risk factors the risk of significant reversed causality as the cause of associations can be dismissed on grounds of plausibility. It would be unlikely that reflux symptoms caused obesity, especially considering the significant difference in effect between sexes. It would be even more unlikely that reflux symptoms caused severe obesity combined with the use of HRT. For the positive association between tobacco smoking and reflux, reversed causality is very unlikely to have caused the effect, 1. because exposure is longitudinal, 2. because it would imply that reflux symptoms caused increased smoking frequency, which is not plausible. Concerning the effect of alcohol use, major effects of reversed causality are unlikely, 1. because alcohol exposure is a longitudinal variable, 2. because of the consistent unity relative risk estimates at all levels of exposure, suggesting a null effect of alcohol on the risk of reflux. Concerning table salt use reversed causality is not a likely explanation for the association with reflux, 1. as the salt variables are longitudinal, 2. since it seems unlikely that frequent reflux symptoms would cause increased intake of table salt. Concerning the cross-sectional negative association between dietary fibers in bread and reflux, it is possible that it was influenced by reversed causality, which would imply that frequent occurrence of reflux symptoms

would cause an increase in the consumption of bread poor in dietary fiber content, which does not sound very likely, but could nevertheless be plausible. It is further unlikely that reversed causality explains the negative association between physical exercise and reflux because, 1. physical exercise is a longitudinal variable, 2. it would imply that reflux symptoms cause increased physical exercise, which is unreasonable as physical exercise is known to trigger reflux episodes by causing rapid fluctuations in abdominal pressure [24]. The data concerning the effect of tea-drinking on reflux is vulnerable to reversed causality, although the consistent unity relative risks at both exposure levels, rather suggest a null-effect of tea drinking on the risk of frequent reflux symptoms.

Precision

If we could eliminate all systematic error, the error that would remain is so called random error, or the error occurring by chance. Terms used to describe the influence of random error on any measurement are precision or reliability. A high level of precision means a low influence of random error. Precision in any measurement is generally determined by the variability within the measured variable and the sample size. In papers I, III and IV of this thesis, sample size is very large, not only permitting good precision in the main analyses, but also allowing extensive sub-group analyses of principal importance (for example severely obese women with estrogen-only HRT). The study described in paper II is of much smaller sample size, which may have been sufficient for the main analyses of the effect of BMI on the risk for esophagitis, but slightly too small for sufficient precision in the analyses concerning HRT. Moreover, it cannot be completely ruled out that the total lack of association between BMI and esophagitis seen among men in this study may have been due to random error concealing a true, but weak, association.

FINDINGS AND IMPLICATIONS

Prevalence of reflux symptoms

Paper I in this thesis demonstrates a total prevalence of heartburn or regurgitation of 31.4% among adults during a period of 12 months preceding data collection. The study further revealed a sex difference concerning age-stratified prevalence of reflux symptoms that has not been described previously. Among women there was a gradual increase in prevalence of both minor and severe symptoms, from the lowest prevalence in the youngest age group to the highest prevalence in the oldest age group. Among men, on the other hand, the prevalence of both minor and severe symptoms increased gradually from the youngest age group until the 51 to 70 age groups, where prevalence peaked, only to decline in the oldest age group. The study further discloses that the lower prevalence of severe reflux symptoms reported among men compared to women in the oldest age groups can not be explained by confounding by BMI, tobacco smoking, or intake of alcohol, table salt, coffee, dietary fibers in bread, nor by the level of physical exercise.

Prevalence studies of Barrett's esophagus indicate that this condition may be more common among men than among women [30, 185], particularly in older age groups. It is, furthermore, well known that the development of Barrett's esophagus, due to greater mucosal acid resistance compared to the original squamous-cell epithelium, may reduce, or even eliminate symptoms of gastroesophageal reflux [30, 186]. Hence, these observations suggest that the lower prevalence of reflux symptoms among older men

compared to women may be explained by an age-related increase in prevalence of symptom-protective intestinal metaplasia among men, while not to the same extent among women. This is also well in line with the striking male predominance in the incidence of esophageal adenocarcinoma.

Obesity and female sex-hormones

In paper II we found a strong and dose-dependent association between increasing BMI and esophagitis among women, but no association among men. Furthermore, the use of HRT tended to increase the effect of obesity on the risk of esophagitis, although the p-value ($p = 0.08$) for the interaction term did not fully support a significant effect-modification. These findings led to the formulation of a three step hypothesis: 1. there is a difference between sexes in the effect of obesity on GERD, with a strong relation in women and none, or a weak relation, among men, 2. female sex-hormones strengthen the association between obesity and GERD and the difference between sexes may be influenced by female sex-hormones, 3. it is likely that estrogens and not gestagens convey this effect.

The chain of thoughts leading to the formulation of this hypothesis starts with that obese women have lower levels of sex hormone binding globulin (SHBG), resulting in a larger proportion of unbound, active estradiol in serum, and also increased synthesis of estrone in the fatty tissue, compared to leaner women [187-189]. Both these mechanisms lead to a significantly increased estrogen activity in obese women [187-189]. It is well established that gastroesophageal reflux symptoms during pregnancy most often commence during the first trimester [97, 190], and has a predominantly hormonally-mediated pathogenesis, although there is also some contribution by the increased abdominal pressure of late pregnancy [94, 95, 97, 98, 190]. Furthermore, it has been demonstrated that women taking sequential oral contraceptives have a reduced lower esophageal sphincter tone [99].

These previous data suggest that the positive association between high BMI and reflux esophagitis observed might be due to the increased estrogen activity among obese women. This hypothesis is further supported by the finding that the risk of reflux esophagitis tends to increase among overweight and obese users of HRT compared to non-HRT users in paper II. Moreover, it has previously been shown that high levels of estrogen activity induces increased expression of nitric oxide synthase and increased nitric oxide synthesis resulting in smooth muscle relaxation in both animal models [191] and in humans [192]. Nitric oxide has in turn been demonstrated to be the predominant relaxing transmitter substance of the lower esophageal sphincter [117-122]. It is therefore tempting, to further reason, that the association between estrogens and gastroesophageal reflux [94, 95, 97-99, 190] might be conveyed by a nitric oxide mediated lowering of smooth muscle tone, or prolongation of transient relaxations, in the lower esophageal sphincter. Finally Piccinini et al. [193], in a small but well designed, randomized, double-blind cross-over study, showed an increase in nitric oxide synthesis in postmenopausal women who used estrogen medication, compared to placebo. An association between high BMI and increased nitric oxide synthesis was also found, which further supports our hypothesis.

In the study described in paper III, we aimed to test the hypothesis above in a large population-based study, which would enable precise analysis of sub-groups of obese HRT users. In this study we found dose-dependent associations between increasing BMI and reflux symptoms both among men and women, although 2-fold stronger for

severely obese women (BMI > 35) compared to severely obese men, thus supporting our hypothesis of a difference between sexes. Moreover, we found that the association between increasing BMI and reflux was significantly stronger among premenopausal (hormonally active) than postmenopausal women after adjustment for HRT-use, and that the use of HRT substantially strengthened the association between BMI and reflux, both findings in clear support of our hypothesis concerning the role of female sex-hormones. Finally, the strongest increase in the risk of reflux symptoms that we found at all was among previously hysterectomized (and thus receiving estrogen-only HRT) women, where the risk of reflux among women with BMI > 35 was 33-fold increased compared to women of normal weight without HRT, which supports our hypothesis that it is estrogens and not gestagens that mediate the effect on GERD.

The study described in paper III further showed that weight-loss significantly decreased the risk of reflux symptoms, which seems feasible if obesity causes reflux, but this clinically important finding has not been described in previous publications. This finding provides convincing support for recommending weight-loss in the primary treatment of overweight reflux patients.

The findings of papers II and III, together with previously published data, provide firm evidence that obesity causes GERD. Moreover, our findings present evidence of a stronger association between obesity and reflux among women, than among men, and of a role for female sex-hormones, most probably estrogen, in augmenting this association.

Tobacco smoking

Paper IV demonstrates a dose-dependent association both between life-time total number of cigarettes smoked and reflux, as well as years of daily smoking (including cigarettes, cigars and pipes) and reflux. For persons having smoked more than 200,000 cigarettes (equivalent to 20 cigarettes per day for 27 years) during their previous life, the risk of having reflux symptoms was significantly 60% higher than for persons who had smoked less than 100 cigarettes totally in life. Correspondingly, persons having smoked daily for more than 20 years had a significant 70% increased risk of symptomatic reflux compared to persons who had smoked daily for less than one year. These results, together with previous findings both of tobacco smoking as a trigger factor for reflux episodes [67, 100-103] and from cross-sectional population-based studies showing a positive association [1, 5, 32], suggest that smoking causes GERD.

Alcohol

In the data described in paper IV there is no association between any level of alcohol exposure and reflux outcome. In spite of the fact that several studies show that the drinking of alcoholic beverages may trigger reflux symptoms [104-108], two out of three previous population-based epidemiological studies [5, 93], and the findings in paper IV, provide convincing evidence against a role for alcohol-drinking in the etiology of GERD, bearing in mind that the single study in support of an association was small and of cross-sectional design [32].

Coffee and tea

In paper IV, the risk of reflux was significantly lower among persons who drank coffee compared to those who did not. This finding can either be explained by a protective effect of ingesting coffee on the risk for reflux, or, which seems more

likely since coffee-drinking has been reported to evoke reflux symptoms [126], by reversed causality, i.e. that persons experiencing reflux symptoms after drinking coffee refrain from it. Weighing all available data, including the only longitudinal study assessing coffee drinking in relation to reflux [93], there is no evidence in favor of coffee causing GERD, although it cannot be ruled out until more longitudinal population-based data are available.

Concerning tea, data from paper IV show no association between any level of exposure to tea-drinking and reflux symptoms. The very few previous reports that have addressed this issue reveal no association [127]. Despite the relative scarcity of evidence, the available data are so consistent that it seems reasonable to conclude that tea does not cause GERD.

Table salt

Table salt (sodium chloride) has to our knowledge not previously been evaluated as a risk factor for GERD. In the study population from Nord-Trøndelag in Norway, heavily salted fish and meat dishes are an inherent part of the traditional diet, which provided us with an important table salt exposure variable not available in many other populations. The consistent dose–response relations reported in paper IV, both between the frequency of eating salted foods and the risk of reflux, and between applying extra salt on regular meals and the risk of reflux, as well as the longitudinal nature of table salt exposure data, indicate that high table salt intake may be a risk factor for symptomatic reflux disease. However, since this study is the first to address the relation between salt and reflux, it can primarily be seen as hypothesis-generating, and more studies, preferably together with a plausible biological mechanism, are needed before a causal association can be established.

Dietary fibers in bread

The data from paper IV concerning dietary fibers in the predominantly consumed type of bread show a dose-dependent decrease in the risk of reflux among study participants predominantly eating bread with a high content of dietary fibers. As these data are of cross-sectional nature, they are sensitive to bias from reversed causality, although such bias here would mean that reflux symptoms dose-dependently make people eat bread with low fiber-content, which seems unlikely. No previous data concerning this association have, to my knowledge, been published, and further studies, using longitudinal data, are necessary to settle the issue. The findings concerning dietary fibers are well in line with the results from previous studies showing that cereal fibers may be protective against esophageal and gastric cardia adenocarcinoma [110], an effect perhaps mediated by decrease in reflux.

Physical exercise

The longitudinal data of recreational physical exercise in paper IV demonstrate a significant reduction in the risk of reflux among persons regularly exercising for more than 30 minutes, compared to persons that do not exercise at all. This finding is well in line with earlier findings by Ruhl et al. [93]. Moreover, the finding is biologically plausible as physical exercise is likely to strengthen the crural diaphragm, the function of which is essential to the anti-reflux barrier. The consistency in results between the two studies addressing this issue, suggests that physical exercise may be a causal factor that protects against reflux.

Summary of etiological factors for GERD

Based on findings from previous studies and from the studies included in this thesis it is likely that genetic factors, obesity, estrogen, tobacco smoking and certain smooth muscle relaxing drugs (nitroglycerines, β -receptor agonists, aminophyllines, anticholinergic drugs and benzodiazepines) may cause GERD. It is, on the same grounds, likely that regular physical exercise may be protective against GERD, and that the consumption of alcohol, tea or aspirin/NSAIDs are not causally related to GERD. As for coffee drinking, intake of dietary fibers, table salt consumption, H. pylori infection, psychological factors and a number of other proposed etiological agents, further studies are needed before any well founded statement regarding causality can be made (Table 16).

Table 16. Summary of GERD etiology. Proposed risk factors and strength of positive (causes GERD) and negative (protects against GERD) associations with GERD*

Proposed risk factor	Positive (+) and negative (-) associations with GERD
Genetic factors	++
Obesity†	++
HRT (estrogen) †	+
HRT+Obesity†	++++
Tobacco smoking†	+
Alcohol†	0
Dietary fat	+ ?
Coffee†	?
Tea†	0
Table salt†	+ ?
Dietary fibers†	- ?
H. pylori infection	- ?
LES-relaxing drugs‡	+
Aspirin/NSAIDs	0 ?
Physical exercise†	-
Psychological factors	?

* The strengths of positive and negative associations are indicated by the number of + or - signs. Absence of association is indicated with 0 and uncertainty is indicated with ?.

† Indicates that the proposed risk factor has been studied in the present thesis.

‡ LES-relaxing drugs indicates nitroglycerines, β -receptor agonists, aminophyllines, anticholinergic drugs and benzodiazepines.

CONCLUSIONS

- Approximately one third of the individuals in the studied population had experienced reflux symptoms during the 12 months preceding data collection.
- Among women the prevalence of reflux symptoms increases linearly with age, while among men the prevalence increases gradually until the age of 50 – 70 years, only to decline among the oldest men.
- There is a dose-dependent association between increasing BMI and GERD.
- The association between body mass and GERD is stronger among women than among men, and stronger among premenopausal women than among postmenopausal women.
- There is a weak to moderately strong association between the use of HRT and symptomatic gastroesophageal reflux among women.
- HRT is a strong and positive effect-modifier of the association between body mass and symptomatic reflux among women.
- The effect of HRT on reflux and on the association between body mass and reflux seems to at least be mediated by estrogens. A possible role for gestagens remains to be investigated.
- Weight-loss decreases the risk of symptomatic reflux, and weight-gain increases the risk.
- There is a dose-dependent association between tobacco smoking and symptomatic reflux.
- The intake of alcohol or tea is not associated to symptomatic reflux.
- Frequent intake of salted fish/meat and the use of extra table salt on regular meals are both associated to symptomatic reflux, although further studies are necessary to confirm this.
- The predominant use of bread high in dietary fiber content seems to be associated to a decreased risk for symptomatic reflux, although this has to be confirmed in further studies.
- There is a negative association between regular physical exercise of at least 30 minutes duration and symptomatic reflux; i.e. regular physical exercise seems to be protective against reflux.

FUTURE STUDIES

H. PYLORI AND GERD

During the last century the incidence of peptic ulcer disease and distal gastric cancer have declined, changes that coincide with the decreasing prevalence of *H. pylori* infection [131, 194]. During the same period of time the prevalence of GERD and Barrett's esophagus, and the incidence of adenocarcinoma of the esophagus seem to have increased [20, 22, 59, 195-198]. In epidemiological studies from Asia the risk of GERD has been demonstrated to be lower among persons infected with *H. pylori*, especially in individuals with CagA-positive strains [132-139]. Moreover a large proportion of *H. pylori*-infected individuals in third world countries, where *H. pylori* prevalence, and especially that of CagA-positive infection, is high and reflux symptom prevalence low, have atrophic pangastritis with diminished acid secretion, and subsequent low risk of having reflux symptoms [131, 134]. This has led to the formulation of a hypothesis stating that the infection with virulent strains, notably CagA-positive ones, of *H. pylori* may through pangastritis with atrophy of the acid-producing mucosal glands, protect against GERD and by extension also against adenocarcinomas of the esophagus and gastric cardia. Recent data from a cohort study by Ye and al. [199] did not show any decrease in the risk of adenocarcinomas of the gastric cardia or esophagus among pernicious anemia patients with long-standing achlorhydria, somewhat challenging the hypothesis in its extension regarding cancer causality.

To test the hypothesis concerning *H. pylori* and GERD we plan to perform a nested case-control study among HUNT 2 participants. Using the same reflux symptom outcome variable as in the case-control studies based on HUNT in this thesis, we will randomly select 500 cases with severe reflux symptoms and 500 control subjects without reflux symptoms. Among these cases and controls we will analyse *H. pylori* status, as well as CagA and Pepsinogen I, a biomarker for atrophic gastritis, for exposure classification. Moreover we will be able to adjust for confounding for all risk factors assessed in the two previous case-control studies based on HUNT data.

STUDIES OF INCIDENT REFLUX SYMPTOMS IN HUNT 3

A third data collection within the HUNT public health survey series is planned to commence in 2005. In this data collection the questions on GERD have been expanded and based on those that answered the reflux question in HUNT 2 who will also participate in HUNT 3, we will for the first time be able to measure the incidence of symptomatic GERD in a population. Moreover, we will be able to study the exposures discussed in this thesis using purely incident reflux as the disease outcome variable, which is likely to shed light on some of the remaining questions in the etiology of GERD.

APPENDIX

Validation questionnaire, Norwegian version

Spørreskjema om brystbrann og sure oppstøt

Mann Kvinne Alder _____

1. I hvilken grad har du hatt disse plagene i de siste 12 månedene?

Brystbrann/sure oppstøt: Ikke plaget litt plaget mye plaget

2. Hvis du har hatt slike plager i de siste 12 månedene, hvor ofte har du hatt: brystbrann? sure oppstøt?

(sett bare ett kryss)

Sjeldnere en månedlig
En eller flere ganger i måneden
En eller flere ganger i uken
Daglig
Mer en tre ganger per dag

(sett bare ett kryss)

Sjeldnere en månedlig
En eller flere ganger i måneden
En eller flere ganger i uken
Daglig
Mer en tre ganger per dag

3. Hvor lenge har du hatt slike plager?

(sett bare ett kryss)

Mindre en ett år
Ett til fem år
Fem til ti år
Mer en ti år

4. I hvilken grad påvirker plagene dine arbeidsevner eller dagliglivets oppgaver?

(sett bare ett kryss)

Aldri, eller i ubetydlig grad
I noen grad
I betydelig grad
Klarer ikke arbeid/dagliglivets oppgaver

5. Lindres plagene dine av magsyrehemmende medisin som feks. Pepcid, Losec, Lanzo, Somac, Nexium, Ranitidin, Zantac, Tagamet, Pepcidin, Ranacid, Ranitidine, Noctone, Cimal, Cimetid, Novaluzid, Balancid, Link eller Titalac?

(sett bare ett kryss)

Aldri, eller i ubetydlig grad
I noen grad
I betydelig grad
Vet ikke

6. Hvis du bruker medisiner, bruker du disse regelmessig eller ved behov?

(sett bare ett kryss)

Regelmessig Ved behov

7. Har du plager med brystbrann eller sure oppstöt om natta?

(sett bare ett kryss)

Aldri eller sjeldnere enn månedlig

Ja, en eller flere ganger i måneden

Ja, hyppigere enn en gang i uka

<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>

Validation questionnaire, Swedish version

Frågeformulär om halsbränna och sura uppstötningar

Man Kvinna Ålder _____

1. I hvilken grad har du haft dessa besvär under de sista 12 månaderna?

Halsbränna/sura oppstötningar: inga besvär lite besvär mycket besvär

2. Om du har haft dessa besvär under de sista 12 månaderna, hur ofta har du haft:

halsbränna?

(sätt bara ett kryss)

Mindre än en gång i månaden

En eller flera gånger i månaden

En eller flera gånger i veckan

Dagligen

Mer än tre gånger per dag

<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>

sura uppstötningar?

(sätt bara ett kryss)

Mindre än en gång i månaden

En eller flera gånger i månaden

En eller flera gånger i veckan

Dagligen

Mer än tre gånger per dag

<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>

3. Hur länge har du haft dessa besvär?

(sätt bara ett kryss)

Mindre än ett år

Ett till fem år

Fem till tio år

Mer än tio år

<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>

4. I hvilken grad påverkar besvären ditt dagliga liv, på jobbet och privat?

(sätt bara ett kryss)

Aldrig, eller i obetydlig grad

I någon grad

I betydelig grad

Klarar inte arbetsliv/vardagsoppgifter

<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>

5. Lindras dina besvär av någon magsyrahämmande medicin som tex. Novaluzid, Link, Losec, Lanzo, Nexium, Pantoloc, Ranitidin, Zantac, Tagamet eller Pepcid?

(sätt bara ett kryss)

Aldrig, eller i obetydlig grad

I någon grad

I betydlig grad

Vet inte

<input type="checkbox"/>
<input type="checkbox"/>
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6. Om du använder mediciner, är det då regelbundet eller vid behov?

(sätt bara ett kryss)

Regelbundet

Vid behov

7. Har du besvär av halsbränna eller sura uppstötningar om natten?

(sätt bara ett kryss)

Aldrig eller mindre än en gång i månaden

Ja, en eller flera gånger i månaden

Ja, oftare än en gång i veckan

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