Mitchell, David Ross (2018) Environmental factors relevant to the rising incidence of gastro-oesophageal reflux disease. MD thesis.

https://theses.gla.ac.uk/30793/

Copyright and moral rights for this work are retained by the author

A copy can be downloaded for personal non-commercial research or study, without prior permission or charge

This work cannot be reproduced or quoted extensively from without first obtaining permission in writing from the author

The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the author

When referring to this work, full bibliographic details including the author, title, awarding institution and date of the thesis must be given

Enlighten: Theses
https://theses.gla.ac.uk/
research-enlighten@glasgow.ac.uk

Environmental factors relevant to the rising incidence of Gastro-oesophageal Reflux Disease

Dr David Ross Mitchell MB ChB, MRCP(UK)(Gastroenterology)

Submitted in fulfilment of the requirements of the

Degree of *Doctor of Medicine*

Institute of Cardiovascular and Medical Sciences

College of Medical, Veterinary and Life Sciences

University of Glasgow

September 2018

Abstract

Gastro-oesophageal reflux disease (GORD) is one of the commonest chronic conditions in the western world with a reported prevalence of 10-20% in Europe and the USA. The disease involves an interplay between factors promoting reflux of gastric juice and failure of defensive forces designed to neutralise the resulting acidity. Transient lower oesophageal relaxations, the acid pocket and the presence of a hiatus hernia are important factors. Acid reflux can cause benign oesophageal injury, including oesophagitis, oesophageal ulceration and peptic structuring, as well as malignant complications like Barrett's oesophagus and oesophageal adenocarcinoma (OAC). GORD, Barrett's and OAC rates have been rising over the last few decades in the Western World and the reasons for this are unclear.

Helicobacter pylori (*H. pylori*) is a common bacterial infection of the stomach present in the majority of the world's human population. It is known to cause chronic gastritis, and can be complicated by the development of peptic ulcer disease, gastric adenocarcinoma and gastric MALT lymphoma. An unexplained observation regarding *H. pylori* infection is its negative association with gastro-oesophageal reflux disease and its malignant complications.

The prevalence of *H. pylori* infection appears to be falling, especially within the Western World. It is possible that *H. pylori* infection is protecting against the development of oesophageal disease from acid reflux and one possible explanation is the infection causing a reduction in gastric acid secretory function. For this to be true, the protective effect from *H. pylori* must be apparent in the majority of those infected. There is little available data on the effect of *H. pylori* infection within the general population. The few previous studies assessing gastric acid secretion have used *H. pylori* infected healthy volunteers, rather than subjects representative of the general population.

The incidence of central obesity is rising in both children and adults across the world. Obesity is strongly associated with gastro-oesophageal reflux and its complications of Barrett's oesophagus and OAC. Central adiposity seems to be of particular importance. The nature of this association is incompletely understood and both mechanical and humoral effects of central obesity may be important.

In the first study we investigated whether the incidences of OAC and gastric adenocarcinoma, as well as their time trends, may be inversely related pointing to a common environmental factor exerting opposite effects on these cancers. We used cross-

sectional data from “Cancer Incidence in Five Continents” (CI5) Volume X and GLOBOCAN 2012. Relevant ICD-10 codes were used to locate oesophageal and gastric cancers anatomically, and ICD-O codes for the histological diagnosis of OAC. For longitudinal analyses, age standardized rates (ASRs) of OAC and total gastric cancer (TGC) were extracted from CI5C-Plus. Estimated (2012) ASRs were available for 51 countries and these showed significant negative correlations between OAC and both TGC (males: correlation coefficient (CC) = −0.38, P =0.006, females: CC= −0.41, P =0.003) and non-cardia gastric cancer rates (males: CC= −0.41, P =0.003 and females: CC= −0.43, P =0.005). Annual incidence trends were analysed for 38 populations through 1989–2007 and showed significant decreases for TGC in 89% and increases for OAC in 66% of these, with no population showing a fall in the latter. Significant negative correlation between the incidence trends of the two cancers was observed in 27 of the 38 populations over the 19–50 years of available paired data. Super-imposition of the longitudinal and cross-sectional data indicated that populations with a current high incidence of OAC and low incidence of gastric cancer had previously resembled countries with a high incidence of gastric cancer and low incidence of OAC. The negative association between gastric cancer and OAC in both current incidences and time trends is consistent with a common environmental factor predisposing to one and protecting from the other.

In our second study we assessed the gastric acid secretory capacity in different anatomical regions in *H. pylori* positive and negative volunteers in a Western population. We studied 31 *H. pylori* positive and 28 *H. pylori* negative volunteers, matched for age, gender and body mass index. Jumbo biopsies were taken at 11 predetermined locations from the gastro-oesophageal junction and stomach. Combined high-resolution pHmetry (12 sensors) and manometry (36 sensors) was performed for 20 min fasted and 90 min postprandially. The squamocolumnar junction was marked with radio-opaque clips and visualised radiologically. Biopsies were scored for inflammation and density of parietal, chief and G cells immunohistochemically.

Under fasting conditions, the *H. pylori* positives had less intragastric acidity compared with negatives at all sensors >1.1 cm distal to the peak lower oesophageal sphincter (LOS) pressure ($p<0.01$). Postprandially, intragastric acidity was less in *H. pylori* positives at sensors 2.2, 3.3 and 4.4 cm distal to the peak LOS pressure ($p<0.05$), but there were no significant differences in more distal sensors. The postprandial acid pocket was thus attenuated in *H. pylori* positives. The *H. pylori* positives had a lower density of parietal and chief cells compared with *H. pylori* negatives in 10 of the 11 gastric locations ($p<0.05$).

17/31 of the *H. pylori* positives were *CagA*-seropositive and showed a more marked reduction in intragastric acidity and increased mucosal inflammation. In conclusion, *H. pylori* positives have reduced intragastric acidity which most markedly affects the postprandial acid pocket.

In the third and final study we investigated the effect of increasing abdominal pressure by waist belt on reflux in patients with reflux disease. We performed a prospective study of patients with oesophagitis (n = 8) or Barrett's oesophagus (n = 6); median age was 56 years and median body mass index was 26.8. Proton pump inhibitors were stopped at least 7 days before the study and H_2 receptor antagonists were stopped for at least 24 hours before. The severity of upper GI symptoms was assessed, and measurements of height, weight, and waist and hip circumference taken. Combined high-resolution pH measurements and manometry were performed in fasted state for 20 minutes and for 90 minutes following a standardized meal. The squamocolumnar junction was marked by endoscopically placed radio-opaque clips. The procedures were performed with and without a waist belt (a weight-lifter belt applied tightly and inflated to a constant cuff pressure of 50 mmHg). Without the belt, intragastric pressure correlated with waist circumference ($r = 0.682$; $P = .008$), with the range in pressure between smallest and largest waist circumference being 15 mmHg. The belt increased intragastric pressure by a median of 6.9 mmHg during fasting ($P = .002$) and by 9.0 mmHg after the meal ($P = .001$). Gastro-oesophageal acid reflux at each of the pH sensors extending 5.5 cm proximal to the peak lower oesophageal sphincter pressure point was increased by approximately 8-fold by the belt (all $P < .05$). Following the meal, the mean number of reflux events with the belt was 4, vs 2 without ($P = .008$). Transient lower oesophageal sphincter relaxations were not increased by the belt, but those associated with reflux were increased (2 vs 3.5; $P = .04$). The most marked effect of the belt was impaired oesophageal clearance of refluxed acid (median values of 23.0 seconds without belt vs 81.1 seconds with belt) ($P = .008$). The pattern of impaired clearance was that of rapid re-reflux after peristaltic clearance. In conclusion we found belt compression increased acid reflux following a meal. The intragastric pressure rise inducing this effect is well within the range associated with differing waist circumference and likely to be relevant to the association between obesity and reflux disease.

Contents

Tables

Figures

Publications

- Mitchell DR, Derakhshan MH, Wirz AA, Ballantyne SA, McColl KEL. Abdominal compression by waist belt aggravates gastroesophageal reflux, primarily by impairing esophageal clearance. Gastroenterology. 2017;152(8):1881-1888.

- Mitchell DR, Derakhshan MH, Wirz AA, Orange C, Ballantyne SA, Going JJ, McColl KE. The gastric acid pocket is attenuated in *H. pylori* infected subjects. Gut. 2017;66(9):1555-1562.

- Derakhshan MH, Arnold M, Brewster DH, Going JJ, Mitchell DR, Forman D, McColl KE. Worldwide Inverse Association between Gastric Cancer and Esophageal Adenocarcinoma Suggesting a Common Environmental Factor Exerting Opposing Effects. Am J Gastroenterol. 2016;111(2):228-39.

- Mitchell DR, Derakhshan MH, Robertson EV, McColl KE. The Role of the Acid Pocket in Gastroesophageal Reflux Disease. J Clin Gastroenterol. 2016 Feb;50(2):111-9.

Acknowledgments

Much of the work presented within this thesis is the result of collaboration with many people, all of whom deserve my gratitude.

Firstly, I wish to thank my supervisor Professor Kenneth E.L. McColl who has delivered superb support and guidance through this journey. His knowledge and experience of this area of research is exceptional, however it was his enthusiasm for the subject which kept me motivated. It has been a real privilege to have been given this opportunity to work closely with him.

I have worked beside Dr Mohammed Derakhshan on all these studies and he deserves special thanks. His work in preparing and analysing our biopsy specimens was vital. He was heavily involved in collection and analysis of the cross-sectional and longitudinal cancer registry data in the study of the inverse association between gastric and oesophageal adenocarcinoma.

I would like to thank Dr James Going for his expert advice in the histopathological analysis of our specimens and in his role as my secondary supervisor.

Sister Angela Wirz deserves great thanks for all the time and assistance she provided in her role as research nurse. She was involved in patient recruitment as well as assisting with the physiological studies.

I would like to thank Dr Stuart Ballantyne for his radiological expertise and to the radiography staff at Gartnavel General Hospital who were always available promptly to take X-rays at vital stages of the study protocol.

My thanks to David H. Brewster at the Scottish Cancer Registry and to David Forman and Melina Arnold at the International Agency for Cancer Research who we collaborated with in our work on the inverse association between gastric and oesophageal adenocarcinoma using cancer registry data.

I would like to acknowledge Clare Orange for her assistance with the preparation and staining of our biopsy specimens.

Many thanks also to the endoscopy nursing staff at Gartnavel General Hospital who ensured the safety and comfort of our volunteers during their endoscopic procedure, and for assisting me with the endoscopy and biopsies.

My special thanks to Dorothy Ronney who has provided immense administrative support as part of our research team, and in her role with the University.

In addition, I would like to thank Janice Arnott for her administrative support and her assistance with our study volunteers.

I am thankful to all our volunteers who have given their valuable time and effort to be part of these studies.

Finally, I would like to thank my family for their support, encouragement and understanding during my research period. I am particularly grateful to my parents, David and Lorna, and my wife Rachel, as without their support this thesis would not have been completed.

Author’s declaration

I declare that, except where explicit reference is made to the contribution of others, that this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Printed Name: _____

Signature: _____

CHAPTER 1

Rising Incidence of Gastro-oesophageal Reflux Disease and its Complications

1.1 Introduction

Gastro-oesophageal reflux disease (GORD) is one of the commonest chronic conditions in the western world with a reported prevalence of 10-20% in Europe and the USA.(1) A common definition is “a condition which develops when the reflux of stomach contents causes troublesome symptoms and/or complications.”(2) The classical symptoms of GORD are heartburn and acid regurgitation, and the condition can be diagnosed based on the occurrence of these symptoms at least twice per week. Other less common symptoms include dysphagia, odynophagia, nausea and extra-oesophageal symptoms such as chronic cough, hoarseness and asthma. Complications of GORD include Barrett’s oesophagus and oesophageal adenocarcinoma. Epidemiological data shows a rising incidence of GORD and these malignant complications.

1.2 Pathophysiology

1.2.1 Anatomy of the gastro-oesophageal junction

The understanding of the pathophysiology of GORD has constantly evolved, and it is now accepted that the condition involves an interplay between factors promoting reflux of gastric juice and failure of defensive forces designed to neutralise the resulting acidity. The gastro-oesophageal junction functions as a barrier to prevent acid reflux and is anatomically complex. It is composed of an intrinsic and extrinsic sphincter. The intrinsic sphincter is made up of a ring of smooth muscle approximately 3cm in length. The muscle fibres split at the distal end to from short transverse clasps around the lesser curve of the stomach and long muscle loops around the greater curve, known as the gastric sling.(3) The external sphincter is provided by contraction of the diaphragmatic crura during normal respiration.

The distal end of the oesophagus is attached to the diaphragm by the phrenoesophageal membrane. This membrane inserts circumferentially into the oesophageal musculature very close to the squamocolumnar junction (SCJ).(4) There is a 3-4cm portion of the distal oesophagus which lies below the diaphragm within the abdominal cavity.

1.2.2 Role of Transient Lower Oesophageal Sphincter Relaxations

Transient lower oesophageal sphincter relaxations (TLOSRs) are the most common events which allow the defences of the gastro-oesophageal junction (GOJ) to be breached. These TLOSRs are defined as lower oesophageal sphincter (LOS) relaxations not induced by swallowing.(5) They play an important physiological role by allowing venting of gas from the stomach following a meal when intragastric pressure is increased.(6) Complete relaxation of the LOS and proximal movement of the GOJ above the crural diaphragm occur simultaneously to facilitate the release of gas (7) and this provides an ideal time for acid reflux to occur. It has been shown in healthy volunteers that 80-100% of acid reflux events are associated with TLOSRs.(8) In GORD patients, the overall number of TLOSRs is not increased compared to controls, however the number of TLOSRs associated with reflux events is increased.(9) A positive pressure gradient between the stomach and the GOJ lumen is required for acid reflux to occur during TLOSRs and this can be provided by abdominal straining or the inspiratory phase of respiration.(10)

Whilst TLOSRs explain why acid reflux is more frequent in the period following a meal, it does not explain why the refluxate is acidic. This paradox of acid reflux occurring at a time when the intragastric environment is least acidic due to the buffering effect of the meal was explained by the discovery of the acid pocket. This was first described in 2001 when Fletcher *et al* discovered an area of low pH immediately distal to the cardia using dual pH electrode pull-through studies 15 minutes after a meal.(11) The authors hypothesised that there was a local pocket of acid close to the gastro-oesophageal junction which escaped the buffering effect of the meal. It was proposed that this pocket is the source of acid in postprandial refluxate. The finding of regional differences of acidity in the stomach in the postprandial period has been confirmed in several subsequent studies.(12, 13)

1.2.3 Role of hiatus hernia

A hiatus hernia is a condition in which elements of the abdominal cavity herniate through the oesophageal hiatus into the mediastinum.(14) This usually involves the stomach and can be a sliding hernia or para-oesophageal hernia. Sliding hernia are more common, and result from disruption of the GOJ due to dilatation of the diaphragmatic hiatus and circumferential laxity of the phrenoesophageal ligament. This leads to dysfunction of the

GOJ, which can allow acidic gastric juice leading to reflux. There is no link between the less common para-oesophageal hernias and GORD.

A sliding hiatal hernia can be diagnosed by upper GI endoscopy when the SCJ is positioned greater than 2cm proximal to the diaphragmatic hiatus. It can also be diagnosed by barium studies, or by high resolution manometry where a double pressure peak is seen, with the proximal peak representing the internal sphincter and the distal peak caused by the diaphragmatic crura.

The correlation between reflux disease and hiatus hernia is well established. GORD patients with oesophagitis are more likely to have a hiatus hernia than those without oesophagitis.(15) An increasing hiatus hernia size correlates with an increased oesophageal acid exposure time, an increased number of long reflux events and a prolonged acid clearance time.(16) Increasing hiatal hernia size is the main predictor of reflux oesophagitis and severity of gastro-oesophageal reflux.(17)

The presence of a hiatus hernia is thought to disrupt several of the normal anti-reflux mechanisms. A study from the 1960s using simultaneous barium and manometry techniques showed early retrograde flow of previously swallowed barium situated within the hiatus hernia during a subsequent swallow. This can happen as the internal sphincter relaxes immediately after the swallow. The barium will flow along the pressure gradient from the hiatus hernia, through the relaxed sphincter, and into the distal oesophagus. In addition, as the intrinsic sphincter has moved above the diaphragm, it no longer benefits from the compressive effect of intra-abdominal pressure.(18) The extrinsic sphincter provided by the diaphragmatic crura remains closed preventing the acid from leaving the hiatal sac distally into the stomach. It has been shown that the more distal extrinsic sphincter remains intact during swallow-associated LOS relaxations, but completely relaxes during TLOSRs.(19)

This demonstrated re-reflux of barium from the hiatal sac was also shown to occur with gastric acid in a subsequent study. Mittal *et al* in 1987 observed that in hiatus hernia patients, a small amount of acid became trapped in the hiatal hernia sac and this acid refluxed back into the oesophagus during subsequent swallow induced relaxations of the LOS. This repeated re-reflux contributed to impaired oesophageal clearance.(20) This is another important mechanism linked to GORD and Jones *et al* found that impaired oesophageal clearance strongly correlated with oesophagitis and hiatus hernia.(16)

Sloan *et al* introduced the concept of early and late retrograde flow. They found late retrograde flow of barium 5-10 seconds after a swallow occurred in control subjects without hiatus hernia and subjects with a reducing hiatus hernia. However, subjects with a non-reducing hernia had early retrograde flow. They suggested that in the subjects without a hernia or with a hernia which had reduced into its original position, the distal portion of the oesophagus remained below the diaphragm and therefore benefited from intra-abdominal pressure helping to maintain a closed sphincter and prevent reflux until the sphincter was opened by the food bolus. However, in subjects with a non-reducing hernia, early retrograde flow occurred as the intrinsic sphincter was intra-thoracic and therefore intra-gastric pressure easily exceeded intra-sphincteric pressure. In this study the non-reducing hernia group had significantly greater acid clearance times than controls.(21)

In addition to the effect on oesophageal acid clearance, a hiatus hernia can also alter the length and position of the acid pocket. Beaumont *et al* found patients with a large hiatus hernia had a longer acid pocket and it was situated above the diaphragm for a longer period compared to those with a small hiatus hernia. As a result, the proximal border of the acid pocket was situated above the SCJ for 50% of the time in large hiatus hernia patients compared to 16% in patients with a small hiatus hernia.(13)

Whilst acid reflux is most common after a meal, it can occur at other times, and this must involve mechanisms other than TLOSRs. Van Herwaarden *et al* described other mechanisms which disrupt the GOJ in patients with hiatus hernia include lower resting LOS pressure, swallow-associated normal LOS relaxations, deep inspiration and straining.(22) Subjects with hiatus hernia have a lower maximal pressure within the high pressure zone of the GOJ.(23)

1.3 Complications of gastro-oesophageal reflux disease

Acid reflux can cause a spectrum of oesophageal injury, from oesophagitis, oesophageal ulceration, peptic structuring, Barrett's oesophagus and oesophageal adenocarcinoma (OAC).

Erosive oesophagitis is diagnosed at upper gastrointestinal (GI) endoscopy. The most validated classification system is the Los Angeles classification of oesophagitis.(24) It includes four grades (A-D) based on the length and circumferential extent of mucosal breaks. In severe oesophagitis ulceration can be present. A benign peptic stricture can

develop within the distal oesophagus due to chronic oesophageal injury and can present as dysphagia requiring endoscopic dilatation.

Barrett's oesophagus is a condition in which metaplastic columnar epithelium with goblet cells replaces the stratified squamous epithelium which normally lines the distal oesophagus. It occurs as a consequence of chronic inflammation due to reflux disease and is a predisposing factor for the development of oesophageal adenocarcinoma.

The earliest estimation of Barrett's oesophagus prevalence was an autopsy study from 1986-1987 which found an estimated prevalence of 376 cases per 100,000 population. This was much higher than the population based study they performed in parallel to the autopsy study which found a prevalence rate of 22.6 cases per 100 000 population.(25) The authors suggested that only a small proportion of this disease is actually diagnosed. Further studies, both population-based and endoscopic studies, would suggest that the prevalence of Barrett's oesophagus is between 0.5-2.0% of unselected individuals, and 5-15% of individuals with GORD.(26) An Italian study of 1533 adults from the general population who underwent upper GI endoscopy found a prevalence rate of 1.3%. Of the subjects with Barrett's oesophagus, 46.2% did not report reflux symptoms.(27)

It is estimated that Barrett's oesophagus increases the risk of oesophageal adenocarcinoma by approximately 30-125 fold compared to the general population.(28, 29) Non-dysplastic Barrett's mucosa can progress to low-grade and high-grade dysplasia before adenocarcinoma develops. The annual incidence of OAC in Barrett's patients varies from 0.3% to 0.6%. The combined incidence of high grade dysplasia and OAC is 0.9 to 1.0%.(30, 31)

Oesophageal adenocarcinoma is the main histological type of oesophageal cancer in the Western World. In 2012 there were an estimated 52,000 oesophageal adenocarcinomas diagnosed worldwide and the vast majority of these were in Northern and Western Europe, North America and Australia.(32) This is in contrast to squamous cell cancers which are by far the commonest type of oesophageal cancer worldwide.

Oesophageal adenocarcinomas are predominantly found in the distal third of the oesophagus and the main risk factors for developing this cancer are GORD, obesity and cigarette smoking whilst oesophageal squamous cell cancers develop more proximally within the oesophagus, and alcohol, diet and smoking are more significant risk factors.

The UK has the highest reported incidence rate of oesophageal adenocarcinoma at 7.2 per 100 000 person-years in men and 2.5 per 100 000 person-years in women. There is an

obvious male predominance in oesophageal cancer incidence, stronger than any other non-sex specific cancer. In North America the incidence in men is more than eight times greater than women.(32) White men have the highest risk of developing oesophageal adenocarcinoma. Another North American study found the cancer was three times more common in white men than black men, and 7.6 times more common in white men compared to white women.(33)

There is a strong association between symptoms of GORD (heartburn and acid regurgitation) and risk of oesophageal adenocarcinoma. A pooled analysis of 5 case-control studies found individuals with symptoms for at least 30 years had a 6.2-fold higher risk for developing oesophageal adenocarcinoma.(34) Approximately 40% of patients who develop oesophageal adenocarcinoma are not known to have GORD prior to diagnosis.(35)

1.4 Rising incidence of gastro-oesophageal reflux disease

Evidence to support the rising incidence of GORD exists, although it has not been extensively documented. The HUNT study was a Norwegian population-based cohort study which showed that patient-reported symptoms of reflux disease increased from 31.4% between 1995-1997 to 40.9% between 2006-2009.(36) A systematic review comparing GORD prevalence in population-based studies conducted before and after 1995 found a statistically significant increase. This was true using studies from North America, Europe and East Asia.(37)

However, there is more convincing data for the increasing incidence of the malignant complications of GORD. Prach *et al* found an increase in incidence of Barrett's oesophagus from 1.4 new cases per 1000 upper gastrointestinal endoscopies in 1980–1981 to 42.7 new cases per 1000 upper gastrointestinal endoscopies in 1992–1993 in Scotland.(38) Conio *et al* also observed a strong increase in the incidence of BO from 0.37 to 10.5 cases per 100 000 person years in Minnesota but the authors suggested that the similar 22 fold increase in number of endoscopies performed may explain the increase.(39) A Dutch cohort study of 386,002 patients showed the incidence of Barrett's oesophagus increased from 14.3 per 100,000 person years in 1997 to 23.1 per 100,000 person years in 2002.(40) When controlled for the change in endoscopy numbers of the same time period, the increase was even more significant.

Oesophageal adenocarcinoma has been one of the fastest increasing malignancies in many countries and is the fastest rising solid cancer in the western world.(41) This increase

appears to have begun in the 1970s in Europe, North America and Australia.(42) It has now overtaken squamous cell carcinoma as the commonest oesophageal cancer in many countries within the Western World.(32) One of the first studies to highlight this rise was a North American study which found incidence rates of oesophageal adenocarcinoma rose by 100% from 1976 to 1987. The average annual increase in white men in this study was 9.4%, and a similar rise of 9.8% was calculated for black men.(33) Using the same North American database, another study discovered a six-fold increase in oesophageal adenocarcinoma incidence from 1975 to 2001.(43)

A large epidemiological study of cancer registries from eight Western countries found the average annual increase ranged from 3.5% per year in Scotland to 8.1% per year in Hawaii between 1960 and 1990.(42) Increases in incidence of the cancer in females has mirrored the rise in males, however it remains 3 to 9 times lower than male cancer incidence.

The previously discussed Dutch cohort study that showed a rise in Barrett's oesophagus also found an increased incidence of oesophageal adenocarcinoma. The cancer incidence rose from 1.7 per 100,000 person years in 1997 to 6.0 per 100,000 person years in 2012.(40)

A North American study using data from the Surveillance, Epidemiology and End Results (SEER) program found an increase in oesophageal adenocarcinoma from 1.01 per 100,000 person-years in 1975-79 to 5.69 per 100,000 person-years in 2000-2004 in white men, a 463% increase. A similar rise of 335% was seen in white women. This study did not analyse data among blacks and other races due to a lack of oesophageal adenocarcinoma diagnoses within this population.(41)

1.5 Conclusion

GORD is a common chronic condition which occurs due to failure of the barrier function of the GOJ. Understanding of the mechanisms leading to this failure are improving. TLOSRs play a key role, as does the presence of a sliding hiatus hernia. A reduced LOS resting pressure, impairment of oesophageal clearance, oesophageal hypersensitivity and the acid pocket are also involved. Whilst GORD itself is a benign condition, it can lead to the malignant complications of Barrett's oesophagus and oesophageal adenocarcinoma. GORD, Barrett's and oesophageal adenocarcinoma rates have been rising over the last few decades in the Western World and the reasons for this are unclear.

CHAPTER 2

Helicobacter pylori and Gastro-oesophageal Reflux Disease

2.1 Introduction

Helicobacter pylori (*H. pylori*) is a common bacterial infection of the stomach present in the majority of the world's human population. It is known to cause chronic gastritis and can be complicated by the development of peptic ulcer disease, gastric adenocarcinoma and gastric MALT lymphoma. An unexplained observation regarding *H. pylori* infection is its negative association with gastro-oesophageal reflux disease and its complications of Barrett's oesophagus and oesophageal adenocarcinoma. This chapter will look at our current knowledge of the effect of *H. pylori* infection on gastric acid secretion in the general population and discuss potential mechanisms for this negative association.

2.2 *Helicobacter pylori* infection

H. pylori was first discovered in 1984 by Australian scientists, Robert Warren and Barry Marshall. They announced their discovery in the paper titled "Unidentified Curved Bacilli in the Stomach of Patients with Gastritis and Peptic Ulceration" which was published in the Lancet.(44) They described the bacteria as S-shaped or curved gram-negative rods, 3 μm x 0.5 μm in size. They noted it's similarity to *Campylobacter* and suggested the name *Campylobacter pyloridis* initially. They found a close correlation between antral gastritis and the presence of the bacteria, linking this as a causal factor for the first time. Finally they hypothesised that peptic ulceration may be due to this bacteria, at a time when the aetiology of peptic ulceration was unknown.(44) In 2005 they jointly received the Nobel Prize in Physiology/Medicine for the discovery of "the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease".

H. pylori infection is primarily acquired during childhood and the transmission occurs through an oral-oral or fecal-oral route primarily within families and particularly in the setting of poor sanitation and hygiene.(45) *H. pylori* has evolved to thrive in the harsh environment of the human stomach. It secretes urease, an enzyme that converts urea into bicarbonate and ammonia, which neutralises the gastric acid. In the majority of cases, colonized *H. pylori* persists in the stomach over the lifetime of the individual host unless eradicated with antibiotics.(46)

2.2.1 Epidemiology

H. pylori is estimated to infect more than half of the entire human population. A recent systematic review of studies of *H. pylori* prevalence from 62 countries found the highest rates of *H. pylori* are in Africa, with a pooled prevalence rate of 70.1%.(47) The lowest rates are found in Australia with a prevalence rate of 24.6% in the general population, however 76% of the indigenous community were infected. The regions with the highest prevalence of *H. pylori* infection are West Asia, South America and Africa, whilst Western Europe, North America and Oceania all have rates lower than 40%.(47)

The prevalence of *H. pylori* infection appears to be decreasing in many parts of the world. The same recent systematic review looked at two time periods (1970-1999 and 2000-2016) and found that *H. pylori* prevalence was lower after the year 2000 in Europe, North America and Oceana. The greatest change was in North America which saw *H. pylori* prevalence drop from 42.7% before 2000 to 26.6% after.(47)

A study from Finland looked at prevalence rates of gastritis, as a surrogate marker for *H. pylori* infection, in patients undergoing diagnostic upper GI endoscopy within a single hospital in 1977, 1985 and 1992. They found an 18% fall in prevalence of gastritis between 1977 and 1992. The greatest reduction was in the 20-49 age group where there was a decrease in prevalence of 38%.(48)

A large retrospective study from Belgium looked at rates of *H. pylori* infection from more than 22,000 patients from 1998 to 2007.(49) They found the overall proportion of infected patients fell from 43% in 1988 to 29% in 2007. They also grouped patients by their ethnic origin and found those from Western Europe had the lowest rate of infection (31.2%) and patients originally from Turkey and North Africa had the highest rates of infection (71.1% and 68.5% respectively).

Even in Eastern countries with higher *H. pylori* prevalence there is evidence of falling rates of the infection. A South Korean study of over 15,000 subjects found a fall in *H. pylori* prevalence from 66.9% in 1998 to 59.6% in 2005. The highest rates remained in the low income group and in subjects from the provinces.(50) In the Guangzhou province in China, a study found that the overall *H. pylori* seroprevalence rate had decreased from 62.5% in 1993 to 47% in 2003.(51)

The falling prevalence of *H. pylori* infection in Western countries is likely to reflect reduced overcrowding, improved sanitation, improved access to clean water and improved socioeconomic status.

2.2.2 Histopathology

The primary histological feature of *H. pylori* infection is infiltration of the lamina propria by plasma cells, lymphocytes and occasional eosinophils. Active chronic superficial gastritis indicates the presence of neutrophils within the lamina propria in addition to these chronic inflammatory cells. Degenerative changes in gastric surface epithelial cells can occur, including cellular oedema, apical mucin loss and microerosions.(52) Lymphoid aggregates are commonly found and are located close to the muscularis mucosa.(53)

The *H. pylori* organisms can often be easily recognised as curved or S-shaped bacilli on routine haematoxylin and eosin (H&E) staining within the surface mucus layer. They are most often seen within the gastric pits. Special stains can be used to make recognition of the infection easier, including the modified Giemsa stain. The organisms are usually most numerous in the gastric antral mucosa with a resulting higher intensity of associated gastritis in this area.

Chronic gastritis is known to progress in a proportion of patients to atrophic gastritis. Atrophic gastritis refers to loss of parietal and chief cells in the gastric body mucosa. This leads to increased space between the glands which become occupied by inflammatory cells or loose connective tissue. The degree of glandular loss can be graded as mild, moderate or severe as described in the Sydney system for the classification of chronic gastritis.(54) Atrophy of the antral mucosa can be more difficult to recognise as the mucosa is composed of mucus glands and often contains a more intense inflammatory cell infiltrate. The grading of the degrees of gastric atrophy shows considerable interobserver variation, especially antral mucosa.(55) Atrophic gastritis can be patchy and multifocal in the early stages. The lesser curvature tends to be affected first with subsequent spread and coalescence of atrophic areas from the antrum up towards the incisura angularis and beyond. This spread is known as the atrophic front.(56)

There is a subgroup of *H. pylori* infected patients in whom bacterial colonisation and gastritis primarily affects the gastric body, with the antrum being relatively spared. This is accompanied by hypochlorhydria and increased atrophic gastritis in the stomach body.(57)

Intestinal metaplasia commonly accompanies *H. pylori* associated atrophic gastritis. *H. pylori* organisms are rarely found in areas of intestinal metaplasia in the stomach, and in patients with severely atrophic gastritis and extensive intestinal metaplasia, the organism can disappear.

2.2.3 *CagA*

The *CagA* gene, which encodes the *CagA* (*Cytotoxin-associated gene A*) protein, is found within a specific chromosomal region called the *cag* pathogenicity island (PAI) in some *H. pylori* strains. It is thought that this is involved in the translocation of *CagA* into the cytoplasm of gastric epithelial cells. Approximately 30–40% of *H. pylori* strains isolated in Western countries do not carry the *cag* PAI and thus are *cagA*-negative, whereas almost all the *H. pylori* isolates from East Asian countries contain the *cag* PAI and are thus *cagA*-positive.(58) It has been found that *cagA*-PAI positive strains of *H. Pylori* cause peptic ulceration and gastric cancer more frequently that *cag*A-PAI negative strains.(59)

2.2.4 Complications of *H. pylori* infection

2.2.4.1 Peptic ulcer disease

H. pylori infection is a common cause of both gastric and duodenal ulceration. Up to 10% of patients infected with *H. pylori* may develop peptic ulcers.(60) Gastric ulcers are thought to occur due the direct effect of the organism on the gastric mucosa. The infection causes mucus depletion and microerosions of the gastric surface epithelial cells which may permit acid, pepsin, bacterial antigens and toxins into the underlying mucosa, leading to the formation of a gastric ulcer.(52) In subjects with duodenal ulcers, the infection produces a predominantly antral gastritis which stimulates gastrin production, causing increased amounts of acid to be produced by the well-maintained and non-inflamed gastric secretory cell mass of the oxyntic mucosa. The gastrin-mediated negative feedback control of acid secretion is lost(61) and the increased acid load passes into the duodenum, damaging the mucosa, causing duodenal ulceration and gastric metaplasia. *H. pylori* may colonise this gastric metaplasia and the consequent inflammation may contribute to the ulceration.

2.2.4.2 Gastric cancer

H. Pylori was classed as a type 1 carcinogen in 1994 by the International Agency for Research in Cancer after large epidemiological studies suggested a strong association with non-cardia gastric cancers.(62) Patients with a corpus predominant gastritis or pan-gastritis with patchy but widespread atrophy and intestinal metaplasia seem to be at particular risk of gastric carcinoma.(63) Approximately 89% of all gastric cancers can be

attributable to *H. pylori* infection.(47) *Cag-A* positive strains of the infection have been associated with an increased risk of gastric cancer.(59) There is increasing evidence that eradication of *H. pylori* infection reduces the risk of gastric cancer.(64)

2.2.4.3 Gastric MALT lymphoma

Epidemiologic studies have shown strong associations between *H. pylori* infection and the presence of gastric MALT lymphomas.(65) Furthermore, eradication of the infection causes regression of most localized gastric MALT lymphomas.(66)

2.3 Negative association between *H. pylori* and GORD

An unexplained observation regarding *H. pylori* infection is its negative association with gastro-oesophageal reflux disease and its complications of Barrett's oesophagus and oesophageal adenocarcinoma. A systematic review published in the BMJ in 2003 examined the prevalence of *H. pylori* infection in patients with GORD. The pooled estimate of the odds ratio from the 20 studies included in the review was 0.60 (95% CI, 0.47-0.78), indicating a lower prevalence of the infection in GORD patients.(67) Substantial heterogeneity was found between the studies, with location being an important factor in this. Further analysis of the studies based on location found studies from the Far East and North America had stronger odds ratios, with studies from Western Europe being equivocal for this association.(67)

A Korean case-control study of 5615 subjects undergoing endoscopy showed the prevalence of *H. pylori* infection was lower in cases of erosive reflux oesophagitis than in controls (38.5% vs 58.2%, $p<0.001$).(68) A large Japanese cross-sectional study of over 10,000 subjects found a negative correlation between *H. pylori* and erosive reflux oesophagitis, but not with non-erosive reflux disease.(69) They also found a negative association between erosive reflux oesophagitis and the pepsinogen I/II ratio, a serological marker for gastric fundic gland atrophy, supporting a protective effect of atrophic gastritis in GORD.

There is more epidemiological data on the inverse association between *H. pylori* infection and the malignant complications of GORD. A North American case-control study of 533 men recruited from the colorectal cancer screening programme and 80 men diagnosed with Barrett's oesophagus found *H. pylori* infection was inversely associated with Barrett's

oesophagus (OR 0.53; 95% CI, 0.29-0.97). The association was stronger with the *CagA* positive strain (OR 0.36; 95% CI, 0.14-0.90).(70) An Irish case-control study published in Gut in 2007 found *H. pylori* seropositivity was associated with a greater than 50% reduction in risk of reflux oesophagitis, Barrett's oesophagus and oesophageal adenocarcinoma.(71) A meta-analysis of 49 studies examining the effect of *H. pylori* infection on Barrett's oesophagus, found a protective effect despite obvious heterogeneity. Four studies were identified that did not have obvious selection and information bias, and these showed a protective effect with a relative risk of 0.46 (95% CI, 0.35-0.60).(72) In addition, seven studies examined the effect of *CagA* positivity which found an even greater protective effect with a relative risk of 0.38 (95% CI, 0.19-0.78).(72) An earlier meta-analysis found similar results with a pooled odds ratio of 0.64 (95% CI, 0.43-0.94) for the relationship between Barrett's oesophagus and *H. pylori* prevalence, and 0.39 (95% CI, 0.21-0.76) for the relationship with *CagA* positive strains.(73) This meta-analysis also included ten studies examining the association between *H. pylori* infection and oesophageal adenocarcinoma and found a negative association with an odds ratio of 0.51 (95% CI, 0.31-0.82).(73)

2.4 Potential mechanisms to explain negative association

It has been postulated that the negative association between *H. pylori* infection and GORD, Barrett's and oesophageal adenocarcinoma may represent the gastric infection protecting against these oesophageal disorders. If so, the falling incidence of the infection in the general population might explain the rising incidence of the oesophageal disorders. One mechanism by which the infection might protect against oesophageal disease is by reducing the ability of the gastric mucosa to secrete acid and pepsin which are the constituents of gastric juice which can induce oesophageal damage. Relatively little is currently known about the effect of chronic *H. pylori* infection on gastric secretory function in the 90% of infected patients who do not develop gastric or duodenal complications. If the degree of reduction in oesophageal disease in the *H. pylori* infected population is due to the infection reducing gastric acid secretion, then this suppression of acid secretion would need to be apparent in the majority of infected subjects.

There is also uncertainty over the mechanism of *H. pylori* infection reducing acid secretion. Potential mechanisms include loss of glands due to gastric atrophy and a reduction in acid output from the glands due to mucosal inflammation. It is known that the

pattern of inflammation and atrophy in *H. pylori* infection does not occur uniformly throughout the stomach and is more marked at the junction between oxyntic mucosa of the stomach body and antral mucosa. This is potentially relevant as differences in secretory function at the proximal border between oxyntic mucosa and cardiac mucosa could contribute to a protective effect from GORD.

2.5 Measurement of gastric acid secretion

Gastric acid secretion has been measured using many different methods. One of the first validated methods was to perform nasogastric aspiration of gastric juice. The position of the nasogastric tube within the stomach was determined either by fluoroscopy or by the water recovery test.[74] The latter method ensured the tube was in the most dependent part of the stomach corpus, allowing the maximum volume of gastric juice to be aspirated and therefore minimise losses by gastric emptying. It also obviated the requirement for X-ray exposure. The potential mesurements of gastric secretion that can be made by nasogastric aspiration include basal acid output (BAO), maximal acid output (MAO) and peak acid output (PAO). BAO is generally a small volume which can fluctuate throughout the course of the day, making it of limited value. MAO and PAO are measured by stimulating gastric acid secretion, and are better measures of parietal cell mass. Histamine [75, 76], pentagastrin, tetragastrin, gastrin-17, gastrin-releasing peptide, bombesin and meal stimulation have all been used for this purpose. The samples, which are usually collected over 15 minute periods, are titrated to a pH of 7 using an alkaline solution to determine the hydrogen ion concentration. The acid output is calculated by multiplying the hydrogen ion concentration by the volume of gastric aspirate. MAO is the acid output obtained over a period of one hour following stimulation, whilst PAO uses the highest measurements obtained within that hour.

The Endoscopic Gastrin Test (EGT) is a newer method for measuring gastric acid secretion developed in Japan.[77] It involves subjects being given an injection of tetragastrin or pentagastrin prior to the endoscopy. At endoscopy any pooled gastric acid on initial intubation is aspirated and discarded. The gastric acid produced between 20 and 30 minutes after injection is then aspirated and collected in a bottle placed between the endoscope and the aspirator. Titration is performed to determine the hydrogen ion concentration. The acid output is calculated by multiplying this with the volume of gastric aspirate, and it is expressed as H^+mEq/10 min.

Nasogastric placement of pH-measuring electrodes can be used as a measure of gastric acidity. They are usually antimony electrodes which are smaller and cheaper than glass electrodes. Most studies have been performed using either one or two sensors. Studies using two sensors usually have one in the oesophagus to measure reflux, and one in the stomach. The gastric electrode can be positioned either using fluoroscopy or by concurrent manometry analysis of the LOS. It has been shown that by advancing the pH probe beyond the LOS the distal end usually ends up in the gastric fundus. 24 hour pH results have been found to be highly reproducible at this site.[78] The main disadvantage to this method is that there is no measurement of the actual volume of acid produced. The measured pH is that of gastric juice which, in addition to hydrochloric acid, is made up of other substances like pepsin and mucous, and can be affected by saliva and bicarbonate secretions.

2.6 Gastric acid secretion in the healthy population

2.6.1 Effect of *H. pylori* infection

There are few studies of gastric acid secretion specifically looking at the healthy population. Peterson *et al* studied 63 *H. Pylori* positive healthy volunteers and 73 *H. pylori* negative healthy volunteers.(79) The acid secretory studies were carried out over a 15 year period, from 1974 to 1989; therefore pentagastrin, histamine and human gastrin heptadecapeptide were all used at various times on different subjects for gastric acid stimulation. The main finding was that BAO in *H. pylori* positive healthy volunteers was 2.8 mmol/h compared to 4.4 mmol/h in *H. pylori* negative healthy volunteers, which was a statistically significant finding. The study did not find any difference in PAO or meal-stimulated acid output.

One of the largest prospective studies was performed in Japan by Iijima *et al* and involved 157 Japanese subjects who had previously had a normal upper GI endoscopy.[80] The majority were completely asymptomatic, whilst 20 subjects had symptoms thought to be unrelated to the upper GI tract, mainly lower abdominal pain and change in bowel habit. They also included 36 healthy volunteers between the ages of 20 and 39 due to the small number of young subjects in the initial study group. They used the Endoscopic Gastrin Test to measure gastric acid secretion. They found the mean EGT value in *H. pylori* positive males was 1.6 mEq/10min, compared to 3.9 mEq/10min in *H. pylori* negative males. In women the difference was less prominent but still statistically significant.

Tarnasky *et al* measured acid secretion by NG tube aspiration under basal conditions and in response to peptone meal stimulation and pentagastrin.[81] The study started with 30 healthy males, however only 22 managed to complete the acid secretion tests. It was not stated the reasons for failure to complete the study. Two subjects were also excluded due to conflicting results from the *H. pylori* breath tests and serology. Therefore data from twenty subjects, 50% of which were *H. pylori* positive, were available for analysis. They found the BAO in *H. pylori* negative subjects was 5.7 mmol/h compares to 3.3 mmol/h in *H. pylori* positives, although this was statistically non-significant. There was no difference in pentagastrin stimulated PAO.

Smith *et al* looked at retrospective data from 95 healthy males who had undergone a 24-hour study of pH measurements from hourly intragastric acid aspiration. All tests were performed at the Royal Free Hospital in London; however, it was not clear over what period these tests had been performed. All the subjects were young, with an age range of 19 to 26. Only eight were *H. pylori* positive. They found no difference in the 24 hour intragastric acidity between the two groups of patients.[82]

Gillen *et al* compared *H. pylori* infected and uninfected healthy volunteers, as well as *H. pylori* positive duodenal ulcer patients.[83] They measured BAO, MAO to increasing doses of G-17 and MAO to increasing doses of CCK-8, by nasogastric aspiration. They found no significant differences in these between 20 infected and 35 uninfected healthy volunteers. They found that the 15 duodenal ulcer patients had significantly higher acid secretion, both under basal conditions and in response to G-17 and CCK-8 stimulation. They also investigated the concentration of gastrin needed to achieve 50% of the maximal acid response, termed C_{50}. Interestingly this showed *H. pylori* positive healthy volunteers required 164.5 ng/l gastrin, compared to 82.2 ng/l in *H. pylori* negative subjects and 69.5ng/l in duodenal ulcer patients. This gives evidence of a decreased sensitivity to gastrin stimulation in *H. pylori* infected healthy volunteers compared to duodenal ulcer patients as the reason for increased acid secretion and subsequent pathology in the latter group. Despite the lack of difference found between the two groups of healthy volunteers in this study, the reduced acid secretion found in the previously mentioned studies could be explained by *H. pylori* associated chronic active superficial gastritis affecting the stomach body, leading to reduced sensitivity to gastrin stimulation.

2.6.2 Effect of *H. pylori* eradication

Four studies have measured gastric acid secretion in healthy volunteers before and after *H. pylori* eradication. Feldman *et al* looked at 24 healthy volunteers aged between 28 and 54 who were all found to be *H. pylori* positive.[84] They underwent NG tube aspiration under basal conditions, as well as meal stimulation with a liquidised steak meal infused through the NG tube, and intragastric titration to a pH of 3. Subjects also had gastric biopsies performed through the NG tube under fluoroscopic guidance. The volunteers then had *H. pylori* eradication with lansoprazole, amoxicillin and clarithromycin for 2 weeks and acid secretion tests were repeated 4 weeks later. 67% were found to have successfully eradicated *H. pylori*. It was found that the basal acidity was 20 mmol/l higher after successful eradication, and that there was no change in acidity if eradication failed. There was no change seen for meal stimulated gastric acid secretion. All the subjects had a pan-gastritis based on the biopsy results, with resolution of the gastritis in subjects in whom *H. pylori* was successfully eradicated.

Gutierrez published a similar study in the Scandinavian Journal of Gastroenterology in 1997.[85] There were 11 *H. pylori* positive healthy subjects included, aged from 21 to 49. The study took place in Columbia. Gastric acid secretion was measured by NG aspiration, and this was carried out before and then 5 to 15 weeks after *H. pylori* eradication. The PAO increased from 14.6 mmol/h to 29.0 mmol/h after eradication, and this was a statistically significant finding. BAO increased from 3.4 mmol/h to 5.4 mmol/h, however the p value was 0.07.

Verdu *et al* measured 24 hour intragastric pH in 18 *H. pylori* positive healthy individuals.[86] The pH electrode was placed 5cm distal to the cardia, determined by fluoroscopy. Recordings were carried out on four occasions, before and after *H. pylori* eradication, and on and off omeprazole. They found no difference in the mean 24-hour pH value before (pH 1.2) and after (pH 1.3) eradication, whilst off omeprazole. They did, however, find that nocturnal pH fell from 1.6 whilst infected with *H. pylori* to a pH of 1.2 post-eradication ($p = 0.005$).

The fourth study was an American study published in 1991.[87] Five *H. pylori* positive healthy subjects from Houston had their acid secretion measured by aspirating 10ml aliquots every hour nasogastrically and measuring the pH. These were all young subjects between the ages of 21 and 25. This was repeated after *H. pylori* eradication. The study

found that the integrated intragastric acidity before and after eradication of *H. pylori* did not change.

2.6.3 Effect of aging

The remaining studies measuring gastric acid secretion looked at the effect of aging. The largest of these studies was published in Gastroenterology in 1996.[88] 206 volunteers underwent a gastric secretory study which involved an NG tube placed in the antrum. BAO and PAO were measured, and pentagastrin was used as the stimulant. The volunteers were split up into 3 groups, young (18-34), middle-aged (35-64) and elderly (>65). *H. pylori* infection rates differed between the three groups, with 81.8% of volunteers in the elderly group infected, 58.2% in the middle-aged group and 45.2% in the young group. Despite this there was no difference in BAO between the three groups. However, there was a difference in PAO between the elderly group and the other two groups. The PAO was 19 mmol/h in the elderly compared to 29.9 mmol/h in middle-aged group ($p=0.002$) and 29.3 mmol/h in young group ($p=0.004$). This paper also looked at gastric histology, by passing biopsy forceps through the NG tube under fluoroscopic guidance and taking one biopsy from the upper body. This showed that 7 subjects with *H. pylori* associated chronic atrophic gastritis (CAG) had a greatly reduced BAO and PAO of 1.0 and 6.8 mmol/h respectively. 78 subjects had chronic active superficial gastritis (CASG), and they had a slightly reduced PAO compared to subjects with normal histology ($p<0.05$) but no difference in BAO. This paper also commented on smoking habit and found that current smokers had a significantly higher BAO and PAO compared to non-smokers. Multiple regression analysis suggested that age has no effect on gastric acid output, and the reduction in PAO in the elderly was due to the higher prevalence of *H. pylori* associated CASG and CAG, as well as lower prevalence of smoking. It is worth noting that only 22 out of 206 volunteers were in the elderly group.

A Japanese study of 110 healthy volunteers was published in Gut one year later.[89] Gastric acid secretion was measured by NG tube aspiration and pentagastrin stimulation was used. The volunteers were comprised of those who had previously had gastric acid studies in the early 1970s and those who were investigated in the early 1990s. They were then divided into eight groups depending on *H. pylori* status and age. This study found that gastric acid secretion was lower in elderly subjects, both in the 1970s and 1990s. *H. pylori* infection was shown to decrease BAO and MAO, especially in the elderly population. It also

showed that acid secretion had increased in both elderly and non-elderly subjects over the 20-year period, and suggested that this was not only due to reduced *H. pylori* prevalence.

The final two studies were small studies. One showed no difference between young and old subjects, unless they had evidence of atrophy.[90] The other found elderly patients had an increased MAO to Gastrin-17 and meal-stimulated gastric acid secretion.[91]

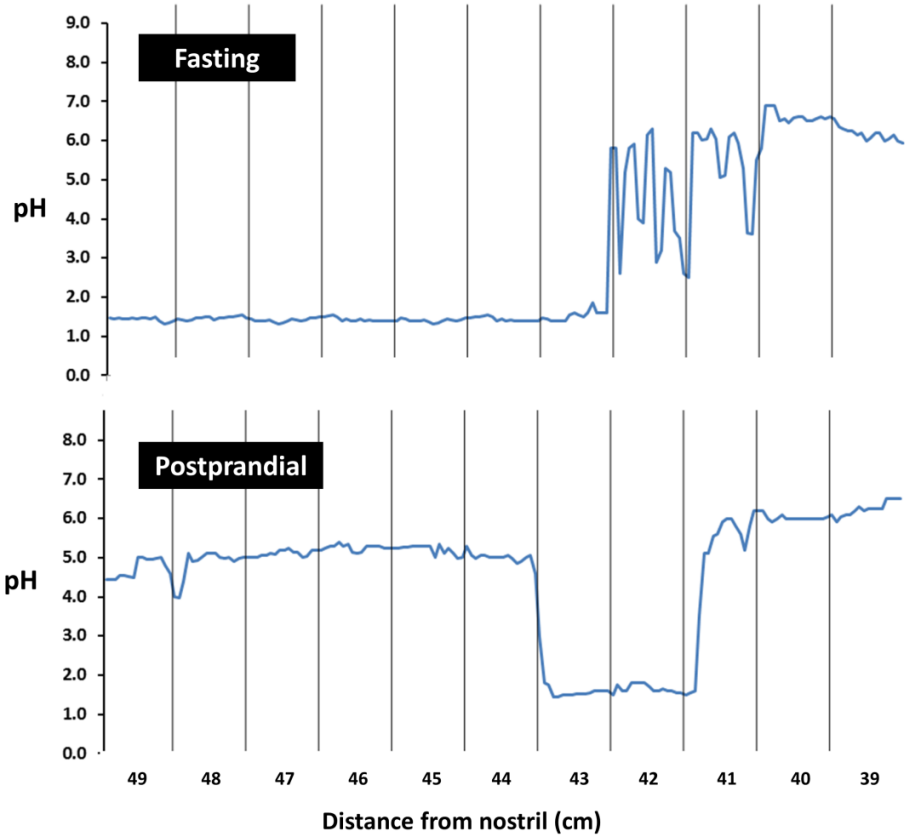
2.7 Role of the acid pocket

It is thought that refluxed acid in the postprandial period originates from gastric contents close to the gastro-oesophageal junction, and this area has been termed the acid pocket. The acid pocket was first described in 2001 when Fletcher *et al* discovered an area of low pH immediately distal to the cardia using dual pH electrode pull-through studies 15 minutes after a meal. The pH at this point was 1.6, lower than the more expected buffered intra-gastric pH of 4.4.(11) (Figure 2.1) The authors hypothesised that there was a local pocket of acid close to the gastro-oesophageal junction which escaped the buffering effect of the meal. It was proposed that this pocket is the source of acid in postprandial refluxate. The finding of regional differences of acidity in the stomach in the postprandial period has been confirmed in a number of subsequent studies.(12, 13) In actual fact the acid pocket was first described over a century ago in the context of the study of peptic ulcers. An American paper by Cannon published in 1898 states: "*In the fundus food near the periphery was acid; food 2cm from the gastric wall showed the original alkalinity.*"(92) A British paper by Hurst published in 1911 observes "*As no peristalsis and consequently no churning of the contents occurs high in the fundus, the outer layer of chyme remains constantly very acidic. A cardiac ulcer is therefore bathed in acid gastric juice at a very early stage in digestion.*"(93)

The acid pocket forms due to the buffering effect of food within the stomach. The acidity falls within the main stomach body where mixing of food and gastric juice is at its greatest. The proximal stomach relaxes following a meal and acts as a reservoir for food. (16) Acid in this area will therefore escape the buffering effect of the meal. The lack of mixing will also allow gastric juice to pool and form a layer of acid on top of the gastric contents. In-vitro experiments have been performed to mimic the movement of food within these two areas of the stomach. When a blended meal and acid is allowed to settle, acid was shown to form a separate layer floating on top. Gentle agitation caused this acid layer to

disappear and measurement of the pH through the mixture proved that mixing had started to occur.(11)

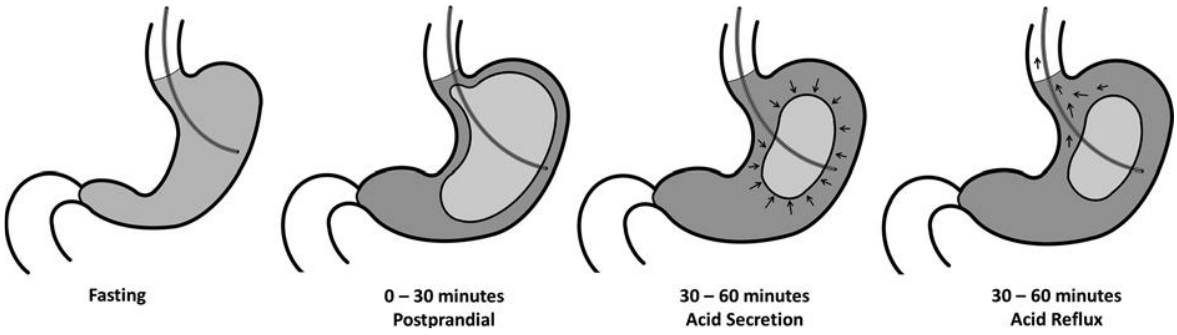
FIGURE 2.1 Example of a pH tracing recorded during the catheter pull-through technique in 1 subject while fasting and again after a meal. The postprandial recording shows a region of high acidity corresponding to the location of the pH step-up point observed under fasting conditions.



The acid pocket was first detected by stationary pull-through pHmetry, however this simply gives us a one-dimensional understanding of the acid pocket. Interestingly, in many studies of the acid pocket, another area of similar acidity is commonly detected by a more distal pH sensor. (94, 95) It is thought that this distal sensor will be pressed up against the gastric mucosa of the greater curvature.(78) The 'acid coat' theory has been proposed as an explanation for this. As acid is secreted from the gastric mucosa in response to a meal, the periphery of the gastric lumen will be most acidic due to its

proximity to the acid source. The acidity will progressively decrease towards the centre of the lumen within the intragastric contents due to a buffering effect. (Figure 2.2)

FIGURE 2.2 A schematic of the "acid coat" forming in the postprandial period. The "acid coat" (dark grey area) forms nearest the gastric mucosa and surrounds the buffered intragastric contents (light grey area).



Two factors will play a role in the evolution of this acid coat. Continuing gastric acid secretion will maintain the acidity around the periphery of the gastric lumen, and may start to overcome the buffering effect more centrally. In addition, gastric emptying means the volume of the intragastric content continues to fall. Complete gastric emptying can take up to two hours in healthy people(96) and as this process continues the buffering capacity will be reduced. Eventually the intragastric environment will return to the fully acidic state seen in the fasting period.

An 'acid film' has been proposed to exist by Pandolfino *et al* who showed the pH transition point moves proximally into the high-pressure zone of the LOS, even extending across the SCJ after a meal in GORD patients. Manometry confirmed that although the LOS pressure was lower in GORD patients, the fact that the LOS still had a pressure associated with it showed that it remained closed. The authors argued that a volume of fluid would not be able to cross the LOS, suggesting instead that only a film of acid could extend through this.(12)

2.7.1 Effect of *H. pylori* infection on the acid pocket

If the acid pocket forms due to secretion of acid from the gastric mucosa of the proximal stomach, then any factors which reduce gastric secretion, either locally or throughout the stomach, may affect the development of the acid pocket. *H. pylori* infection is the major cause of gastric mucosal atrophy and reduced acid secretion. Loss of gastric secretary cells does not occur uniformly throughout the stomach but may be more marked at the periphery of the acid-secreting mucosa.(97) The term "atrophic front" describes atrophic gastritis initially seen at the border between the antrum and the oxyntic mucosa of the gastric body and moving proximally with time. It is thought to advance quicker up the lesser curve.(56) Mucosal atrophy and intestinal metaplasia have been shown to occur more frequently on the lesser curvature compared to the greater curvature.(98) The overall reduction in gastric acid secretion throughout the oxyntic mucosa could result in reduced acidity of the acid pocket. It is also plausible that *H. pylori* infection could cause a similar process starting at the cardia and extending distally, which would potentially have a more direct effect on the acid pocket. However, this effect in the proximal stomach has not been studied previously. The falling prevalence of *H. pylori* infection in many countries (48, 99) might therefore be contributing to the rising incidence of GORD and its complications.

2.8 Conclusion

There is a negative association between *H. pylori* infection and GORD. The infection also has an inverse association with Barrett's oesophagus and oesophageal adenocarcinoma, the complications of chronic GORD. Greater than 50% of the world's population is infected with *H pylori*. The prevalence of *H. pylori* infection appears to be falling, especially within the Western World. It is possible that *H. pylori* infection is protecting against the development of oesophageal disease from acid reflux and one possible explanation is the infection causing a reduction in gastric acid secretory function. For this to be true, the protective effect from *H. pylori* must be apparent in the majority of those infected. There is little available data on the effect of *H. pylori* infection within the general population. The few previous studies assessing gastric acid secretion have used *H. pylori* infected healthy volunteers, rather than subjects representative of the general population.

CHAPTER 3

Obesity and gastro-oesophageal reflux disease

3.1 Introduction

In this chapter I will discuss the evidence for the rising incidence of obesity over the last three decades and how this is a global phenomenon. I will then review the evidence for the association between obesity and gastro-oesophageal reflux disease, Barrett's oesophagus and oesophageal adenocarcinoma. Finally, I will review the potential mechanisms behind the association, acknowledging that there are both mechanical and humeral mechanisms.

3.1 Obesity and its rising incidence

Obesity is generally defined as excess bodyweight. The body mass index (BMI) is used to express weight adjusted for height and is calculated as weight in kilograms divided by height in metres squared. The World Health Organisation (WHO) classification defines being overweight as having a BMI greater than 25 and obesity as a BMI greater than 30. Over the last three decades there has been a dramatic increase in rates of obesity in the Western World.

In the United Kingdom comprehensive data from health surveys shows the prevalence of obesity in adults in 1980 was 6% for men and 8% for women. The proportion of the population defined as obese trebled by 2002, with 23% of men and 25% of women affected.(100) The largest increases were in the younger age groups, with a doubling of obesity prevalence seen in both men and women in the age range 25-34 years.

In the United States, the prevalence of obesity in adults increased from 15% in 1980 to 33% in 2004.(101) In addition, the prevalence of overweight children increased from 6% to 19%.(102) The National Health and Nutrition Examination Survey (NHANES) carried out by the Centers for Disease Control and Prevention (CDC) in the US shows the prevalence of obesity in the 1960s was 11% for men and 16% among women. There was little change up to 1980, until the survey between 1988 and 1994 showed prevalence had risen to 21% in men and 26% in women. A further increase was found in 2003-2004 with the prevalence rising to 32% in men and 34% in women.(103)

The increase in obesity has also been documented in children. Padez *et al* looked at obesity trends in Portuguese children aged 7-9 years from 1970 up to 2002. The study found an increase in BMI over this period, with the greatest increase in the period 1992-2002. The prevalence of obesity in 2002 was 11.3%.(104) Luo *et al* looked at data from the China Health and Nutrition Survey from 1989 to 1997. They found the overall prevalence of obesity in pre-school children aged 2-6 years increased from 4.2% in 1989 to 6.4% in 1997. The greatest increase was in children living in urban areas, where the prevalence increased from 1.5% in 1989 to 12.6% in 1997.(105)

3.2 Association between obesity and gastro-oesophageal reflux disease

In addition to the known associations with type 2 diabetes mellitus, cardiovascular disease and non-alcoholic fatty liver disease, obesity is also strongly associated with gastro-oesophageal reflux disease and its' complications. A meta-analysis by Hampel *et al* found nine studies which examined the relationship of BMI with GORD symptoms, and six of the studies showed statistically significant associations.(106) Obese subjects were found to have a 2.0-fold increased risk of GORD symptoms, and 2.8-fold increased risk of oesophageal adenocarcinoma. No studies were found which examined the relationship between obesity and Barrett's oesophagus. A more recent met-analysis by Corley *et al* identified 20 studies consisting of 18,346 patients.(107) The pooled estimates for an association between GORD and increased BMI were heterogeneous. When stratified by country of origin, seven studies from the United States demonstrated an association between BMI and GORD with an odds ratio of 1.57 for the overweight category and 2.15 for the obese category. The results from European studies were heterogeneous with some individual studies showing an association and some showing no association.(107)

El-Serag *et al* performed a cross-sectional study of 206 consecutive patients undergoing 24-hour pH measurements. They found a BMI in the obese range was associated with a significant increase in the percentage of time oesophageal pH<4, number of acid reflux episodes and number of reflux episodes lasting longer than 5 minutes.(108)

A North American cross-sectional study posted a validated self-reported questionnaire to a random sample of the general population of Olmsted County, Minnesota. 1524 subjects (72%) responded, with 69% of the subjects with a BMI greater than 30 reporting symptoms compatible with GORD. The study demonstrated that obese subjects had an odds ratio of 2.8 for experiencing at least weekly reflux symptoms. Obesity was the strongest risk factor which the study assessed, surpassing family history, smoking, and alcohol intake.(109)

Ruhl *et al* used NHANES data to follow up 12,349 subjects for a median of 18.5 years and performed a multivariate survival analysis. Reflux disease was recorded as patients requiring hospital admission with a diagnosis of oesophagitis or uncomplicated hiatus hernia. They found for every increase in BMI of $5kg/m^2$ the hazard ratio for developing GORD was 1.22.(110)

Nandurkar *et al* used data from validated questionnaires on GORD, energy expenditure, dietary intake, and measures of personality and life event stress completed by 211 community subjects. They used logistic regression analysis to analyse potential risk factors for GORD and found BMI was associated with GORD independently of lifestyle factors known to induce acid reflux, such as a high fat diet or exercise.(111)

It has been suggested that central abdominal fat deposition leading to an increase in intra-abdominal pressure is more important in causing GORD, Barrett's oesophagus and oesophageal adenocarcinoma than simple obesity. This could help explain the increased incidence of these diseases in men as excess abdominal fat is characteristic of male-pattern obesity. A systematic review and meta-analysis of the relationship between central adiposity and erosive oesophagitis by Singh *et al* included studies if they reported on visceral adipose tissue area, waist-hip ratio or waist circumference. 19 studies were identified which reported on the association between central obesity and erosive oesophagitis. The pooled odds ratio was 1.87 (95% CI, 1.51-2.31) indicating a significantly higher risk of erosive oesophagitis with increased central adiposity.(112) The association between central adiposity persisted after adjusting for BMI in an analysis restricted to 8 studies.(112)

The same meta-analysis also looked at the association between central adiposity and the complications of GORD, namely Barrett's oesophagus and oesophageal adenocarcinoma.

15 studies examined the association between central adiposity and Barrett's oesophagus and found a significantly higher risk with central adiposity with a pooled odds ratio of 1.98 (95% CI, 1.52-2.57).(112) Five of the studies adjusted for BMI, and the pooled odds ratio remained significant with a pooled odds ratio of 1.88 (95% CI, 1.20-2.95). The relationship was stronger for long-segment Barrett's oesophagus as compared with short segment. There was also a significantly higher risk of oesophageal adenocarcinoma with central adiposity. From 6 relevant studies, meta-analysis found a pooled odds ratio of 2.51 (95% CI, 1.56-4.04) for the risk of developing oesophageal adenocarcinoma with central adiposity. There was insufficient data to assess the BMI-independent effect.(112)

A case-control study conducted in California with 320 cases of Barrett's oesophagus, 316 patients with GORD and 317 controls found a positive association between Barrett's oesophagus and an abdominal circumference greater than 80cm, independent of BMI, compared with population controls (odds ratio 2.24, 95% CI, 1.21-4.15). (113) Another North American case-control study including 193 patients with Barrett's oesophagus and 211 matched population controls found all measures of central adiposity were strongly related to Barrett's. A high waist/hip ratio (Greater than 0.9 in males and greater than 0.85 in females) had an adjusted odd ratio of 2.4 (95% CI, 1.4-3.9) for Barrett's with an even stronger odds ratio of 4.3 (95% CI, 1.9-9.9) for long-segment Barrett's oesophagus. Waist/thigh ratio and waist circumference also had statistically significant associations with Barrett's whilst BMI had an odds ratio of 2.0 for long segment Barrett's and a non-significant association with visible Barrett's cases.(114)

A more recent meta-analysis analysing the effect of BMI on oesophageal and gastric cardia adenocarcinoma identified 22 case-control and cohort studies which included almost 8000 cancer cases. The overall relative risk was 2.34 (95% CI, 1.95-2.81), with a stronger association with oesophageal adenocarcinoma which had a relative risk of 2.73 (95% CI, 2.16-3.46).(115)

3.4 Potential mechanisms of association between obesity and GORD

The reason for the increased incidence of GORD in obese patients is likely to be multifactorial, and both mechanical and humoral effects may be involved. The presence of

a hiatus hernia is an independent risk factor for the development of GORD. There is evidence that hiatus hernia is more common with increasing BMI.(116) In a retrospective study of 181 patients undergoing workup for bariatric surgery, 37.0% had evidence of a hiatus hernia on an upper GI contrast study.(117) In a prospective study of 1224 patients undergoing upper GI endoscopy, the BMI was significantly higher in the 20% of patients found to have a hiatus hernia.(118) The increased prevalence of hiatus hernia in obese subjects is likely to contribute to the increased incidence of GORD in these subjects. Pandolfino *et al* used high-resolution manometry to show that obesity was significantly associated with separation of the manometric LOS component and crural diaphragm component of the gastro-oesophageal junction.(10)

In addition, central obesity has been shown to increase intra-abdominal pressure(119) and intragastric pressure. A prospective cross-sectional study of 322 patients found a 10% increase in intragastric pressure for each unit increase in BMI.(120) This is likely due to mechanical pressure on the stomach from the increased mass of abdominal fat. A similar increase in intragastric pressure has been observed in pregnant women and in patients with ascites.(121, 122) This increase in intragastric pressure is generally greater than the increase in intra-oesophageal pressure, leading to an increased gastro-oesophageal pressure gradient (GOPG).(10) The increase in GOPG in obese subjects compared to lean subjects may overcome the lower oesophageal sphincter (LOS) pressure. It has been shown that the LOS pressure is not increased in obese subjects.(123, 124)

Transient lower oesophageal sphincter relaxations (TLOSRs) are the predominant event leading to gastro-oesophageal reflux and are triggered by gastric distension. Wu *et al* recruited 28 asymptomatic obese patients referred for weight-loss surgery and compared them with age and sex matched overweight subjects (BMI 25-30) and normal weight subjects (BMI 20-25). Two-hour postprandial oesophageal manometry and pH monitoring revealed the obese and overweight groups had an increased rate of TLOSRs. The obese group had 7.3 in 2 hours compared with 3.8 in the overweight group and 2.1 in the normal weight group ($p<0.001$). All three groups had comparable LOS pressure, LOS length, and peristaltic function.(125) A smaller study comparing 7 health controls with 7 morbidly obese patients using 24 hour ambulatory oesophageal manometry and pH monitoring found a substantial increase in the number of TLOSRs in the obese group compared to the controls in the postprandial period.(126)

A study of gastric emptying of a mixed solid/liquid meal using a double-isotope technique in 31 obese patients and 31 controls found significantly reduced gastric emptying of both the solid and liquid component of the meal. There was a significant correlation between BMI and gastric emptying ($r = 0.44$, $p<0.01$).(127) This potentially means acidic gastric contents are present within the stomach for a greater length of time and therefore available to reflux for a greater length of time.

Other potential mechanisms thought to play a role in GORD including a lower oesophageal sphincter pressure, gastric volume and content, and crural diaphragm function have also been studied in obese patients and have not been shown to differ from controls.(124, 128, 129)

Humoral changes in obesity may also be involved. One study of gastric acid secretion comparing 13 grossly obese patients with 16 age-matched controls of normal body weight found obese patients had a higher maximal gastric acid response to graded pentagastrin (36.6 +/- 2.9 mmol/hr, compared to 27.1 +/- 2.4 mmol/hr in controls; $p<0.05$), and required a lower dose of pentagastrin to reach maximal acid output.(130)

In another functional study, obese patients had higher outputs of bile and pancreatic enzymes, as well as higher plasma levels of pancreatic polypeptide, in the resting state compared to healthy controls.(128) Therefore the content of the gastric juice in obese patients may be altered such that it is more damaging to oesophageal mucosa in the event of gastro-oesophageal reflux.

There is also evidence of altered vagal function in obese patients. Vagal stimulation by modified sham feeding led to reduced gastric acid secretion in obese patients compared to subjects with normal body weight and a similar parietal cell mass.(130) Obese patients also had a 50% reduction in pancreatic enzyme secretion, bile acid emptying and gastrin release.(128)

Nilsson *et al* found a strong association between BMI and endoscopically-proven reflux oesophagitis in women, but not in men. In addition they found this association was even stronger in females using oestrogen replacement.(131) A population-based case-control study by the same group using results of public health surveys from 65,363 adults found the risk of reflux among severely obese men was 3.3-fold higher compared to those with normal body weight, and 6.3-fold higher in severely obese women.(132) They found this association was strongest in pre-menopausal women, and the use of oestrogen replacement

strengthened the association in post-menopausal women. The authors argued that oestrogen may play an important role in GORD.

Visceral fat is known to produce hormones known as adipocytokines, including adiponectin, leptin, IL-6, and TNF-a. Obesity causes changes in the circulating levels of these enzymes. Leptin is a peptide hormone secreted by adipocytes and circulating levels of leptin are directly proportional to body mass fat.(133) Leptin has been observed to enhance the anti-apoptotic and growth-promoting effects of acid in oesophageal adenocarcinoma cells in culture.(134) A case-control trial showed that Barrett's oesophagus is associated with an increase in proinflammatory cytokines and leptin.(135) The work in this area supports a role for inflammation in the development of Barrett's and oesophageal adenocarcinoma, however more work is required.

3.5 Conclusion

The incidence of central obesity is rising in both children and adults across the world. Obesity is strongly associated with gastro-oesophageal reflux and its complications of Barrett's oesophagus and oesophageal adenocarcinoma. Central adiposity seems to be of particular importance. The nature of this association is incompletely understood and both mechanical and humoral effects of central obesity may be important.

CHAPTER 4

Study of the inverse association between gastric cancer and oesophageal adenocarcinoma

4.1 Introduction

Over the past three decades, oesophageal adenocarcinoma (OAC) has been one of the fastest increasing malignancies in many countries.(33) The cancer is thought to be the result of gastro-oesophageal reflux damaging the distal oesophagus and causing columnar metaplasia often with intestinal phenotype. This Barrett's mucosa has an increased risk of progressing to dysplasia and adenocarcinoma.

The environmental factors causing the recent marked increase in incidence of OAC are unclear. Central obesity is associated with both reflux and OAC but a study indicates that this can only explain 6.5% of the rise in incidence of OAC.(136) In addition, obesity does not explain some of the geographical variations in the rising incidence of the cancer.(137)

Smoking is another well-established risk factor for oesophageal adenocarcinoma.(138) However, the prevalence of smoking in the Western world has decreased over the past few decades and thus this cannot account for the rise in incidence of oesophageal adenocarcinoma. Another possible explanation is that *H. pylori* infection has been protecting against acid reflux, and thus OAC, and this is being lost by the falling incidence of the infection.(139) There is a well-established negative association between *H. pylori* infection and both gastro-oesophageal reflux and OAC.(71, 140) The negative association between *H. pylori* and OAC is independent of the other risk factors of OAC including smoking and BMI.(141) A proposed mechanism for the protective effect of *H. pylori* is that the gastritis induced by it may cause atrophy and reduced acid secretory capacity of the gastric mucosa. As the acidity of the gastric juice is its main damaging component, reduction of this by *H. pylori* would protect against reflux-induced oesophageal damage and associated adenocarcinoma.(142)

H. pylori infection may produce different patterns of gastritis which are associated with different disease outcomes. When the *H. pylori* gastritis spares the acid secreting body region of the stomach, it results in increased gastrin release and accompanying increased acid secretion which is the key condition leading to duodenal ulceration.(143) When the *H. pylori* gastritis involves the entire stomach and causes atrophy and reduced acid secretion, there is a markedly increased incidence of gastric adenocarcinoma for all regions of the stomach except the cardia adjoining the gastro-oesophageal junction.(144) There is little information on the pattern of *H. pylori* gastritis and gastric secretory status in the 90% of *H.pylori*-positive subjects who do not develop either ulcer disease or gastric cancer.

If *H. pylori* infection does protect against OAC by reducing gastric acid secretion, there should be a negative association between non-cardia gastric cancer (NCGC) and OAC at a population level as the gastric mucosal changes predisposing to gastric cancer would be the same as those protecting from OAC.

4.2 Aims

This study examined the relationship between the current incidences of the two cancers in different geographical regions of the world. In addition, we studied the relation between changes in incidence of the two cancers over time.

4.3 Materials & Methods

4.3.1 Cross-Sectional Studies

Based on data from Cancer Incidence in Five Continents Vol. X (CI5X) and GLOBOCAN 2012, age-specific and age-standardized (world population) incidence rates (WASR) were estimated for OAC and gastric cancer by topographical subsite (cardia and non-cardia) in 2012. This method has been described in more detail by Arnold *et al*.(32) and Colquhoun *et al*.(145) In brief, sex- and age-specific (<65; ≥65 years) proportions of OAC were computed for all countries included in CI5X except for those with no cases of OAC in one of four substrata (male, female; <65, ≥65 years) (N=51). Similarly, the proportions of cardia cancer (C16.0) and NCGC (C16.1-6) cases out of all gastric cancers with known topography (C16.0-6) were calculated for each country included in CI5X and stratified by sex and the same broad age groups, provided there were two or more cases of cardia cancer (CGC) and NCGC within each sex and age group stratification. Where there were multiple datasets (from different regional cancer registries within a country), cases and populations were pooled to obtain estimated national proportions. The histological types of oesophageal cancer and the topographical classification of gastric cancers were defined according to the third edition of the International Classification of Diseases for Oncology (ICD-O-3) which was presented in Cancer Incidence in Five Continents Vol. IX (CI5IX). For this analysis, the national or regional proportions of CGC/NCGC and OAC cases determined in the previous steps were applied to the 2012 gastric and OAC incidence estimates in GLOBOCAN 2012. Age-standardized incidence rates were computed using the world standard population.

4.3.2 Longitudinal Studies

For the longitudinal study, WASR of OAC and total gastric cancer (TGC) were extracted from Cancer Incidence in Five Continents-Plus (CI5-plus). Cardia and non-cardia subsite data were not used because there were few longitudinal data for the specific subsite and because there have been continuous changes in the subsite assignation practices for gastric cancer in histopathology laboratories**.** A total of 38 populations around the world were selected for this study based on a) availability of data of oesophageal cancer recorded by histology, b) a time period of at least 19 years ending in 2007, c) if separate datasets were available for ethnic groups, at least one dataset per ethnic group was selected.

4.3.3 Statistical Analyses

For the cross-sectional study, Spearman's rho correlations were used to explore the relationship between WASR of the two cancers. In the longitudinal study, in addition to Spearman's rho correlations for pairwise correlation of OAC and TGC, time trends for individual registries, regression modelling was used to estimate the degree of incidence changes over time as described by Kim *et al*.(146) Briefly, using Joinpoint Programme version 4.1.0 (National Cancer Institute, USA) we analysed the time trend data for each cancer, in each registry, in men and women individually (38 x2 x2= 152 datasets). The programme fitted the simplest Joinpoint model that the data allowed. The models used the log of the WASR for calculating the average annual percent change (AAPC) in rates and their corresponding 95% confidence intervals. When any comparisons were made between different populations, the AAPC was always limited to the same 19-year period (1989-2007). When correlations were made between the change of cancer incidence between the two cancers within the same population, we present this in two different forms, firstly for the full length of available longitudinal data for each population and secondly, limiting it to the same common observation period (1989-2007).

4.4 Results

4.4.1 Cross-sectional Study of National data

Incidence rate estimates were available for 51 countries. (Table 4.1) There was a wide range in the rates of both gastric cancer and OAC across these different countries. The incidence of OAC varied from 0.23 per 100,000 person-years to 7.24. The incidence of TGC varied from 2.84 to 62.26 and that of NCGC from 1.75 to 58.64. In general terms, gastric cancer was highest in East Asia and lowest in Western Europe and North America whereas OAC showed the opposite trend. In all 51 countries except the UK, OAC incidence rates were lower than the TGC rates and in 43 of 51 countries also lower than the NCGC incidence rate.

Statistically significant negative correlations between the incidence rates of OAC and both TGC (CC= -0.38, p=0.006) and NCGC incidence (CC= -0.41, p=0.003) were observed. The wide range in incidence of both OAC and TGC together with their inverse correlation resulted in a more than 200-fold range in the ratios of TGC to OAC across the different countries (more than 200:1 for the Republic of Korea to less than 1:1 for the UK)

Despite the strong negative correlation between the incidence rates of the two cancers, inspection of the data points indicated that in two respects the correlation was not linear (Figure 4.1). Firstly, the incidence of OAC seemed to be at a similar low level for all countries with moderate or high gastric cancer incidence and with a progressive rise in incidence at lower gastric cancer levels. The incidence of TGC below which the rise in OAC was apparent was approximately 15 per 100,000p/y in men (Figure 4.1) and 7.5 per 100,000p/y in women (Figure 4.2). Secondly, when the incidence of TGC was low, the level of OAC incidence varied considerably between countries with some showing marked elevation, some moderate and some no elevation. The equivalent figures for women showed a similar pattern though this was less clear due to much lower incidence rates (Figure 4.2).

In order to investigate further the geographical distribution of the countries according to their TGC and OAC incidence rates, we sub-divided them into 4 groups (Figure 4.1b). In men, the first 3 groups consisted of countries with low TGC incidence (<15 per 100,000 p/y) and OAC incidence high (>5) - Group A, medium (1.5-5) - Group B, and low (<1.5) – Group C. The fourth group (D) consisted of countries with high rates of gastric cancer (>15) and low OAC (<1.5).

Table 4.1: Age-standardised incidence rate (world) of gastric cancer and oesophageal adenocarcinoma in different countries in cross-sectional study

	Men					Women		
Country	**TGC**	**Cardia**	**NCGC**	**OAC**	**TGC**	**Cardia**	**NCGC**	**OAC**
UK	6.44	3.89	2.55	7.24	3.15	1.46	1.69	1.36
Netherlands	7.61	3.39	4.22	7.05	3.9	1.03	1.69	1.36
Ireland	8.83	3.64	5.19	5.36	4.42	1.42	3.0	1.01
New Zealand	6.69	3.27	3.42	3.96	3.83	0.88	2.94	0.56
USA	5.33	2.6	2.73	3.62	2.7	0.66	2.04	0.42
Belgium	8.04	4.96	3.08	3.53	3.83	1.36	2.48	0.57
Australia	6.72	3.44	3.27	3.41	3.14	0.99	2.15	0.49
Denmark	8.32	5.79	2.53	3.07	3.05	1.27	1.78	0.76
Canada	6.95	3.26	3.69	2.99	3.12	0.83	2.29	0.42
Switzerland	5.01	2.21	2.8	2.59	3.58	0.89	2.69	0.49
Norway	5.61	2.41	3.2	2.32	3.82	0.91	2.91	0.43
Malta	11.08	6.1	4.98	2.3	5.53	1.2	4.33	0.11
Germany	10.66	3.84	6.82	2.16	5.44	1.01	4.43	0.28
Czech Rep	10.21	2.81	7.41	2.16	5.27	0.75	4.52	0.29
Uruguay	14.36	7.61	6.75	2.07	6.7	2.06	4.64	0.61
Argentina	9.87	2.79	7.08	1.98	4.19	0.71	3.48	0.44
Austria	9.2	4.89	4.31	1.97	4.83	1.42	3.41	0.23
Brazil	13.08	4.26	8.82	1.96	5.96	1.16	4.8	0.59
Finland	6.72	4.86	1.86	1.8	3.94	2.06	1.88	0.3
France	6.97	3.03	3.94	1.56	2.8	0.48	2.32	0.33
Spain	10.97	2.91	8.05	1.27	5.11	0.66	4.45	0.18
Columbia	18.89	6.93	11.95	1.11	8.97	2.18	6.8	0.21
Lithuania	22.73	2.99	19.75	1.09	8.02	0.61	7.41	0.15
Turkey	17.92	4.49	13.43	1.09	10.93	2.34	8.59	0.21
Israel	9.71	4.03	5.68	0.94	4.9	1.45	3.45	0.2
Puerto Rico	5.28	1.37	3.91	0.94	3.11	0.48	2.64	0.26
Ukraine	22.39	4.36	18.03	0.9	9.14	1.29	7.85	0.16
Latvia	23.05	4.97	18.08	0.86	8.68	0.93	7.74	0.18
Bulgaria	14.51	2.71	11.8	0.86	6.99	0.88	6.12	0.12
Croatia	14.47	7.5	6.97	0.85	6.27	2.39	3.88	0.12
Iran	20.6	13.37	7.22	0.85	9.72	5.4	4.32	0.85
Belarus	29.14	3.37	25.77	0.84	12.22	0.95	11.27	0.1
China	32.77	11.97	20.8	0.83	13.1	3.37	9.73	0.26
Russia	24.45	4.74	19.71	0.79	10.8	1.58	9.21	0.13
Slovakia	13.95	3.43	10.52	0.79	6.58	0.92	5.66	0.15
Costa Rica	21.43	4.65	16.78	0.75	13.66	1.48	12.18	0.15
Chile	23.29	7.35	15.94	0.74	9.19	1.99	7.2	0.26
Slovenia	15.37	5.01	10.36	0.65	6.42	1.02	5.4	0.1
Italy	10.89	2.43	8.46	0.61	5.92	0.63	5.29	0.09
Serbia	11.94	6.69	5.25	0.59	5.68	2.94	2.75	0.14
Philippines	4.81	2.03	2.78	0.56	2.88	0.89	1.99	0.14
Egypt	2.84	1.09	1.75	0.52	2.27	0.55	1.72	0.28
Poland	13.19	7.87	5.32	0.50	4.95	2.08	2.86	0.09
Estonia	19.5	2.96	16.54	0.44	10.31	1.03	9.28	0.07
India	8.56	3.26	5.3	0.43	3.68	1.41	2.27	0.13
Japan	45.75	4.73	41.01	0.42	16.46	1.25	15.21	0.07
Saudi Arabia	3.84	1.36	2.48	0.42	2.41	0.72	1.69	0.15
Singapore	10.85	3.18	7.67	0.34	5.83	1.31	4.52	0.07
Ecuador	20.69	3.5	17.19	0.33	13.43	0.95	12.48	0.21
Thailand	3.77	1.36	2.14	0.27	2.49	0.80	1.68	0.08
Rep of Korea	62.26	3.62	58.64	0.23	24.67	1.07	23.60	0.04

Figure 4.1: Correlations between incidence rates (WASR) of OAC and gastric cancer in cross-sectional study in men. 1a: OAC versus total gastric cancer, 1b: visual clustering of countries divided into groups A-D. **Note:** Each dot represents a dataset from a country.

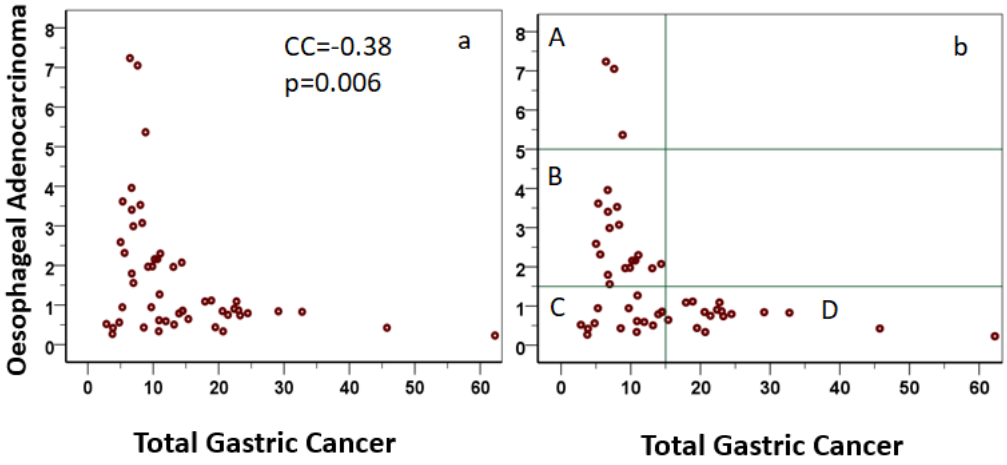
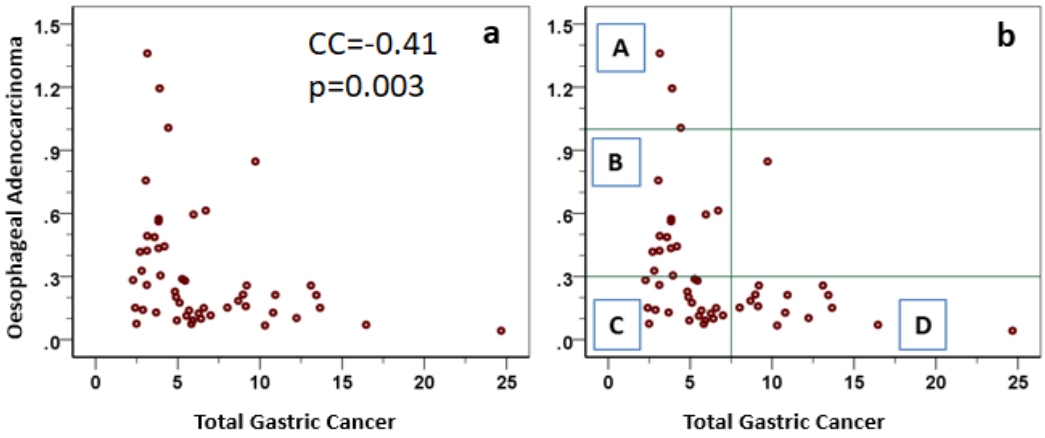


Figure 4.2: Correlations between incidence rates (WASR) of OAC and gastric cancer in cross-sectional study in women. 1a: OAC versus total gastric cancer, 1b: visual clustering of countries divided into groups A-D. **Note:** Each dot represents a dataset from a country.



In men, incidence rate in the different groups showed distinct geographical patterns (Figure 4.3). Group A with the highest rate of OAC and low (<15) TGC was limited to the UK, the Netherlands and Ireland. Group B with moderate OAC and low TGC (<15) comprised Northern America, Australia, New Zealand, Germany, Belgium, Denmark, Finland, Norway, France, Brazil and Uruguay. Area C with low OAC and low TGC (<15) was a very heterogeneous group from around the world (Thailand, Philippines, India, Bulgaria, Croatia, Egypt, Israel, Italy, Spain, Poland, Serbia, Slovakia, Singapore); Group D with low OAC and moderate to high TGC includes South American countries, Eastern Europe, Korea, China, Japan, Russia, Iran and Turkey.

In women, sub-dividing all countries into groups based on their rates of oesophageal and gastric cancer incidence demonstrated similar geographical patterns (Figure 4.4)

Figure 4.3: Maps of countries colour-coded with visual clustering of populations with different combinations of OAC and gastric cancer in cross-sectional study in men.
Note: The colour coding is based on groups A-D. Group A: (red) TGC<15 & OAC≥5, Group B (yellow): TGC<15 & 1.5≤OAC<5, Group C (green): TGC<15 & OAC<1.5, Group D (blue): TGC≥15 & OAC<1.5. The grey code indicates no data available.

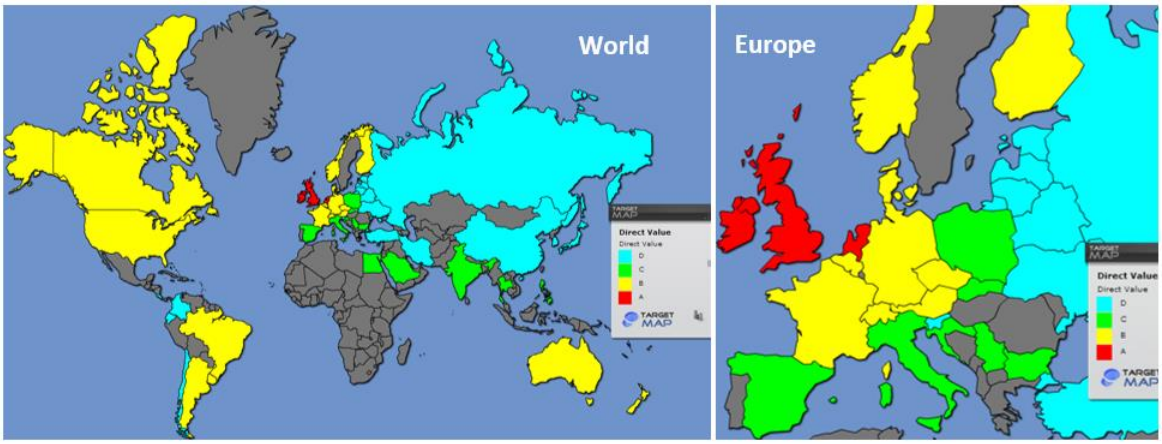
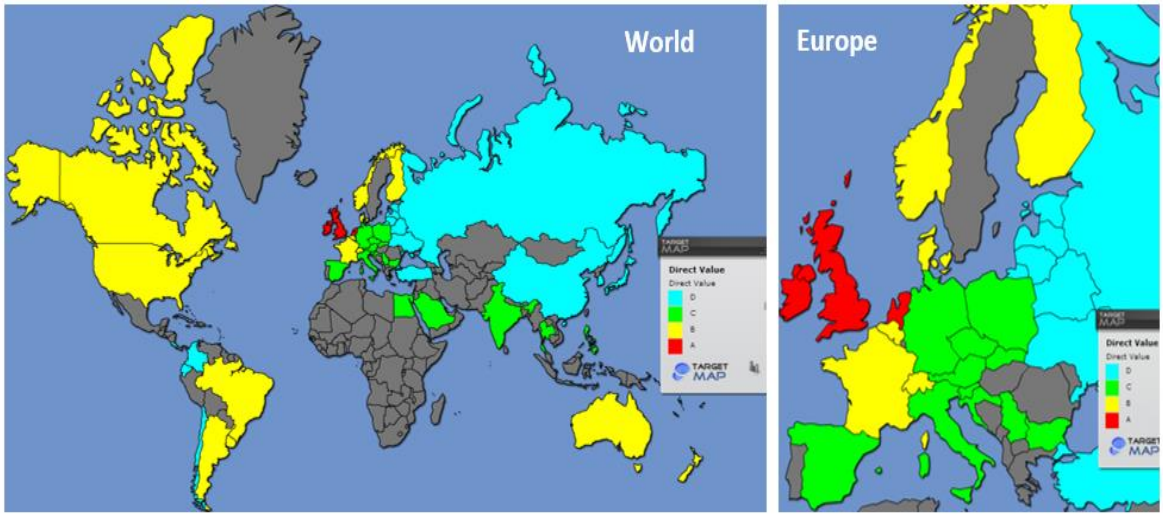


Figure 4.4: Maps of countries colour-coded with visual clustering of populations with different combinations of OAC and gastric cancer in cross-sectional study in women. Note: The colour coding is based on groups A-D. Group A: (red) TGC<15 & OAC≥5, Group B (yellow): TGC<15 & 1.5≤OAC<5, Group C (green): TGC<15 & OAC<1.5, Group D (blue): TGC≥15 & OAC<1.5. The grey code indicates no data available.



4.4.2 Longitudinal Study

4.4.2.1 Changes in incidence of oesophageal adenocarcinoma and gastric cancer

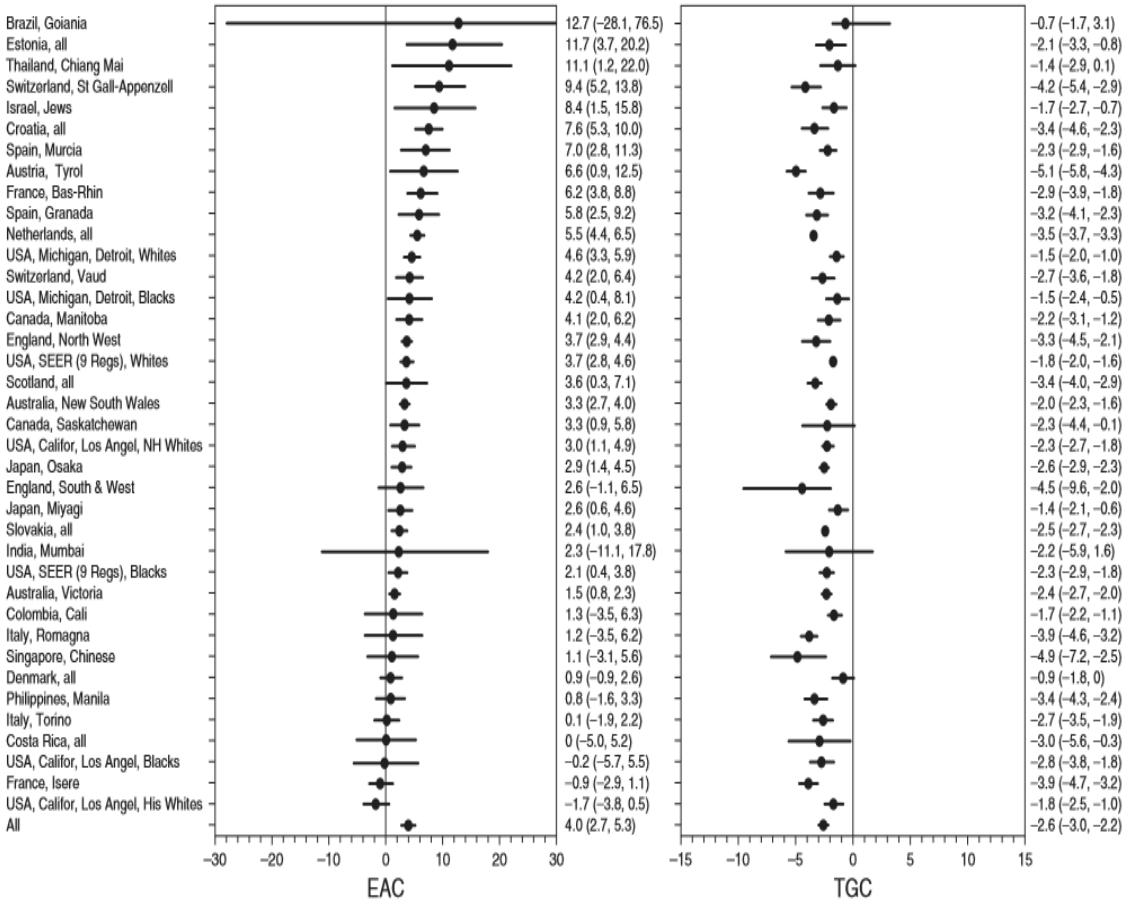
The incidence (WASR) of OAC and TGC at the start year (different for each registry), year 1989 (earliest time common for all populations) and year 2007 (end year) were reported in Table 4.2, for men and women separately.

To explore the rate of change in incidence of OAC and TGC during the period of 1989- 2007 in males, we calculated average annual percentage change (AAPC) for each of the 38 populations individually, and presented these in Figure 4.5, ordered by the most recent incidence of OAC. During this time period, most populations (34/38, 89%) had experienced a significant decrease in the incidence of TGC, with these 34 showing a range of AAPC from -1.4% (95% CI: -2.1, -0.6 in Japan, Miyagi) to -5.1% (95% CI: -5.8, -4.3) in Austria, Tyrol. Twenty-five of 38 (66%) populations showed a significant increase in incidence of OAC during the period of observation and no population showed a significant fall in incidence (Figure 4.5). Annual increases in OAC incidence ranged from 1.5% (95% CI: 0.8, 2.3 in Victoria, Australia) to 11.7% (95% CI: 3.7, 20.2 in Estonia).

Table 4.2: List of recruited registries in longitudinal study, and incidence rates (WASR) of total gastric cancer and oesophageal adenocarcinoma at the start, year 1989, and end of period.

Study Population	Full-range Period (yrs)	Men						Women					
		OAC (start)	OAC (1989)	OAC (2007)	TGC (start)	TGC (1989)	TGC (2007)	OAC (start)	OAC (1989)	OAC (2007)	TGC (start)	TGC (1989)	TGC (2007)
Scotland, all	1975 -2007 (33)	2.36	3.42	7.47	21.17	18.56	9.72	0.83	1.05	1.49	10.64	7.90	4.57
England, North West	1979 -2007 (29)	1.58	3.70	7.07	22.32	17.76	10.12	0.46	0.86	1.34	10.01	7.74	4.08
England, South & West	1979 -2007 (29)	1.40	3.46	5.84	17.21	14.79	7.02	0.30	0.48	1.11	6.99	3.77	2.72
Netherlands, all	1989 -2007 (19)	2.26	2.26	5.42	15.68	15.68	8.88	0.58	0.58	0.93	6.11	6.11	3.98
USA, Michigan, Detroit, Whites	1973 -2007 (35)	0.67	1.74	4.39	10.13	9.56	6.42	0.10	0.22	0.64	4.49	3.76	2.93
USA, SEER (9 Regs), Whites	1975 -2007 (33)	0.61	1.83	3.74	9.31	8.04	5.55	0.22	0.27	0.60	7.02	5.09	3.35
Switzerland, St Gall-Appenzell	1983 -2007 (25)	0.93	0.76	3.22	12.09	12.23	6.41	0.32	0.35	0.53	6.14	5.32	3.92
USA, Calif, Los Angel, Non-His Whites	1973 -2007 (35)	0.35	1.61	2.77	9.92	7.99	5.52	0.10	0.22	0.51	4.03	3.19	2.43
Australia, New South Wales	1983 -2007 (25)	0.71	1.54	2.67	12.36	10.82	7.54	0.00	0.26	0.50	8.03	6.46	3.68
Australia. Victoria	1983 -2007 (25)	1.23	1.74	2.64	14.84	12.33	7.97	0.12	0.20	0.45	5.27	4.40	3.47
Canada, Manitoba	1958 -2007 (50)	0.08	1.28	2.64	25.6	12.60	8.33	0.05	0.26	0.42	4.45	3.28	2.26
Denmark, all	1978 -2007 (30)	0.81	1.76	2.54	15.05	10.29	7.68	0.19	0.19	0.40	3.88	3.88	3.86
Switzerland, Vaud	1988 -2007 (20)	1.48	1.48	2.47	10.63	10.63	7.22	0.13	0.13	0.36	11.3	4.88	3.22
France, Bas-Rhin	1975 -2007 (33)	0.88	0.83	2.12	17.58	12.78	8.42	0.00	0.11	0.35	8.14	7.27	4.33
Canada, Sascatchhevan	1968 -2007 (40)	0.13	1.20	1.99	14.2	8.51	5.93	0.00	0.00	0.31	18.15	19.07	13.53
France, Isere	1979 -2007 (29)	0.33	1.98	1.51	13.26	14.81	7.36	0.04	0.05	0.28	6.81	3.54	3.01
USA, Calif, Los Angel, His Whites	1973 -2007 (35)	0.04	1.47	1.47	14.98	13.43	9.53	0.09	0.14	0.27	7.68	5.77	5.62
Spain, Murcia	1983 -2007 (25)	0.74	1.21	1.44	17.88	15.67	10.46	0.09	0.02	0.24	5.96	5.02	3.31
Spain, Granada	1985 -2007 (23)	0.58	0.39	1.16	17.77	14.92	10.02	0.03	0.03	0.22	16.40	16.40	6.93
USA, Michigan, Detroit, Blacks	1973 -2007 (35)	0.30	0.48	1.03	16.24	16.10	12.08	0.04	0.05	0.19	14.78	6.19	5.20
Colombia, Cali	1983 -2007 (25)	0.19	0.68	0.87	32.46	33.40	25.82	0.00	0.07	0.18	7.82	6.31	4.81
Israel, Jews	1963 -2007 (45)	0.06	0.08	0.86	24.84	13.26	9.58	0.00	0.05	0.17	8.63	7.96	7.01
Italy, Torino	1985 -2007 (23)	0.41	0.55	0.82	20.25	19.18	10.65	0.00	0.00	0.16	8.94	9.15	5.40
Austria, Tyrol	1988 -2007 (20)	0.42	0.42	0.79	28.24	28.24	11.24	0.04	0.09	0.16	6.69	6.22	4.78
Slovakia, all	1973 -2007 (35)	0.15	0.35	0.78	42.30	25.06	16.43	0.00	0.04	0.15	8.29	5.40	3.80
USA, SEER (9 Regs), Blacks	1975 -2007 (33)	0.17	0.46	0.74	15.30	15.04	10.43	0.00	0.00	0.14	23.97	23.97	10.48
Japan, Miyagi	1978 -2007 (30)	0.68	0.43	0.69	82.51	84.41	65.72	0.05	0.19	0.10	8.44	7.09	5.20
Croatia, all	1988 -2007 (20)	0.26	0.26	0.63	28.98	28.98	17.36	0.02	0.04	0.09	19.41	10.89	7.19
Costa Rica, all	1980 -2007 (28)	0.55	0.52	0.63	48.40	39.53	24.65	0.08	0.08	0.08	11.87	11.87	7.22
USA, Calif, Los Angel, Blacks	1973 -2007 (35)	0.37	0.68	0.59	15.53	13.76	8.66	0.00	0.05	0.08	26.73	16.45	11.43
Estonia, all	1968 -2007 (40)	0.04	0.09	0.56	57.27	35.25	23.29	0.16	0.03	0.06	8.62	6.67	4.30
Brazil, Goiania	1988 -2007 (20)	0.45	0.45	0.51	25.46	25.46	25.86	0.01	0.06	0.06	52.05	30.70	17.26
Philippines, Manila	1983 -2007 (25)	0.32	0.34	0.45	14.16	11.72	6.91	0.00	0.02	0.04	17.21	15.12	7.86
Italy, Romagna	1988 -2007 (20)	0.44	0.44	0.45	41.78	41.78	20.34	0.23	0.05	0.03	38.26	34.52	22.21
Japan, Osaka	1963 -2007 (45)	0.00	0.22	0.41	107.06	70.42	44.19	0.15	0.25	0.03	23.39	17.19	14.11
Singapore, Chinese	1968 -2007 (40)	0.00	0.28	0.38	45.07	33.48	14.90	0.00	0.00	0.03	4.48	4.79	4.17
Thailand, Chiang Mai	1983 -2007 (25)	0.00	0.06	0.11	8.25	8.09	6.84	0.08	0.05	0.02	5.10	4.08	2.58
India, Mumbai	1978 -2007 (30)	0.29	0.04	0.08	7.11	6.68	4.46	0.11	0.11	0.00	12.46	12.46	12.27

Figure 4.5: Average Annual Percentage Changes (AAPC) of OAC versus TGC in men during short common period (1989-2007) of registration in populations.



We investigated any differences between the 13 populations showing no significant rise in OAC and the 25 populations showing a rise. The populations which showed no significant change in OAC showed a rate of decrease in TGC (AAPC range -4.9% to 0.7%) similar to those that showed increase in OAC (AAPC range = -5.1% to -1.4%), (p=0.504). Likewise, the registries showing no rise in OAC did not differ from those showing an increase in that the two groups had a similar most recent (2007) TGC incidence (9.5 vs 9.6/1000,000py; p=0.584).

4.4.2.2 Correlation of Incidence trends of oesophageal adenocarcinoma and gastric cancer

We looked for any correlation between the incidence trends for both cancers for those 38 populations with sufficient longitudinal data and included the full observation period of paired data (i.e. not limited to 1989-2007) (Table 4.3). In men, significant negative

correlations between incidence rate trends of OAC and TGC over time were present in 28 of the 38 populations when compared over preceding 19-50 years and evaluated in pairs. Positive correlations were not observed for any population.

When the calculation of correlations between OAC and TGC incidence trends was limited to the common but shorter observation period (last 19 years of 1989-2007), the magnitude of correlations was weaker in some of the populations. Only 19 of 38 populations showed significant negative correlations for men and 7 of 38 for women (Table 4.4).

Table 4.3: Correlations between incidence (WASR) trends of oesophageal adenocarcinoma and total gastric cancer in two time periods of full range and 1989-2007 in men

Study Population	Population	Full Range				1989 - 2007	
	(2007)	Period (yrs)	CC	*P* value		CC	*P* value
Scotland, all	2485606	1975 -2007 (33)	-0.961	0.000		-0.913	0.000
England, North West	3223560	1979 -2007 (29)	-0.926	0.000		-0.800	0.000
England, South & West	3442830	1979 -2007 (29)	-0.924	0.000		-0.774	0.000
Netherlands, all	8100293	1989 -2007 (19)	-0.961	0.000		-0.971	0.000
USA, Michigan, Detroit, Whites	1390555	1973 -2007 (35)	-0.921	0.000		-0.821	0.000
USA, SEER (9 Regs), Whites	10682176	1975 -2007 (33)	-0.972	0.000		-0.834	0.000
Switzerland, St Gall-Appenzell	263298	1983 -2007 (25)	-0.590	0.002		-0.519	0.023
USA, Calif, Los Angel, Non-His Whites	1446148	1973 -2007 (35)	-0.843	0.000		-0.520	0.023
Australia, New South Wales	3420484	1983 -2007 (25)	-0.888	0.000		-0.810	0.000
Australia. Victoria	2574901	1983 -2007 (25)	-0.810	0.000		-0.593	0.007
Canada, Manitoba	588875	1958 -2007 (50)	-0.777	0.000		-0.402	0.088
Denmark, all	2704655	1978 -2007 (30)	-0.930	0.000		-0.766	0.000
Switzerland, Vaud	323759	1988 -2007 (20)	-0.402	0.079		-0.370	0.119
France, Bas-Rhin	534515	1975 -2007 (33)	-0.536	0.001		-0.458	0.049
Canada, Sascatchhevan	495639	1968 -2007 (40)	-0.724	0.000		-0.083	0.734
France, Isere	585746	1979 -2007 (29)	0.045	0.816		0.129	0.599
USA, Calif, Los Angel, His Whites	2220592	1973 -2007 (35)	-0.457	0.006		-0.076	0.756
Spain, Murcia	714667	1983 -2007 (25)	-0.394	0.051		-0.382	0.107
Spain, Granada	438332	1985 -2007 (23)	-0.442	0.035		-0.396	0.093
USA, Michigan, Detroit, Blacks	475552	1973 -2007 (35)	-0.271	0.116		0.193	0.428
Colombia, Cali	1000036	1983 -2007 (25)	0.010	0.964		0.029	0.906
Israel, Jews	2674800	1963 -2007 (45)	-0.637	0.000		-0.173	0.479
Italy, Torino	435148	1985 -2007 (23)	-0.341	0.112		0.048	0.844
Austria, Tyrol	342794	1988 -2007 (20)	-0.530	0.016		-0.475	0.040
Slovakia, all	2621095	1973 -2007 (35)	-0.818	0.000		-0.463	0.046
USA, SEER (9 Regs), Blacks	1724091	1975 -2007 (33)	-0.592	0.000		-0.434	0.063
Japan, Miyagi	1140676	1978 -2007 (30)	-0.383	0.036		-0.574	0.010
Croatia, all	2137984	1988 -2007 (20)	-0.737	0.000		-0.713	0.001
Costa Rica, all	2227538	1980 -2007 (28)	-0.008	0.967		-0.419	0.074
USA, Calif, Los Angel, Blacks	450132	1973 -2007 (35)	-0.357	0.035		-0.155	0.525
Estonia, all	617828	1968 -2007 (40)	-0.420	0.007		-0.471	0.042
Brazil, Goiania	588132	1988 -2007 (20)	0.048	0.842		0.098	0.690
Philippines, Manila	2993487	1983 -2007 (25)	-0.371	0.068		-0.185	0.448
Italy, Romagna	577247	1988 -2007 (20)	-0.074	0.755		-0.192	0.431
Japan, Osaka	4366616	1963 -2007 (45)	-0.915	0.000		-0.572	0.011
Singapore, Chinese	1324700	1968 -2007 (40)	-0.558	0.000		-0.188	0.441
Thailand, Chiang Mai	741784	1983 -2007 (25)	-0.281	0.173		-0.109	0.658
India, Mumbai	7479777	1978 -2007 (30)	-0.425	0.019		-0.567	0.011

Table 4.4: Correlations between incidence (WASR) trends of oesophageal adenocarcinoma and total gastric cancer in two time periods of full range and 1989-2007 in women

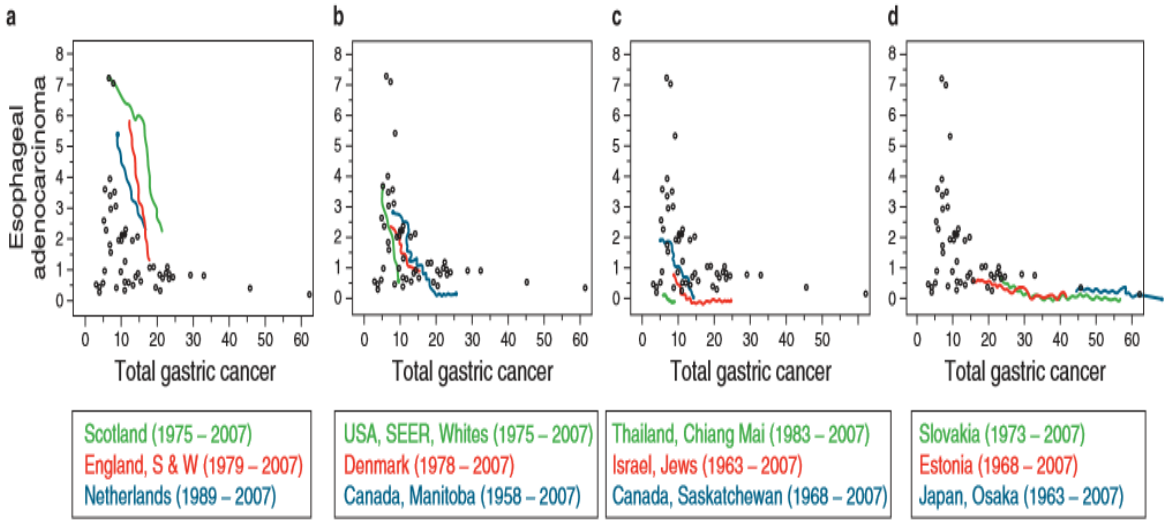
Study Population	Period (yrs)	Full Range			1989-2007	
		CC	*P* value		CC	*P* value
Scotland, all	1975 -2007 (33)	-0.860	0.000		-0.400	0.090
England, North West	1979 -2007 (29)	-0.778	0.000		-0.452	0.052
England, South & West	1979 -2007 (29)	-0.944	0.000		-0.849	0.000
Netherlands, all	1989 -2007 (19)	-0.853	0.000		-0.860	0.000
USA, Michigan, Detroit, Whites	1973 -2007 (35)	-0.716	0.000		-0.384	0.104
USA, SEER (9 Regs), Whites	1975 -2007 (33)	-0.863	0.000		-0.612	0.005
Switzerland, St Gall-Appenzell	1983 -2007 (25)	-0.270	0.191		-0.130	0.595
USA, Calif, Los Angel, Non-His Whites	1973 -2007 (35)	-0.730	0.000		-0.408	0.083
Australia, New South Wales	1983 -2007 (25)	-0.680	0.000		-0.573	0.010
Australia. Victoria	1983 -2007 (25)	-0.610	0.001		-0.476	0.040
Canada, Manitoba	1958 -2007 (50)	-0.486	0.000		-0.175	0.474
Denmark, all	1978 -2007 (30)	-0.731	0.000		-0.246	0.309
Switzerland, Vaud	1988 -2007 (20)	-0.104	0.662		0.011	0.966
France, Bas-Rhin	1975 -2007 (33)	-0.523	0.002		-0.234	0.334
Canada, Sascatchhevan	1968 -2007 (40)	-0.270	0.091		0.168	0.492
France, Isere	1979 -2007 (29)	-0.032	0.870		0.118	0.631
USA, Calif, Los Angel, His Whites	1973 -2007 (35)	-0.419	0.012		-0.310	0.196
Spain, Murcia	1983 -2007 (25)	-0.014	0.949		0.122	0.620
Spain, Granada	1985 -2007 (23)	-0.348	0.104		-0.222	0.360
USA, Michigan, Detroit, Blacks	1973 -2007 (35)	-0.295	0.085		-0.283	0.241
Colombia, Cali	1983 -2007 (25)	-0.373	0.066		-0.336	0.160
Israel, Jews	1963 -2007 (45)	-0.477	0.001		0.086	0.726
Italy, Torino	1985 -2007 (23)	-0.451	0.031		-0.251	0.301
Austria, Tyrol	1988 -2007 (20)	-0.159	0.504		-0.251	0.301
Slovakia, all	1973 -2007 (35)	-0.607	0.000		-0.383	0.106
USA, SEER (9 Regs), Blacks	1975 -2007 (33)	-0.429	0.013		-0.353	0.138
Japan, Miyagi	1978 -2007 (30)	-0.171	0.367		0.237	0.328
Croatia, all	1988 -2007 (20)	-0.155	0.514		-0.272	0.260
Costa Rica, all	1980 -2007 (28)	0.143	0.468		0.244	0.314
USA, Calif, Los Angel, Blacks	1973 -2007 (35)	-0.316	0.064		-0.075	0.761
Estonia, all	1968 -2007 (40)	-0.428	0.006		-0.243	0.316
Brazil, Goiania	1988 -2007 (20)	0.071	0.767		-0.077	0.755
Philippines, Manila	1983 -2007 (25)	0.149	0.478		0.019	0.940
Italy, Romagna	1988 -2007 (20)	-0.470	0.037		-0.421	0.073
Japan, Osaka	1963 -2007 (45)	-0.667	0.000		-0.292	0.225
Singapore, Chinese	1968 -2007 (40)	-0.237	0.141		-0.096	0.697
Thailand, Chiang Mai	1983 -2007 (25)	-0.463	0.020		-0.514	0.024
India, Mumbai	1978 -2007 (30)	-0.297	0.111		-0.529	0.020

4.4.3 Relationship of longitudinal and cross-sectional data

We investigated the relationship between the cross-sectional data of most recent incidences of the two cancers and the longitudinal incidence trends. On the cross-sectional scatter plot figure showing the correlation between current incidence of OAC and TGC across different countries we superimposed a line through the correlation dot plots for the available time points over preceding years. This allowed us to examine the changes in incidence of both TGC and OAC cancer over time and how it related to their current incidences in different countries. To facilitate visual inspection we did this separately for each of the four categories of countries i.e. groups A, B, C and D based on incidence

pattern of the two cancers (Figure 4.6). For the longitudinal data we selected the 3 populations from each of groups A, B, C and D with the longest observation periods. The resulting plots indicated that the countries with current low incidence of TGC and high, medium or low incidence of OAC had previously resembled countries with a current high incidence of gastric cancer and low incidence of OAC; as the former had decreased the latter had increased markedly, (group A) moderately (group B) or not at all (group C).

Figure 4.6: Longitudinal data superimposed on cross-sectional cancer incidences in men. The dots indicate the cross-sectional data of the most recent OAC and TGC incidences for the different countries shown in Figure 4.1. The superimposed colour lines represent longitudinal data for selected populations. The populations were selected by having the longest available longitudinal data. The time line of the longitudinal data is always from right to left. Note: Incidence of OAC and TGC plotted are per 100,000py.



4.5 Discussion

Our study has demonstrated an inverse association between the incidence of OAC and gastric cancer. This is apparent with respect to both the current incidences of these two cancers across different countries and with respect to changing incidence of the two cancers within the same populations over time. The inverse association is intriguing in view of the apparent similarities between these two cancers. Both OAC and TGC arise from epithelia of closely

adjacent, indeed abutting regions of the upper gastrointestinal tract, both are the result of chronic damage and inflammation exerted by their luminal environment and both usually show very similar and often indistinguishable histological appearance.

Our cross-sectional study showed that both cancers were similar in having a wide range in current incidence rates (20-30 fold in males) across the different countries but different in having contrasting geographical patterns. The longitudinal studies showed that the incidences of both TGC and OAC had changed markedly over recent decades, but these changes were in opposite directions and there was a statistically significant inverse association between the changing incidence rates of the two cancers in 74% of the registries.

Combining the cross-sectional and longitudinal data provided an overall picture of what has been happening to these two cancers over time and in different regions of the world. Previously, most countries had a high incidence of gastric cancer and a low incidence of OAC. Since then, the incidence of gastric cancer has fallen in all countries and as it has reached low levels it has been accompanied by varying degrees of increase in OAC.

What is the explanation for the opposing incidences and time trends of these two cancers? Could differences between countries in classification of junctional cancers into oesophageal versus gastric locations and/or changes in classification of these cancers over time explain some of the observations? In the cross-sectional study, we found that the inverse association remained strong and indeed became slightly stronger by excluding cancers occurring at the gastric cardia and thus more likely to be misclassified. The longitudinal data only provided information on TGC. Spurious inverse association between incidence trends of the two cancers due to changing classification of cardia junctional cancers would only be likely to be a significant issue in countries with a very high incidence of gastric cancer and low incidence of OAC as misclassification of a small proportion of the former could substantially increase the latter.(147) Such misclassification, however, could not explain the strong inverse incidence trends as they were most apparent in countries with lower gastric cancer incidence.(148)

The marked changes in incidence of TGC and OAC over a short time scale indicate the influence of a changing environmental factor. In addition, the inverse association between the changing incidences indicates that the environment factor is exerting opposite effects on these two cancers. The environmental factors that are thought to explain the falling incidence of TGC include a falling incidence of *H. pylori* atrophic gastritis, dietary changes and reduced smoking.(149) Could the falling incidence of any of these be associated with an

increase in OAC and thus explain the inverse association in the incidence trends of the two cancers? Smoking is not a candidate as it is a similar risk factor for both cancers.(138, 150) Dietary changes might be important. There is some evidence that increased intake of vitamins and reduced salt consumption may have contributed to the falling incidence of gastric cancer.(151, 152) These specific dietary factors would not in themselves explain the increase in OAC and indeed increased vitamin consumption may protect from OAC.(153) However, increased caloric intake and associated obesity is a well-established risk factor for OAC.(153) It is therefore possible that changes in the diet comprising both a fall in salt content and increased caloric content could produce a fall in total TGC and rise in OAC. However, a recent analysis indicated that increasing obesity may only account for 6.5% of the increase in incidence of OAC and suggesting the role of additional environmental factors.(136) Another environmental factor that might exert opposite effects on the incidence of the two cancers is *H. pylori* atrophic gastritis which is the most important etiological factor for non-cardia gastric cancer.(154) In countries with a high incidence of gastric cancer there is also a high incidence of atrophic gastritis and associated impaired gastric acid secretion.(155) In contrast, in subjects without *H. pylori*, gastric acid is maintained and shows no decline with increasing age.(88) In countries with a high incidence of gastric cancer the high prevalence of *H. pylori* atrophic gastritis will protect from OAC as any gastro-oesophageal refluxate will have reduced ability to damage the oesophagus due to its reduced acidity. Epidemiological studies have shown consistent associations between *H. pylori* and both TGC and OAC being positive with respect to the former and negative with respect to the latter.(141) As the prevalence of *H. pylori* atrophic gastritis falls, it will be accompanied by a fall in gastric cancer but potentially also a rise in OAC due to increasing gastric acidity. The prevalence of *H. pylori* infection has fallen over recent decades in association with improved living conditions.(49)

Interactions between *H. pylori* and dietary factors might also be important. There is some evidence that a high-vitamin, low salt diet may protect from the development of atrophic gastritis in *H. pylori*-infected subjects.(156) Improved living conditions, with accompanying fall in *H. pylori* prevalence as well as increased vitamin and reduced salt intake could together markedly reduce atrophic gastritis with resultant fall in TGC and increase in OAC. In the previous study by Anderson *et al*. showing a strong negative association between *H. pylori* and oesophageal adenocarcinoma, the association persisted even after correcting for atrophic gastritis detected by serum pepsinogens.(71) However, it is recognized that serum pepsinogens are insensitive markers of atrophy.(157) In addition, *H. pylori* body-predominant gastritis is associated with reduced gastric acid secretion

independent of atrophy.(158) Furthermore, body-predominant gastritis is an important risk factor for gastric cancer,(159) so even without significant atrophy it might both promote gastric cancer and protect from oesophageal adenocarcinoma.

Though there were strong inverse associations between the two cancers with respect to both current incidences and time trends, there was a group of countries with a low incidence of both cancers. This was a heterogeneous group consisting of Thailand, Philippines, Singapore, India, Egypt, Israel, Bulgaria, Croatia, Italy, Spain, Poland, Serbia, and Slovenia. Due to the limited availability of longitudinal data for many of these populations, it was difficult to determine whether their low incidence of both cancers was due to the absence of a rise in OAC as their incidence of TGC fell, or whether they had never had a high incidence of TGC and were somehow protected from both cancers. It is possible that genetic and/or environmental factors present in some of these populations might inhibit the progression from inflammation to neoplasia which is a common final step in the pathways leading to both TGC and OAC. A lack of increase in OAC despite TGC falling to a low level might be due to the genetic and/or environmental factors protecting from gastro-oesophageal reflux, which is an essential early step in the pathway to OAC. Comparative studies of countries with low vs. high incidence of OAC despite low incidence of TGC may shed new light on the aetiology and pathogenesis of OAC.

An important question is whether countries where gastric cancer incidence is still high but falling such as Japan and South East Asia will encounter a rise in OAC like that recently experienced in Western countries. The current incidence of gastric cancer in these countries is still at a level which when present in Western countries was not yet associated with any rise in OAC. However, the current incidence of gastric cancer in Japan and South East Asia is still at a level which when present in Western countries was not yet associated with any rise in OAC and it will be interesting to observe what happens when this point is reached.

Strengths of our study include the use of high quality global surveillance data. Limitations also need however to be recognised. Incidence rates used in the cross-sectional analysis were based on country-, age- and sex-specific proportions of OAC from CI5X, which were then applied to GLOBOCAN 2012 data. Hence, they represent estimates of the true incidence rates within each country and should be interpreted with caution. Our inclusion criteria for the cross-sectional study furthermore resulted in a selection of 51 mostly high-income countries, which may not be fully representative on the global level. As pathological practices and classifications of histological subtypes will have changed over time, this should also be kept in mind when interpreting the results, especially from the longitudinal analyses.

Also, registries covering different time periods (19 to 50 years) were included in some of the longitudinal analysis, making them not directly comparable.

In conclusion, this study demonstrates a strong inverse association between gastric cancer and OAC with respect to both their current incidences and time trends and which is consistent with *H. pylori* gastritis predisposing to the former and protecting from the latter. Our study also points to marked differences between populations with respect to the degree of increase in incidence of OAC observed as gastric cancer falls to a low level and understanding the factors responsible for these differences will be key to developing preventative strategies for OAC in the future.

CHAPTER 5

Study of the effect of *Helicobacter pylori* infection on intragastric acidity

5.1 Introduction

Helicobacter pylori (H. pylori) is a common bacterial infection of the stomach present in the majority of the world's human population and resulting in varying degrees of inflammation of the underlying gastric mucosa. The infection is acquired in early childhood and usually persists indefinitely unless specifically eradicated.(46)

One of the major medical advances of the past 25 years has been the discovery that this common infection plays an important role in the aetiology of duodenal and gastric ulcers and also of gastric cancer.(160) Eradicating the infection produces a long-term cure for the majority of patients with peptic ulcers unrelated to NSAID therapy. There is also increasing evidence that eradication of the infection reduces the risk of gastric cancer.(64)

An unexplained observation regarding the infection is its negative association with gastro-oesophageal reflux disease (GORD) and its complications of Barrett's oesophagus and oesophageal adenocarcinoma, with these disorders being less than half as common in infected subjects.(142, 161) It has been postulated that this negative association may represent the gastric infection protecting against these oesophageal disorders. If so, the falling incidence of the infection in the general population might explain the rising incidence of these oesophageal diseases.

One mechanism by which the infection might protect against oesophageal disease is by reducing the ability of the gastric mucosa to secrete acid and pepsin which are the constituents of gastric juice which can induce oesophageal damage. The infection is known to exert varying effects on gastric secretory function. In subjects with duodenal ulcers, the infection produces a non-atrophic gastritis with well-maintained gastric secretory cell mass which secretes increased amounts of acid due to the infection inhibiting the gastrin-mediated negative feedback control of acid secretion.(162) In patients who develop gastric cancer, the infection induces an atrophic gastritis with loss of gastric secretory cells and thus reduced acid secretion. Only approximately 1 in 10 *H.pylori* infected subjects develop complicating ulcer disease or gastric cancer and relatively little is known about the effects of the chronic infection on gastric secretory function in the 90% of infected subjects without these complications.(60) If the degree of reduction in oesophageal disease in the *H.pylori* infected population is due to the infection reducing gastric acid secretion, then this suppression of acid secretion would need to be apparent in the majority of infected subjects.

Recent evidence indicates that it is the acidity of the gastric contents close to the gastro-oesophageal junction (GOJ), referred to as the acid pocket, which refluxes and causes oesophageal damage.(163) It is also known that loss of gastric secretory cells due to *H.pylori*-induced atrophic gastritis does not occur uniformly throughout the stomach but may be more marked at the periphery of the acid secreting mucosa.(97) In assessing any potential protective effect of the infection against oesophageal damage, it is important to examine the structure and secretory function of different anatomical regions of the stomach as well as its overall secretory capacity.

5.2 Aims

The aim of this study was to assess gastric acid secretory status in different anatomical regions of the stomach and in subjects representative of the majority of the *H. pylori* infected population.

5.3 Methods and Materials

5.3.1 Subjects

Study participants were volunteers from the general population of the West of Scotland. Subjects who were currently taking, or had recently taken, proton pump inhibitors, were currently using H_2 receptor antagonists or had ever received *H. pylori* eradication therapy were excluded. Recruitment was by general advertisement and from the NHS Scotland SHARE database.

5.3.2 Study Design

5.3.2.1 Study Day 1: Clinical measurements and Urea breath test

The presence and severity of any gastrointestinal symptoms was assessed using the Short-Form Leeds Dyspepsia Questionnaire(164) and a medication history was recorded. Measurements of height, weight, waist and hip circumference were taken. Volunteers were tested for *H. pylori* infection by C^{14} urea breath test. Fasting serum and plasma samples were stored at -20˚C and later tested for *H. pylori CagA* IgG using ELISA (Genesis Diagnostics Ltd, Littleport, UK).

5.3.2.2 Study day 2: Upper gastrointestinal endoscopy

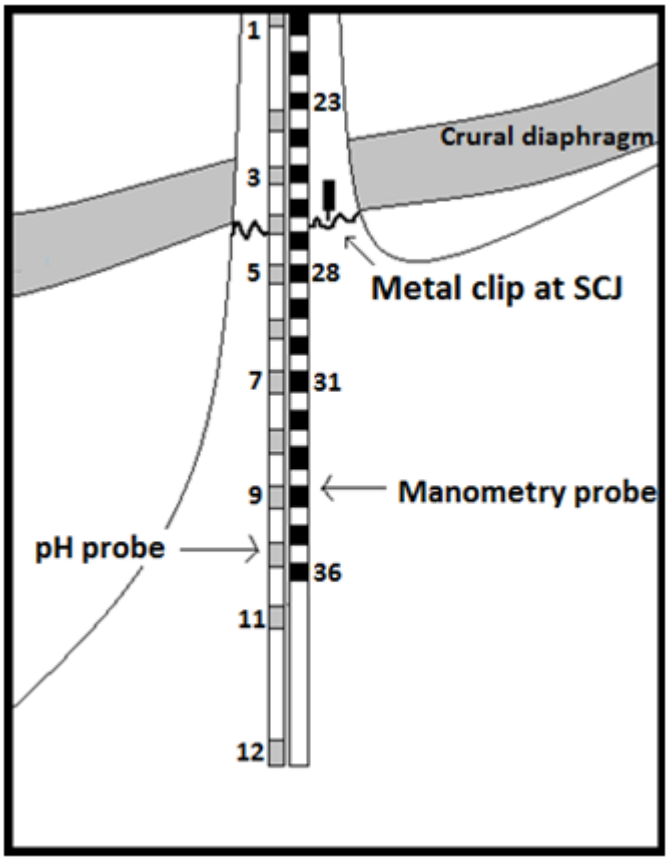
Volunteers attended after an overnight fast for upper gastrointestinal endoscopy. They were offered topical lidocaine throat spray or conscious sedation with midazolam 1-3mg. Biopsies were taken using large capacity biopsy forceps (Radial Jaw™ 4; Boston Scientific, Hemel Hempstead, UK) with a jaw span of 8mm. Two junctional biopsies were taken perpendicular to the squamocolumnar junction (SCJ), one from lesser and one from greater curve, and targeted to include squamous mucosa at the proximal end. Three further junctional biopsies were taken longitudinally below the SCJ, aiming for end-to-end biopsies starting at 6, 12 and 18mm distal to the SCJ down the lesser curve. In addition, six further gastric biopsies were taken from gastric fundus, mid-body on greater curve, mid-body on lesser curve, distal body on greater curve, incisura angularis and antrum. Finally, two small metal radio-opaque clips were attached to the SCJ using a single use rotatable clip fixing device (QuickClip 2™; Olympus, Southend-on-Sea, UK).

Biopsies were immediately placed onto non-adherent dental wax and oriented flat. More detailed information concerning the two-stage orientation method has been described elsewhere.(165) The specimens were later embedded in agar on the filter paper without further manipulation. Staining was performed with conventional H&E, as well as monoclonal antibodies to H^+/K^+ATPase, pepsinogen I and gastrin.

5.3.2.3 Study Day 3: Combined manometry and pH study

The volunteers attended after an overnight fast for combined high-resolution manometry and pH studies. The combined probe was passed pernasally and positioned so that the most proximal pH sensor was 5cm above the lower oesophageal sphincter (LOS), with the remaining eleven sensors lying across the sphincter and within the proximal stomach. The relative positions of the 12-sensor pH catheter, 36 sensor manometer and SCJ are shown in Figure 5.1**.** Manometry and pH data were recorded concurrently for a 20-minute fasting period. Subjects then consumed a standardised meal over ten minutes [400g Waitrose spaghetti bolognese ready meal and 100ml water (500kcal; 55.2g carbohydrate, 27.8g protein, 17.6g fat)]. Following this, manometry and pH recordings were continued for a further 90 minutes. An X-ray was taken before and after the meal to visualise the metal clips at the SCJ.

Figure 5.1: Schematic diagram of the relative positions of the 12-sensor pH catheter, 36 sensor manometer and squamocolumnar junction (SCJ) (identified by attached metal clip).



5.3.3 Equipment

5.3.3.1 High-resolution pHmetry

pH recordings were taken using a high-resolution pH catheter (Synectics Medical Ltd, Enfield, UK). This was a custom-made pH probe composed of 12 antimony pH electrodes with the most distal electrode situated 5mm from the tip of the catheter, with the other eleven electrodes 35, 46, 57, 68, 79, 90, 101, 112, 123, 134 and 169mm proximal to the tip. The probe was calibrated prior to each study using pH buffer solution (Synmed Ltd, Enfield, UK) at pH 7.01 and pH 1.07. Recordings were captured using Polygram Net software (Synectics Medical Ltd, Enfield, UK).

5.3.3.2 High-resolution manometry

Manometry was performed using a high resolution solid-state catheter with 7.5mm spacing between 36 circumferential sensors (Given Imaging, Hamburg, Germany). Calibration was performed prior to each study and In vivo calibration was carried out on a weekly basis and applied to each study to compensate for thermal drift. Recordings were captured with ManoScan 360 high-resolution Manometry System and analysed with ManoView ESO v3.0.1 software (Given Imaging, Hamburg, Germany).

5.3.3.3 Combined probe

The manometry and pH catheters were combined using two thin strips of Leukoplast Sleek waterproof tape (BSN Medical, Pinetown, SA) such that manometry sensor 25 was immediately adjacent to pH sensor 3.

5.3.4 Data analysis

5.3.4.1 Intragastric acid

The 90-minute postprandial period was split into three 30-minute periods for analysis. The median pH for each of the 12 pH sensors was calculated for the twenty-minute fasting period and the three 30-minute postprandial periods. Acid exposure at the GEJ was also examined by calculating the % of time pH <4.

5.3.4.2 Manometry

Manometric characteristics were analysed in detail during fasting and the same three postprandial periods. For each two-minute period, one inspiratory point and one expiratory point was chosen from the longest period without interference from swallowing, coughing or transient lower oesophageal sphincter relaxations (TLOSRs). The mean pressure in inspiration and expiration was calculated for each of the 36 sensors over the twenty-minute fasting period and thirty-minute postprandial periods. The peak LOS pressure was taken as the sensor showing the highest mean pressure. The position of the SCJ was derived from the position of the metal clips relative to the combined manometry and pH sensors seen on X-ray.

5.3.4.3 Histopathological Assessment

Glandular height: The vertical height of epithelium starting from lamina propria to tip of gland were measured in 3 well-oriented and representative fields and expressed as "Total Thickness of Epithelium". To measure the "Glandular Height", the same method was limited to areas of gland containing secretory cells, but not superficial foveolar epithelial cells. All results were expressed as median (IQR) in mm.

Inflammatory scoring: The intensity of inflammatory infiltrate by polymorphonuclear (PMN) and mononuclear (MN) cells was scored semi-quantitively (0=none; 1=mild; 2=moderate; 3=severe) as recommended in the Updated Sydney Classification of Gastritis.(54) A combined inflammatory score was calculated as the sum of these two scores. Intestinal metaplasia (IM) was scored by estimating the proportion of epithelial surface covered by goblet cells.

Immunohistochemistry: The oriented biopsies, double embedded in agar and paraffin, were cut in standard 4-micron thickness and immunostained individually for parietal cell, chief cell and G cells. For parietal cells, we used a commercial mouse monoclonal anti-H+/K+ ATPase (Ab 2866, Abcam, Cambridge, UK) diluted at 1:20,000. For Chief cells, a mouse monoclonal anti-pepsinogen 1 antibody (Ab 50123, Abcam, Cambridge, UK) was used at dilution of 1:4000. The G cells were stained with anti-gastrin (Ab-16035, Abcam, Cambridge, UK) diluted at 1:200. A Thermo Quanto Detection Kit (TL-125-OHD, Thermo Fisher, UK) was used as secondary antibody.

To calculate the density of parietal cells, chief cells and G cells, absolute number of stained cells were counted at a magnification of 125X in 3 well-oriented and representative fields (1 mm^2 each) and expressed as mean cell number per 1 mm^2 area in each patient. All selected areas must have had complete glands located in sagittal plane, in which the lamina propria was in bottom and luminal side of epithelium was in top.

5.3.5 Statistical analysis

All continuous data are expressed as medians and interquartile ranges unless otherwise stated. Comparison of variables between groups was made using the Mann-Whitney *U* test. Biopsy inflammatory scores are presented as crosstabulations and compared using Fisher's exact test. Significance for all statistical tests was set as p value <0.05.

5.3.6 Ethics

The study protocol was approved by the West of Scotland Ethics Committee and all volunteers provided informed written consent.

5.4 Results

5.4.1 Subjects

Of the 137 subjects assessed for eligibility for the study, 49 were excluded due to current or recent use of PPI therapy (n=9) or history of previous *H. pylori* eradication therapy (n=8) or declining to participate following full explanation of the study protocol (n=32). Eighty-eight subjects proceeded to the urea breath test, of which 31 were *H. pylori* positive, and all of these went on to complete the full study protocol. Of the 57 testing *H. pylori* negative, 28 went on to complete the study due to 1 withdrawing consent after the endoscopy and 28 not being selected to proceed in order to maintain matching of the positive and negative groups with respect to age, gender and BMI.

The 31 *H. pylori* positive and 28 *H. pylori* negative subjects who completed the study were well matched with respect to age (55 vs 56 years; p=0.95), gender (18/31 vs 18/28 males; p=0.84) and BMI (26.3 vs 26.8 kg/m2; p=0.72). There were seven current smokers in the *H. pylori* positive group compared with one current smoker in the *H. pylori* negative group (p=0.035).

The median dyspepsia score for *H. pylori* positives was 2.0 (range 0–9) compared with 0 (range 0–3) for the *H. pylori* negative subjects (p=0.002). Out of 31 *H. pylori* positives, 17 (54.8%) of them were taking no medication compared with 10/29 (35.7%) of the *H. pylori* negative subjects. The most frequent medications were antihypertensives, statins, antidepressants and inhalers for asthma. No subject was taking medications known to affect gastric secretion.

At endoscopy, four *H. pylori* positive subjects had a hiatus hernia (2–4 cm in length), one subject had Los Angeles (LA) grade A reflux oesophagitis and one subject had Barrett's mucosa of 3 cm. None of the *H. pylori* negatives had a hiatus hernia, although two subjects had reflux oesophagitis (LA grades A and B).

5.4.2 Gastro-oesophageal acidity

5.4.2.1 Fasting period

Under fasting conditions, the *H. pylori* positive subjects had less intragastric acidity compared to the *H. pylori* negatives at all sensors more than 1.1cm distal to the peak LOS pressure. The fall from neutral oesophageal pH to highly acidic intragastric pH also occurred more abruptly in the *H. pylori* negatives. At the sensor 3.3cm distal to the peak LOS pressure, the median pH in the *H. pylori* negatives had fallen to 2.27 compared to 6.13 in the positives (p<0.001). The radio-opaque clips indicated that this pH sensor was 1.8cm distal to the SC junction. At the most distal pH sensor, 6.6cm below the peak LOS pressure, the median pH in the *H. pylori* negatives was 1.62 compared to 2.39 in the positives (p=0.003), indicating that even this far into the stomach the acidity is significantly less in *H. pylori* infected subjects. Table 5.1 shows the fasting median pH values and interquartile ranges for all sensors in both groups.

Table 5.1. Median (IQR) pH values at sensors relative to peak LOS pressure comparing *H. pylori* negative (n=28) and positive (n=31) groups during 20-minute fasting period.

Sensor location	***H. pylori* negative**	***H. pylori* positive**	**P value**
5cm proximal	7.20 (0.70)	7.19 (0.74)	0.933
1.1cm proximal	7.33 (0.78)	7.37 (0.62)	0.525
Peak LOS pressure	7.34 (0.79)	7.28 (0.51)	0.499
1.1cm distal	7.06 (1.63)	7.13 (0.51)	0.213
2.2cm distal	5.79 (4.26)	6.94 (1.38)	0.004
3.3cm distal	2.27 (2.58)	6.13 (5.06)	<0.001
4.4cm distal	1.70 (1.16)	4.11 (4.95)	<0.001
5.5cm distal	1.68 (0.66)	2.88 (3.66)	<0.001
6.6cm distal	1.62 (3.66)	2.39 (3.06)	0.003

Figure 5.2 shows the median pH of both groups at each sensor below peak LOS pressure and illustrates both the sudden decrease in pH in *H pylori* negatives and the increased acidity in *H pylori* negatives compared to positives at all sensors more than 1.1cm distal to peak LOS pressure. The mean distance of the SCJ and distal border of the LOS from the peak LOS pressure is also shown on the graph. This shows the difference between the two groups begins at the first sensor below the SCJ, and this sensor is located within the distal end of the LOS.

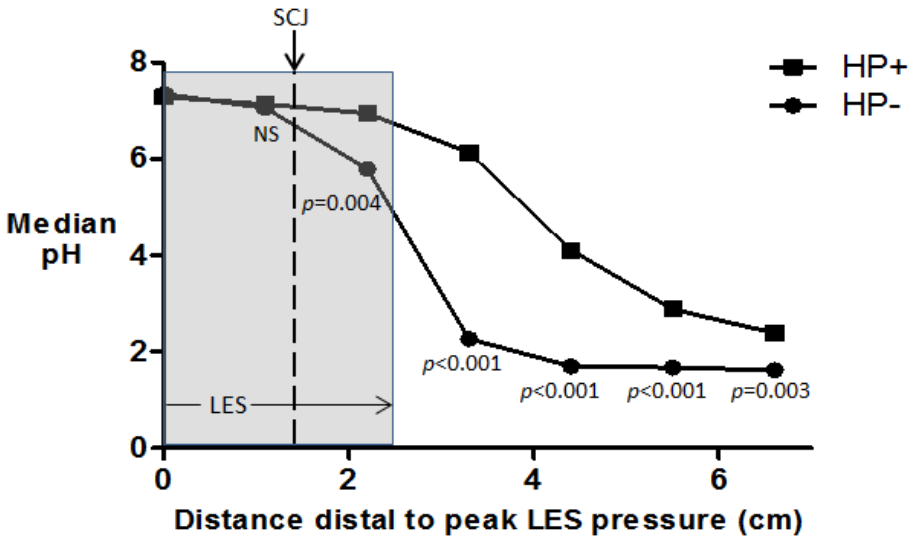


Figure 5.2: Median pH for 20-minute fasting period relative to lower oesophageal sphincter (LOS) and squamocolumnar junction (SCJ) in *H. pylori* positive (HP+) and negative (HP-) subjects.

pH values represent the negative logarithm of the hydrogen ion activity, which relates closely to the hydrogen ion (H^+) concentration. We transformed our pH data into hydrogen ion activity to show the actual differences in acidity between the two groups. In the *H. pylori* positives, the H^+ activity at 4.4 cm distal to peak LOS pressure (the first sensor with pH <5) is 0.1 mmol/l and this increases to 4.1 mmol/l at 6.6cm distal. In *H. pylori* negatives the H^+ activity is 20.2 mmol/l at 4.4cm and 24.3 mmol/l at 6.6cm. The data for all intragastric sensors is given in table 5.2.

Table 5.2. Median (IQR) hydrogen ion activity (mmol/l) at sensors relative to peak LOS pressure comparing *H. pylori* negative (n=28) and positive (n=31) groups during 20-minute fasting period.

Sensor location	***H. pylori* negative**	***H. pylori* positive**	**P value**
1.1cm distal	$1.0x10^{-4}$ ($3.4x10^{-3}$)	$7.4x10^{-5}$ ($9.1x10^{-5}$)	0.15
2.2cm distal	$2.9x10^{-3}$ (6.3)	$1.1x10^{-3}$ ($1.3x10^{-3}$)	0.003
3.3cm distal	5.6 (19.6)	$7.4x10^{-4}$ (6.3)	<0.001
4.4cm distal	20.2 (26.2)	0.1 (16.2)	<0.001
5.5cm distal	21.1 (26.3)	1.3 (16.2)	<0.001
6.6cm distal	24.3 (28.7)	4.1 (25.1)	0.003

5.4.2.2 Postprandial periods

Throughout the three postprandial periods, intragastric acidity was significantly less in the *H. pylori* positives at the pH sensors placed 2.2, 3.3 and 4.4cm distal to the peak pressure of the LOS but no significant difference was detected by the more distal sensors placed at 5.5 and 6.6cm distal to this reference point (Table 5.3). In the first postprandial period, which starts immediately after meal has been consumed up until 30 minutes later, the lowest median pH value is 2.46 at 3.3cm distal to the peak LOS pressure in the *H. pylori* negative group. In the *H. pylori* positives, the lowest median pH is 4.26, also at 3.3cm distal to peak LOS pressure. In the second postprandial period (30-60 minutes after the meal) in the *H. pylori* negative group the median pH has fallen further to 1.59 at 3.3cm distal to peak LOS pressure. In the *H. pylori* positives, the pH has fallen to 2.07, but there is still a significant difference between the two groups (p=0.009). In the third and final postprandial period (60-90 minutes after the meal) the results are similar, with the lowest median pH being 1.61 at 3.3cm distal to peak LOS pressure in *H. pylori* negatives and 2.01 for *H. pylori* positives at 4.4cm distal to peak LOS pressure. These three sensors detecting a significant difference in gastric acidity between the two groups were those closest to the GOJ with the most proximal of them being only 0.6cm distal to the SCJ. Figure 5.3 shows the median pH values in the first postprandial period (0-30 minutes) for the two groups at each sensor below the peak LOS pressure. It also shows the mean distance of the SCJ and distal border of the LOS from the peak LOS pressure. This illustrates that the first sensor showing significant differences between the two groups is the sensor immediately below the SCJ, and this is within the distal end of the lower oesophageal sphincter. The graph

also reveals the formation of an acid pocket which has developed in the *H. pylori* negative subjects and is attenuated in the *H. pylori* positives.

Table 5.3 Median (IQR) pH values at sensors relative to peak LOS pressure comparing *H. pylori* negative (n=28) and positive (n=31) groups during the three 30-minute postprandial periods.

	0-30 minutes			**30-60 minutes**			**60-90 minutes**		
Sensor location	***HP-***	***HP+***	***p* value**	***HP-***	***HP+***	***p* value**	***HP-***	***HP+***	***p* value**
5cm proximal	7.28 (0.79)	7.03 (0.72)	0.274	7.18 (0.81)	6.98 (0.77)	0.499	7.13 (0.85)	7.04 (0.67)	0.861
1.1cm proximal	7.20 (0.96)	7.29 (0.68)	0.443	7.06 (1.42)	7.00 (0.75)	0.705	7.13 (1.77)	6.96 (1.27)	0.786
Peak LOS pressure	6.83 (0.62)	6.94 (0.66)	0.339	6.76 (1.02)	6.88 (0.48)	0.391	6.56 (1.27)	6.77 (0.58)	0.245
1.1cm distal	5.90 (1.88)	6.74 (1.18)	0.063	5.25 (4.19)	6.40 (1.72)	0.053	6.43 (4.80)	6.38 (2.21)	0.306
2.2cm distal	3.17 (3.07)	5.55 (2.84)	0.005	1.95 (1.00)	3.21 (4.46)	0.005	2.20 (2.82)	3.82 (4.40)	0.024
3.3cm distal	2.46 (2.75)	4.26 (2.84)	0.006	1.59 (1.08)	2.07 (2.29)	0.009	1.61 (0.82)	2.30 (3.08)	0.010
4.4cm distal	4.09 (3.17)	4.87 (1.60)	0.025	1.81 (2.09)	2.93 (3.25)	0.032	1.67 (0.94)	2.01 (2.10)	0.031
5.5cm distal	4.62 (1.21)	4.79 (1.36)	0.309	2.13 (2.02)	3.48 (2.89)	0.062	1.74 (1.45)	2.36 (2.74)	0.078
6.6cm distal	4.60 (1.17)	4.68 (0.96)	0.313	3.39 (2.19)	4.10 (2.23)	0.158	2.08 (1.58)	3.87 (2.35)	0.184

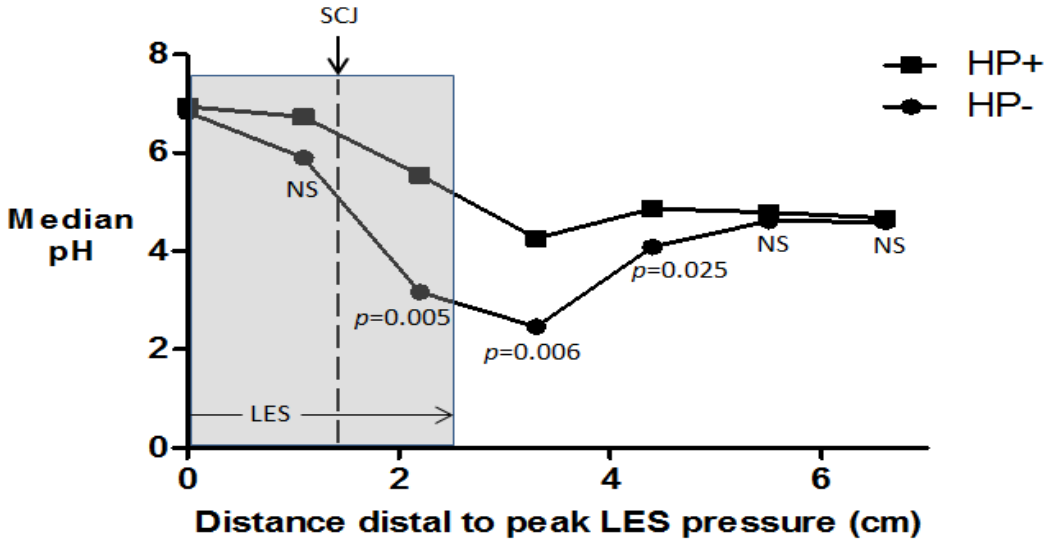


Figure 5.3: Median pH for 0-30 minute period after meal relative to lower oesophageal sphincter (LOS) and squamocolumnar junction (SCJ) in *H. pylori* positive (HP+) and negative (HP-) subjects.

The % of time pH<4 for each of the three postprandial periods was significantly greater in the *H. pylori* negatives versus positive subjects for the electrodes extending 4.4cm distal to the peak LOS pressure in the first postprandial period (0-30 minutes). In addition, the % time pH<4 was also significantly greater at the sensor immediately at peak LOS pressure and extending 1.1cm above the peak LOS pressure, indicating increased intrasphincteric reflux in this group. This was also evident in the second postprandial period, with the *H. pylori* negatives having a median %time pH<4 of 3.7% at the peak LOS pressure and 2.1% 1.1cm proximally. In *H. pylori* positives this was 0.9% at peak LOS pressure and 0.3% 1.1cm proximal. In the third postprandial period the differences between the two groups at the intrasphincteric sensors did not reach statistical significance. There was little evidence of trans-sphincteric acid reflux at the sensor placed 5cm proximal in either group at any stage (Table 5.4).

Table 5.4: Median (IQR) percentage time pH<4 at sensors relative to peak LOS pressure comparing *H. pylori* negative (n=28) and positive (n=31) groups during the three 30-minute postprandial periods.

	0-30 minutes			**30-60 minutes**			**60-90 minutes**		
Sensor location	***HP-***	***HP+***	***p* value**	***HP-***	***HP+***	***p* value**	***HP-***	***HP+***	***p* value**
5cm proximal	0.2 (0.4)	0.0 (0.7)	0.384	0.0 (0.7)	0.0 (0.5)	0.354	0.0 (1.2)	0.0 (0.5)	0.280
1.1cm proximal	3.0 (2.8)	0.0 (1.3)	0.005	2.1 (14.3)	0.3 (2.8)	0.046	2.0 (6.4)	0.6 (0.7)	0.088
Peak LOS pressure	4.2 (6.5)	0.6 (2.0)	<0.001	3.7 (15.3)	0.9 (4.4)	0.017	2.7 (8.7)	1.2 (7.4)	0.162
1.1cm distal	15.4 (30.8)	1.8 (19.3)	0.003	33.9 (67.0)	5.2 (33.0)	0.021	7.6 (77.1)	10.1 (25.5)	0.264
2.2cm distal	62.9 (49.7)	22.6 (51.8)	0.001	90.8 (28.7)	63.2 (81.1)	0.002	81.1 (51.7)	55.1 (84.7)	0.026
3.3cm distal	64.9 (45.7)	46.4 (66.1)	0.004	99.7 (9.3)	91.5 (49.0)	0.017	99.2 (3.2)	91.0 (59.1)	0.009
4.4cm distal	44.2 (69.2)	15.1 (53.0)	0.032	99.0 (18.7)	88.9 (77.9)	0.111	100.0 (3.0)	99.4 (20.7)	0.043
5.5cm distal	24.3 (47.4)	12.9 (48.5)	0.375	96.2 (37.3)	86.1 (88.2)	0.083	100.0 (1.2)	99.8 (80.1)	0.105
6.6cm distal	13.7 (46.4)	9.9 (21.7)	0.355	82.8 (72.6)	38.5 (99.3)	0.104	99.8 (9.0)	96.8 (61.0)	0.099

5.4.2.3 *CagA*

Seventeen of the *H. pylori* positives were *CagA* seropositive and fourteen *CagA* seronegative. In the fasting period, there were significant differences between *H. pylori* negatives and *CagA* seronegative *H. pylori* positives at the sensors 4.4, 5.5 and 6.6cm distal to peak LOS pressure. For *CagA* seropositives there were significant differences at sensors 2.2, 3.3, 4.4, 5.5 and 6.6cm distal to peak LOS pressure. Between *CagA* seronegatives and seropositives, there were significant differences at two sensors, at 2.2cm and 6.6cm distal to peak LOS pressure.

In the first postprandial period (0-30 minutes after meal) there were significant differences between *CagA* seropositives and *H. pylori* negatives at the sensors 1.1 to 4.4 cm distal to peak LOS pressure. Similarly, in the second postprandial period (30-60 minutes), *CagA* seropositives were significantly different to *H. pylori* negatives at sensors 2.2 to 5.5cm distal to peak LOS pressure. At the sensor 3.3cm distal to peak LOS pressure there was a significant difference in median pH between *CagA* seronegatives and seropositives. In the final postprandial period (60-90 minutes) significant differences existed between *H. pylori* negatives and *CagA* seropositives at sensors 2.2 to 5.5cm distal to peak LOS pressure. There were no significant differences between *CagA* seropositives and negatives, or *CagA* seronegatives and *H. pylori* negatives. (Table 5.5)

5.4.3 Conventional H&E Staining

5.4.3.1 Inflammation

The *H. pylori* positives had a greater combined inflammatory cell infiltrate at each of the 11 biopsy sites compared to the *H. pylori* negatives (Table 5.6). The increased combined inflammatory cell infiltrate in the *H. pylori* positives consisted of a mixture of PMN cells and MN cells and tended to be more intense close to the SCJ, lesser curve, distal stomach, incisura and antrum compared to the gastric fundus and mid-body ($p<0.05$ for each). The *H. pylori* negatives had a MN cell infiltrate limited to the SCJ and to a lesser extent at the antrum and angularis incisura, but its intensity was less than that of the *H. pylori* positives at these sites. (Table 5.7) There was minimal evidence of PMN cell infiltrate at any location in the *H. pylori* negatives. (Table 5.8)

Table 5.5: Median (IQR) pH in *H. pylori* negatives (n=28), *H. pylori* positive *CagA* negatives (n=14) and *H. pylori* positive *CagA* positives (n=17) during 20-minute fasting and three 30-minute postprandial periods. **Note**: *Indicates statistically different from *H. pylori* negatives. ‡Indicates statistically different from *H. pylori* positive *CagA* negatives ($p<0.05$).

	Fasting				**0-30 minutes**				**30-60 minutes**				**60-90 minutes**		
Sensor location	***HP-***	***HP+ CagA-***	***HP+ CagA+***		***HP-***	***HP+ CagA-***	***HP+ CagA+***		***HP-***	***HP+ CagA-***	***HP+ CagA+***		***HP-***	***HP+ CagA-***	***HP+ CagA+***
5cm proximal	7.20 (0.70)	7.22 (0.68)	7.06 (0.64)		7.28 (0.79)	7.11 (0.80)	6.97 (0.64)		7.18 (0.81)	7.01 (0.83)	6.93 (0.61)		7.13 (0.85)	7.09 (0.90)	7.00 (0.85)
1.1cm proximal	7.33 (0.78)	7.65 (0.75)	7.32 (0.53)		7.20 (0.96)	7.55 (0.67)	7.20 (0.59)		7.06 (1.42)	6.97 (1.46)	7.00 (0.60)		7.13 (1.77)	6.96 (0.79)	6.95 (0.83)
Peak LOS pressure	7.34 (0.79)	7.52 (0.51)	7.18 (0.31)		6.83 (0.62)	7.02 (0.77)	6.89 (0.63)		6.76 (1.02)	6.93 (1.02)	6.80 (0.39)		6.56 (1.27)	6.79 (0.70)	6.77 (0.56)
1.1cm distal	7.06 (1.63)	7.13 (1.65)	7.13 (0.40)		5.90 (1.88)	6.66 (4.46)	6.74* (1.10)		5.25 (4.19)	6.36 (2.52)	6.55 (1.79)		6.43 (4.80)	5.96 (2.60)	6.48 (1.21)
2.2cm distal	5.79 (4.26)	6.19 (4.53)	7.13*‡ (0.70)		3.17 (3.07)	4.38 (3.76)	6.25* (1.84)		1.95 (1.00)	2.19 (3.02)	5.72* (4.69)		2.20 (2.82)	3.37 (4.28)	5.86* (4.65)
3.3cm distal	2.27 (2.58)	3.16 (4.94)	6.76* (3.22)		2.46 (2.75)	3.58 (2.67)	5.16* (1.92)		1.59 (1.08)	1.86 (1.85)	2.61*‡ (3.73)		1.61 (0.82)	2.08 (1.32)	2.86* (4.06)
4.4cm distal	1.70 (1.16)	3.60* (4.99)	4.11* (4.09)		4.09 (3.17)	4.48 (1.51)	5.28* (1.78)		1.81 (2.01)	2.54 (1.70)	3.85* (3.67)		1.67 (0.94)	1.89 (1.75)	2.19* (3.39)
5.5cm distal	1.68 (0.66)	2.18* (2.26)	4.17* (4.17)		4.62 (1.21)	4.70 (1.31)	4.97 (1.61)		2.13 (2.02)	2.99 (2.64)	4.36* (3.16)		1.74 (1.45)	1.84 (1.94)	2.56* (2.78)
6.6cm distal	1.62 (3.66)	1.80 (1.46)	4.11*‡ (4.72)		4.60 (1.17)	4.66 (0.77)	4.68 (1.13)		3.39 (2.19)	3.76 (2.10)	4.35 (2.23)		2.08 (1.58)	2.15 (2.09)	3.18 (3.56)

Table 5.6: Cross-tabulation table showing the number of subjects within the *H. pylori* negative (*HP*-) and positive (*HP*+) groups with each combined inflammatory score (0-6) at the 11 different gastric biopsy locations.

Combined Inflammatory score	**Across SCJ (greater curve)**		**Across SCJ (lesser curve)**		**6mm distal SCJ**		**12mm distal SCJ**		**18mm distal SCJ**	
	HP-	*HP*+	*HP*-	*HP*+	*HP*-	*HP*+	*HP*-	*HP*+	*HP*-	*HP*+
0	8	0	1	0	15	0	25	0	26	0
1	10	0	16	0	8	1	2	1	1	4
2	9	0	6	0	1	3	0	6	0	7
3	1	7	0	7	0	10	0	8	1	9
4	0	11	0	11	0	11	0	11	0	5
5	0	11	0	9	0	5	0	4	0	4
6	0	1	0	0	0	0	0	0	0	1
Fisher's Exact test	$p<0.001$		$p<0.001$		$p<0.001$		$p<0.001$		$p<0.001$	

Combined Inflammatory score	**Fundus**		**Mid-body, lesser curve**		**Mid-body, greater curve**		**Distal body, greater curve**		**Incisura angularis**		**Antrum**	
	HP-	*HP*+	*HP*-	*HP*+	*HP*-	*HP*+	*HP*-	*HP*+	*HP*-	*HP*+	*HP*-	*HP*+
0	25	0	25	0	27	0	24	0	17	0	14	0
1	1	5	2	0	0	2	2	0	9	0	9	0
2	0	6	0	6	0	8	0	7	1	0	0	0
3	1	12	0	11	0	8	0	5	0	6	1	1
4	0	5	0	6	1	5	1	7	0	3	0	6
5	0	1	0	4	0	4	1	8	0	13	0	11
6	0	2	0	4	0	4	0	4	0	9	0	8
Fisher's Exact test	$p<0.001$		$p<0.001$		$p<0.001$		$p<0.001$		$p<0.001$		$p<0.001$	

Table 5.7: Cross-tabulation table showing the number of subjects within the *H. pylori* negative (*HP*-) and positive (*HP*+) groups with each chronic inflammatory score (0-3) at the 11 different gastric biopsy locations.

MN score	**Across SCJ (above greater curve)**		**Across SCJ (above lesser curve)**		**6mm distal SCJ**		**12mm distal SCJ**		**18mm distal SCJ**			
	HP-	*HP+*	*HP-*	*HP+*	*HP-*	*HP+*	*HP-*	*HP+*	*HP-*	*HP+*		
0	4	0	1	0	15	0	25	0	26	0		
1	10	0	16	0	8	3	2	7	1	9		
2	10	15	6	15	1	19	0	17	1	15		
3	0	15	0	12	0	7	0	6	0	6		
Fisher's Exact test	p<0.001		p<0.001		p<0.001		p<0.001		p<0.001			
MN score	**Fundus**		**Mid-body lesser curve**		**Mid-body greater curve**		**Distal body greater curve**		**Incisura angularis**		**Antrum**	
	HP-	*HP+*	*HP-*	*HP+*	*HP-*	*HP+*	*HP-*	*HP+*	*HP-*	*HP+*	*HP-*	*HP+*
0	25	0	25	0	27	0	24	0	17	0	14	0
1	1	11	2	6	0	10	2	7	9	0	9	0
2	1	17	0	17	1	13	1	11	1	9	1	6
3	0	3	0	8	0	8	1	13	0	22	0	20
Fisher's Exact test	p<0.001		p<0.001		p<0.001		p<0.001		p<0.001		p<0.001	

Table 5.8: Cross-tabulation table showing the number of subjects within the *H. pylori* negative (*HP-*) and positive (*HP+*) groups with each acute inflammatory score (0-6) at the 11 different gastric biopsy locations.

PMN score	Across SCJ (above greater curve)		Across SCJ (above lesser curve)		6mm distal SCJ		12mm distal SCJ		18mm distal SCJ			
	HP-	*HP+*	*HP-*	*HP+*	*HP-*	*HP+*	*HP-*	*HP+*	*HP-*	*HP+*		
0	16	0	23	0	23	0	27	1	27	6		
1	1	9	0	11	0	14	0	16	1	15		
2	0	20	0	15	0	15	0	13	0	8		
3	0	1	0	1	0	0	0	0	0	1		
Fisher's Exact test	p<0.001		p<0.001		p<0.001		p<0.001		p<0.001			
PMN score	**Fundus**		**Mid-body lesser curve**		**Mid-body greater curve**		**Distal body greater curve**		**Incisura angularis**		**Antrum**	
	HP-	*HP+*	*HP-*	*HP+*	*HP-*	*HP+*	*HP-*	*HP+*	*HP-*	*HP+*	*HP-*	*HP+*
0	26	5	27	0	27	2	26	0	27	0	23	0
1	1	18	0	17	0	16	0	13	0	6	1	2
2	0	6	0	10	1	9	2	14	0	16	0	16
3	0	2	0	4	0	4	0	4	0	9	0	8
Fisher's Exact test	p<0.001		p<0.001		p<0.001		p<0.001		p<0.001		p<0.001	

5.4.3.2 Intestinal Metaplasia

Intestinal metaplasia was identified in 14 of the 31 *H. pylori* positive subjects. In 7 of these it was limited to one or more of the biopsies from mid-body lesser curve, distal body greater curve, incisura angularis and antrum. In 3 of the subjects it was present in at least one of the above sites and in the biopsies close to the SCJ. In a further 3 it was limited to the region close to the SCJ. In 1 subject it was present in each biopsy except for one of the biopsies from the SCJ.

Intestinal metaplasia was identified in only four of the 28 *H. pylori* negative subjects. In three of these it was only seen in the biopsies across the SCJ and in the fourth subject it was only seen in the biopsy from the fundus.

5.4.3.3 Gastric Gland Thickness

The thickness of the gastric secretory glands was significantly reduced in the *H. pylori* positive versus negative subjects throughout the gastric mucosa except for the biopsies taken across the SCJ. Median glandular thickness was greatest in *H. pylori* negatives from biopsies at the mid-body of the greater curve and from 18mm distal to SCJ, which was more proximal along the greater curvature. The degree of reduction in median glandular thickness was similar throughout the oxyntic gastric mucosa (Table 5.9).

Table 5.9: Median glandular thickness in millimetres (IQR) at each biopsy location comparing *H. pylori* negatives (n=28) and positives (n=31).

Biopsy location	***H. pylori* negatives**	***H. pylori* positives**	***P* value**
Across SCJ, Greater curve	0.30 (0.20–0.30)	0.25 (0.20–0.30)	0.515
Across SCJ, Lesser curve	0.28 (0.0–0.30)	0.20 (0.10–0.30)	0.461
6mm distal SCJ	0.35 (0.30–0.40)	0.30 (0.20–0.30)	0.006
12mm distal SCJ	0.40 (0.40–0.45)	0.30 (0.30–0.35)	<0.001
18mm distal SCJ	0.45 (0.40–0.50)	0.35 (0.30–0.40)	<0.001
Fundus	0.43 (0.40–0.45)	0.40 (0.35–0.40)	0.008
Mid-body, Lesser curve	0.40 (0.40–0.45)	0.35 (0.30–0.40)	<0.001
Mid-body, Greater curve	0.45 (0.40–0.45)	0.35 (0.30–0.40)	<0.001
Distal body, Greater curve	0.40 (0.35–0.49)	0.30 (0.25–0.35)	<0.001
Incisura Angularis	0.33 (0.30–0.40)	0.25 (0.20–0.30)	<0.001
Antrum	0.20 (0.13–0.30)	0.20 (0.0–0.20)	0.041

5.4.4 Immunohistochemistry

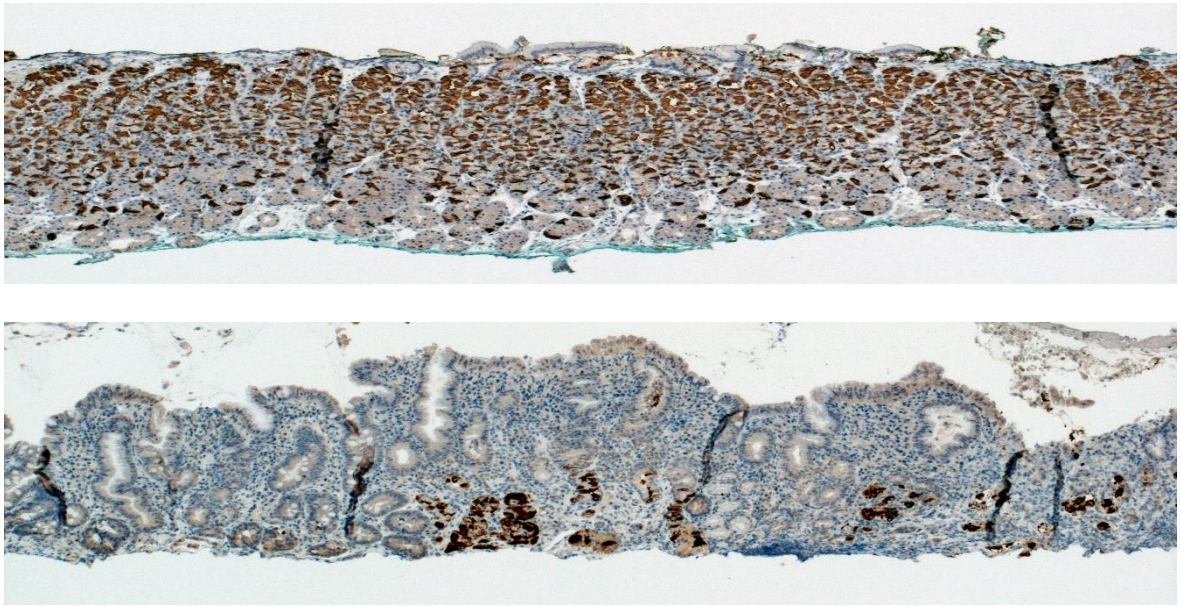
5.4.4.1 Parietal and Chief Cell Density

The median parietal and chief cell density in *H. pylori* negatives was greater in biopsies taken from the gastric body (i.e. fundus, mid-body lesser cure and greater curve) compared to biopsies taken from the gastro-oesophageal junction (i.e. across SCJ and distal to SCJ). The *H. pylori* positives had a significant reduction in density of both parietal and chief cells compared to *H. pylori* negatives, and this was seen at each of the 11 intragastric locations assessed except for the SCJ greater curve where the difference did not achieve statistical significance (Table 5.10). The degree of reduction was similar for the two cell types. Representative biopsies stained for parietal cells from an *H. pylori* negative and positive subject are shown in figure 5.4.

Table 5.10: Median densities (IQR) of parietal and chief cells at each biopsy location comparing *H. pylori* negatives (n=28) and positives (n=31).

	Parietal cell density (cells/mm^2)				**Chief cell density (cells/mm^2)**		
Biopsy location	***H.pylori* -**	***H.pylori* +**	***P* value**		***H.pylori* -**	***H.pylori* +**	***P* value**
Across SCJ, Greater curve	67 (0-162)	17 (10-39)	0.185		94 (0-156)	22 (3-52)	0.150
Across SCJ, Lesser curve	50 (14-127)	9 (0-51)	0.012		89 (17-139)	22 (0-62)	0.017
6mm distal SCJ	231 (175-286)	144 (59-190)	<0.001		245 (203-272)	129 (52-190)	<0.001
12mm distal SCJ	317 (300-362)	193 (137-250)	<0.001		379 (312-404)	206 (125-299)	<0.001
18mm distal SCJ	357 (334-383)	241 (201-283)	<0.001		404 (374-421)	273 (194-353)	<0.001
Fundus	347 (285-401)	258 (220-292)	<0.001		421 (384-451)	310 (255-389)	<0.001
Mid-body, Lesser curve	361 (316-381)	235 (166-290)	<0.001		401 (367-419)	285 (206-367)	<0.001
Mid-body, Greater curve	356 (318-398)	250 (201-297)	<0.001		420 (372-441)	305 (243-354)	<0.001
Distal body, Greater curve	322 (293-349)	107 (25-263)	<0.001		365 (296-398)	136 (17-292)	<0.001
Incisura Angularis	203 (124-250)	12 (0-87)	<0.001		215 (98-296)	7 (0-99)	<0.001
Antrum	40 (6-67)	7 (0-18)	0.002		22 (1-85)	0 (0-5)	<0.001

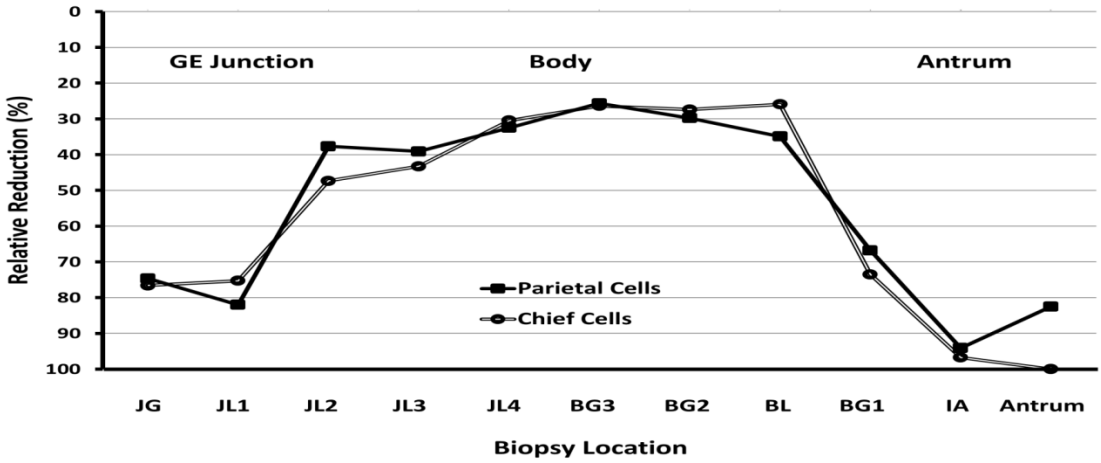
Figure 5.4 Biopsies from gastric body stained with monoclonal antibody to H^+/K^+ ATPase



The depletion of both cells in the *H. pylori* positives versus negatives was more marked in the biopsies taken from the distal gastric mucosa (i.e. antrum, incisura angularis, and distal body greater curve) being reduced by 67-100% compared to that observed in the more central region of the oxyntic mucosa (fundus and mid-body) at 26-35% (Fig. 5.5).

In addition, the length of mucosa extending distal to the SCJ which contained no detectable parietal cells was greater in the *H. pylori* positives versus negatives (1.5mm vs 1.0mm; p=0.013). However, the degree of reduction in specialised cell density in the biopsies taken 6mm and 12mm distal to the SCJ (38-47%) was not dissimilar from that observed in the more central oxyntic mucosa (i.e. fundus and mid-body) (26-35%) (Fig. 5.5).

Fig 5.5: Relative reduction in parietal and chief cell densities at different gastric locations in *H. pylori* infected versus non-infected



Note: At the GE junction and distal stomach these cells are reduced by 80% whereas in the mid-body reduction was about 30%. Biopsy locations: **JG**: across SCJ above greater curve; **JL1**: across SCJ above lesser curve; JL**2**: 6mm distal SCJ; JL**3**: 12mm distal SCJ; JL**4**: 18mm distal SCJ; BG**3**: Fundus; BL: mid-body lesser curve; BG**2**: mid-body greater curve; BG**1**: distal body greater curve; IA: incisura angularis; Ant: antrum.

5.4.4.2 G Cell Density

The density of G cells was reduced in the antrum of the *H. pylori* positive versus negative subjects [48 (IQR: 31-86) vs. 91 (64-129), $p<0.001$], but the converse was seen with respect to the biopsies taken from the distal body region [0 (IQR: 0-32) vs 0 (0-0), $p=0.007$].

5.4.4.3 *CagA*

Seventeen of the *H. pylori* positives were *CagA* seropositive and fourteen *CagA* seronegative. The *CagA* seropositives had a greater combined inflammatory cell infiltrate at 3 of the 11 biopsy sites compared to *CagA* seronegatives. These sites were 6mm distal to SCJ, 18mm distal to SCJ and distal body on greater curvature. At the other 8 sites there was no statistical difference found between *CagA* seropositives and seronegatives. (Table 5.11)

CagA seropositives had a significantly reduced parietal cell density compared to *CagA* seronegatives at only one of the biopsy sites and this was across the SCJ (above greater

curve). Similarly, only one biopsy site showed reduced chief cell density in the *CagA* seropositive site compared to the seronegatives, this time 18mm distal to SCJ. (Table 5.12)

Table 5.11: Cross-tabulation table comparing the number of *H. pylori* positive *CagA* negative (*HP+ CagA-*) and *H. pylori* positive *CagA* positive (*HP+ CagA+*) subjects with each combined inflammatory score (0-6) at all gastric biopsy locations.

Combined Inflammatory score	**Across SCJ (above greater curve)**		**Across SCJ (above lesser curve)**		**6mm distal SCJ**		**12mm distal SCJ**		**18mm distal SCJ**			
	HP+ CagA-	*HP+ CagA+*	*HP+ CagA-*	*HP+ CagA+*	*HP+ CagA-*	*HP+ CagA+*	*HP+ CagA-*	*HP+ CagA+*	*HP+ CagA-*	*HP+ CagA+*		
0	0	0	0	0	0	0	0	0	0	0		
1	0	0	0	0	1	0	0	1	2	2		
2	0	0	0	0	3	0	5	1	5	2		
3	3	4	2	5	1	9	4	4	5	4		
4	5	6	5	6	6	5	4	7	0	5		
5	5	6	4	5	2	3	0	4	0	4		
6	0	1	0	0	0	0	0	0	1	0		
Fisher's Exact test	p=1.000		p=0.449		p=0.009		p=0.084		p=0.034			
Combined Inflammatory score	**Fundus**		**Mid-body lesser curve**		**Mid-body greater curve**		**Distal body greater curve**		**Incisura angularis**		**Antrum**	
	HP+ CagA-	*HP+ CagA+*	*HP+ CagA-*	*HP+ CagA+*	*HP+ CagA-*	*HP+ CagA+*	*HP+ CagA-*	*HP+ CagA+*	*HP+ CagA-*	*HP+ CagA+*	*HP+ CagA-*	*HP+ CagA+*
0	0	0	0	0	0	0	0	0	0	0	0	0
1	2	3	0	0	1	1	0	0	0	0	0	0
2	3	3	4	2	5	3	7	0	0	0	0	0
3	7	5	6	5	1	7	1	4	2	4	1	0
4	1	4	2	4	4	1	3	4	3	0	4	2
5	0	1	1	3	2	2	2	6	7	6	6	5
6	1	1	1	3	1	3	1	3	2	7	2	6
Fisher's Exact test	p=0.803		p=0.579		p=0.158		p=0.012		p=0.120		p=0.343	

Table 5.12: Median densities (IQR) of parietal and chief cells at each biopsy location comparing *H. pylori* negatives (n=28), *H. pylori* positive *CagA* negatives (n=14) and *H. pylori* positive *CagA* positives (n=17)
Note: *Indicates statistically different from *H. pylori* negatives. ‡Indicates statistically different from *H. pylori* positive *CagA* negatives ($p<0.05$).

	Parietal cell density				**Chief cell density**		
Biopsy location	***HP-***	***HP+ CagA-***	***HP+ CagA+***		***HP-***	***HP+ CagA-***	***HP+ CagA+***
Across SCJ (above greater curve)	67 (162)	30 (59)	12 (19)‡		94 (156)	39 (109)	17 (35)
Across SCJ (above lesser curve)	50 (113)	8 (50)*	9 (56)*		89 (122)	5 (45)*	26 (66)*
6mm distal SCJ	231 (111)	146 (142)*	141 (129)*		245 (69)	104 (155)*	139 (152)*
12mm distal SCJ	317 (62)	193 (135)*	188 (124)*		379 (92)	201 (190)*	211 (170)*
18mm distal SCJ	357 (49)	253 (65)*	233 (127)*		404 (47)	310 (107)*	253 (163)*‡
Fundus	347 (116)	226 (88)*	263 (63)*		421 (67)	327 (140)*	397 (124)*
Mid-body lesser curve	361 (65)	223 (123)*	247 (150)*		401 (52)	354 (204)*	245 (190)*
Mid-body greater curve	356 (80)	255 (116)*	250 (89)*		420 (69)	333 (164)*	287 (118)*
Distal body greater curve	322 (56)	165 (255)*	84 (190)*		366 (102)	266 (336)*	59 (221)*
Incisura angularis	203 (126)	39 (155)*	10 (34)*		215 (198)	68 (172)*	0 (28)*
Antrum	40 (61)	12 (24)*	5 (17)*		22 (84)	0 (7)*	0 (0)*

5.5 Discussion

In our volunteers recruited from the general population of the West of Scotland, those with *H. pylori* infection had less intragastric acidity both under fasting conditions and following a meal compared to uninfected volunteers matched for age, gender and BMI**.** In addition, those with the infection had a reduced density of both acid secreting parietal cells and pepsin producing chief cells compared to those uninfected. These findings indicate that *H. pylori* infection within our Western population is associated with a less acidic and proteolytic intragastric environment.

The reduced intragastric acidity in the *H. pylori* positive subjects was apparent throughout the stomach under fasting conditions. After the meal, however, the reduced acidity in the *H. pylori* positives was evident within the first few centimetres distal to the GEJ but no significant difference in acidity was apparent in the main body of the stomach. There was also evidence of increased acidity after the meal in the *H. pylori* negatives right at the SCJ junction and extending 2cm above it indicating increased intrasphincteric acid reflux. We and others have previously reported that the proximal region of the stomach close to the GEJ largely escapes the buffering effect of ingested food and may remain highly acidic after a meal.(13, 166) This phenomenon has been called the acid pocket and is thought to be important in GORD induced oesophageal damage after a meal when reflux is most common. It is therefore interesting that it is at this region close to the GEJ where the reduced acidity was most apparent in the *H. pylori* infected subjects.

What is the reason for the reduced acidity in the *H. pylori* positives after a meal, being most marked close to the GEJ? There was no evidence that the depletion in parietal cell density in the *H. pylori* positives was more pronounced over the few centimetres close to the GEJ compared to other regions in the stomach. Inflammation may also inhibit gastric secretory function(158) and this was slightly increased close to the GEJ and in the distal stomach compared to the mid-body gastric mucosa. The elevation of intragastric pH following the meal in the *H. pylori* positives being most marked close to the GEJ may simply reflect the relative intragastric distribution of gastric juice and ingested food. Following a meal, the food occupies the centre of the stomach and the secreted gastric juice, the region close to the stomach wall which secretes it. Impaired acid secretion will elevate the pH of the gastric juice and this will be most apparent close to the stomach wall. In contrast, the central region of the stomach will reflect the pH of the food and thus will be relatively unaffected by

changes in the acidity of secreted juice. The effect of *H. pylori* on intragastric pH after the meal being most evident close to the GEJ may be due to this region being close to the wall of the stomach.

Whatever the explanation for the changes in acidity between *H. pylori* positives and negatives being most marked close to the GEJ, after the meal, the observation is likely to be important with respect to the propensity of gastro-oesophageal reflux producing oesophageal damage. It is well recognised that gastric juice which refluxes into the oesophagus is that present close to the GEJ and also that reflux most commonly occurs during the postprandial period when TLOSRs are most frequent.(167)

The reduction in parietal cell density observed in the *H. pylori* positive subjects was associated with a similar reduction in chief cell density. This is consistent with the infection and inflammation causing a loss in gastric glands and also with the previous literature showing that the development of parietal and chief cells is intimately linked.(168) We did not measure the secretion of pepsin and other digestive enzymes produced by the chief cells but their reduced density is likely to be associated with reduced secretory capacity after the meal. Reduction in gastric juice peptic activity has previously been reported in *H.pylori* infected subjects.(169) The peptic activity of the gastric juice is as important, and arguably more important than its acidity, with respect to the ability to damage oesophageal mucosa and therefore the reduction in both specialised cells is likely to represent a substantial reduction in the damaging capacity of reflux gastric juice in *H.pylori* infected subjects.(170)

There was a reduction in the density of G cells in the antrum of the *H. pylori* positives indicating a depletion of antral as well as oxyntic glands. In contrast, G cell density in the distal body mucosa of the *H. pylori* positives was higher than in the *H. pylori* negative subjects. This can be explained by the distal acid secreting body mucosa, which does not have G cells, being replaced by an antral-like mucosa that contains G cells (a process that has been called “antralization”). This process can be associated with the development of pseudo-pyloric metaplasia, also called spasmolytic polypeptide expressing metaplasia (SPEM).(171, 172) This is consistent with our observation that the reduction in parietal and chief cell densities in *H. pylori* positives was most pronounced in the distal body mucosa. Together these findings are likely to represent the previously reported proximal progression of the junction between the antrum and body type mucosa leading to shrinkage in the surface area of the stomach covered by oxyntic mucosa in *H. pylori* atrophic gastritis.(173)

There are few previous studies assessing gastric secretory function in *H. pylori* infected healthy volunteers in the Western world. In a retrospective analysis of 95 healthy, young male volunteers (age 19-26 years) Smith *et al* reported that the 8 seropositive for *H.pylori* had similar intragastric acidity to the other 87.(82) In a retrospective analysis of 136 healthy volunteers, Peterson *et al* reported reduced basal acid output but no significant difference in gastrin stimulated peak acid output or meal stimulated acid output assessed by intragastric titration in *H.pylori* seropositives.(79) In a prospective study of 206 healthy volunteers, Feldman *et al* in 1996 reported reduced gastrin stimulated peak acid output and reduced basal pepsin output in those with *H.pylori* detected histologically in gastric biopsies.(88) In 1998, our own group reported a reduced acid secretory response to gastrin stimulation in 20 *H.pylori* positive versus 24 *H.pylori* negative healthy volunteers.(83) Several studies in the Japanese population have reported reduced gastric secretory function in *H.pylori* positive healthy volunteers.(80, 174)

Our current study differs from previously published studies in several important respects. Firstly, we aimed to study subjects representative of the general population infected with *H. pylori* rather than asymptomatic healthy volunteers. Secondly, by using intragastric pH sensors, we avoided the use of non-physiological gastric stimuli, gastric aspiration or intragastric titration which may not be representative of the subjects usual gastric functioning. Thirdly, we focused on the middle-aged population rather than young students as the former is the population in whom reflux disease manifests itself. Finally, and probably most critically, we employed a technique which allowed us to assess the acidity in different regions of the stomach and in particular close to the GEJ.

Our observation that gastric acidity was reduced most markedly close to the GEJ is interesting in the light of the previously reported but unexplained observations by Feldman *et al* in 1999. They observed that in healthy volunteers, eradication of *H.pylori* did not alter basal or meal-stimulated gastric acid secretion assessed by intragastric titration but did result in a 2-3 fold increase in gastro-oesophageal acid reflux.(84) In the light of our current study, the observed increase in gastro-oesophageal acid reflux may have been explained by the *H.pylori* infection reducing intragastric acidity close to the GEJ.

Is our finding of reduced gastric secretory function in the *H. pylori* infected population a peculiar feature of our West of Scotland population or relevant to the wider Western community? *H.pylori* induced atrophic gastritis and reduced acid secretory function is associated with gastric cancer and the prevalence of the two correlates at a population level.(175) The incidence of gastric cancer in Scotland is 9.7 /100,00py and similar to that

of Western European and North American countries and substantially lower than that of Eastern European and Far Eastern countries.(176) This would suggest that our findings of reduced acid secretory function is representative of what is happening in Western countries.

Though our study demonstrates that the *H. pylori* infected general adult population has less intragastric acidity than the uninfected population, this association does not necessarily indicate that the reduced intragastric acidity is caused by the infection. However, causal association seems highly likely as *H. pylori* gastritis is recognised to cause loss of gastric glands and impaired secretory function. In addition, the more marked changes in gastric secretory function in those with the more virulent *CagA* strain supports it being caused by the infection. Confirming causality by an intervention study has potential problems as *H. pylori*-induced loss of gastric glands is generally regarded as being irreversible.

In summary, our current study indicates that *H. pylori* infected population volunteers have reduced intragastric acidity compared to uninfected controls and that this is most marked close to the GEJ. This observation may explain the negative association between the infection and GEJ disease and its complications.

CHAPTER 6

Study of the effect of increasing intra-abdominal pressure by waist compression on gastro-oesophageal reflux disease

6.1 Introduction and Aims

Central obesity is strongly associated with gastro-oesophageal reflux and its complications of Barrett's oesophagus and oesophageal adenocarcinoma.(106, 177) The nature of this association is incompletely understood and both mechanical and humoral effects of central obesity may be important.

Both BMI and waist circumference (WC) show a strong positive correlation with intra-gastric pressure (IGP) and the gastro-oesophageal pressure gradient (GOPG).(10, 178) Abdominal compression by a waist belt also increases these pressures and thus reproduces the manometric characteristics associated with central obesity.(7, 179) Previous investigators have examined the effect of waist belt compression on the manometric characteristics of the lower oesophageal sphincter (LOS) in both healthy volunteers and patients with reflux disease. The rise in IGP caused by the waist belt is accompanied by a rise in LOS pressure though sometimes of a lesser magnitude.(180, 181) Waist belt compression in short term studies does not result in the development of, or aggravation of, hiatus hernia or an increased separation of the intrinsic and extrinsic components of the LOS.(23, 182)

Surprisingly, there is a paucity of information on the effects of waist belt compression on gastro-oesophageal acid reflux itself despite this being the main mediator of oesophageal damage. Lee *et al* recently examined the effect of waist belt compression on gastro-oesophageal pH in healthy volunteers without reflux disease.(183) The belt caused the location of the pH transition point where the pH changes from gastric to oesophageal pH to migrate 2cm more proximally within the LOS and this was most apparent after a meal. The belt did not cause the pH transition point to extend above the squamocolumnar junction (SCJ) onto oesophageal mucosa. There was an increase in short segment reflux detected 1.3cm above the SCJ but none detected at any of the 7 pH sensors spaced at 1 cm increments proximal to this. In these subjects with a normal LOS there was, therefore, little evidence that the waist belt significantly increased oesophageal acid exposure.

The aim of this study is to investigate the effects of waist belt compression in patients known to have reflux disease.

6.2 METHODS & MATERIALS

6.2.1 Subjects

Study subjects were patients with typical symptoms of gastro-oesophageal reflux disease (GORD) and at least Los Angeles (LA) Grade B reflux oesophagitis or Barrett's oesophagus on upper GI endoscopy. Proton pump inhibitors (PPIs) were stopped at least 7 days prior to the study and H_2 receptor antagonists were stopped for at least 24 hours.

6.2.2 Study design

6.2.2.1 Study Day 1: Clinical measurements

The severity of upper gastrointestinal symptoms was assessed using the Short-Form Leeds Dyspepsia Questionnaire.(164) Medication history was recorded. Measurements of height, weight, waist and hip circumference were taken.

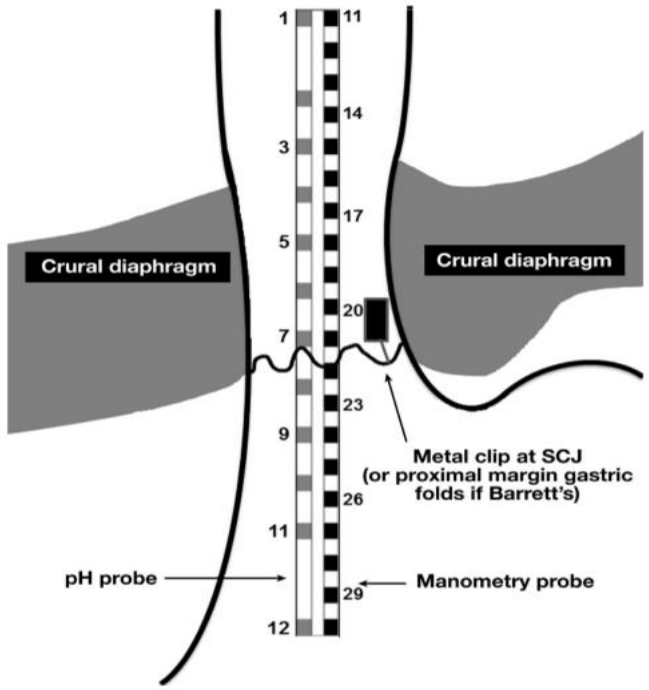
6.2.2.2 Study day 2: Upper gastrointestinal endoscopy

Volunteers attended after an overnight fast for upper gastrointestinal endoscopy. They were offered topical lidocaine throat spray or conscious sedation with midazolam 1-3mg. The upper gastrointestinal tract was inspected. The distance from incisors to SCJ was measured. If a hiatus hernia was present, the distance to the diaphragmatic impression was also noted. Two small metal radio-opaque clips were attached to the SCJ using a single use rotatable clip fixing device (QuickClip 2™; Olympus, Southend-on-Sea, UK). In subjects with Barrett's oesophagus the clips were attached to the most proximal margin of the gastric folds.

6.2.2.3 Study Days 3 and 4: Combined manometry and pH study with and without waist belt

The volunteers attended fasted for a further two study days. On days, a combined high-resolution manometer and pH probe was passed pernasally and positioned so that the pH sensors were lying across the LOS and extending at least 5.5 cm above the LOS. The relative positions of the 12-sensor pH catheter, 36-sensor manometer and SCJ is shown in Figure 6.1.

Figure 6.1: Diagrammatic representation of relative positions of the pH probe, manometer probe, squamocolumnar junction marked by radio-opaque clip and crural diaphragm. The marks on the probes indicate the sensor numbering of each probe.



One of the study days was performed without the application of the waist belt. Manometry and pH data were recorded concurrently for a 20-minute fasting period with the subjects sitting upright at a 60-degree angle. They then consumed a standardised meal over ten minutes [400g Waitrose spaghetti bolognese ready meal and 100ml water (500kcal; 55.2g carbohydrate, 27.8g protein, 17.6g fat)]. Following this, manometry and pH recordings were continued for a further 90 minutes. An X-ray was taken before and after the meal to visualise the metal clips at the SCJ.

On the other study day, the above procedure was repeated but with the application of a waist belt throughout the whole recording period. A weight-lifter belt (Nike, USA) was applied tightly with a blood pressure cuff placed under the belt. This was inflated to a constant cuff pressure of 50mmHg. The order of the study days with and without the waist belt was alternated in random fashion. Any upper GI symptoms experienced during the tests were recorded with respect to time, location, duration and character.

6.2.3 Equipment

6.2.3.1 High-resolution pHmetry

pH recordings were taken using a high-resolution pH catheter (Synectics Medical Ltd, Enfield, UK). This was a custom-made pH probe composed of 12 antimony pH electrodes with the most distal electrode situated 5mm from the tip of the catheter, with the other eleven electrodes 35, 46, 57, 68, 79, 90, 101, 112, 123, 134 and 169mm proximal to the tip. The probe was calibrated prior to each study using pH buffer solution (Synmed Ltd, Enfield, UK) at pH 7.01 and pH 1.07. Recordings were captured using Polygram Net software (Synectics Medical Ltd, Enfield, UK).

6.2.3.2 High-resolution manometry

Manometry was performed using a high resolution solid-state catheter with 7.5mm spacing between 36 circumferential sensors (Given Imaging, Hamburg, Germany). Calibration was performed prior to each study and In vivo calibration was carried out weekly and applied to each study to compensate for thermal drift. Recordings were captured with ManoScan 360 high-resolution Manometry System and analysed with ManoView ESO v3.0.1 software (Given Imaging, Hamburg, Germany).

6.2.3.3 Combined probe

The manometry and pH catheters were combined using two thin strips of Leukoplast Sleek waterproof tape (BSN Medical, Pinetown, SA) such that manometry sensor 21 was immediately adjacent to pH sensor 7.

6.2.4 Data analysis

6.2.4.1 Acid exposure

Acid exposure was examined by calculating the percentage of time pH was less than 4 for each sensor across the LOS and up to 5.5cm proximal to LOS in the 20-minute fasting period and the 90-minute postprandial period. Location of the pH transition point was defined by the position of the pH sensor recording a drop in median pH of at least one unit from proximal to distal and correcting for 1.1 cm spacing as previously described.(184) Reflux events were defined as a drop in pH to below 4 and lasting at least 1 second. The

total number of reflux events were counted within the 20-minute fasting period and 90-minute postprandial period.

6.2.4.2 Manometric parameters

Manometric characteristics were analysed in detail during fasting and after the meal. For each two-minute period, one inspiratory point and one expiratory point was chosen from the longest period without interference from swallowing, coughing or transient lower oesophageal sphincter relaxations (TLOSRs). The mean pressure in inspiration and expiration was calculated for each of the 36 sensors in the fasting period and postprandial period. The peak LOS pressure was taken as the sensor showing the highest mean pressure. IGP was also calculated on inspiration and expiration and was defined as the mean pressure of the first three sensors immediately distal to the LOS. Intra-oesophageal pressure (IOP) was defined as the mean pressure of three consecutive sensors located 6, 6.75 and 7.5 cm proximal to the peak LOS pressure.

6.2.4.3 Measurement of manometric locations

All measurements were made using data collected in the expiratory phase of respiration. The upper border of the LOS was defined as the most proximal sensor where the pressure was at least 2mmHg above IGP. The lower border of the LOS was defined as the most distal sensor where the pressure was at least 2mm Hg above IGP. The pressure inversion point (PIP) was defined as the transition point from the abdominal pressure compartment (positive wave deflection) into the thoracic pressure compartment (negative wave deflection). The position of the SCJ was derived from the position of the metal clips relative to the combined manometry and pH sensors seen on X-ray. In the event of clips being visible at different levels, the mid-point between the two clips was used as the position of the SCJ. All measurements (in cm) were determined from the nares.

6.2.5 Statistical analysis

All continuous data are expressed as medians and interquartile ranges unless otherwise stated. Comparison of variables between related groups was made using the Wilcoxon Signed Rank test. For all correlations between two continuous variables, the Spearman

Rho bivariate correlations were used. Significance for all statistical tests was set as p value <0.05.

6.2.6 Ethics

The study protocol was approved by the West of Scotland Ethics Committee and all volunteers provided informed written consent.

6.3 Results

Fifteen subjects completed the study protocol, but one had to be excluded due to a technical issue resulting in loss of the manometry data for one study day. Thus 14 subjects were included in the final analysis. The median age of the group was 56 years (range 24-76) and all subjects were male. The median BMI was 26.8 (range 22-42) and the median WC was 101cm (range 79-142cm). At endoscopy, 11/14 had evidence of a hiatus hernia (length 2-4cm). 8/14 had reflux oesophagitis (either LA grade B or C) and 6/14 had Barrett's 0esophagus (median length 3.5cm, range 1-9cm).

6.3.1 Effect of belt on Intragastric Pressure and GOPG

During fasting the belt increased IGP and GOPG during both inspiration and expiration (Table 6.1). On inspiration, the median IGP was 13.5mmHg without the belt versus 19.9mmHg with the belt (p=0.005) and the GOPG was 13.7mmHg versus 18.6 mmHg (p=0.041). On expiration, the median IGP was 9.8mmHg without the belt compared to 16.7mmHg with the belt (p=0.002) and the GOPG was 5.0mmHg versus 9.1mmHg (p=0.035).

Following the meal, the belt also increased IGP on both inspiration and expiration (Table 6.1). On inspiration, the IGP without the belt was 13.5mmHg versus 23.3mmHg with the belt (p=0.001) and the GOPG was 16.2 versus 22.5mmHg (p=0.008). On expiration, the IGP was 10.8mmHg without the belt compared to 19.8mmHg with the belt on (p=0.001) and the GOPG was 8.0mmHg versus 11.9mmHg (p=0.016). The greater increase in the IGP than GOPG was due to the belt also causing an increase in intra-oesophageal pressure.

Without the belt there was no difference in IGP fasting versus after the meal [9.8mmHg (IQR 8.9) versus 10.8mmHg (IQR 7.2); p=0.084). With the belt the IGP was greater after

the meal compared with under fasting conditions [19.8mmHg (IQR 7.6) versus 16.7mmHg (IQR 9.5); p=0.002].

6.3.2 Effect of belt on LOS

During the fasting period the belt increased median peak LOS pressure on expiration relative to atmospheric pressure, being 23.9mmHg with the belt off versus 27.5mmHg with the belt on (p=0.030) (Table 6.1). However, there was a fall in the median peak LOS pressure relative to the IGP on inspiration apparent after the meal, being 27.1mmHg with the belt off and 17.8mmHg with the belt on (p=0.041).

The belt did not cause any significant changes in the LOS with respect to the distance between its upper border and nares, its length, or the position of the PIP, peak LOS pressure or SCJ relative to upper border of the LOS (Table 6.2). In addition, the belt did not influence the number of subjects with a double peak manometric pattern. When fasted, 5 subjects had a double peak pattern without the belt and 7 with the belt and after the meal, 6 without the belt and 7 with it.

Table 6.1: Effect of waist belt on manometric parameters in fasting and postprandial states in expiration and inspiration

	Fasting			Postprandial		
Expiration	**Belt Off**	**Belt On**	***p* value**	**Belt Off**	**Belt On**	***p* value**
Median Peak LOS pressure (IQR)	23.9 (8.4)	27.5 (11.9)	0.030	25.3 (9.6)	30.9 (12.8)	0.177
Median LOSP vs IGP (IQR), mm Hg	12.6 (7.2)	11.3 (9.4)	0.826	14.2 (12.7)	10.7 (13.7)	0.158
Median IOP (IQR), mm Hg	5.2 (5.0)	6.3 (4.9)	0.124	4.3 (4.7)	6.8 (5.5)	0.004
Median IGP (IQR), mm Hg	9.8 (8.9)	16.7 (9.5)	0.002	10.8 (7.2)	19.8 (7.6)	0.001
Median GOPG (IQR)	5.0 (4.8)	9.1 (5.9)	0.035	8.0 (3.2)	11.9 (7.4)	0.016
Inspiration						
Median Peak LOS pressure (IQR)	33.2 (12.9)	39.5 (17.6)	0.433	41.3 (21.5)	41.2 (4.5)	0.778
Median LOSP vs IGP (IQR), mm Hg	20.2 (17.5)	20.0 (15.4)	0.124	27.1 (18.8)	17.8 (16.2)	0.041
Median IOP (IQR), mm Hg	-0.9 (3.7)	0.5 (2.0)	0.158	-0.2 (4.8)	0.9 (4.0)	0.022
Median IGP (IQR), mm Hg	13.5 (7.6)	19.9 (11.5)	0.005	13.5 (5.8)	23.3 (8.1)	0.001
Median GOPG (IQR)	13.7 (7.7)	18.6 (11.5)	0.041	16.2 (6.6)	22.5 (4.9)	0.008

LOS = Lower oesophageal sphincter, LOSP = Lower oesophageal sphincter pressure, IGP = Intragastric pressure, IOP = Intra-oesophageal pressure, GOPG = Gastro-oesophageal sphincter pressure, IQR = Interquartile range.

Table 6.2: Effect of waist belt on relative locations of anatomical structures of the gastro-oesophageal junction.

	Fasting			Postprandial		
	Belt Off	**Belt On**	***p* value**	**Belt Off**	**Belt On**	***p* value**
Upper border LOS (cm from nares)	43.38(4.81)	43.38 (4.00)	0.271	41.75 (3.63)	42.21 (2.40)	0.330
LOS length, cm	3.75 (1.50)	3.38 (1.88)	0.218	3.00 (2.06)	2.88 (1.38)	0.636
PIP (cm from upper border LOS)	0.43 (0.93)	0.43 (1.80)	0.801	0.60 (2.01)	0.54 (0.88)	0.245
Peak LOSP (cm from upper border LOS)	1.13 (0.75)	1.13 (0.75)	0.809	1.25 (0.69)	1.13 (0.56)	0.598
SCJ (cm from upper border LOS)	1.12 (1.80)	1.10 (1.90)	0.241	0.88 (1.40)	0.48 (1.79)	0.124
pH transition point (cm from upper border LOS)	2.18 (1.55)	1.53 (2.80)	0.220	0.78 (1.51)	-0.64 (3.37)	0.003
pH transition point (cm from SCJ)	0.83 (2.61)	1.05 (2.51)	0.444	0.00 (1.02)	-1.17 (2.89)	0.016

LOS = Lower oesophageal sphincter, PIP = Pressure inversion point, LOSP = Lower oesophageal sphincter pressure, SCJ = Squamocolumnar junction.

6.3.3 Effect of belt on gastro-oesophageal reflux

The waist belt caused a marked increase in gastro-oesophageal reflux during the 90 minutes following the meal (Table 6.3). Acid exposure at each of the 5 pH sensors extending 5.5cm proximal to the peak LOS pressure point was significantly increased with versus without the belt with the percentage time pH <4 being increased by approximately 8 times at each position. Both with and without the belt acid exposure progressively increased with proximity to the peak LOS pressure point so that with the belt the pH was less than 4 at 1.1cm above the peak LOS pressure point for 49.7% of the time following the meal compared to 7.3% without the belt (p=0.03). The waist belt also increased the acid exposure at the peak LOS pressure point (66.1% versus 18.4%, p=0.056) and 1.1 cm distal to it (89.6% versus 59.4%, p=0.026).

The waist belt also increased acid reflux after the meal relative to the clip marking the SCJ or in the case of the 6 patients with Barrett's, the proximal extent of the gastric folds. At 1.1cm proximal to the clip, the percentage time pH<4 was 41.4% (IQR 61.1) with the belt versus 7.0% (IQR 18.9) without it (p<0.05); at 2.2cm proximal 12.5% (IQR 44.0) versus 1.3% (IQR 8.4; p=0.01); at 3.3cm proximal 11.3% (IQR 21.2) versus 0.7% (IQR 6.5; p<0.02) and at 4.4cm proximal 4.5% (IQR 9.9) versus 0.3% (IQR 2.8; p<0.01).

Following the meal, the median number of reflux events with the belt was twice that without the belt [2 (IQR 2) vs 4 (IQR 6); p=0.008] (Table 6.4). The median number of TLOSRs was not different but the number accompanied by acid reflux was increased with the belt [2 (IQR 2) vs 3.5 (IQR 5); p=0.041]. The median time from onset of TLOSR until return of the LOS to stable tone and original position was not different with the belt off versus on [46.0s (IQR 10.4) vs 44.8s (IQR 14.4); p=0.279]. The most marked effect of the belt was to reduce the rate of oesophageal clearance of refluxed acid with the median time being 23.0 seconds without the belt versus 81.1 seconds with the waist belt (p=0.008). Examining the pH plots of the long reflux events occurring after the meal with the belt revealed evidence of attempted clearance of acid followed by immediate re-reflux of acid (Figure 6.2). There was no difference in the median amplitude of distal oesophageal contractions with or without the waist belt [85.8mmHg (IQR 32.8) vs 79.5mmHg (IQR 48.1); p=0.387] (Table 6.4)

During the fasting period there was no difference in oesophageal acid exposure with versus without the belt. However, the acidity at the peak LOS pressure point and at the

intragastric sensors located 1.1cm, 2.2cm, and 3.3cm distal to it was greater with versus without belt. ($p < 0.02$ for each). (Table 6.3)

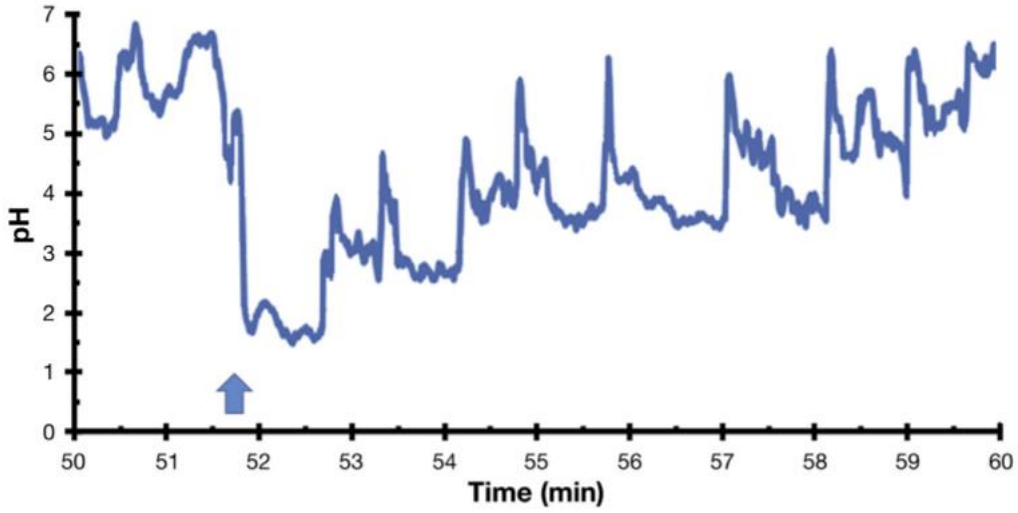
Table 6.3: Median (IQR) percentage time pH<4 at sensors relative to peak LOS pressure comparing subjects with and without waist belt.

Sensor Location	Fasting			Postprandial		
	Belt Off	Belt On	*p* value	Belt Off	Belt On	*p* value
5.5cm proximal	0 (0)	0 (0)	0.285	0.2 (1.4)	2.5 (9.6)	0.038
4.4cm proximal	0 (0.1)	0 (0)	1.000	0.5 (2.6)	4.7 (14.3)	0.002
3.3cm proximal	0 (0.1)	0 (0.6)	0.341	1.0 (5.0)	8.0 (31.4)	0.013
2.2cm proximal	0.1 (1.5)	0.1 (1.3)	0.415	3.5 (10.1)	12.4 (41.3)	0.009
1.1cm proximal	0.3 (3.8)	0.4 (9.0)	0.286	7.3 (15.0)	49.7 (52.0)	0.030
Peak LOS pressure	2.6 (6.9)	5.2 (41.5)	0.016	18.4 (38.6)	66.1 (42.3)	0.056
1.1cm distal	18.3 (48.2)	55.6 (79.3)	0.019	59.4 (48.5)	89.6 (14.2)	0.026
2.2cm distal	53.4 (57.4)	95.1 (15.8)	0.005	86.7 (19.9)	85.3 (26.2)	0.701
3.3cm distal	88.6 (66.4)	99.8 (5.0)	0.016	88.3 (36.2)	89.8 (31.9)	0.722

Table 6.4: Effect of waist belt on mechanism of acid exposure across the LOS during 90-minute postprandial period

	Belt Off	Belt On	*p* value
Median no. of reflux events (IQR)	2 (2)	4 (6)	0.008
Median no. TLOSRs (IQR)	7 (3.3)	6 (5.3)	0.279
Median no. TLOSRs associated with reflux (IQR)	2 (2)	3.5 (5)	0.041
Average clearance time (IQR), seconds	23.0 (63.4)	81.1 (110.6)	0.008
Median no. peristalsis to clear acid (IQR)	1.0 (1)	1.5 (2)	0.074
Median peristaltic distal oesophageal pressure (IQR), mmHg	79.5 (48.1)	85.8 (32.8)	0.387

Figure 6.2: An example of an oesophageal pH recording at sensor 5.5cm above peak LOS pressure from one of the study subjects wearing a belt during the postprandial period. Following the initial reflux event (marked by arrow) there is clearance of acid by a peristaltic wave but this is followed immediately by re-reflux.



6.3.4 Effect of Belt on Gastro-oesophageal pH Step-Up Point

The belt caused the location of the point where acidity changes from gastric pH to oesophageal pH (pH transition point) to move proximally after the meal with respect to the LOS upper border and peak pressure as well as the SCJ (Table 6.2). Without the belt the pH transition point was 0.78cm distal to the upper border LOS but with the belt it was 0.64cm proximal to it (p=0.003). Likewise, without the belt the pH transition point was precisely at the level of the clip marking the squamocolumnar junction (or in the 6 subjects with circumferential Barrett's the proximal extent of the gastric folds) but with the belt it was 1.17cm proximal to it (p=0.016). This meant that with the belt on the distal oesophagus was constantly exposed to the level of acidity normally only seen in the stomach.

There was no significant difference in the position of the pH step up with and without the belt during the 20-minute fasting period (Table 6.2).

6.3.5 Correlation of WC with both the intragastric pressure and GOPG

Without the belt, there was a strong correlation between the WC of the 14 patients included in the study and their fasting IGP both on expiration (r=0.682, p=0.008) and inspiration (r=0.581, p=0.029). There was also a positive correlation with fasting GOPG on inspiration (r=0.640, p=0.014) but this was not seen on expiration. No significant correlations were apparent in the 90-minute period following the meal.

6.4 Discussion

Our study indicates that in reflux patients, waist belt constriction causes a marked increase in gastro-oesophageal reflux most evident after a meal. The effect of the belt was most marked close to the gastro-oesophageal junction where the pH of the distal oesophagus lined, or normally lined, by squamous mucosa became like that of the proximal stomach.

As previously reported the belt caused a rise in the IGP, which in the empty stomach is equivalent to intra-abdominal pressure, and also an increase in GOPG.(179, 183) The rise in GOPG was less than in IGP and this can be explained by the fact that the belt also caused an increase in intra-oesophageal pressure.

The belt also raised peak LOS pressure which has previously been observed both in healthy volunteers and reflux patients.(185) Mittal *et al* observed that the rise in LOS pressure with abdominal compression was associated with tonic contraction of crural diaphragm EMG activity.(181) In our current study after the meal the belt caused a greater rise in the IGP than in LOS pressure causing a significant fall in LOS pressure relative to the IGP which is the pressure gradient preventing reflux. This fall in LOS pressure relative to the IGP has been reported by some but not all investigators.(23, 183) The fall in LOS pressure relative to the IGP in our current study was only apparent after the meal and involved patients with reflux disease and in these respects differed from previous studies. Consistent with previous reports we found no evidence that the belt, at least in the short term of our study, caused any increased separation of the two components of the LOS which would be indicative of promoting hiatus hernia formation.(182)

We extended previous work by monitoring the effect of the belt on actual acid reflux. We found that the belt markedly increased acid exposure following the meal at each of the pH sensors placed at 1.1cm increments and extending 5.5cm proximal to the peak LOS

pressure point. At each of these locations the belt increased oesophageal acid exposure by approximately 8-fold relative to that without the belt. Without the belt the amount of acid increased with proximity to the LOS and the 8-fold increase with the belt caused the pH of the most distal oesophagus to be < 4 for 49.7% of the time following the meal. The belt also caused a marked increase in acid exposure after the meal when measured relative to the clip marking the SCJ or proximal extent of gastric folds.

Our combined high-resolution pH and manometry system allowed us to examine the mechanism of the increased oesophageal acid exposure induced by the belt. After the meal the belt doubled the number of reflux episodes. There was no increase in the number of TLOSRs but there was an increase in those associated with reflux. The most marked effect of the belt was impairment of oesophageal acid clearance which was approximately 4 times longer than without the belt. This impaired clearance was often related to re-reflux of acid occurring immediately after an oesophageal peristaltic clearance wave.

The pH pattern of the impaired oesophageal clearance with the belt in our study is similar to that previously reported in patients with hiatus hernia. Mittal *et al* in 1987 observed that in hiatus hernia patients oesophageal acid clearance by a swallow was often followed by rapid re-reflux due to retrograde flow of contents from the hiatal sac during the swallow induced relaxation of the LOS.(20) Jones *et al* also found that impaired oesophageal clearance was strongly correlated with oesophagitis and hiatus hernia.(16) In hiatus hernia patients reflux of barium trapped in the hiatal sac following a swallow has also been observed and shown to be most marked in non-reducing hernias.(18, 21) The vast majority of the reflux patients in our study had hiatus hernias and the belt is thus aggravating the impaired oesophageal clearance associated with hiatus hernia.

The waist belt also caused the pH step up point (where the pH changes from gastric to oesophageal) to move proximally by 1-2cm within and even above the LOS and again this was most marked following the meal. We were also able to see the effect of the belt on the location of the pH step-up point relative to the location of the SCJ or in the case of the 6 patients with circumferential Barrett's the proximal extent of the gastric folds. Without the belt the pH step-up was at the level of the SCJ (or proximal gastric folds in Barrett's patients) but with the belt was displaced 1-2cm above it. The cause of this proximal displacement of the pH step-up point is unclear but might be due to marked impaired distal oesophageal acid clearance. In hiatus hernia patients, the impaired clearance is most marked near to the gastro-oesophageal junction.(20)

We considered the possibility that the belt might cause some artefactual evidence of distal oesophageal acid reflux by increasing the duration and/or magnitude of proximal migration of the gastro-oesophageal junction during TLOSRs. This could increase acid detected by the distal oesophageal sensors due to their contact with the acidic gastric mucosa. However, our analysis indicated that the time for restitution of normal tone and position of the LOS following TLOSRs was the same with versus without the belt. This excludes the prolonged acid clearance, which was the main effect of the belt, from being attributed to prolonged proximal migration of the gastro-oesophageal junction during TLOSRs. Although we could not measure the amplitude of migration of the gastro-oesophageal junction during TLOSRs, a previous study by Kahrilas *et al* showed that abdominal compression did not influence the proximal movement of the gastro-oesophageal junction during peristalsis in healthy volunteers or subjects with hiatus hernia.(182)

The increase in oesophageal acid exposure produced by the belt in our current study is substantially more than observed in the earlier study in healthy volunteers without reflux disease or hiatus hernia.(183) This indicates that the reflux promoting effect of the belt is much more significant in patients with impaired LOS function.

The increase in oesophageal acid exposure induced by the belt was confined to the 90-minute period after the meal and several factors may explain this. The actual IGP with the belt on was higher after the meal than fasted despite these pressures being similar without the belt. In addition, most of the increase in reflux occurred during TLOSRs and its subsequent impaired clearance and TLOSRs mainly occur after meals. Though there was no increased oesophageal acid exposure with the belt during the fasting period the acidity of the most proximal stomach close to the gastro-oesophageal junction was increased and the reason for this is not clear.

The acid exposure of the distal oesophagus in our reflux subjects with the belt was equivalent to that of the proximal stomach. The proximal region of the stomach escapes the buffering effect of food and remains highly acidic after a meal. If this degree of acid exposure of the distal oesophagus were prolonged it would be likely to result in columnar metaplasia as the squamous mucosa transforms to a type more suited to a gastric rather than oesophageal luminal environment. Six of our patients did have Barrett's oesophagus.

Our findings are likely to be relevant to the mechanism of the association between central obesity and reflux disease. Increasing WC is accompanied by an increase in intra-abdominal and intra-gastric pressure.(178) Even with the relatively small number of

subjects in our current study there was a strong and highly significant correlation between WC and IGP. The range in IGP between the smallest and largest WC was 17mmHg and this is greater than the average rise in intragastric pressure produced by the waist belt of 6.5mmHg fasted and 9.5mmHg after the meal. It would appear, therefore, that much of the association between WC and reflux could be explained by the effects on intra-abdominal pressure.

Our findings are also relevant to potential adverse effects of tight waist bands or clothing in subjects with impaired LOS function. As both central obesity and tight waist band increase intra-abdominal pressure it would seem appropriate to advise reflux patients to both lose weight and avoid such clothing. Our findings suggest that it will be particularly important to avoid tight waist belts after meals when their reflux promoting effects are most pronounced. However, caution needs to be taken in extrapolating the findings of our short-term study to long-term use of waist constricting clothing.

CHAPTER 7

Discussion and Future Work

7.1 Discussion

The studies included within this thesis all add to the current understanding of the environmental factors contributing to the rising incidence of gastro-oesophageal reflux disease and its malignant complications of Barrett's oesophagus and oesophageal adenocarcinoma.

Gastro-oesophageal reflux disease is one of the commonest chronic conditions and evidence suggests that the incidence has been increasing over the past few decades.(1) Chronic GORD can lead to Barrett's metaplasia of the distal oesophagus which can progress to oesophageal adenocarcinoma, and the incidence of these conditions appears to be rising also. There has been a marked 3-4 fold rise in the incidence of oesophageal adenocarcinoma in the West over the past 30 years(41, 43). Our epidemiological study of national cancer registries suggests that Scotland has the highest recorded incidence of oesophageal adenocarcinoma in the world. The rapid rise in incidence of oesophageal cancer indicates that it is the result of environmental changes and identifying these will be an essential step in the development of preventative measures.

We showed the incidence of oesophageal adenocarcinoma varies considerably across different countries. A similar variation exists in the incidence of gastric adenocarcinoma. We observed a very strong negative association between these two cancers, with oesophageal adenocarcinoma only rising above 2 per 100,000-person years in populations where non-cardia gastric cancer is below 10 per 100,000-person years. In addition, we found a very strong negative correlation in the time trends of the two cancers in the West. In Scotland, gastric cancer showed a 3-fold fall in incidence over the same time period as a 3-4 fold rise in oesophageal adenocarcinoma. This trend was apparent in many populations. Oesophageal cancer has risen significantly in the majority of the populations over an 18-year period, whilst gastric adenocarcinoma rates have fallen in the majority of populations.

The inverse association between the changing incidences of the two cancers may indicate that a single environmental factor is responsible and is exerting opposite effects on the two cancers. The environmental factors that are thought to explain the falling incidence of gastric adenocarcinoma include a falling incidence of *H. pylori* atrophic gastritis, dietary changes and reduced smoking.(149) Smoking is a similar risk factor for both cancers and as smoking rates have generally been decreasing this could not explain the rising incidence of oesophageal cancer.(138, 150) There is some evidence that increased intake of vitamins and

reduced salt consumption may have contributed to the falling incidence of gastric cancer.(151, 152) These specific dietary factors would not in themselves explain the increase in oesophageal adenocarcinoma and indeed increased vitamin consumption may protect against oesophageal adenocarcinoma.(153) However, increased caloric intake and associated obesity is a well-established risk factor for OAC.(153) It is therefore possible that changes in the diet comprising both a fall in salt content and increased caloric content could produce a fall in gastric cancer and rise in oesophageal adenocarcinoma. However, a recent analysis indicated that increasing obesity may only account for 6.5% of the increase in incidence of oesophageal adenocarcinoma.(136) Another environmental factor that might exert opposite effects on the incidence of the two cancers is *H. pylori* atrophic gastritis which is the most important etiological factor for non-cardia gastric cancer.(154) In countries with a high incidence of gastric cancer there is also a high incidence of atrophic gastritis and associated impaired gastric acid secretion.(155) This will protect from oesophageal adenocarcinoma as any gastro-oesophageal refluxate will have reduced ability to damage the oesophagus due to its reduced acidity.

H. pylori infection of the stomach, discovered in 1984, is estimated to infect more than half of the world's population.(47) Numerous epidemiological studies have found decreasing *H. pylori* incidence rates in many parts of the world.(47-51) The infection causes a chronic gastritis which can progress to atrophic gastritis with intestinal metaplasia. This can lead to the development of complications such as gastric and duodenal ulcer disease, gastric adenocarcinoma and MALT lymphoma. *H. pylori* strains which are *cagA*-PAI positive cause peptic ulceration and gastric cancer more frequently that *cag*A-PAI negative strains.(59) The well documented inverse association between *H. pylori* infection and presence of GORD, Barrett's oesophagus and oesophageal adenocarcinoma could be explained by the gastric infection protecting against these oesophageal diseases. If so, the falling incidence of the infection in the general population might explain the rising incidence of the oesophageal disorders. One mechanism by which the infection might protect against oesophageal disease is by reducing the ability of the gastric mucosa to secrete acid and pepsin which are the constituents of gastric juice which can induce oesophageal damage.

We studied the effect of *H. pylori* infection on intragastric acidity in the West of Scotland population as if the infection is conferring protection against oesophageal adenocarcinoma, it must be protecting against oesophageal disease in the vast majority of the population

rather than just the 10% who develop complications such as peptic ulcer disease. If the protection is due to the infection reducing gastric acid secretion, then this suppression of acid secretion would need to be apparent in the majority of infected subjects.

Our study of subjects from the general population of the West of Scotland found that those with *H. pylori* infection had less intragastric acidity both under fasting conditions and following a meal compared to uninfected subjects matched for age, gender and body weight. This adds to our understanding of why there is an inverse association between the infection and gastro-oesophageal reflux disease. Following a meal, the reduced acidity was found close to the GOJ, compared to fasting when the reduced acidity was throughout the stomach. Parietal cell density was reduced throughout the gastric mucosa and not just in the area close to the GOJ, so could not explain this finding. Inflammation was found to be slightly increased close to the GOJ, as well as in the distal stomach compared to the mid-body gastric mucosa. This is a potential reason for the reduced acidity in this area as inflammation can inhibit gastric secretory function.(158) However this would not explain why this reduced acidity is only seen in the postprandial period and not in the fasting period.

The acid pocket, described as a local pocket of acid close to the gastro-oesophageal junction which escaped the buffering effect of the meal, may explain these postprandial findings. The phenomenon likely represents the relative intragastric distribution of gastric juice and ingested food. Following a meal, the food occupies the centre of the stomach whilst newly secreted gastric juice will be close to the stomach wall. Impaired acid secretion will elevate the pH of the gastric juice which will therefore be most apparent close to the stomach wall. In contrast, the central region of the stomach will reflect the pH of the food and thus will be relatively unaffected by changes in the acidity of secreted juice. At the GOJ the stomach wall is closer together therefore the pH probe will more likely to be close to the wall where the pH is lower. Therefore, the effect of *H. pylori* on gastric secretion is likely to be greatest here. It is plausible that this effect offers the main protection against acid refluxing from this acid pocket

At present, *H. pylori* is being treated in virtually all subjects with the infection despite the great majority not having any clinical disease arising from it. The growing evidence that the infection may be exerting a protective effect on acid reflux and oesophageal adenocarcinoma suggests this approach may not be correct. This is particularly important

now that the risk of oesophageal adenocarcinoma has exceeded that of gastric cancer in the UK.

The findings of this study do not necessarily indicate that the reduced intragastric acidity is caused by the infection. However, causal association seems highly likely as *H. pylori* gastritis is recognised to cause loss of gastric glands and impaired secretory function. In addition, the more marked changes in gastric secretory function in those with the more virulent *CagA* strain supports it being caused by the infection. Confirming causality by an intervention study has potential problems as *H. pylori*-induced loss of gastric glands is generally regarded as being irreversible.

The third and final study described within this thesis adds to our understanding of the association between obesity and GORD. The incidence of obesity is rising around the world, and is especially well documented in the United States and the UK.(100-103) Obesity is clearly associated with GORD and oesophageal adenocarcinoma.(106, 115) The nature of this association is incompletely understood and both mechanical and humoral effects of central obesity may be important.

We looked at the effect of waist belt compression on intra-abdominal pressure. Consistent with previous studies, we found a strong correlation between waist circumference and IGP, suggesting this is an important mechanism in explaining the association. The waist belt increased IGP and the GOPG as documented in previous studies. However, we extended previous work by monitoring the effect of the belt on actual acid reflux and found that the belt markedly increased acid exposure following the meal up to 5.5cm proximal to the peak LOS pressure point by approximately 8-fold relative to that without the belt. The mechanism causing this appeared to be an increase in the number of TLOSRs associated with acid reflux in addition to impairment of oesophageal acid clearance, which was approximately 4 times longer with the waist belt applied.

Our study indicates that in reflux patients, waist belt constriction causes a marked increase in gastro-oesophageal reflux most evident after a meal. The effect of the belt was most marked close to the gastro-oesophageal junction where the pH of the distal oesophagus lined, or normally lined, by squamous mucosa became like that of the proximal stomach.

Our combined high-resolution pH and manometry system allowed us to examine the mechanism of the increased oesophageal acid exposure induced by the belt. After the meal the belt doubled the number of reflux episodes. There was no increase in the number of TLOSRs but there was an increase in those associated with reflux. The most marked effect

of the belt was impairment of oesophageal acid clearance which was approximately 4 times longer than without the belt. This impaired clearance was often related to re-reflux of acid occurring immediately after an oesophageal peristaltic clearance wave.

The pH pattern of the impaired oesophageal clearance with the belt in our study is similar to that previously reported in patients with hiatus hernia. Mittal *et al* in 1987 observed that in hiatus hernia patients oesophageal acid clearance by a swallow was often followed by rapid re-reflux due to retrograde flow of contents from the hiatal sac during the swallow induced relaxation of the LOS.(20) Jones *et al* also found that impaired oesophageal clearance was strongly correlated with oesophagitis and hiatus hernia.(16) In hiatus hernia patients reflux of barium trapped in the hiatal sac following a swallow has also been observed and shown to be most marked in non-reducing hernias.(18, 21) The vast majority of the reflux patients in our study had hiatus hernias and the belt is thus aggravating the impaired oesophageal clearance associated with hiatus hernia.

The waist belt also caused the pH step up point (where the pH changes from gastric to oesophageal) to move proximally by 1-2cm within and even above the LOS and again this was most marked following the meal. We were also able to see the effect of the belt on the location of the pH step-up point relative to the location of the SCJ or in the case of the 6 patients with circumferential Barrett's the proximal extent of the gastric folds. Without the belt the pH step-up was at the level of the SCJ (or proximal gastric folds in Barrett's patients) but with the belt was displaced 1-2cm above it. The cause of this proximal displacement of the pH step-up point is unclear but might be due to marked impaired distal oesophageal acid clearance. In hiatus hernia patients, the impaired clearance is most marked near to the gastro-oesophageal junction.(20)

The increase in oesophageal acid exposure produced by the belt in our current study is substantially more than observed in the earlier study in healthy volunteers without reflux disease or hiatus hernia.(183) This indicates that the reflux promoting effect of the belt is much more significant in patients with impaired LOS function.

Our findings are likely to be relevant to the mechanism of the association between central obesity and reflux disease. Increasing WC is accompanied by an increase in intra-abdominal and intra-gastric pressure.(178) Even with the relatively small number of subjects in our current study there was a strong and highly significant correlation between WC and IGP. The range in IGP between the smallest and largest WC was 17mmHg and this is greater than the average rise in intragastric pressure produced by the waist belt of

6.5mmHg fasted and 9.5mmHg after the meal. It would appear, therefore, that much of the association between WC and reflux could be explained by the effects on intra-abdominal pressure.

Our findings are also relevant to potential adverse effects of tight waist bands or clothing in subjects with impaired LOS function. As both central obesity and tight waist band increase intra-abdominal pressure it would seem appropriate to advise reflux patients to both lose weight and avoid such clothing. Our findings suggest that it will be particularly important to avoid tight waist belts after meals when their reflux promoting effects are most pronounced. However, caution needs to be taken in extrapolating the findings of our short-term study to long-term use of waist constricting clothing.

7.2 Suggestions for future work

Several questions arise from our epidemiological study. Future work in countries with a high incidence of gastric adenocarcinoma should assess if the changing epidemiology of gastric and oesophageal adenocarcinoma mirrors the changes seen in the Western World. Predicting and highlighting this change early will be important in preparing health services for a rise in reflux disease and oesophageal adenocarcinoma.

The study highlighted a heterogeneous group of countries with low levels of both oesophageal and gastric adenocarcinoma. It is unclear whether these countries have always had low levels of these cancers, or whether they have seen gastric adenocarcinoma levels fall similar to other countries throughout the world, without the rise in oesophageal adenocarcinoma levels seen elsewhere. Studies comparing patients from countries with low incidence of both cancers e.g. Italy and Spain with patients from countries with high level of oesophageal adenocarcinoma and low levels of gastric adenocarcinoma could shed new light on the aetiology and pathogenesis of oesophageal adenocarcinoma.

Understanding how *H. pylori* infection protects against acid reflux and oesophageal adenocarcinoma by modifying the acid secreting capacity of the gastric mucosa closest to the oesophagus may allow more targeted approaches to the treatment of these conditions to be developed. At present, treatments suppress the secretion of acid by the entire stomach and are consequently associated with adverse effects including increased risks of infection, impaired absorption of calcium and iron and rebound acid hypersecretion due to reflux hypergastrinaemia induced oxyntic mucosal hyperplasia. The real panacea for gastro-

oesophageal reflux disease treatment would be the development of a drug or technique which prevents acid reflux with no side-effects. Targeting the acid in the proximal stomach in the postprandial period without affecting overall gastric acidity would be an attractive prospect.

H. pylori testing and eradication is currently indicated for patients presenting to primary care with dyspeptic symptoms. In this setting dyspepsia is defined broadly to include people with heartburn, acid regurgitation, nausea, and bloating in addition to those with recurrent epigastric pain. The general population who have had previous *H. pylori* eradication would be an interesting group to assess in terms of gastric mucosa histopathology, as well as a physiological assessment of their GOJ function and presence of acid reflux. Determining the number of years of infection, as well as the length of time post-eradication could be correlated with the findings of gastric mucosal status and acid reflux. This could give us more evidence of the effect of *H. pylori* eradication in a population with low levels of gastric cancer and increasing incidence of oesophageal adenocarcinoma.

The dynamic nature of the GOJ and the SCJ within it means this is a difficult area to study. Advancement in technology should be used to gain greater understanding of the pathophysiology of GORD. As the technology available for oesophageal manometry and pH measurement advances our understanding of the pathophysiology of GORD will improve. Impedance is becoming more commonly used in research and clinical care. This would give further information of the types of reflux which occur in the groups of patients we studied

In our study using a tight waist belt applied for 2 hours, the manometric changes across the GOJ are similar to those seen in obese patients, however on removal of the waist belt the changes are reversed. IT would be interesting to assess whether the physiological and manometric changes which occur in obese patients are reversed by weight loss. It may be that the changes at the GOJ which lead to hiatus hernia development are permanent and therefore medical and surgical treatments are required for severe GORD even if weight loss is achieved.

References

1. El-Serag HB. Time trends of gastroesophageal reflux disease: a systematic review. Clin Gastroenterol Hepatol. 2007;5(1):17-26.
2. Vakil N, van Zanten SV, Kahrilas P, Dent J, Jones R, Globale K. [The Montreal definition and classification of gastroesophageal reflux disease: a global, evidence-based consensus paper]. Z Gastroenterol. 2007;45(11):1125-40.
3. Liebermann-Meffert D, Allgower M, Schmid P, Blum AL. Muscular equivalent of the lower esophageal sphincter. Gastroenterology. 1979;76(1):31-8.
4. Friedland GW. Progress in radiology: historical review of the changing concepts of lower esophageal anatomy: 430 B.C.--1977. AJR Am J Roentgenol. 1978;131(3):373-8.
5. Holloway RH, Penagini R, Ireland AC. Criteria for objective definition of transient lower esophageal sphincter relaxation. Am J Physiol. 1995;268(1 Pt 1):G128-33.
6. Holloway RH, Kocyan P, Dent J. Provocation of transient lower esophageal sphincter relaxations by meals in patients with symptomatic gastroesophageal reflux. Digestive diseases and sciences. 1991;36(8):1034-9.
7. Lee YY, Whiting JG, Robertson EV, Derakhshan MH, Wirz AA, Smith D, et al. Kinetics of transient hiatus hernia during transient lower esophageal sphincter relaxations and swallows in healthy subjects. Neurogastroenterol Motil. 2012;24(11):990-e539.
8. Schoeman MN, Tippett MD, Akkermans LM, Dent J, Holloway RH. Mechanisms of gastroesophageal reflux in ambulant healthy human subjects. Gastroenterology. 1995;108(1):83-91.
9. Ribolsi M, Holloway RH, Emerenziani S, Balestrieri P, Cicala M. Impedance-high resolution manometry analysis of patients with nonerosive reflux disease. Clin Gastroenterol Hepatol. 2014;12(1):52-7.
10. Pandolfino JE, El-Serag HB, Zhang Q, Shah N, Ghosh SK, Kahrilas PJ. Obesity: a challenge to esophagogastric junction integrity. Gastroenterology. 2006;130(3):639-49.
11. Fletcher J, Wirz A, Young J, Vallance R, McColl KEL. Unbuffered highly acidic gastric juice exists at the gastroesophageal junction after a meal. Gastroenterology. 2001;121(4):775-83.
12. Pandolfino JE, Zhang Q, Ghosh SK, Post J, Kwiatek M, Kahrilas PJ. Acidity surrounding the squamocolumnar junction in GERD patients: "acid pocket" versus "acid film". The American journal of gastroenterology. 2007;102(12):2633-41.
13. Beaumont H, Bennink RJ, de Jong J, Boeckxstaens GE. The position of the acid pocket as a major risk factor for acidic reflux in healthy subjects and patients with GORD. Gut. 2010;59(4):441-51.
14. Kahrilas PJ, Kim HC, Pandolfino JE. Approaches to the diagnosis and grading of hiatal hernia. Best Pract Res Clin Gastroenterol. 2008;22(4):601-16.
15. Berstad A, Weberg R, Froyshov Larsen I, Hoel B, Hauer-Jensen M. Relationship of hiatus hernia to reflux oesophagitis. A prospective study of coincidence, using endoscopy. Scand J Gastroenterol. 1986;21(1):55-8.
16. Jones MP, Sloan SS, Jovanovic B, Kahrilas PJ. Impaired egress rather than increased access: an important independent predictor of erosive oesophagitis. Neurogastroenterol Motil. 2002;14(6):625-31.
17. Jones MP, Sloan SS, Rabine JC, Ebert CC, Huang CF, Kahrilas PJ. Hiatal hernia size is the dominant determinant of esophagitis presence and severity in gastroesophageal reflux disease. Am J Gastroenterol. 2001;96(6):1711-7.
18. Longhi EH, Jordan PH, Jr. Pressure relationships responsible for reflux in patients with hiatal hernia. Surg Gynecol Obstet. 1969;129(4):734-48.
19. Mittal RK, Fisher MJ. Electrical and mechanical inhibition of the crural diaphragm during transient relaxation of the lower esophageal sphincter. Gastroenterology. 1990;99(5):1265-8.

20. Mittal RK, Lange RC, McCallum RW. Identification and mechanism of delayed esophageal acid clearance in subjects with hiatus hernia. Gastroenterology. 1987;92(1):130-5.
21. Sloan S, Kahrilas PJ. Impairment of esophageal emptying with hiatal hernia. Gastroenterology. 1991;100(3):596-605.
22. van Herwaarden MA, Samsom M, Smout AJ. Excess gastroesophageal reflux in patients with hiatus hernia is caused by mechanisms other than transient LES relaxations. Gastroenterology. 2000;119(6):1439-46.
23. Kahrilas PJ, Lin S, Chen J, Manka M. The effect of hiatus hernia on gastro-oesophageal junction pressure. Gut. 1999;44(4):476-82.
24. Lundell LR, Dent J, Bennett JR, Blum AL, Armstrong D, Galmiche JP, et al. Endoscopic assessment of oesophagitis: clinical and functional correlates and further validation of the Los Angeles classification. Gut. 1999;45(2):172-80.
25. Cameron AJ, Zinsmeister AR, Ballard DJ, Carney JA. Prevalence of columnar-lined (Barrett's) esophagus. Comparison of population-based clinical and autopsy findings. Gastroenterology. 1990;99(4):918-22.
26. Westhoff B, Brotze S, Weston A, McElhinney C, Cherian R, Mayo MS, et al. The frequency of Barrett's esophagus in high-risk patients with chronic GERD. Gastrointest Endosc. 2005;61(2):226-31.
27. Zagari RM, Fuccio L, Wallander MA, Johansson S, Fiocca R, Casanova S, et al. Gastro-oesophageal reflux symptoms, oesophagitis and Barrett's oesophagus in the general population: the Loiano-Monghidoro study. Gut. 2008;57(10):1354-9.
28. Solaymani-Dodaran M, Logan RF, West J, Card T, Coupland C. Risk of oesophageal cancer in Barrett's oesophagus and gastro-oesophageal reflux. Gut. 2004;53(8):1070-4.
29. Williamson WA, Ellis FH, Jr., Gibb SP, Shahian DM, Aretz HT, Heatley GJ, et al. Barrett's esophagus. Prevalence and incidence of adenocarcinoma. Arch Intern Med. 1991;151(11):2212-6.
30. Thomas T, Abrams KR, De Caestecker JS, Robinson RJ. Meta analysis: Cancer risk in Barrett's oesophagus. Aliment Pharmacol Ther. 2007;26(11-12):1465-77.
31. Desai TK, Krishnan K, Samala N, Singh J, Cluley J, Perla S, et al. The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. Gut. 2012;61(7):970-6.
32. Arnold M, Soerjomataram I, Ferlay J, Forman D. Global incidence of oesophageal cancer by histological subtype in 2012. Gut. 2015;64(3):381-7.
33. Blot WJ, Devesa SS, Kneller RW, Fraumeni JF, Jr. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. JAMA. 1991;265(10):1287-9.
34. Cook MB, Corley DA, Murray LJ, Liao LM, Kamangar F, Ye W, et al. Gastroesophageal reflux in relation to adenocarcinomas of the esophagus: a pooled analysis from the Barrett's and Esophageal Adenocarcinoma Consortium (BEACON). PLoS One. 2014;9(7):e103508.
35. Vaughan TL, Fitzgerald RC. Precision prevention of oesophageal adenocarcinoma. Nat Rev Gastroenterol Hepatol. 2015;12(4):243-8.
36. Ness-Jensen E, Lindam A, Lagergren J, Hveem K. Changes in prevalence, incidence and spontaneous loss of gastro-oesophageal reflux symptoms: a prospective population-based cohort study, the HUNT study. Gut. 2012;61(10):1390-7.
37. El-Serag HB, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut. 2014;63(6):871-80.
38. Prach AT, MacDonald TA, Hopwood DA, Johnston DA. Increasing incidence of Barrett's oesophagus: education, enthusiasm, or epidemiology? Lancet. 1997;350(9082):933.
39. Conio M, Cameron AJ, Romero Y, Branch CD, Schleck CD, Burgart LJ, et al. Secular trends in the epidemiology and outcome of Barrett's oesophagus in Olmsted County, Minnesota. Gut. 2001;48(3):304-9.
40. van Soest EM, Dieleman JP, Siersema PD, Sturkenboom MC, Kuipers EJ. Increasing incidence of Barrett's oesophagus in the general population. Gut. 2005;54(8):1062-6.
41. Brown LM, Devesa SS, Chow WH. Incidence of adenocarcinoma of the esophagus among white Americans by sex, stage, and age. J Natl Cancer Inst. 2008;100(16):1184-7.

42. Edgren G, Adami HO, Weiderpass E, Nyren O. A global assessment of the oesophageal adenocarcinoma epidemic. Gut. 2013;62(10):1406-14.
43. Pohl H, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. J Natl Cancer Inst. 2005;97(2):142-6.
44. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984;1(8390):1311-5.
45. Everhart JE. Recent developments in the epidemiology of Helicobacter pylori. Gastroenterol Clin North Am. 2000;29(3):559-78.
46. Goh KL, Chan WK, Shiota S, Yamaoka Y. Epidemiology of Helicobacter pylori infection and public health implications. Helicobacter. 2011;16 Suppl 1:1-9.
47. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. Gastroenterology. 2017;153(2):420-9.
48. Sipponen P, Helske T, Jarvinen P, Hyvarinen H, Seppala K, Siurala M. Fall in the prevalence of chronic gastritis over 15 years: analysis of outpatient series in Finland from 1977, 1985, and 1992. Gut. 1994;35(9):1167-71.
49. Miendje Deyi VY, Vanderpas J, Bontems P, Van den Borre C, De Koster E, Cadranel S, et al. Marching cohort of Helicobacter pylori infection over two decades (1988-2007): combined effects of secular trend and population migration. Epidemiol Infect. 2011;139(4):572-80.
50. Yim JY, Kim N, Choi SH, Kim YS, Cho KR, Kim SS, et al. Seroprevalence of Helicobacter pylori in South Korea. Helicobacter. 2007;12(4):333-40.
51. Chen J, Bu XL, Wang QY, Hu PJ, Chen MH. Decreasing seroprevalence of Helicobacter pylori infection during 1993-2003 in Guangzhou, southern China. Helicobacter. 2007;12(2):164-9.
52. Hui PK, Chan WY, Cheung PS, Chan JK, Ng CS. Pathologic changes of gastric mucosa colonized by Helicobacter pylori. Hum Pathol. 1992;23(5):548-56.
53. Wyatt JI, Rathbone BJ. Immune response of the gastric mucosa to Campylobacter pylori. Scand J Gastroenterol Suppl. 1988;142:44-9.
54. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol. 1996;20(10):1161-81.
55. Offerhaus GJ, Price AB, Haot J, ten Kate FJ, Sipponen P, Fiocca R, et al. Observer agreement on the grading of gastric atrophy. Histopathology. 1999;34(4):320-5.
56. El-Zimaity HM. Gastric atrophy, diagnosing and staging. World J Gastroenterol. 2006;12(36):5757-62.
57. El-Omar EM, Oien K, El-Nujumi A, Gillen D, Wirz A, Dahill S, et al. Helicobacter pylori infection and chronic gastric acid hyposecretion. Gastroenterology. 1997;113(1):15-24.
58. Ito Y, Azuma T, Ito S, Miyaji H, Hirai M, Yamazaki Y, et al. Analysis and typing of the vacA gene from cagA-positive strains of Helicobacter pylori isolated in Japan. J Clin Microbiol. 1997;35(7):1710-4.
59. Parsonnet J, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. Gut. 1997;40(3):297-301.
60. Kuipers EJ. Review article: exploring the link between Helicobacter pylori and gastric cancer. Aliment Pharmacol Ther. 1999;13 Suppl 1:3-11.
61. el-Omar EM, Penman ID, Ardill JE, Chittajallu RS, Howie C, McColl KE. Helicobacter pylori infection and abnormalities of acid secretion in patients with duodenal ulcer disease. Gastroenterology. 1995;109(3):681-91.
62. Hansen S, Melby KK, Aase S, Jellum E, Vollset SE. Helicobacter pylori infection and risk of cardia cancer and non-cardia gastric cancer. A nested case-control study. Scand J Gastroenterol. 1999;34(4):353-60.
63. Lee A, Dixon MF, Danon SJ, Kuipers E, Megraud F, Larsson H, et al. Local acid production and Helicobacter pylori: a unifying hypothesis of gastroduodenal disease. Eur J Gastroenterol Hepatol. 1995;7(5):461-5.

64. Ford AC, Forman D, Hunt RH, Yuan Y, Moayyedi P. Helicobacter pylori eradication therapy to prevent gastric cancer in healthy asymptomatic infected individuals: systematic review and meta-analysis of randomised controlled trials. BMJ. 2014;348:g3174.
65. Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, et al. Helicobacter pylori infection and gastric lymphoma. N Engl J Med. 1994;330(18):1267-71.
66. Fischbach W, Goebeler-Kolve ME, Dragosics B, Greiner A, Stolte M. Long term outcome of patients with gastric marginal zone B cell lymphoma of mucosa associated lymphoid tissue (MALT) following exclusive Helicobacter pylori eradication therapy: experience from a large prospective series. Gut. 2004;53(1):34-7.
67. Raghunath A, Hungin AP, Wooff D, Childs S. Prevalence of Helicobacter pylori in patients with gastro-oesophageal reflux disease: systematic review. BMJ. 2003;326(7392):737.
68. Chung SJ, Lim SH, Choi J, Kim D, Kim YS, Park MJ, et al. Helicobacter pylori Serology Inversely Correlated With the Risk and Severity of Reflux Esophagitis in Helicobacter pylori Endemic Area: A Matched Case-Control Study of 5,616 Health Check-Up Koreans. J Neurogastroenterol Motil. 2011;17(3):267-73.
69. Minatsuki C, Yamamichi N, Shimamoto T, Kakimoto H, Takahashi Y, Fujishiro M, et al. Background factors of reflux esophagitis and non-erosive reflux disease: a cross-sectional study of 10,837 subjects in Japan. PLoS One. 2013;8(7):e69891.
70. Rubenstein JH, Inadomi JM, Scheiman J, Schoenfeld P, Appelman H, Zhang M, et al. Association between Helicobacter pylori and Barrett's esophagus, erosive esophagitis, and gastroesophageal reflux symptoms. Clin Gastroenterol Hepatol. 2014;12(2):239-45.
71. Anderson LA, Murphy SJ, Johnston BT, Watson RG, Ferguson HR, Bamford KB, et al. Relationship between Helicobacter pylori infection and gastric atrophy and the stages of the oesophageal inflammation, metaplasia, adenocarcinoma sequence: results from the FINBAR case-control study. Gut. 2008;57(6):734-9.
72. Fischbach LA, Nordenstedt H, Kramer JR, Gandhi S, Dick-Onuoha S, Lewis A, et al. The association between Barrett's esophagus and Helicobacter pylori infection: a meta-analysis. Helicobacter. 2012;17(3):163-75.
73. Rokkas T, Pistiolas D, Sechopoulos P, Robotis I, Margantinis G. Relationship between Helicobacter pylori infection and esophageal neoplasia: a meta-analysis. Clin Gastroenterol Hepatol. 2007;5(12):1413-7, 7 e1-2.
74. Hassan MA, Hobsley M. Positioning of subject and of nasogastric tube during a gastric secretion study. British medical journal. 1970;1(5694):458-60.
75. Lawrie JH, Forrest AP. The Measurement of Gastric Acid. Postgrad Med J. 1965;41:408-17.
76. Kay AW. Effect of large doses of histamine on gastric secretion of HCl; an augmented histamine test. British medical journal. 1953;2(4827):77-80.
77. Iijima K, Ohara S, Sekine H, Koike T, Kubota Y, Kato K, et al. A new endoscopic method of gastric acid secretory testing. The American journal of gastroenterology. 1998;93(11):2113-8.
78. Fackler WK, Vaezi MF, Richter JE. Ambulatory gastric pH monitoring: proper probe placement and normal values. Alimentary pharmacology & therapeutics. 2001;15(8):1155-62.
79. Peterson WL, Barnett CC, Evans DJ, Jr., Feldman M, Carmody T, Richardson C, et al. Acid secretion and serum gastrin in normal subjects and patients with duodenal ulcer: the role of Helicobacter pylori. Am J Gastroenterol. 1993;88(12):2038-43.
80. Iijima K, Ohara S, Koike T, Sekine H, Shimosegawa T. Gastric acid secretion of normal Japanese subjects in relation to Helicobacter pylori infection, aging, and gender. Scand J Gastroenterol. 2004;39(8):709-16.
81. Tarnasky PR, Kovacs TO, Sytnik B, Walsh JH. Asymptomatic H. pylori infection impairs pH inhibition of gastrin and acid secretion during second hour of peptone meal stimulation. Digestive diseases and sciences. 1993;38(9):1681-7.
82. Smith JT, Pounder RE, Nwokolo CU, Lanzon-Miller S, Evans DG, Graham DY, et al. Inappropriate hypergastrinaemia in asymptomatic healthy subjects infected with Helicobacter pylori. Gut. 1990;31(5):522-5.

83. Gillen D, el-Omar EM, Wirz AA, Ardill JE, McColl KE. The acid response to gastrin distinguishes duodenal ulcer patients from Helicobacter pylori-infected healthy subjects. Gastroenterology. 1998;114(1):50-7.
84. Feldman M, Cryer B, Sammer D, Lee E, Spechler SJ. Influence of H. pylori infection on meal-stimulated gastric acid secretion and gastroesophageal acid reflux. Am J Physiol. 1999;277(6 Pt 1):G1159-64.
85. Gutierrez O, Melo M, Segura AM, Angel A, Genta RM, Graham DY. Cure of Helicobacter pylori infection improves gastric acid secretion in patients with corpus gastritis. Scandinavian journal of gastroenterology. 1997;32(7):664-8.
86. Verdu EF, Armstrong D, Idstrom JP, Labenz J, Stolte M, Dorta G, et al. Effect of curing Helicobacter pylori infection on intragastric pH during treatment with omeprazole. Gut. 1995;37(6):743-8.
87. Prewett EJ, Smith JT, Nwokolo CU, Hudson M, Sawyerr AM, Pounder RE. Eradication of Helicobacter pylori abolishes 24-hour hypergastrinaemia: a prospective study in healthy subjects. Alimentary pharmacology & therapeutics. 1991;5(3):283-90.
88. Feldman M, Cryer B, McArthur KE, Huet BA, Lee E. Effects of aging and gastritis on gastric acid and pepsin secretion in humans: a prospective study. Gastroenterology. 1996;110(4):1043-52.
89. Kinoshita Y, Kawanami C, Kishi K, Nakata H, Seino Y, Chiba T. Helicobacter pylori independent chronological change in gastric acid secretion in the Japanese. Gut. 1997;41(4):452-8.
90. Katelaris PH, Seow F, Lin BP, Napoli J, Ngu MC, Jones DB. Effect of age, Helicobacter pylori infection, and gastritis with atrophy on serum gastrin and gastric acid secretion in healthy men. Gut. 1993;34(8):1032-7.
91. Goldschmiedt M, Barnett CC, Schwarz BE, Karnes WE, Redfern JS, Feldman M. Effect of age on gastric acid secretion and serum gastrin concentrations in healthy men and women. Gastroenterology. 1991;101(4):977-90.
92. Cannon W. Journal of Boston Society of Medical Sciences. 1898;2(6):59-66.
93. Hurst A. The sensibility of the alimentary canal: Oxford University Press, London; 1911.
94. Hila A, Bouali H, Xue S, Knuff D, Castell DO. Postprandial stomach contents have multiple acid layers. Journal of clinical gastroenterology. 2006;40(7):612-7.
95. Clarke AT, Wirz AA, Seenan JP, Manning JJ, Gillen D, McColl KE. Paradox of gastric cardia: it becomes more acidic following meals while the rest of stomach becomes less acidic. Gut. 2009;58(7):904-9.
96. Burton DD, Kim HJ, Camilleri M, Stephens DA, Mullan BP, O'Connor MK, et al. Relationship of gastric emptying and volume changes after a solid meal in humans. Am J Physiol Gastrointest Liver Physiol. 2005;289(2):G261-6.
97. Sakaki N, Kozawa H, Egawa N, Tu Y, Sanaka M. Ten-year prospective follow-up study on the relationship between Helicobacter pylori infection and progression of atrophic gastritis, particularly assessed by endoscopic findings. Aliment Pharmacol Ther. 2002;16 Suppl 2:198-203.
98. Sekine H, Iijima K, Koike T, Abe Y, Imatani A, Kato K, et al. Regional differences in the recovery of gastric acid secretion after Helicobacter pylori eradication: evaluations with Congo red chromoendoscopy. Gastrointestinal endoscopy. 2006;64(5):678-85.
99. de Vries AC, Meijer GA, Looman CW, Casparie MK, Hansen BE, van Grieken NC, et al. Epidemiological trends of pre-malignant gastric lesions: a long-term nationwide study in the Netherlands. Gut. 2007;56(12):1665-70.
100. Rennie KL, Jebb SA. Prevalence of obesity in Great Britain. Obes Rev. 2005;6(1):11-2.
101. Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999-2000. JAMA. 2002;288(14):1723-7.
102. Ogden CL, Flegal KM, Carroll MD, Johnson CL. Prevalence and trends in overweight among US children and adolescents, 1999-2000. JAMA. 2002;288(14):1728-32.
103. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. JAMA. 2006;295(13):1549-55.

104. Padez C, Fernandes T, Mourao I, Moreira P, Rosado V. Prevalence of overweight and obesity in 7-9-year-old Portuguese children: trends in body mass index from 1970-2002. Am J Hum Biol. 2004;16(6):670-8.
105. Luo J, Hu FB. Time trends of obesity in pre-school children in China from 1989 to 1997. Int J Obes Relat Metab Disord. 2002;26(4):553-8.
106. Hampel H, Abraham NS, El-Serag HB. Meta-analysis: obesity and the risk for gastroesophageal reflux disease and its complications. Ann Intern Med. 2005;143(3):199-211.
107. Corley DA, Kubo A. Body mass index and gastroesophageal reflux disease: a systematic review and meta-analysis. Am J Gastroenterol. 2006;101(11):2619-28.
108. El-Serag HB, Ergun GA, Pandolfino J, Fitzgerald S, Tran T, Kramer JR. Obesity increases oesophageal acid exposure. Gut. 2007;56(6):749-55.
109. Locke GR, 3rd, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ, 3rd. Risk factors associated with symptoms of gastroesophageal reflux. Am J Med. 1999;106(6):642-9.
110. Ruhl CE, Everhart JE. Overweight, but not high dietary fat intake, increases risk of gastroesophageal reflux disease hospitalization: the NHANES I Epidemiologic Followup Study. First National Health and Nutrition Examination Survey. Ann Epidemiol. 1999;9(7):424-35.
111. Nandurkar S, Locke GR, 3rd, Fett S, Zinsmeister AR, Cameron AJ, Talley NJ. Relationship between body mass index, diet, exercise and gastro-oesophageal reflux symptoms in a community. Aliment Pharmacol Ther. 2004;20(5):497-505.
112. Singh S, Sharma AN, Murad MH, Buttar NS, El-Serag HB, Katzka DA, et al. Central adiposity is associated with increased risk of esophageal inflammation, metaplasia, and adenocarcinoma: a systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2013;11(11):1399-412 e7.
113. Corley DA, Kubo A, Levin TR, Block G, Habel L, Zhao W, et al. Abdominal obesity and body mass index as risk factors for Barrett's esophagus. Gastroenterology. 2007;133(1):34-41; quiz 311.
114. Edelstein ZR, Farrow DC, Bronner MP, Rosen SN, Vaughan TL. Central adiposity and risk of Barrett's esophagus. Gastroenterology. 2007;133(2):403-11.
115. Turati F, Tramacere I, La Vecchia C, Negri E. A meta-analysis of body mass index and esophageal and gastric cardia adenocarcinoma. Ann Oncol. 2013;24(3):609-17.
116. Menon S, Trudgill N. Risk factors in the aetiology of hiatus hernia: a meta-analysis. Eur J Gastroenterol Hepatol. 2011;23(2):133-8.
117. Che F, Nguyen B, Cohen A, Nguyen NT. Prevalence of hiatal hernia in the morbidly obese. Surg Obes Relat Dis. 2013;9(6):920-4.
118. Stene-Larsen G, Weberg R, Froyshov Larsen I, Bjortuft O, Hoel B, Berstad A. Relationship of overweight to hiatus hernia and reflux oesophagitis. Scand J Gastroenterol. 1988;23(4):427-32.
119. Sugerman HJ, DeMaria EJ, Felton WL, 3rd, Nakatsuka M, Sismanis A. Increased intra-abdominal pressure and cardiac filling pressures in obesity-associated pseudotumor cerebri. Neurology. 1997;49(2):507-11.
120. El-Serag HB, Tran T, Richardson P, Ergun G. Anthropometric correlates of intragastric pressure. Scand J Gastroenterol. 2006;41(8):887-91.
121. Navarro-Rodriguez T, Hashimoto CL, Carrilho FJ, Strauss E, Laudanna AA, Moraes-Filho JP. Reduction of abdominal pressure in patients with ascites reduces gastroesophageal reflux. Dis Esophagus. 2003;16(2):77-82.
122. Ulmsten U, Sundstrom G. Esophageal manometry in pregnant and nonpregnant women. Am J Obstet Gynecol. 1978;132(3):260-4.
123. Mercer CD, Wren SF, DaCosta LR, Beck IT. Lower esophageal sphincter pressure and gastroesophageal pressure gradients in excessively obese patients. J Med. 1987;18(3-4):135-46.
124. O'Brien TF, Jr. Lower esophageal sphincter pressure (LESP) and esophageal function in obese humans. J Clin Gastroenterol. 1980;2(2):145-8.
125. Wu JC, Mui LM, Cheung CM, Chan Y, Sung JJ. Obesity is associated with increased transient lower esophageal sphincter relaxation. Gastroenterology. 2007;132(3):883-9.
126. Schneider JH, Kuper M, Konigsrainer A, Brucher B. Transient lower esophageal sphincter relaxation in morbid obesity. Obes Surg. 2009;19(5):595-600.

127. Maddox A, Horowitz M, Wishart J, Collins P. Gastric and oesophageal emptying in obesity. Scand J Gastroenterol. 1989;24(5):593-8.
128. Wisen O, Johansson C. Gastrointestinal function in obesity: motility, secretion, and absorption following a liquid test meal. Metabolism. 1992;41(4):390-5.
129. Harter RL, Kelly WB, Kramer MG, Perez CE, Dzwonczyk RR. A comparison of the volume and pH of gastric contents of obese and lean surgical patients. Anesth Analg. 1998;86(1):147-52.
130. Wisen O, Rossner S, Johansson C. Gastric secretion in massive obesity. Evidence for abnormal response to vagal stimulation. Dig Dis Sci. 1987;32(9):968-72.
131. Nilsson M, Lundegardh G, Carling L, Ye W, Lagergren J. Body mass and reflux oesophagitis: an oestrogen-dependent association? Scand J Gastroenterol. 2002;37(6):626-30.
132. Nilsson M, Johnsen R, Ye W, Hveem K, Lagergren J. Obesity and estrogen as risk factors for gastroesophageal reflux symptoms. JAMA. 2003;290(1):66-72.
133. Morton GJ. Hypothalamic leptin regulation of energy homeostasis and glucose metabolism. J Physiol. 2007;583(Pt 2):437-43.
134. Beales IL, Ogunwobi OO. Leptin synergistically enhances the anti-apoptotic and growth-promoting effects of acid in OE33 oesophageal adenocarcinoma cells in culture. Mol Cell Endocrinol. 2007;274(1-2):60-8.
135. Garcia JM, Splenser AE, Kramer J, Alsarraj A, Fitzgerald S, Ramsey D, et al. Circulating inflammatory cytokines and adipokines are associated with increased risk of Barrett's esophagus: a case-control study. Clin Gastroenterol Hepatol. 2014;12(2):229-38 e3.
136. Kong CY, Nattinger KJ, Hayeck TJ, Omer ZB, Wang YC, Spechler SJ, et al. The impact of obesity on the rise in esophageal adenocarcinoma incidence: estimates from a disease simulation model. Cancer Epidemiol Biomarkers Prev. 2011;20(11):2450-6.
137. Kroep S, Lansdorp-Vogelaar I, Rubenstein JH, Lemmens VE, van Heijningen EB, Aragones N, et al. Comparing trends in esophageal adenocarcinoma incidence and lifestyle factors between the United States, Spain, and the Netherlands. Am J Gastroenterol. 2014;109(3):336-43; quiz 5, 44.
138. Drahos J, Xiao Q, Risch HA, Freedman ND, Abnet CC, Anderson LA, et al. Age-specific risk factor profiles of adenocarcinomas of the esophagus: A pooled analysis from the international BEACON consortium. Int J Cancer. 2016;138(1):55-64.
139. Blaser MJ. Disappearing microbiota: Helicobacter pylori protection against esophageal adenocarcinoma. Cancer Prev Res (Phila). 2008;1(5):308-11.
140. Cremonini F, Di Caro S, Delgado-Aros S, Sepulveda A, Gasbarrini G, Gasbarrini A, et al. Meta-analysis: the relationship between Helicobacter pylori infection and gastro-oesophageal reflux disease. Aliment Pharmacol Ther. 2003;18(3):279-89.
141. de Martel C, Llosa AE, Farr SM, Friedman GD, Vogelman JH, Orentreich N, et al. Helicobacter pylori infection and the risk of development of esophageal adenocarcinoma. J Infect Dis. 2005;191(5):761-7.
142. McColl KE, Watabe H, Derakhshan MH. Role of gastric atrophy in mediating negative association between Helicobacter pylori infection and reflux oesophagitis, Barrett's oesophagus and oesophageal adenocarcinoma. Gut. 2008;57(6):721-3.
143. Hansen S, Vollset SE, Derakhshan MH, Fyfe V, Melby KK, Aase S, et al. Two distinct aetiologies of cardia cancer; evidence from premorbid serological markers of gastric atrophy and Helicobacter pylori status. Gut. 2007;56(7):918-25.
144. Gajperia C, Barbiere JM, Greenberg D, Wright K, Lyratzopoulos G. Recent incidence trends and sociodemographic features of oesophageal and gastric cancer types in an English region. Aliment Pharmacol Ther. 2009;30(8):873-80.
145. Colquhoun A, Arnold M, Ferlay J, Goodman KJ, Forman D, Soerjomataram I. Global patterns of cardia and non-cardia gastric cancer incidence in 2012. Gut. 2015;64(12):1881-8.
146. Kim HJ, Fay MP, Feuer EJ, Midthune DN. Permutation tests for joinpoint regression with applications to cancer rates. Stat Med. 2000;19(3):335-51.
147. Blaser MJ, Saito D. Trends in reported adenocarcinomas of the oesophagus and gastric cardia in Japan. Eur J Gastroenterol Hepatol. 2002;14(2):107-13.

148. Ekstrom AM, Signorello LB, Hansson LE, Bergstrom R, Lindgren A, Nyren O. Evaluating gastric cancer misclassification: a potential explanation for the rise in cardia cancer incidence. J Natl Cancer Inst. 1999;91(9):786-90.
149. Sonnenberg A. Review article: historic changes of Helicobacter pylori-associated diseases. Aliment Pharmacol Ther. 2013;38(4):329-42.
150. Peleteiro B, Castro C, Morais S, Ferro A, Lunet N. Worldwide Burden of Gastric Cancer Attributable to Tobacco Smoking in 2012 and Predictions for 2020. Dig Dis Sci. 2015;60(8):2470-6.
151. Gonzalez CA, Lujan-Barroso L, Bueno-de-Mesquita HB, Jenab M, Duell EJ, Agudo A, et al. Fruit and vegetable intake and the risk of gastric adenocarcinoma: a reanalysis of the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study after a longer follow-up. Int J Cancer. 2012;131(12):2910-9.
152. Tsugane S. Salt, salted food intake, and risk of gastric cancer: epidemiologic evidence. Cancer Sci. 2005;96(1):1-6.
153. Kubo A, Corley DA. Meta-analysis of antioxidant intake and the risk of esophageal and gastric cardia adenocarcinoma. Am J Gastroenterol. 2007;102(10):2323-30; quiz 31.
154. Plummer M, Franceschi S, Vignat J, Forman D, de Martel C. Global burden of gastric cancer attributable to Helicobacter pylori. Int J Cancer. 2015;136(2):487-90.
155. Kamada T, Haruma K, Ito M, Inoue K, Manabe N, Matsumoto H, et al. Time Trends in Helicobacter pylori Infection and Atrophic Gastritis Over 40 Years in Japan. Helicobacter. 2015;20(3):192-8.
156. Inoue M, Tajima K, Kobayashi S, Suzuki T, Matsuura A, Nakamura T, et al. Protective factor against progression from atrophic gastritis to gastric cancer--data from a cohort study in Japan. Int J Cancer. 1996;66(3):309-14.
157. Peitz U, Wex T, Vieth M, Stolte M, Willich S, Labenz J, et al. Correlation of serum pepsinogens and gastrin-17 with atrophic gastritis in gastroesophageal reflux patients: a matched-pairs study. J Gastroenterol Hepatol. 2011;26(1):82-9.
158. Derakhshan MH, El-Omar E, Oien K, Gillen D, Fyfe V, Crabtree JE, et al. Gastric histology, serological markers and age as predictors of gastric acid secretion in patients infected with Helicobacter pylori. J Clin Pathol. 2006;59(12):1293-9.
159. Meining A, Kompisch A, Stolte M. Comparative classification and grading of Helicobacter pylori gastritis in patients with gastric cancer and patients with functional dyspepsia. Scand J Gastroenterol. 2003;38(7):707-11.
160. Sitas F. Twenty five years since the first prospective study by Forman et al. (1991) on Helicobacter pylori and stomach cancer risk. Cancer Epidemiol. 2016;41:159-64.
161. Kandulski A, Malfertheiner P. Helicobacter pylori and gastroesophageal reflux disease. Curr Opin Gastroenterol. 2014;30(4):402-7.
162. McColl KE, el-Omar EM, Gillen D. The role of H. pylori infection in the pathophysiology of duodenal ulcer disease. J Physiol Pharmacol. 1997;48(3):287-95.
163. Kahrilas PJ, McColl K, Fox M, O'Rourke L, Sifrim D, Smout AJ, et al. The acid pocket: a target for treatment in reflux disease? Am J Gastroenterol. 2013;108(7):1058-64.
164. Fraser A, Delaney BC, Ford AC, Qume M, Moayyedi P. The Short-Form Leeds Dyspepsia Questionnaire validation study. Aliment Pharmacol Ther. 2007;25(4):477-86.
165. Derakhshan MH, Robertson EV, Yeh Lee Y, Harvey T, Ferrier RK, Wirz AA, et al. In healthy volunteers, immunohistochemistry supports squamous to columnar metaplasia as mechanism of expansion of cardia, aggravated by central obesity. Gut. 2015;64(11):1705-14.
166. Fletcher J, Wirz A, Young J, Vallance R, McColl KE. Unbuffered highly acidic gastric juice exists at the gastroesophageal junction after a meal. Gastroenterology. 2001;121(4):775-83.
167. Scheffer RC, Wassenaar EB, Herwaarden MA, Holloway RH, Samsom M, Smout AJ, et al. Relationship between the mechanism of gastro-oesophageal reflux and oesophageal acid exposure in patients with reflux disease. Neurogastroenterol Motil. 2005;17(5):654-62.
168. Choi E, Roland JT, Barlow BJ, O'Neal R, Rich AE, Nam KT, et al. Cell lineage distribution atlas of the human stomach reveals heterogeneous gland populations in the gastric antrum. Gut. 2014;63(11):1711-20.

169. Newton JL, James OF, Williams GV, Allen A. The diurnal profile of gastric pepsin activity is reduced with Helicobacter pylori infection. Dig Dis Sci. 2004;49(7-8):1103-8.
170. Tobey NA, Hosseini SS, Caymaz-Bor C, Wyatt HR, Orlando GS, Orlando RC. The role of pepsin in acid injury to esophageal epithelium. Am J Gastroenterol. 2001;96(11):3062-70.
171. Rubio CA, Jaramillo E, Suzuki G, Lagergren P, Nesi G. Antralization of the gastric mucosa of the incisura angularis and its gastrin expression. Int J Clin Exp Pathol. 2009;2(1):65-70.
172. Goldenring JR, Nam KT, Wang TC, Mills JC, Wright NA. Spasmolytic polypeptide-expressing metaplasia and intestinal metaplasia: time for reevaluation of metaplasias and the origins of gastric cancer. Gastroenterology. 2010;138(7):2207-10, 10 e1.
173. Graham DY, Kato M, Asaka M. Gastric endoscopy in the 21st century: appropriate use of an invasive procedure in the era of non-invasive testing. Dig Liver Dis. 2008;40(7):497-503.
174. Haruma K, Kamada T, Kawaguchi H, Okamoto S, Yoshihara M, Sumii K, et al. Effect of age and Helicobacter pylori infection on gastric acid secretion. J Gastroenterol Hepatol. 2000;15(3):277-83.
175. Weck MN, Brenner H. Prevalence of chronic atrophic gastritis in different parts of the world. Cancer Epidemiol Biomarkers Prev. 2006;15(6):1083-94.
176. Derakhshan MH, Arnold M, Brewster DH, Going JJ, Mitchell DR, Forman D, et al. Worldwide Inverse Association between Gastric Cancer and Esophageal Adenocarcinoma Suggesting a Common Environmental Factor Exerting Opposing Effects. Am J Gastroenterol. 2016;111(2):228-39.
177. Steffen A, Schulze MB, Pischon T, Dietrich T, Molina E, Chirlaque MD, et al. Anthropometry and esophageal cancer risk in the European prospective investigation into cancer and nutrition. Cancer Epidemiol Biomarkers Prev. 2009;18(7):2079-89.
178. de Vries DR, van Herwaarden MA, Smout AJ, Samsom M. Gastroesophageal pressure gradients in gastroesophageal reflux disease: relations with hiatal hernia, body mass index, and esophageal acid exposure. Am J Gastroenterol. 2008;103(6):1349-54.
179. Derakhshan MH, Robertson EV, Fletcher J, Jones GR, Lee YY, Wirz AA, et al. Mechanism of association between BMI and dysfunction of the gastro-oesophageal barrier in patients with normal endoscopy. Gut. 2012;61(3):337-43.
180. Kaye MD, Rein R, Johnson WP, Showalter JP. Responses of the competent and incompetent lower oesophageal sphincter to pentagastrin and abdominal compression. Gut. 1976;17(12):933-9.
181. Mittal RK, Fisher M, McCallum RW, Rochester DF, Dent J, Sluss J. Human lower esophageal sphincter pressure response to increased intra-abdominal pressure. Am J Physiol. 1990;258(4 Pt 1):G624-30.
182. Kahrilas PJ, Wu S, Lin S, Pouderoux P. Attenuation of esophageal shortening during peristalsis with hiatus hernia. Gastroenterology. 1995;109(6):1818-25.
183. Lee YY, Wirz AA, Whiting JG, Robertson EV, Smith D, Weir A, et al. Waist belt and central obesity cause partial hiatus hernia and short-segment acid reflux in asymptomatic volunteers. Gut. 2014;63(7):1053-60.
184. Robertson EV, Derakhshan MH, Wirz AA, Lee YY, Seenan JP, Ballantyne SA, et al. Central obesity in asymptomatic volunteers is associated with increased intrasphincteric acid reflux and lengthening of the cardiac mucosa. Gastroenterology. 2013;145(4):730-9.
185. Dodds WJ, Hogan WJ, Miller WN, Stef JJ, Arndorfer RC, Lydon SB. Effect of increased intraabdominal pressure on lower esophageal sphincter pressure. Am J Dig Dis. 1975;20(4):298-308.