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BILE IN THE OESOPHAGUS CONTRIBUTES TO THE DEVELOPMENT AND COMPLICATIONS OF GASTRO- OESOPHAGEAL REFLUX DISEASE.

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ABSTRACT

Objective: To clarify the relationship between duodenogastro- oesophageal reflux (DGOR) and gastro- oesophageal reflux disease (GORD) and its complications.

Methods: As persons who have had their gallbladders removed have been shown to have an increased incidence of duodenogastric reflux, one would expect them to have an increased incidence of DGOR. Two epidemiological studies, one case-control and one population based, attempted to show an association between cholecystectomy and oesophageal cancer. Furthermore, patients with reflux symptoms twice weekly or more, for at least 6 months, and healthy volunteers were recruited and examined. Upper gastrointestinal endoscopy, circadian oesophageal acidity values, bilirubin levels, oesophageal motility and a study of gastric emptying using a scintigraphic method, were performed to assess DGOR, GORD and foregut motility parameters.

Results: A 30% increase in standard incidence ratio was found for cholecystectomised patients as regards to the risk for developing adenocarcinoma of the oesophagus. This increase was not seen for squamous cell carcinoma of the oesophagus. Neither did non-operated patients with gall-stone disease show any increased risk for the two cancers. The amount of bilirubin detected in the oesophagus showed a significant correlation to impaired oesophageal motility, as measured by the degree of efficiency of its peristaltic contractions. In a multivariate analysis it was found that this effect was correlated to bile reflux but not to acid reflux. Gastric emptying parameters, proximal and total, showed no differences in patients with DGOR compared to a normal material. No correlation was found between the degree of acid or bile reflux in the oesophagus and gastric emptying parameters. Finally, a noramal control group was described for combined ambulatory recordings of pH, bilirubin and oesophageal motility.

Conclusions: DGOR is of importance in GORD. An increased risk for adenocarcinoma of the oesophagus following cholecystectomy may result from an increase in DGOR. This increased risk is small and does not necessitate any change in our current management of gall stone disease. Impaired oesophageal motility seen with GORD is associated with DGOR but not with acid reflux, however it does not improve after correction for DGOR. It is not clear if this impairment is due to structural changes in the oesophageal wall as a result of DGOR or a pre-existing condition. There seems to be no general disturbance of foregut motility with DGOR and no correlation between gastric emptying and biliary reflux. DGOR should be taken into consideration when treating patients with reflux disease.

LIST OF PUBLICATIONS

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        oesophagus and gastric cardia.
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        Gastroenterology 2001;121:548-53.

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IV.   Gastric emptying and duodeno-gastroesophageal reflux in gastroesophageal
        reflux disease.
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        PM, Jacobsson H, Näslund E
        Digestive and Liver Disease 2002; accepted for publication

V.   Normal values for ambulatory combined 24-h pH, bile and manometric
        monitoring of the oesophagus in males and females.
        Freedman J, Lindqvist M, Melcher A, Granström L, Näslund E
        In manuscript.

CONTENTS

LIST OF ABBREVIATIONS

CI	Confidence Interval
CLO	Columnar Lined Oesophagus
COX2	Cyclooxygenase 2
DGER/DGOR	Duodeno Gastro Oesofageal Reflux
DGR	Duodeno Gastric Reflux
GERD/GORD	Gastro Oesophageal Reflux Disease
GOR	Gastro Oesophageal Reflux
H_2	Histamine 2 receptor
HIDA	^{99m}Tc-N-(2,6-diethylphenylcarbomoylmethyl) iminodiacetic acid
IM	Intestinal Metaplasia
LSBE/O	Long Segment Barrett's Oesophagus/Esophagus
MMC	Migrating Myoelectric Complex
OR	Odds ratio
PPI	Proton Pump Inhibitor
SIR	Standard Incidence Ratio
SISRs	Swallowing Induced Sphincter Relaxations
SSBE/O	Short Segment Barrett's Oesophagus/Esophagus
TLOSRs	Transient Lower Oesophageal Sphincter Relaxations

1 THESIS, MAIN SECTION

1.1 INTRODUCTION

Reflux of duodenal juice to the oesophagus seems to be an important factor in development of oesophagitis and its complications. This introduction will give a short historic overview and then continue with aspects of gastro oesophageal reflux disease (GORD) and the role of duodenal juice in causing disease.

1.1.1 The Distant Past

Bile has been considered an important factor in health and disease since ancient times. In the Tamil civilization of 12000 BC, the ancient Siddha system of medicine is based on three bodily humours, consisting of Vatha, Pittha and Kapha (Wind, Bile and Phlegm). Pittha corresponded to heat and was located in the solar plexus and represented by the sun. This link between bile and heat can also be seen in Hippocratic medicine, originating 400 BC, where the humoural theory tells us of four bodily humours; yellow bile, black bile, phlegm and blood. The yellow bile represented the element of fire and resided in the liver and was associated with a choleric, that is angry and impulsive, personality. In Aeschylus' *The Libation-bearers,* 458 BC, Electra, in order to convey that she feels like a person in mortal agony, recites: "Over my heart, too, there surges a wave of bile, and I have the symptoms of a person pierced through by a lance." Electra's description of biliary reflux to the oesophagus causing oesophagitis and retrosternal pain brings us to modern medicine.

The concept of oesophagitis being caused by gastric reflux was first reported by Quincke in the last decade of the 19:th century and elaborated by Winkelstein in 1933[1]. Until then, oesophagitis was considered to be caused by either irritants (mechanical, thermal, chemical), infections such as syphilis or tuberculosis, or as complications to other diseases such as cardiospasm, diverticula or neoplasms.

Dissatisfaction with gastric reflux as the sole agent causing oesophageal erosions was raised as oesophagitis is frequently seen after total and subtotal gastrectomy, with removal of the acid-producing part of the stomach[2]. The significance of bile in reflux oesophagitis in primates was clearly demonstrated by Gillison et al. [3], where rhesus monkeys underwent surgery to establish reflux of acid and bile with acid. The monkeys subjected to bile reflux had much more severe oesophagitis.

1.1.2 Gastro Oesophageal Reflux Disease

The term GORD describes the clinical manifestations of gastro oesophageal reflux with typical symptoms and findings of tissue damage secondary to reflux. Typical symptoms are heartburn and regurgitation but atypical symptoms such as non-cardiac chest pain, upper respiratory symptoms (laryngitis, sinusitis, globus hystericus), asthma, hiccups and anaemia can occur. Tissue damage causes erosions, ulcers, strictures or epithelial metaplasia. There is no consensus on how frequent symptoms have to be for the diagnosis of GORD.

1.1.2.1 Epidemiology

The overall prevalence of GORD in the western world is estimated at 20-40%. In Asia, a prevalence of 5-20% has been reported[4], and in sub-Saharan Africa the disease is very uncommon as is adenocarcinoma of the oesophagus[5]. In the west, there have been reports that the incidence of GORD is increasing[6, 7].

Daily symptoms of reflux are experienced by 4-7% of the western population and in up to 20% these symptoms occur weekly[7, 8]. In a population study in Olmsted county, in the USA, as many as 59% reported occasional heartburn/regurgitation[9]. In Sweden 25% of the population report occasional symptoms of reflux[10]. Erosive oesophagitis is seen in 2%, with complications such as epithelial metaplasia in 0.4% and adenocarcinoma of the oesophagus in 4/100 000[8]. This cancer-form has been strongly associated with reflux disease[11] and has the most rapid rise in incidence of all cancers, and now surpasses squamous cell carcinoma as the most frequent form of oesophageal cancer in the west[12].

The prevalence of GORD is not sex or age dependant in adults[13].

1.1.2.2 Reflux mechanisms

Gastro oesophageal reflux occurs when the complex anatomical and physiological structure of the oesophagogastric junction fails. The structure is composed of the lower oesophageal sphincter, the diafragmal crurae, the phreno-oesophageal ligament, the acute angle of His, the intra abdominal segment of the oesophagus and the mucosal rosette (Figure 1).
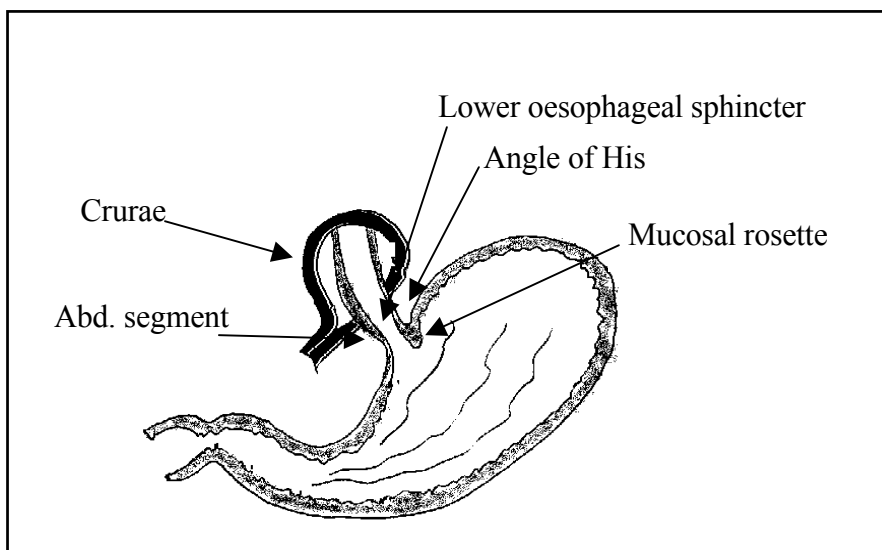

Figure 1.
Anatomical aspects of the gastro- oesophageal junction

1.1.2.2.1 Sphincter relaxation.

Reflux may occur as a result of a chronic low pressure in the lower oesophageal sphincter (LOS), swallowing induced sphincter relaxations (SISRs), originating from the oesophagus, or during transient lower oesophageal sphincter relaxations[14] (TLOSRs), originating from the stomach. TLOSRs are thought to be the main cause of reflux disease[15].

It is thought that the purpose of TLOSRs is to vent air from a distended stomach. These relaxations occur most frequently in the upright position[16] and are triggered by distension of the stomach, especially in the cardiac region[17]. TLOSRs occurs equally in patients with reflux disease as in asymptomatic subjects, but more often associated with acid reflux in the former[18]. TLOSRs are more prolonged than SISRs[19] and are neurally mediated with concurrent inhibition of the diaphragmatic crurae[20].

Low LOS pressures are frequently seen with erosive GORD. Reflux can occur as a result of increased abdominal pressure, such as bending over or coughing, when LOS pressure falls below 10 mmHg. Whether the impaired contractility of the sphincter is caused by reflux or is a primary motility disorder is not known. Aliviating the reflux symptoms with medication will not, however, improve LOS pressure[21].

1.1.2.2.2 Hiatal Hernia

A hernia of the diafragmal hiatus causes several of the antireflux mechanisms to be lost, such as the abdominal segment of the oesophagus, the acute angle of HIS and the mucosal rosette. Further, the crurae apply their pressure below the LOS thereby losing synergism with the sphincter. Reflux episodes are caused by straining, deep inspiration and normal SISRs rather than by TLOSRs[22]. In a study by Kahrilas et al.[23], TLOSRs induced by gastric distension, were more frequent in patients with hiatal hernia than in those without and in controls.

1.1.2.2.3 Delayed gastric emptying

Impairment in gastric emptying is seen with obstruction of gastric outlet and with conditions of reduced gastric motility, such as diabetes mellitus, Parkinson's disease and following surgery with vagotomy. In GORD, there seems to be a delay in emptying of the proximal stomach but not in total gastric emptying[24]. There are, however, some earlier studies that have found evidence for delayed total gastric emptying[25, 26] but also for the absence of impaired gastric emptying in GORD[27, 28]. If proximal gastric emptying was a major cause of GORD one would expect these patients to have a greater reflux volume than controls. An oesophageal aspiration study on patients with GORD could not however demonstrate any increase in reflux volume compared to controls, although patients seem to have more volume reflux in a supine posture than controls when upright[29].

1.1.2.3 Protective mechanisms

1.1.2.3.1 Pre-epithelial defence

The pre-epithelial defence consists of neutralising mucous from salivary glands and distal oesophageal glands as well as oesophageal motility, performing clearance of the lumen. A defect in any of these factors increase the risk of oesophageal damage, as seen in those with achalasia[30] and scleroderma[31]. It is however not known if saliva plays an important role in oesophageal protection in man[32]. Approximately 20% of patients with erosive oesophagitis have impaired oesophageal motility, as measured by oesophageal body motility, and an impaired lower oesophageal sphincter, but whether this is secondary to reflux is not known[33, 34]. The impaired motility does not, however, improve after medical or surgical treatment of reflux[35, 36].

1.1.2.3.2 Epithelial defence

The oesophageal epithelium is very resistant to acid exposure. Perfusion with 1.1 M HCl for 30 minutes, as done in the Bernstein test, does not affect the epithelial integrity. The oesophagus lacks the mucous that protects the cylindrical epithelium of the more distal gastrointestinal tract, instead it relies on a resistant apical membrane and layers of tight junctions with intracellular glycoproteins. If these structures are overcome and hydrogen ions penetrate the epithelial cells, hydrogen exchanging membrane pumps come into action. One is a Na^+/H^+ exchanger and another is a Na^+-dependent Cl^-/HCO_3^- exchanger[37]. When these pumps come into action, there is an accumulation of fluid in the extra-cellular space that can be seen in light and electron microscopes[38] (Figure 2).

1.1.2.3.3 Post epithelial defence

The neutralising capability of the sub-mucosal microcirculation in the oesophageal lining is called the post epithelial defence. This is where hydrogen ions are ultimately transported away from the epithelium.

1.1.2.4 Mucosal damage

Damage to the epithelial integrity causes an inflammatory response, oesophagitis. The most common cause is reflux of gastric content, but other causes are possible.

1.1.2.4.1 Traumatic

Traumatic erosions of the mucosa can be seen with foreign bodies and food impaction. This can occur without a structural abnormality of the oesophagus but is more common with a stenosis, such as ring strictures after prolonged reflux disease or after restrictive obesity surgery[158].

1.1.2.4.2 Infectious

Oesophagitis can be caused by HIV[39], CMV, herpes simplex[40] and candida albicans[41].

1.1.2.4.3 Allergic

Allergic reactions such as eosinofilic oesophagitis[42] and dermatological conditions such as epidermolysis bullosa[43] can cause oesophagitis.
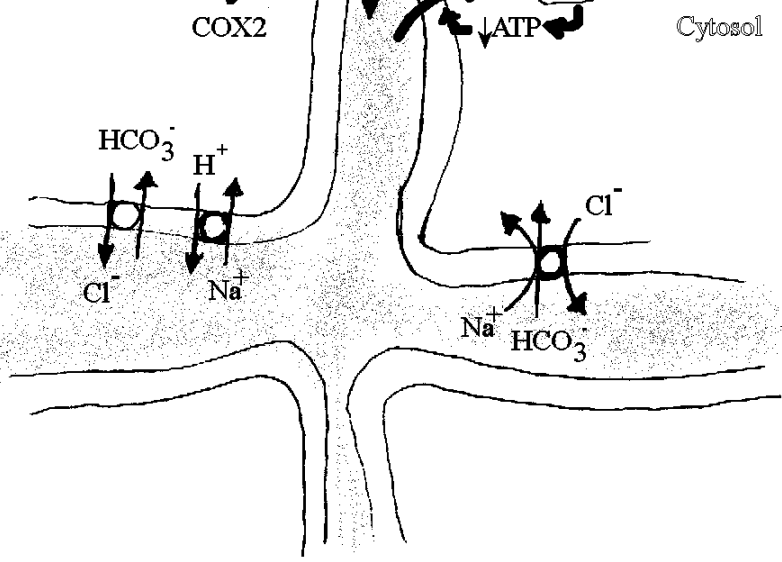
Endogenous substances capable of inducing oesophagitis are hydrochloric acid, pepsin, trypsin, phosopholipases and bile acids. These substances cause reflux oesophagitis, the most common form of oesophagitis. Studies on the toxicity of individual substances have usually been performed on the isolated rabbit oesophagus.
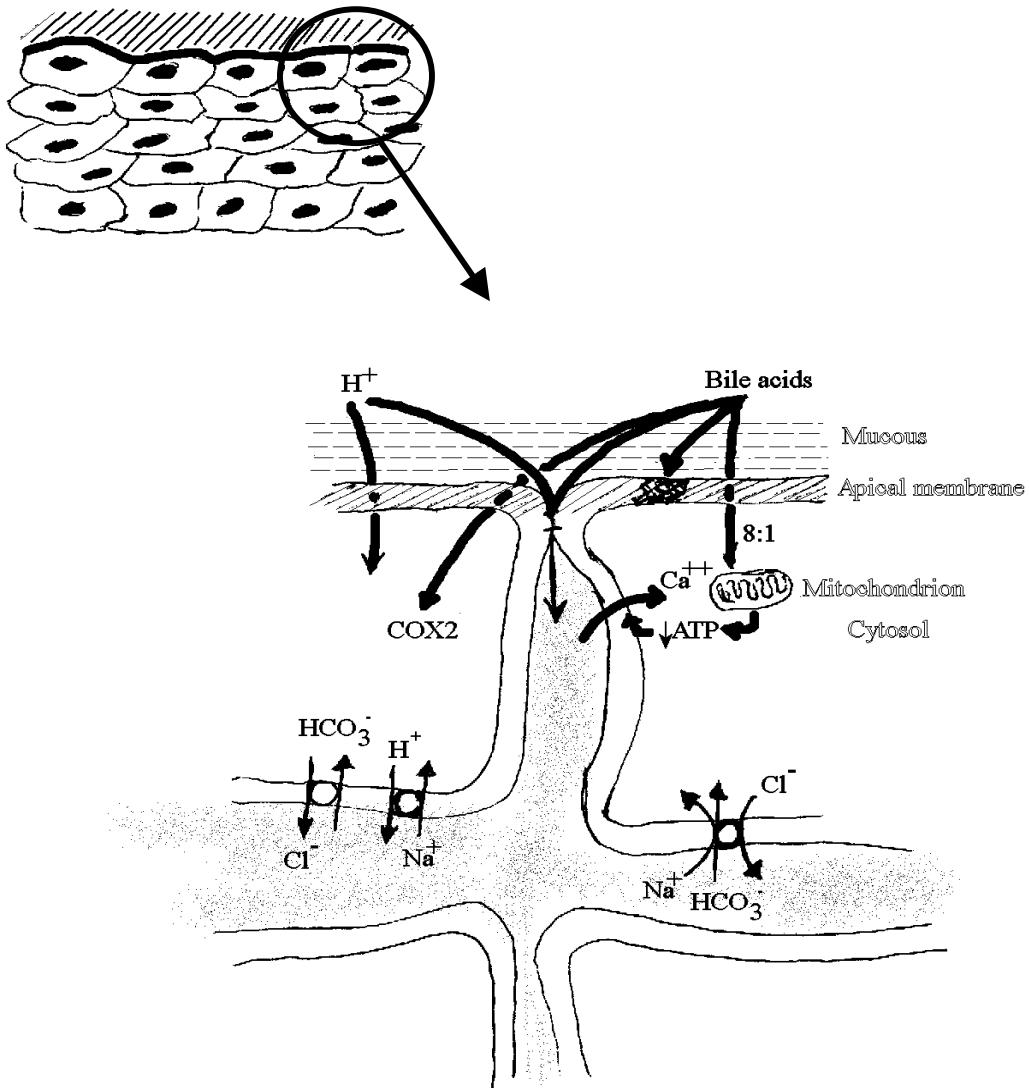
H^+ Bile acids

Mucous

Apical membrane

8:1

Ca^{++} Mitochondrion

COX2   ↓ATP   Cytosol

HCO_3^- H^+

Cl^- Na^+

Cl^-

Na^+ HCO_3^-

Figure 2. *Illustration of different mechanisms of acid- and bile mediated cytotoxicity.*

Hydrochloric acid: The apical membrane of the stratified oesophageal epithelium is very resistant to hydrogen ions. With a luminal pH of 2, little or no acidification of the epithelial cytosol occurs[45, 46]. When the epithelium is damaged however hydrochloric acid is very effective in causing heartburn as is tested with the Bernstein test[47], where the oesophageal mucosa is flushed with 0.1 M hydrochloric acid for 30 minutes or until symptoms of heartburn or chest pain occurs. HCl also acts as a facilitator for pepsin, trypsin and unconjugated bile acids.

Pepsin: Pepsin is a proteolytic enzyme excreted into the stomach. It is activated in an acid milieu, and increases cellular permeability enabling hydrogen ions to enter into the intercellular space, which is less resistant than the apical membrane[46, 48, 49]. Pepsin is not proteolytic at neutral pH.

Trypsin: Trypsin is a proteolytic pancreatic enzyme that is inactivated at acidic pH. It increases mucosal permeability and facilitates acid damage[48]. Treatment with a trypsin inhibitor diminished oesophagitis in rats with surgically induced DGOR[50].

Lipase: Pancreas lipase also has the ability to increase oesophageal mucosal permeability at a neutral pH[51].

Bile: Conjugated and deconjugated bile salts as well as lysolecithin, formed by phospholipase action on lecithin, are the main harmful components of bile. Bile salts are excreted into the bile ducts in the conjugated form. The primary bile acids cholate and chenodeoxycholate are conjugated with taurin or glycin at a ratio of 1:3. The conjugation greatly increases the solubility of the bile acids. In the distal gut, intestinal bacteria can transform primary bile acids into secondary bile acids such as deoxycholic and lithocholic acid. Bile acid can enter mucosal cells when in a lipofilic form. This occurs in a pH interval of 2-5 for the conjugated bile acids and at neutral pH for unconjugated bile salts. This pH-dependency also influence precipitation. Conjugated bile acids precipitate, and are thereby rendered harmless, at a pH below 1.5. Unconjugated bile acids precipitate below a pH of 3-4. Bile acids are concentrated in the epithelium at levels as high as eight times the lumenal concentration[52, 53], probably by intracellular ionisation and membrane entrapment[54, 55] (Figure 2).

In different animal models, bile acids have been shown to cause toxic damage to the oesophageal epithelium after a few hours of exposure at concentrations of 1-10 mmol/l[46, 48, 56] and after 5-8 days exposure at concentrations of 100-200 μmol/l[57, 58]. The toxic mechanisms are not fully known and several theories have been put forth. In animal studies, it has been shown that cytotoxicity is mediated through mitochondrial dysfunction with depleted stores of ATP resulting in calcium influx and cell death[58-61]. Another theory is that bile cause cell damage by its detergent property and thereby disruption of cellular membranes, this mechanism is less likely as active uptake and disruption of cellular stability occurs in concentrations lower than those needed for micelle formation or loss of membrane structures[62, 63]. Bile has also been shown to induce cyclooxygenase-2, and thereby promote inflammatory activity in the oesophagus[64].

1.1.3 Duodeno Gastro Oesophageal Reflux

Reflux of hydrochloric acid is generally accepted as the main cause of oesophagitis but can by no means explain all cases of reflux disease. Oesophagitis is seen in patients after total and subtotal gastrectomy, where acid reflux seems unlikely, and resolves after duodenal diversion[65-67], indicating duodenal juice as an important factor. Resolution of oesophagitis with remaining abnormal acid reflux has also been described after duodenal diversion as an antireflux procedure, again indicating DGOR as an pathogen[68]. Further, modern proton pump inhibiting drugs effectively abolish gastric acid secretion but only heal 87-89% of patients with oesophagitis[69]. It is also well known that 10-25% of patients with erosive oesophagitis have normal pH-studies, again indicating another explanation for injury[70, 71]. Interest in the subject goes back a long time, with pioneering work done as early as 1950 by Ferguson et al.[72] in cats and dogs, and in 1972 working on primates Gillison et al.[3] showed that duodenal juice indeed causes severe oesophageal damage.

The overwhelming majority of patients with GORD have not been subjected to any gastric surgery to explain reflux of duodenal contents to the oesophagus, thus other reasons must be sought.

1.1.4 Detection of duodenal juice.

1.1.4.1.1 Aspiration techniques

Aspiration techniques have been used and allows very accurate analyses of the components of the refluxate[29, 73-75]. However, this technique is resource-consuming and difficult to perform over extended periods of time. Because aliquots are collected, peaks in concentration may be lost. It is very difficult to perform in an ambulatory setting and its relation to normal physiology can be questioned.

1.1.4.1.2 Scintigraphic techniques

This method is practical since it involves minimal discomfort for the patient, but it has several drawbacks. ^{99m}Tc-N-(2,6-diethylphenylcarbomoylmethyl) iminodiacetic acid (HIDA) scintigraphy is limited by its relative insensitivity for oesophageal reflux and the use of radioactive isotopes. There is also a problem of patient mobility, overlap of organs and the intermittent nature of bile reflux[76]. This technique has also a limited time span of examination and can no be used in an ambulatory setting..

1.1.4.1.3 pH monitoring

Since duodenal juice is alkaline, it has been thought that registration of alkaline reflux to the stomach and oesophagus could be a good marker for DGER. This is been the case, since small amounts of duodenal reflux can be buffered in an acid stomach, in fact it has been shown that most bouts of DGER occurs under normal or acid pH in the oesophagus. An alkaline shift may also occur as a result of influx of alkaline saliva or bicarbonates from submucosal oesophageal glands[77].

1.1.4.1.4 Ambulatory bilirubin monitoring

A system for ambulatory detection of bilirubin was validated in 1993 by Bechi et al.[78]. This system consists of a fibreoptic probe carrying light signals into the oesophagus and back to an optoelectonic system via a fibreoptic bundle. Bilirubin is detected between a mirror and the fibreoptic tip at the end of the probe, by light absorption at 453 nm where bilirubin has an absorption peek. Data is collected in a portable data logger and later analysed on a computer. This technique allows ambulatory detection of bile in the oesophagus in much the same manner as pH detection. Never the less there are some limitations. Some coloured foodstuffs interfere with the measurements and small pieces of food might get stuck between the mirror and the tip of the fibreoptic bundle. At low pH, bilirubin forms dimers with different optic properties, causing an underestimation of bilirubin concentration of at least 30 percent[79].

1.1.4.2 Duodeno Gastric Reflux

Reflux of duodenal juice frequently occurs after surgery involving the pylorus but is also seen with an intact pylorus. Healthy individuals have bile reflux to the stomach more often during supine than upright posture (for a median of 25% vs 4% of the time, respectively) with a large inter-individual variation[80]. Duodeno gastric reflux (DGR) occurs late in phase III of the migrating myoelectric complex (MMC), the fasting small bowel motility pattern. Half of these pressure waves are retrograde[81]. Bile is mostly deviated to the gallbladder during this period and is seen as reflux in only 17% of healthy individuals[82]. DGR is more frequent after cholecystectomy[83, 84] and in studies on dogs, the mechanism for this seems to be an increased number of pyloric relaxations[85]. In a study by Fiorucci et al.[86] it was found that GORD-patients have significantly more duodenogastric reflux than controls, 85 vs. 59%. In addition, intragastric bile acid concentrations were 6-8 times higher in GORD-patients than in controls.

Interestingly, reflux pass the LOS is seen during the MMC of the stomach only in patients with GORD[87], linking both DGR and GOR to the MMC, perhaps indicating a more widespread motility dysfunction in patients with DGOR.

1.1.4.3 Gastro Oesophageal Reflux

There are no indications that patients with DGOR have another mechanism of gastro oesophageal reflux than patients with GORD. Please see section 1.1.2.

1.1.4.4 Duodenal Juice and Oesophagitis

It is still controversial if DGOR is of clinical relevance. Kauer et al.[88] found that patients with more advance stages of reflux disease have increased exposure to bile. Almost all patients with Barrett's oesophagus had biliary reflux, half of the patients with erosive oesophagitis had DGOR but only a few normal subjects had detectable bile in their oesophagi. Oesophageal aspiration studies have found levels of bile acids in sufficient concentration to cause mucosal damage in patients with oesophagitis[73, 74]. Even unconjugated bile acids have been found in patients with advanced disease, which is a little surprising since this unconjugation of bile salts demands the presence of intestinal bacteria which are usually absent from the stomach and the duodenum. However, bacterial overgrowth of the stomach can occur after effective treatment with proton pump inhibitors[89]. The deconjugating capacity of Helicobacter pylori, although unknown, has been put forth as a possible explanation.

1.1.4.5 Duodenal Juice and Metaplasia

Metaplasia of the distal oesophagus with columnar cells, also referred to as columnar lined oesophagus (CLO), is strongly correlated with reflux disease[90], even if all patients with severe reflux, as measured by pH-monitoring, do not develop metaplasia. Patients with severe oesophagitis without metaplasia and those with metaplasia have equal acid exposure suggesting that other factors beside hydrochloric acid may be of importance[91]. Metaplasia can be of fundic, gastric or intestinal type. Intestinal metaplasia, is associated with an increased risk for adenocarcinoma of the oesophagus[92]. It has been estimated that the increase in risk is 30-125 times greater than that of an age-matched population[93]. Some investigators suggest non-intestinal subtypes to be harmless, but others have found that these often progress to the dangerous intestinal type[94].

The prevalence of CLO varies in different geographical regions and is difficult to appraise since many of those with CLO are without symptoms. Never the less it has been suggested that CLO of more than 3 cm occurs in 0.5 to 5% of the population[95].

Most patients with intestinal metaplasia, also called long segment Barrett's oesophagus (LSBO) which reaches more than 3 cm above the proximal limit of the gastric rugae (as opposed to short segment Barrett's oesophagus (SSBO) of less than 3 cm), have significant DGOR[96-104] suggesting that duodenal contents may be of importance in the development of intestinal metaplasia.

1.1.4.6 Duodenal Juice and Carcinogenesis

Bile acids act in the gut as detergents for the absorption of cholesterol and fat-soluble vitamins. They are also trophic to the gut epithelium and structurally resembling carcinogenic polycyclic aromatic hydrocarbons. This led Cook and Kennaway to suspect that bile acids might have carcinogenic abilities as far back as in the late 1930s[105]. Epidemiological studies have also found a weak association between the continous intestinal perfusion of bile seen after cholecystectomy, and intestinal carcinogenesis, especially right-sided colon cancer[106-109]. The duodenum has some unknown mechanism of protection since it has the highest bile concentrations, but very rarely develops adenocarcinoma.

Bile acids, especially deoxycholic acid[110], are associated with an increase in epithelial proliferation in the colon. Bile acids can activate potein kinase C, which is involved in the carcinogenic pathway[111]. Bile acids, in an acidic environment, stimulate cell proliferation and cyclooxygenase 2 (COX2) expression in metaplastic and malignant oesophageal epithelium[112, 113]. This effect is not seen with bile acids at neutral pH. COX2 may contribute to carcinogenesis by inhibition of apoptosis[114], stimulation of tumour invasiveness[115], stimulation of angiogenesis[116], modulation of inflammatory response and through conversion of procarcinogens to carcinogens. Treatment with cyclooxygenase inhibitors decrease the risk for gastrointestinal cancer[117].

A model for oesophageal carcinogenesis has been developed in rats and studies have shown an increased risk for cancer after surgical deviation of duodenal and pancreatic contents to the oesophagus[118-121]. In this model, acid suppression by proton pump inhibition, further stimulated growth of the oesophageal mucosa in the presence of DGOR, suggesting a more harmful effect of DGOR with a neutral pH[122]. The model has however been questioned and Öberg et al. who could not find any association between carcinogenesis and the composition of the refluxate[123]. In humans, patients with early adenocarcinoma of the oesophagus have a very high prevalence of DGOR[124].

1.2 AIMS

- Patients with Barrett's oesophagus, a pre-malignant lesion, have an increased incidence of DGOR. Therefore the first aim of this study was to examine if there is any association between DGOR and adenocarcinoma of the oesophagus by using cholecystectomy as an indicator of DGOR, as cholecystectomy is associated with an increase in duodenogastric reflux. Two separate epidemiological studies are presented in Paper I and II

- In animal studies, bile in the oesophagus causes epithelial and subepithelial structural damage and might thus influence oesophagheal motility. In humans, it has been shown that GORD is associated with impaired oesophageal motor function, believed to be caused by chronic reflux of acid. Therefore the association between DGOR and oesophageal motility was studied during 24 hours. Paper III

- Disturbances in gastric emptying has been reported in subjects with GORD. A third aim of this study was to asses if subjects with DGOR have disturbed gastric emptying compared to normal subjects, as a disturbance could contribute to the development of DGOR. Paper IV

- Ambulatory measurement of bile in the oesophagus is a relatively new technique. Multiple catheter set-ups are used, and this may affect the results by added discomfort in the tested subject, why reference values for single-catheter set ups may not be valid. The last aim of this study was to establish normal values for the dual-catheter assembly used in this study. Paper V

1.3 SUBJECTS

1.3.1 Paper I

This case-control study comprised the entire population of Sweden under 80 years of age, born in Sweden and still residing there during the period December 1, 1994, through December 31, 1997. All cases of oesophageal and gastric cardia adenocarcinoma and half of the cases of oesophageal squamous cell carcinoma (subjects born on even dates) were eligible. The study included four separate groups of patients. Group 1 were 189 patients with adenocarcinoma of the oesophagus. Group 2 were 262 patients with adenocarcinoma of the gastric cardia. Group 3 were 167 patients with squamous cell carcinoma of the oesophagus. Group 4 were 820 control subjects. Group one and two together constituted 85% of all eligible adenocarcinoma cases in Sweden during the study period. The participation rate among both controls and oesophageal squamous cell carcinoma cases was 73%.

1.3.2 Paper II

A population-based retrospective cohort study was conducted in Sweden based on the Inpatient Register. This register was founded in 1964/1965 and includes information based on the national registration number, a unique identification number assigned to every resident in Sweden. It was thus possible to find all surgical procedures and discharge diagnoses during inpatient care. Information on the date of death or emigration was derived from linkages to the nationwide Register of Causes of Death and the Emigration Registry, respectively.

1.3.3 Paper III

In order to find individuals with and without bile reflux, 39 patients were recruited from referrals for diagnostic endoscopy or evaluation for fundoplication together with 10 normal volunteers without symptoms of GORD.

Forty-nine individuals (median age 48 years (range 21-66), BMI 26 (18-32), 26 female) were recruited for this study. Exclusion criteria were previous upper abdominal surgery, diabetes, connective tissue disease, neurological disorders, severe heart disease, alcohol abuse, psychiatric disorders and pregnancy.

1.3.4 Paper IV

Fifteen patients undergoing evaluation for GORD with reflux of bile (B+) to the oesophagus and 15 subjects without a history of GORD and normal bilirubin monitoring (B-) were included in the study. The latter were healthy individuals without gastrointestinal symptoms and free of any medication. Previous studies have shown that postmenopausal women and men have similar gastric emptying rates in contrast to pre-menopausal women who have a slower rate of gastric emptying. Thus post-menopausal women were grouped with male subjects.

1.3.5 Paper V

There were twenty healthy volunteers, median age of 24 years (21-70), in the study. They had not had any symptoms of gastro- oesophageal reflux disease during the past 6 months, and only sporadic occurrences before that. They were not on any medication and had no prior gastrointestinal surgery. Neither were they diabetic nor suffering from any connective tissue disease or neurological disorder. They were recruited from hospital staff and medical students. One was a smoker, six were women and none were pregnant.

Study	Number of persons studied	Male	Female
Paper I	1 438	1187	251
Adenocarcinoma		165	24
Squamous cell carc.		120	47
Cardiac adenocarc.		223	39
Controls		679	141
Paper II	268 312	87 263	181 049
Paper III	49	23	26
Paper IV	30	19	11
Paper V	20	14	6

Table 1. *Number of individuals in the different studies.*

1.4   METHODS

1.4.1   Paper I

Design.

All cases of adenocarcinoma of the gastric cardia or oesophagus and squamous cell carcinoma of the oesophagus, throughout the country, were identified shortly after diagnosis through contact with treating centres and regional tumour registries. The control persons were randomly selected from 10-year age and sex strata in the entire Swedish population, using the continuously updated computerised population register. The numbers selected in each stratum were adjusted to mimic the age and sex distribution of the oesophageal adenocarcinoma cases.

Tumour classification.

To reduce tumour misclassification, uniform routines for documentation of the tumours were introduced at the participating departments. The biopsies and/or surgical specimens from 97% of the cases were reviewed by one pathologist.

Exposure information.

All cases and controls underwent computer-aided face-to-face interviews by specially trained, professional interviewers. Questions were asked about a history of cholecystectomy and the date for surgery. Information about previous peptic ulcer, its location, and the date for diagnosis, as recalled by the patient was also collected. Ulcers that were first detected within five years of the interview were disregarded. Information was gathered concerning several potentially confounding factors and were adjusted for in a multivariate analysis.

Statistical Analysis

To assess the association between cholecystectomy or history of ulcer disease and oesophageal cancer, logistic regression was used in both univariate and multivariate modelling. Model parameters were estimated by the maximum likelihood method. From these estimates, odds ratios with 95% confidence intervals were computed. In the baseline model, adjustments were made for age and sex, as the frequency distribution of these factors was not necessarily the same in different groups. In the multivariate modelling, adjustments were further made for potential confounding variables; age, sex, reflux symptoms, educational level, body mass index, tobacco smoking, alcohol use, intake of fruit and vegetables, meal size, and physical activity. In one model, reflux symptoms were excluded in the analysis.

1.4.2 Paper II

409 214 persons with a diagnosis of cholelithiasis were identified in the national inpatient registry. Every incident cancer was identified by linkage with the Swedish Cancer Register. 25 936 persons were excluded due to prevalent cancers, since in this analysis we included only first primary cancers. Furthermore 13 809 persons were removed from the study due to invalid data or other inconsistencies uncovered during the record linkage. Among the remaining 369 469, 268 312 were cholecystectomized at the time of, or after the diagnosis of cholelithiasis. They constituted the cholecystectomy cohort. Person-years for this cohort were calculated from the discharge date of the cholecystectomy until the occurrence of a first cancer, death, emigration, or the end of observation (December 31, 1997), whichever occurred first.

The cholelithiasis cohort consisted of 101 156 persons who had a diagnosis of cholelithiasis but were not cholecystectomized, and 66 489 patients who had had a late cholecystectomy after an initial diagnosis of cholelithiasis. In the cholelithiasis cohort, person-years were accumulated from a first diagnosis of cholelithiasis until the occurrence of a cholecystectomy, a first cancer, death, emigration, or the end of observation, whichever occurred first.

Second primary cancers or cancers detected first at autopsy were excluded from all analyses. For analysis, cancer of the esophagus was subdivided by histology into adenocarcinoma and squamous-cell carcinoma of the oesophagus.

Statistical analysis
To assess the association between cholecystectomy and oesophageal cancer, the standardized incidence ratio (SIR), the ratio of the observed to the expected number of cancers, was used to estimate relative risk. The expected number of cancers was calculated by multiplying the observed person-years by age (in 5-year groups), sex, and calendar year-specific cancer incidence rates. The expected rates were derived from the entire Swedish population without a reported cancer and aggregated by 5 calendar years to avoid instability across the calendar year. Confidence intervals of SIRs were calculated assuming that the observed number of cancers followed a Poisson distribution. We excluded all cancers and person-years accrued during the first year of follow-up in the main analyses to avoid influence of selection bias.

1.4.3 Paper III-V

Endoscopy (III)
A complete oesophago-gastro-duodenoscopy was performed at the time of inclusion with or without local anaesthesia or intravenous administration of 1 to 5 mg of midazolam. Subjects excluded proton pump inhibitors or H_2-receptor blockers for at least 2 weeks prior to the examination. Biopsies were taken from the stomach for *Helicobacter pylori* detection using the CLO-test (Ballard Medical Products, Draper, Utah, USA). The endoscopic grading of oesophagitis was assessed by the Los Angeles-classification[157].

24-hour ambulatory combined pH, bilirubin and esophageal manometry (III-V)
All subjects excluded proton pump inhibitors (PPI) and H_2-blockers for a week prior to the examination. The left nostril was anesthetized using lignocaine gel. A calibrated combined pH and 3-channel micro transducer manometry catheter was then introduced and passed to the stomach (Koenigsberg Instruments Inc., Pasadena, CA, USA). A pull-through manometric detection of the lower oesophageal sphincter (LES) was performed with all three pressure sensors. The pH-sensor was positioned 5 cm, and the pressure tip transducers 3, 8 and 13 cm, above the upper border of the LES. The bile detection catheter (Bilitec 2000, Medtronic Functional Diagnostics A/S, Copenhagen, Denmark) was then introduced next to the pH-catheter and positioned at the same level as the pH-sensor. Both catheters were then tethered to the nose and cheek with adhesive tape and connected to the portable recording units. All were instructed to abstain from alcohol, tobacco, carbohydrated drinks and foodstuffs known to disturb readings of the Bilitec system (i.e. coloured food, for example coffee and tomatoes). To minimize the risk of obstructing the reflective mirror of the Bilitec catheter, only liquid food was allowed. They were prompted to keep a diary, noting the time for episodes of reflux symptoms, food intake and laying down. They returned the following day for removal of the catheter assembly.

The stationary manometry, pH- and bile data was analyzed using Polygram for Windows software, version 2.1 (Medtronics Synectics, Stockholm, Sweden). All tracings were also examined visually for exclusion of possibly erroneous recordings. For the bilirubin study, periods with increased absorption, which occurred during eating, with an instantaneous rise to absorption >0.14 were excluded from the analysis. Acid reflux was defined as periods when pH fell below 4, as is generally accepted. Bilirubin reflux was defined as periods when the absorbance was greater than 0.14.

The ambulatory manometry data was analyzed using Multigram for DOS software (Synectics Medtronics, Stockholm, Sweden). All readings were first examined visually to ensure correct baseline settings for detection of esophageal contractions but not pressure changes due to breathing, and were if needed, corrected. The software was set to detect an oesophageal contraction if the pressure rose above 20 mm Hg for a minimum duration of 1 second. The recording was divided into periods of pain, meal, postprandial (0-30 minutes after meal intake), upright and supine. Propagated contractions were defined as a peristaltic wave passing all three pressure sensors. An effective propagation was defined as a propagated contraction with a contractile pressure of at least 30 mm Hg at all three recording points[125].

Statistics

In the study of an association between DGOR and oesophageal motility, a univariate regression analysis was performed for percent of time with pH <4, percent of time with bilirubin absorbance >0.14, age and sex with percent effective contractions as the dependent variable. A multivariate linear regression analysis was performed with log (pH), log (bilirubin), age and sex, again with the percent effective contractions as the dependent variable.

In the study of normal variation, median and 5-95 percentiles were calculated and non-parametric tests were used for statistical analyses (Friedman's test and Kruskal-Wallis' where applicable)

Gastric emptying (IV)

A test meal with a technetium-99m labeled, 1300kJ, omelet and an unlabelled low-calorie, 290 kJ drink was ingested after an overnight fast[151]. Tobacco use was not allowed after midnight before the examination. Immediately upon finishing the meal, a soft drink was taken and imaging was started. The persons were investigated in a standing position. Successive 1-minute frontal and dorsal registrations were made every 5 minutes during the first 50 minutes, followed by imaging every 10 minutes for a total of 120 minutes. All were allowed to move between the registrations, but were usually sitting comfortably close to the camera.

The activity in the stomach was outlined by a region of interest in each of the images and geometric mean values calculated. After correction for physical decay, the total activity in the stomach was converted to percent of the maximum count rate recorded in each investigation and the values were plotted against time. The same procedure was done for regions of interest in the proximal and distal stomach.

A linear fit computation by least-square regression was performed and applied to the linear part of the curve. This was manually defined in all investigations. In most subjects the linear phase lasted between 30 and 120 minutes. The linear emptying was defined as the slope of the fitted curve (%/minute). The lag phase (lag 90) and half-emptying time (T_{50}) were defined by the intercepts of the regression line with the 90% and 50% levels, respectively. In addition, the linear fit was used to define the percentage gastric retention of the meal at 60, 90 and 120 minutes by using the values of the regression lines at these positions. The area under the curve (AUC) was calculated for fundic and antral activity over time to assess proximal gastric emptying.

Statistical methods

Comparisons between healthy volunteers and patients with GORD were performed with the Mann-Whitney U-test. A univariate regression analysis was performed for lag 90, T_{50}, gastric emptying rate and fundus emptying rate, with percent of time with pH <4 or percent of time with bilirubin absorbance >0.14 as the dependent variable. The natural log of bilirubin and pH were used for all statistical analysis. A power analysis showed 80 % probability of detecting a 11 minutes difference in lag 90, 0.2 %/minute in gastric emptying rate and 21 minutes in half emptying time. $p < 0.05$ was considered significant.

1.5 RESULTS

1.5.1 Paper I

Cholecystectomy

In this case control study there was no statistically significant associations between cholecystectomy and any of the three studied cancers (Figure 3). An analysis was also made for cancer risk amongst persons with the combination of reflux symptoms and previous cholecystectomy. Although number of observed cases was small, the cholecystectomy did not seem to further enhance the positive association between gastro-oesophageal reflux disease and oesophageal adenocarcinoma. In 118 persons with Barrett's oesophagus, 14 of which had been cholecystectomized, there was no increased risk for oesophageal adenocarcinoma (data not shown).
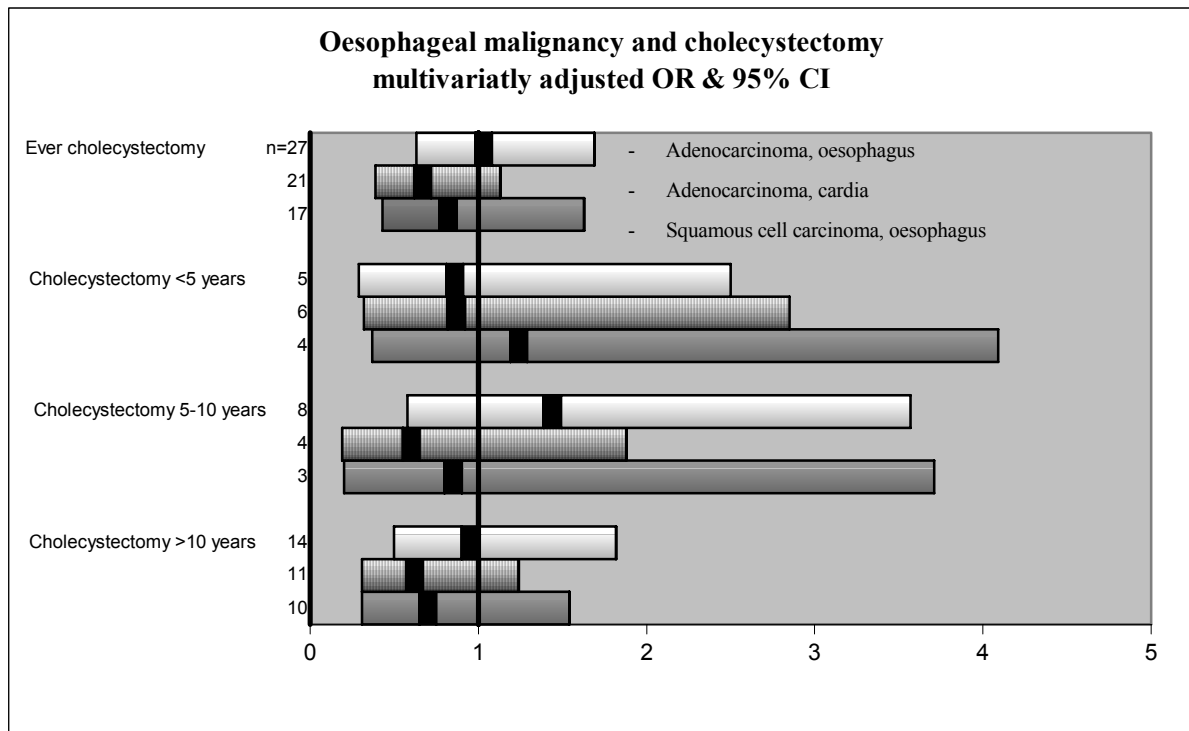
Figure 3. *Odds ratios (OR) and confidence intervals for oesophageal cancers after cholecystectomy. N=number of cases.*

Peptic ulcer disease

In the age- and gender-adjusted analyses there were no conspicuous and statistically significant associations between self-reported previous ulcer disease and risk for any of the studied carcinomas. All ulcers had not been classified as either duodenal or gastric, which accounts for the missing four patients in group 3. After adjustments, however, the odds ratio for oesophageal adenocarcinoma among individuals with a peptic ulcer history was 0.6, relative to persons without such a history. This departure from unity was not statistically significant (95% CI=0.3-1.1). The risk deficit was of similar magnitude regardless of whether the ulcer had a gastric or duodenal location. Reflux was the co-variate that accounted for most of the confounding. The corresponding relative risk estimates for adenocarcinoma of the gastric cardia and oesophageal squamous cell carcinoma were close to unity.

1.5.2 Paper II

The cohort consisted of 268 312 cholecystectomised persons, who together contributed to a 3 151 494 person-year risk. The mean follow-up duration after surgery was 13 years. Cholecystectomy was more common among women than in men in Sweden during the study period. At the time of surgery, men were on average older than women.

Among persons who had been followed from 1 to 32 years following cholecystectomy, there were 53 observed cases of adenocarcinoma of the oesophagus compared to the expected 38 cases derived from the Swedish population. This rendered a 30% increase in risk (SIR = 1.3; 95% CI = 1.0-1.8), similar in men and women, which persisted 10 years after cholecystectomy (SIR = 1.5; 95% CI = 1.0-2.1). The excess risk of oesophageal adenocarcinoma was higher among persons who were 60 or older at cholecystectomy, compared to those who underwent a cholecystectomy at a younger age. No association was found between the risk for squamous-cell carcinoma of the oesophagus and cholecystectomy. After exclusion of the first-year observation after cholecystectomy, the SIR was 0.9 (95% CI = 0.7-1.1). No significant difference for excess risk was observed either for gender or latency interval after surgery (Figure 4).
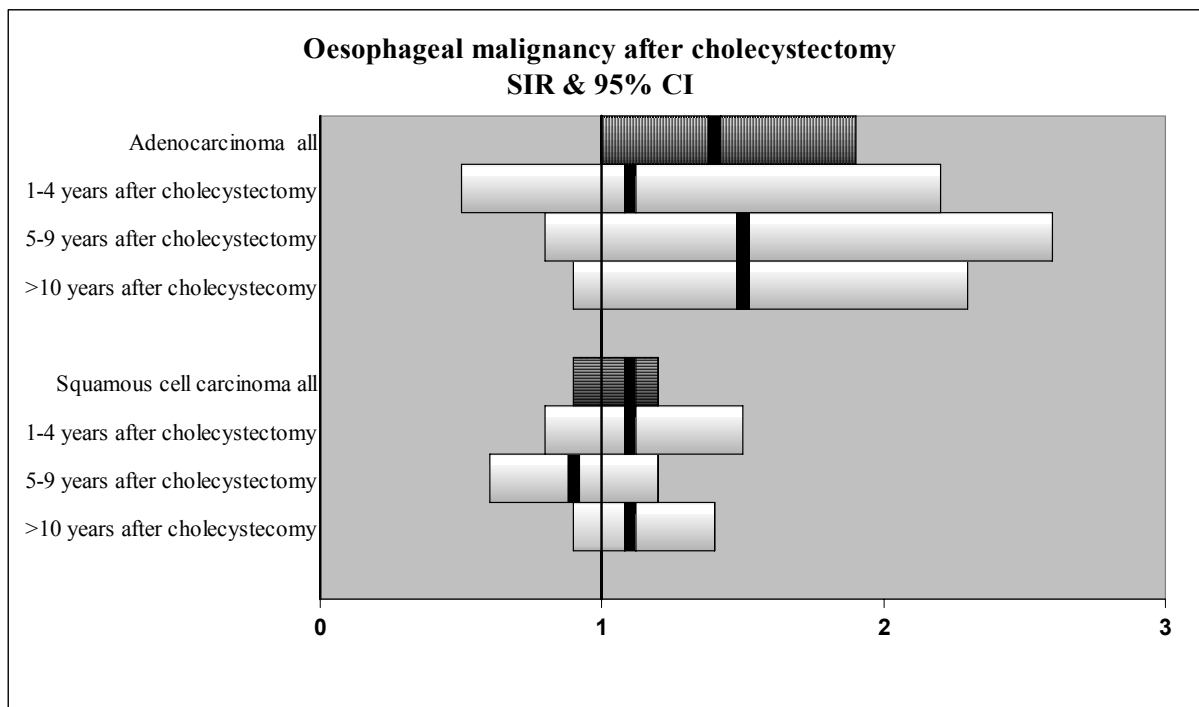

Figure 4. *Standard incidence ratio (SIR) and confidence intervals (CI) for oesophageal malignancy after cholecystectomy.*

As a comparison group, 167 646 persons who had been hospitalised for gallstone disease were also analysed. In total, the cohort contributed with 658 641 person-years at risk. On average, persons included in the gallstone cohort were older (64 years) than those in the cholecystectomy cohort (53 years). The mean follow-up duration was about 5 years. No increased risk of oesophageal adenocarcinoma was observed in this cohort (SIR = 0.9; 95% CI=0.5-1.6). Lack of association was evident across all strata stratified by gender, latency interval after surgery or age at surgery. Similarly, no increased risk of squamous-cell carcinoma of the esophagus was found in the gall stone cohort (SIR = 1.0; 95% CI = 0.7-1.3) (data not shown).

1.5.3 Paper III

Endoscopy and stationary manometry.
Three of the subjects were H. pylori positive and 7 had a distance from the diaphragm to the gastro-oesophageal junction greater than 3 cm. According to the Los Angeles classification of oesophagitis, 29 were classified as normal, 6 as grade A, 12 as grade B and 2 as grade C. The median (range) resting LOS pressure was 13.5 (6-50) mm Hg and length was 4 (1-7) cm.

Results of pH and bile reflux studies
Fourteen persons showed increased bile reflux (of which one had a normal pH study (3.3% of total time with pH<4)), 21 had only acid reflux and 14 had no pathological reflux of acid nor bile into the oesophagus (Figure 5). There was a strong correlation between acid and bile reflux. The percentage of time with pH<4 was not significantly different between the A±B+ and A+B- groups (9.8 (3.3-26.7) and 4.9 (3.5-20.8), respectively, p=0.16).

Figure 5. *Subjects in study stratified by endoscopic appearance and results of pH and bile measurements. ERD=erosive reflux disease. NERD=non-erosive reflux disease.*

Ambulatory manometry.
There was a negative correlation between bilirubin and the percent effective contractions (p=0.008), but not statistically significantly between pH and the percent effective contractions (p=0.06) during the 24-hour study period. There was no significant correlation between age or sex and the percent effective contractions. During the postprandial, upright and supine periods there was a significant negative correlation between bilirubin and the percent effective contractions, but during the meal period no significant correlation was found. In a multivariate analysis only bilirubin could account for the variation in percent effective contractions (p=0.001, R^2=0.22). The result was the same for complete propagated contractions, i.e. when not taking the force of the contraction into account.

Postoperative motility study.
Follow-up 24-hour manometry was done one year after laparascopic fundoplication in 10 patients (median 540 days; range 265-940). All patients were free of reflux symptoms after the operation. Acid exposure was significantly reduced after fundoplication; 7.2 (range 1.7-22.1) percent of time with pH<4 before vs 2.0 (0-7.9) after surgery (p=0.004). DGOR was reduced from 2.8 (range 0-46) percent time with bilirubin absorbance>0.14, to 0.0 (range 0-16) after surgery (not significant, only four patients with DGOR prior to surgery). There was no consistent change in the proportion of effective propagated contractions. In a linear regression model, neither the amount of preoperative bile reflux nor acid reflux effected the postoperative difference in proportion of effective propagated contractions. Subanalyses regarding supine, upright and postprandial periods gave similar results.
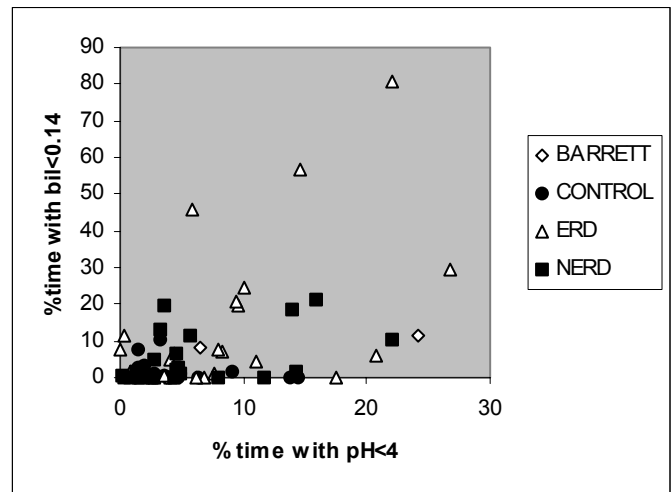
1.5.4 Paper IV

Solid gastric emptying
There was no difference between the B+ and the B- subjects with regard to any of the parameters of gastric emptying studied (lag 90, T_{50}, gastric emptying rate and amount retained in the stomach at 60, 90 and 120 min (figure 4). Similarly, there was no difference in the emptying rate of the gastric fundus to the antrum. In a univariate analysis there was no association between the percent of time with bilirubin >0.14 in the esophagus and any of the gastric emptying parameters. No
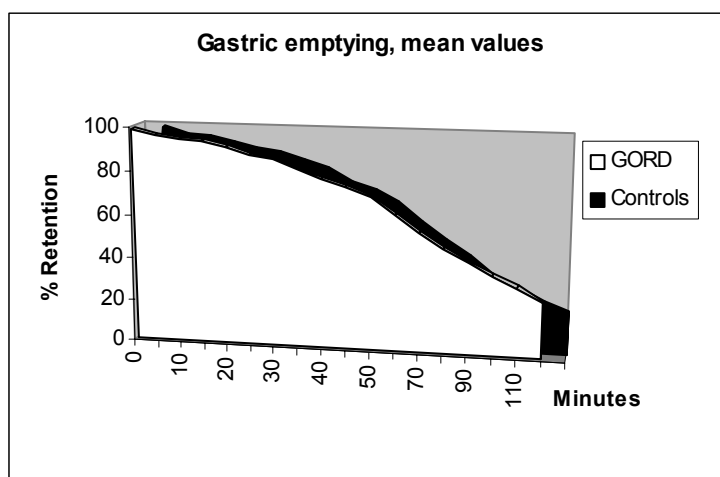

Figure 6. *Mean values for retained gastric activity in patients with reflux disease (GORD) and normal controls after a radioactively labeled test meal.*

association was found between any of the gastric emptying parameters and the percent of time with pH <4 in the esophagus. Of the 15 patients with bile reflux, none had a pathological value for the lag phase, one had a abnormal rate of gastric emptying and two had an abnormal half emptying time when compared to the large cohort of normal subjects previously collected.

24-hour pH- and bilirubin monitoring
The median (range) percent time with bilirubin >0.14 and pH was greater in the B+ subjects (20.8 (7.7-80.7) and 15.3 (0-55), respectively) than in the B- subjects (0 (0-5.8) and 2.7 (1.5-20.8), respectively) (both p<0.05).

24-hour manometry
There was no difference in LES pressure or length in the B+ and B- subjects (pressure; 13.5 (7.0-31.0) vs 18.5 (6.0-28.0) mm Hg, p=0.78, length; 4.0 (2.0-6.0) vs 4.0 (2.0-7.0) cm., p=0.66, respectively). Complete 24-hour recordings were obtained in 10 of the B+ and 14 of the B- subjects, the remaining six were studied before the ambulatory manometry test became available. The percent effective contractions were significantly lower in the B+ group compared to the B- subjects. Similar significant results were obtained if the postprandial, supine or upright periods were examined. There was no correlation between the percent effective contractions in the esophagus, both over the whole 24-hour period and the postprandial period as compared to the gastric emptying rate (%/min) (R^2=0.12, p=0.56 and R^2=0.16, p=0.45, respectively).

1.5.5 Paper V

Acid reflux.

The median percent time with pH<4 for the whole period of study was 3.1% (1.5-14; 5 and 95 percentiles). However two of the male subjects had pH<4, for more than 13% of the study period and neither of them had symptoms of gastro- oesophageal reflux. The reflux episodes occurred mainly in upright stance.

Bile reflux.

The mean distribution of bilirubin detection in the oesophagus was evenly distributed over the day. Bile was detected for a median of 0.1% (0.0-7.7; 5 and 95 percentiles) of the time.

Ambulatory manometry.

The subjects were studied for a median of 20 hours (range 16-22), of which the meal period lasted 44 (range 18-170) minutes. They were supine for a median of 8.9 (range 6-16) hours. Median contractile amplitude in the different parts of the oesophagus was higher in the proximal pressure point, 67 mm Hg, than in the two distal, 51 and 52 mm Hg respectively, p<0.05, the difference was not due to malfunctioning of the recording apparatus. This finding differs from previous studies where pressure is usually highest in the distal point[155, 156].

Parameter	Median (5-95 percentiles)
pH, %time with pH<4	3.1 (1.5-14)
upright	5.5 (2.5-20.7)
supine	0.0 (0.0-3.7)
Bile, %time with absorption>0.14	0.1 (0.0-7.7)
upright	0.2 (0.0-3.8)
supine	0.0 (0.0-14.9)
Manometry	
% peristaltic contractions	82 (66-90)
% efficient contractions	30 (14-45)
contractions/min upright	1.2 (0.6-1.7)
contractions/min supine	0.40 (0.3-0.7)

Table 2. *Normal values for reflux of acid and bile to the oesophagus, and different motility parameters in 20 healthy subjects.*

There was no significant difference in pressure between the studied time periods at the two upper levels. At the lowest pressure point, the amplitude was somewhat higher during supine periods compared to the other periods (p=0.008). Approximately 2% of all contractions were hypertensive, i.e. more then 180 mm Hg. Eighty percent of the contractions were peristaltic, 53% of which were complete, i.e. 43% of the total number of motility patterns. Of these, 65% had a pressure over 30 mm Hg at all three pressure points, giving an efficient peristalsis in a median of 30% (range 4.8-47) of all registered contractile patterns. The percentage of effective contractions was not statistically different in the subdivisions of the total time (meal-postprandial-supine-upright), neither was the percentage of complete propagated contractions. There was no correlation between the percent of time with pH <4 and bilirubin >0.14 and percent effective contractions in these normal subjects.

Gender and age.

Median percent time with pH<4 did not differ for men and women, being 2.8% (range 1.5-14.5) and 3.8% (range 1.4-6.3), respectively. Neither did the percent time with bilirubin >0.14 differ for men and women, being 0.0% (range 0.0-10.2) and 0.3% (range 0.0-7.6), respectively. Females had fewer effective contractions than males during the upright period, 19% (range 4.6-31) vs 32% (range 17-67), p=0.04. In a regression analyses there was no correlation between age the studied oesophageal parameters.

1.6 DISCUSSION

1.6.1 DGOR and oesophageal cancer.

Reflux of duodenal juice into the oesophagus increases the risk for malignant transformation of the oesophageal lining. This has clearly been demonstrated in animal studies[118-121], but is much more difficult to demonstrate in humans. It has been shown that patients with pre-malignant changes in the oesophageal mucosa frequently demonstrate reflux of duodenal juices to the oesophagus[100-104]. It is difficult to demonstrate a causal relationship, as acid exposure is elevated in parallel with bile exposure. The correlation between GORD and oesophageal cancer was addressed in a recently published paper, with a Swedish population-based case-control study, where a strong association between GORD and adenocarcinoma of the oesophagus was demonstrated[11]. In order to address the question of bile reflux, we expanded this study to include patients who were cholecystectomized and thus expected to have an increased risk of DGOR, as it has previously been shown that cholecystectomy is followed by a 3-10 fold increase in duodeno- gastric reflux[83, 84, 126-128], although these results have been challenged[129]. The incidence of GORD after cholecystectomy was not found to be increased in one study[130], but the reverse has been found true in others[131, 132].

We did not find any association between cholecystectomy and cancer of the oesophagus in our case-control study. However, in this study relatively few patients with oesophageal cancer had been cholecystectomised. Thus, to further test our hypothesis that DGOR might be of importance, a population based study was performed and a 30% increase in risk of adenocarcinoma of the oesophagus was found in patients after cholecystectomy. The reason for this increased risk of oesophageal adenocarcinoma, is likely to be due to effects of acid and bile on the oesophageal mucosa, where bile acids have been shown to stimulate cell growth in the foregut epithelium[112, 113].

These results demonstrate the difficulty of using a case-control approach for rare diseases. Although a good control of confounding factors can be achieved, it is difficult to acquire the volume of patients needed. In this case, it was found that using large, population based, national registries, gave better means to demonstrate a significant association, however losing some control over confounding factors and diagnostic accuracy.

The majority of cholecystectomised patients only have small increases in duodeno- gastric reflux, and most of them do not have significant gastro- oesophageal reflux, so it follows that the increase in risk is carried by a limited number of patients. The low incidence of oesophageal adenocarcinoma, and the small increase in risk after cholecystectomy, does not warrant any change in clinical practice when dealing with patients with gall-stone disease. The implication is rather to increase awareness of DGOR as a pathogen in GORD and perhaps making anti-reflux surgery an option for these patients.

1.6.2 DGOR and foregut motility.

1.6.2.1 DGOR and oesophageal motility.

In the material presented in this thesis, 60% of patients with symptoms of gastro- oesophageal reflux at least twice weekly for more than 6 months had erosive disease. This is a somewhat higher figure than previously presented, where around 40% of GORD-patients have erosive disease[133]. This discrepancy could be explained by the fact that the patients in the present material were examined at a referral centre and therefore were somewhat selected. Forty percent of the patients with erosive disease had pathological reflux of bile to the oesophagus which is lower than the 79% described by Vaezi et al.[134], but similar to the results presented by Cuomo et al.[135] and Romagnoli et al.[136].

Los Angeles classification of GORD
A Erosions <5 mm in length
B Erosion >5 mm in length but not spanning mucosal folds.
C Erosions covering less than 75% of the circumference
D Erosions more extensive than C

Figure 7. *Adapeted from Armstrong et al. The endoscopic assessment of esophagitis: a progress report on observer agreement. Gastroenterology 1996*

Previous studies have shown a clear relationship between DGOR and increasing grades of oesophagitis[88, 104, 135]. This does not prove that duodenal juice worsens the oesophagitis, since acid load is increased in parallel. In the present material, the majority of those with erosive disease had oesophagitis grade A and B of the Los Angeles classification. Only 2 patients had grade C, and none had grade D (Figure 7). A possible explanation is that most patients in this country have already received treatment with PPI:s before endoscopy. All patients in the present study were however instructed not to take proton pump inhibitors and histamine receptor blockers in the two weeks prior to examination.

It has previously been shown that oesophageal motility deteriorates with increasing grades of oesophagitis[125, 137]. To assess if DGOR is associated with changes in oesophageal motility, subjects with GORD and normal controls were studied with ambulatory recordings of oesophageal motility, pH and bile reflux. Oesophageal motility parameters were correlated with acid and bile load in the oesophagus (paper III). It was demonstrated that deteriorating oesophageal motility was correlated with bile reflux. When analysed in a multivariate model, percent time with bile reflux, and not acid reflux, was the significant factor. This again implies a possible role for duodenal juice in the pathogenesis of reflux disease. Bile reflux might impair oesophageal motility, by thickening of submucosal collagen[137],which may perpetuate the disease by decreasing acid and bile clearance, thus further enabling mucosal damage and risk of oesophageal carcinogenesis. An alternate interpretation could be the other way around, that is that patients with DGOR might have a disturbed foregut motility causing the reflux pattern. Interestingly, oesophageal motility did not improve after fundoplication. The same results has been reached by other investigators both following surgery[35, 137, 138] and adequate medical treatment[36, 139]. This could imply that structural damage caused by reflux is irreversible, alternatively that these patients have impaired oesophageal motility caused by something else than reflux, and therefore do not improve after correction of reflux.

1.6.2.2 DGOR and gastric emptying.

To investigate if DGOR is associated with disturbances of gastric emptying, subjects with DGOR and normal subjects were studied with a scintigraphic gastric emptying technique and ambulatory recordings of oesophageal pH, bile manometry (paper IV). No correlation was found between the various parameters of gastric emptying and acid or bile reflux. Further, gastric emptying parameters were similar in GORD and in healthy volunteers, implying that there is no general foregut motility disorder accompanying DGOR. Several previous studies have addressed the question of gastric emptying and reflux disease, with some finding evidence of delayed gastric emptying in up to 40% of GORD-patients[25, 26], but others not[27, 28]. More recently, studies have concentrated on proximal gastric retention, because patients with GORD have been shown to have a more pronounced fundic relaxation in response to meals. It has been found that there indeed seems to be a delayed proximal gastric emptying in reflux disease, both in scintigraphic[24] and 3-D ultrasonic[140] studies. These results could not be confirmed in paper III. Further evidence that delayed gastric emptying is not a common problem in GORD is that pharmacological treatment with prokinetics has had very disappointing results[141].

1.6.3 DGOR and clinical practice.

Some authors do not believe that DGOR is of any clinical importance and it has been demonstrated that acid reduction with proton pump inhibitors effectively reduce both acid and bile reflux healing 9 of 10 patients with oesophagitis[36, 102]. It has also been shown that acute reflux symptoms are related to acid, and not biliary reflux[142]. The mechanism by which acid suppression reduces DGOR is thought to be a reduction of intragastric volume. There is a potential danger with this regime in patients with massive bile reflux as unconjugated bile acids are much more harmful at a neutral pH and deconjugation of bile acids has been shown to occur in some patients on continuous medication with PPI:s[89]. The alternative to medical treatment is of course surgery (Figure 8). Fundoplication is well known to effectively inhibit gastro- oesophageal reflux and has been

Figure 8. *Surgical options in treatment of GORD*

put forward as the treatment of choice for patients with DGOR and Barrett's oesophagus[124, 143]. An alternative operation is a diversion of duodenal juice such as a duodenal switch procedure. This treatment effectively inhibits duodenal juices reaching the stomach and oesophagus but does not reduce the production of gastric acid and pepsin. Of great interest, and providing further evidence for the importance of DGOR in GORD, is a publication by Salminen et al.[68], where severe reflux disease was treated by a Roux-en-Y procedure and selective vagotomy, previously described by Fékété et al[144]. All patients were relieved of their symptoms and the oesophagitis healed, but postoperative pH-analyses showed unaltered acid load in the oesophagus. Thus inhibition of biliary, but not acid, reflux promoted healing and symptom relief.
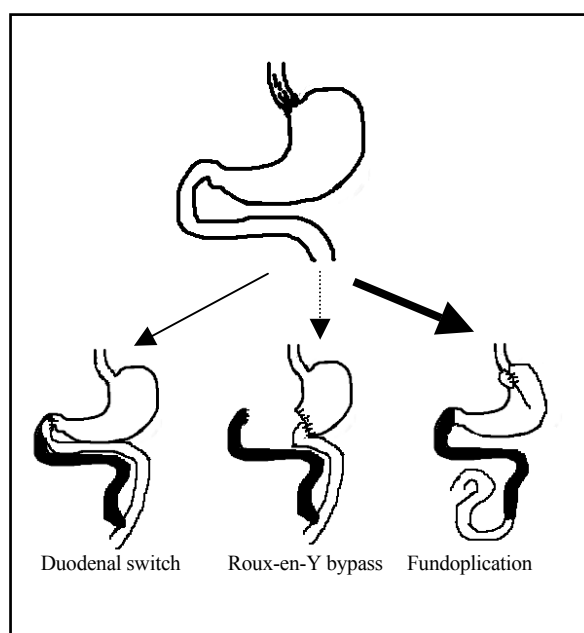
1.6.4 Oesophageal physiology in health.

The final paper (paper V) deals with normal variations of oesophageal pH, bile reflux and motility and includes reference values. When performing simultaneous recordings of these three parameters, at least two catheters had to be used since there is no available catheter incorporating all three functions. The added discomfort of the extra catheter could have some influence on the normal reference values for the different parameters. Using this dual catheter system for simultaneous recordings of pH, motility and bile, normal values for pH were higher than reference values seen in standard pH-metry. The normal variation of bile reflux was similar to that reported by other groups as were the motility parameters excepting the percentage of efficient peristaltic waves, which showed somewhat lower values.

The upper limit for percent time with pH<4 of 14% for the whole period of study was higher than expected and certainly higher than the value of 3.4% used at our department. This discrepancy resulted from two male individuals who had increased upright reflux. In a search in recent literature, a similar pattern has been observed by many authors[145-148] but the reason is unclear. Perhaps male individuals have a lower threshold for nausea caused by the indwelling naso-oesophageal catheters, and therefore increased reflux over the lower oesophageal sphincter. If this be the case, one would expect to find much lower pH-values during sleep, and this was infact found in the present study.

The upper limit of 7.7% for percent time with detected bilirubin in the oesophagus compares favourably with figures previously presented by others[55, 88, 149]. One exception being a Danish study where high levels of bilirubin were detected both in normal persons and following fundoplication of GORD-patients[150]. There may have been some technical problems in this Danish study since 5 of 42 subjects showed obviously erroneous recordings and were excluded. Furthermore, dietary restrictions to inhibit food that could block the reflective tip mirror of the bilitec catheter, and give false high readings were not inforced.

Manometric variables did not appear sexrelated, except for percent efficient contractions, during the supine time interval, where women had less efficient peristalsis. This parallels the slower gastric emptying seen in pre-menopausal women[151]. A possible explanation could be the effects of oestrogen on the activity of the vagal nerve[152] or perhaps an increased activity of nitric oxide synthase[153]. All the women in this study were pre-menopausal. There was no influence of age on the different motility parameters which has previously been reported in a stationary manometric study of oesophageal motility[154]. A possible explanation could be the relatively narrow age span (21-44 and one of 72 years) of the volunteers in paper V.

Studies on normal variation in ambulatory oesophageal motor function are scarce. The number of contractions and contraction amplitudes in the present study are similar to those previously published[137, 155, 156]. The persons in this study were instructed only to take liquid meals, to prevent blocking of the tip mirror of the Bilitec catheter, which could result in an underestimation of the physiological amplitudes during meal periods. Results show that swallowing of solid food results in higher amplitudes[137]. Oesophageal peristalsis was significantly diminished during the supine period where there was a clear reduction in the percentage of peristaltic waves, and also a reduction to half the number of contractions per minute, which is in accordance with previous findings[137, 155, 156].

1.7 MAIN CONCLUSIONS

- There was an increase in risk for adenocarcinoma of the oesophagus after cholecystectomy of about 30%.

- The risk for adenocarcinoma of the oesophagus after cholecystectomy is not high enough to warrant a change in the management of gall stone disease.

- A possible explanation for this risk increase is the increase in duodeno- gastro- oesophageal reflux (DGOR) following cholecystectomy.

- DGOR, but not acid reflux, is associated with less effective oesophageal motility. It is possible that DGOR causes impaired motility thereby perpetuating the disease, possibly increasing the risk of developing adenocarcinoma.

- DGOR is not associated with changes in gastric emptying, indicating otherwise normal foregut motility with DGOR.

- Different catheter set-ups used for studying oesophageal function results in different normal values. This needs to be considered when assessing results from different studies.

- Should treatment of GERD change due to the results of this study? It is possible that surgery should be considered for patients with advanced DGOR as surgery corrects both acid and bile reflux, where medical treatment can be insufficient.

2 ACKNOWLEDGEMENTS

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