Main Title

THE GASTRIC ACID POCKET IS ATTENUATED IN *H. PYLORI* INFECTED SUBJECTS

Short Title:

Acid Pocket and *H. pylori*

David R Mitchell[1], Mohammad H Derakhshan[1], Angela A Wirz[1], Clare Orange[2],

Stuart A Ballantyne[3], James J Going[4], Kenneth E.L. McColl[1]

[1]Section of Gastroenterology, Institute of Cardiovascular & Medical Sciences, University of Glasgow, Glasgow, UK

[2]University Department of Pathology, School of Medicine, University of Glasgow, Glasgow, UK

[3]Department of Clinical Radiology, NHS Greater Glasgow & Clyde, Glasgow, UK

[4]Institute of Cancer Sciences, University of Glasgow, Glasgow, UK

CORRESPONDING AUTHOR:

Prof Kenneth E.L. McColl,
Institute of Cardiovascular & Medical Sciences,
University of Glasgow,
126 University Place, Glasgow, G12 8TA, UK
Email: kenneth.mccoll@glasgow,ac.uk

ABBREVIATIONS:

GEJ: Gastroesophageal Junction,   **SCJ:** Squamocolumnar Junction, **LES:** Lower oesophageal sphincter, ***H.pylori:*** *Helicobacter pylori,* **GERD:** gastroesophageal reflux disease, **TLESRs:** Transient lower esophageal sphincter relaxations, **PMN:** polymorphonuclear cells, **MN:** mononuclear cells, **IM:** Intestinal metaplasia, **NSAID:** Non-steroidal anti-inflammatory drug; **PPI**: Proton pump inhibitor; **BMI**: Body mass index.

Keywords:

Acid pocket, *Helicobacter pylori*, gastric secretion, gastroesophageal junction, atrophic gastritis.

Word count: 4485

ABSTRACT

Objective: Gastric acid secretory capacity in different anatomical regions, including the postprandial acid pocket, was assessed in *H. pylori* positive and negative volunteers in a Western population.

Design: We studied 31 *H.pylori* positive and 28 *H.pylori* negative volunteers, matched for age, gender and BMI. Jumbo biopsies were taken at 11 pre-determined locations from the gastroesophageal junction and stomach. Combined high resolution pHmetry (12 sensors) and manometry (36 sensors) was performed for 20 minutes fasted and 90 minutes 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.

Results: Under fasting conditions, the *H.pylori* positives had less intragastric acidity compared to negatives at all sensors >1.1cm distal to the peak lower oesophageal sphincter (LES) pressure ($p<0.01$). Postprandially, intragastric acidity was less in *H.pylori* positives at sensors 2.2, 3.3 and 4.4cm distal to the peak LES pressure ($p<0.05$), but there was 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 to *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.

Conclusion: In population volunteers, *H.pylori* positives have reduced intragastric acidity which most markedly affects the postprandial acid pocket.

SUMMARY BOX

WHAT IS ALREADY KNOWN?

1. There is a negative association between *H. pylori* infection and both gastroesophageal reflux disease and oesophageal adenocarcinoma.

2. The mechanism of this negative association is unclear but might be related to *H. pylori* reducing gastric acidity.

3. The gastric acid which refluxes into the oesophagus originates from the proximal gastric acid pocket.

NOVEL FINDINGS:

1. In population volunteers, intragastric acidity was less in those with *H. pylori* infection and this was most marked in the proximal stomach close to the gastroesophageal junction.

2. The density of parietal cells and chief cells was reduced in *H. pylori* positives compared to negatives.

3. The reduction in intragastric acidity and severity of inflammation were more marked in CagA positive versus CagA negative *H. pylori* infected subjects.

CLINICAL IMPLICATIONS:

The reduced intragastric acidity close to the gastroesophageal junction in the *H. pylori* infected subjects provides a mechanism for the negative association between the infection and reflux disease and its complications.

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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. [1]

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.[2] 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.[3]

An unexplained observation regarding the infection is its negative association with gastroesophageal reflux disease (GERD) and its complications of Barrett's oesophagus and oesophageal adenocarcinoma, with these disorders being less than half as common in infected subjects. [4, 5] 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.[6] 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

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infection on gastric secretory function in the 90% of infected subjects without these complications.[7]   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 gastroesophageal junction (GEJ), referred to as the acid pocket, which refluxes and causes oesophageal damage.[8] 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.[9]   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.

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

METHODS & MATERIALS

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.

Study design

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 [10] 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).

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).

Biopsy specimen processing

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.[11] The specimens were later embedded in agar on the filter paper without

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further manipulation. Staining was performed with conventional H&E, as well as monoclonal

antibodies to $H^+/K^+ATPase$, pepsinogen I and gastrin.

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 (LES), 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 is shown in

Fig **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.

Equipment

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).

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).

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.

Data analysis

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.

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 (TLESRs). 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 LES pressure was taken as

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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.

Histopathological Assessment

A. Studies using Conventional H&E:

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 [12]. 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.

B. 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.

Quantification of Secretory Cells:

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.

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.

Ethics

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

RESULTS

Of the 137 subjects assessed for eligibility for the study, 49 were excluded due to current or recent use of proton pump inhibitor (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). 88 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 body mass index (BMI) (Fig. S1).

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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/m^2; *p*=0.72). There were 7 current smokers in the *H.pylori* positive group compared to 1 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 to 0 (range 0-3) for the *H.pylori* negative subjects (*p*=0.002).   17/31 (54.8%) of the *H.pylori* positives were taking no medication compared to 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, 4 *H.pylori* positive subjects had a hiatus hernia (2-4cm in length), 1 subject had LA Grade A reflux esophagitis, and one subject had 3cm of Barrett's mucosa. None of the *H.pylori* negatives had a hiatus hernia, although two subjects had reflux esophagitis (LA grade A and B).

Gastroesophageal Acidity

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 LES pressure (Table 1). 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 LES 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 SCJ.

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 LES but no significant difference was detected by the more distal sensors placed at 5.5 and 6.6cm distal to this reference point (Table 1).   These three sensors detecting a

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significant difference in gastric acidity between the two groups were those closest to the GEJ with the most proximal of them being only 0.6cm distal to the SCJ (Fig. 2).

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 3cm distal to the peak LES pressure, at the peak LES pressure and also extending 1.1cm above the peak LES pressure (Table 2)**.**

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Table 1. Median (IQR) pH values at sensors relative to peak LES pressure comparing *H.pylori* negative (n=28) and positive (n=31) groups during 20 minute fasting period and three 30 minute postprandial periods..

Sensor location	Fasting				0-30 minutes				30-60 minutes				60-90 minutes		
	HP-	*HP+*	*p* value		*HP-*	*HP+*	*p* value		*HP-*	*HP+*	*p* value		*HP-*	*HP+*	*p* value
5cm proximal	7.20 (0.70)	7.19 (0.74)	0.933		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	7.33 (0.78)	7.37 (0.62)	0.525		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 LES pressure	7.34 (0.79)	7.28 (0.51)	0.499		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	7.06 (1.63)	7.13 (0.51)	0.213		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	5.79 (4.26)	6.94 (1.38)	0.004		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	2.27 (2.58)	6.13 (5.06)	<0.001		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	1.70 (1.16)	4.11 (4.95)	<0.001		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	1.68 (0.66)	2.88 (3.66)	<0.001		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	1.62 (3.66)	2.39 (3.06)	0.003		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

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Table 2: Median (IQR) percentage time pH<4 at sensors relative to peak LES pressure comparing *H.pylori* negative (n=28) and positive (n=31) groups during 20 minute fasting period and three 30 minute postprandial periods.

Sensor location	Fasting				0-30 minutes				30-60 minutes				60-90 minutes		
	HP-	*HP+*	*p* value		*HP-*	*HP+*	*p* value		*HP-*	*HP+*	*p* value		*HP-*	*HP+*	*p* value
5cm proximal	0.0 (0.0)	0.0 (0.0)	0.271		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	1.1 (2.8)	0.0 (0.1)	0.004		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 LES pressure	1.7 (4.9)	0.0 (1.0)	0.001		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	6.6 (30.6)	1.0 (7.3)	0.008		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	32.1 (65.4)	2.8 (21.7)	0.004		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	75.6 (48.2)	13.5 (75.6)	0.003		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	93.0 (42.3)	42.4 (42.3)	<0.001		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	97.6 (14.0)	60.4 (62.1)	0.001		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	99.5 (4.8)	84.8 (61.6)	0.011		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

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Gastric Histopathology

A. Conventional H&E Staining

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 3).   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 also 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.   There was minimal evidence of PMN cell infiltrate at any location in the *H.pylori* negatives.

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 also in the biopsies close to the SCJ.   In a further 3 it was limited to the region close to the SCJ.   In 1 subjects 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.

Gastric Gland Height

The height 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 (Table 4).

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Table 3: 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	

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Table 4. Median (IQR) of glandular thickness and densities of parietal and chief cells at each biopsy location comparing *H.pylori* negatives (n=28) and positives (n=31).

Biopsy location	Glandular Thickness (mm)			Parietal cell density (cells/mm^2)			Chief cell density (cells/mm^2)		
	H.pylori -	*H.pylori* +	*P* value	*H.pylori* -	*H.pylori* +	*P* value	*H.pylori* -	*H.pylori* +	*P* value
Across SCJ, Greater curve	0.30 (0.20–0.30)	0.25 (0.20–0.30)	0.515	67 (0-162)	17 (10-39)	0.185	94 (0-156)	22 (3-52)	0.150
Across SCJ, Lesser curve	0.28 (0.0–0.30)	0.20 (0.10–0.30)	0.461	50 (14-127)	9 (0-51)	0.012	89 (17-139)	22 (0-62)	0.017
6mm distal SCJ	0.35 (0.30–0.40)	0.30 (0.20–0.30)	0.006	231 (175-286)	144 (59-190)	<0.001	245 (203-272)	129 (52-190)	<0.001
12mm distal SCJ	0.40 (0.40–0.45)	0.30 (0.30–0.35)	<0.001	317 (300-362)	193 (137-250)	<0.001	379 (312-404)	206 (125-299)	<0.001
18mm distal SCJ	0.45 (0.40–0.50)	0.35 (0.30–0.40)	<0.001	357 (334-383)	241 (201-283)	<0.001	404 (374-421)	273 (194-353)	<0.001
Fundus	0.43 (0.40–0.45)	0.40 (0.35–0.40)	0.008	347 (285-401)	258 (220-292)	<0.001	421 (384-451)	310 (255-389)	<0.001
Mid-body, Lesser curve	0.40 (0.40–0.45)	0.35 (0.30–0.40)	<0.001	361 (316-381)	235 (166-290)	<0.001	401 (367-419)	285 (206-367)	<0.001
Mid-body, Greater curve	0.45 (0.40–0.45)	0.35 (0.30–0.40)	<0.001	356 (318-398)	250 (201-297)	<0.001	420 (372-441)	305 (243-354)	<0.001
Distal body, Greater curve	0.40 (0.35–0.49)	0.30 (0.25–0.35)	<0.001	322 (293-349)	107 (25-263)	<0.001	365 (296-398)	136 (17-292)	<0.001
Incisura Angularis	0.33 (0.30–0.40)	0.25 (0.20–0.30)	<0.001	203 (124-250)	12 (0-87)	<0.001	215 (98-296)	7 (0-99)	<0.001
Antrum	0.20 (0.13–0.30)	0.20 (0.0–0.20)	0.041	40 (6-67)	7 (0-18)	0.002	22 (1-85)	0 (0-5)	<0.001

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B. Immunohistochemistry

Parietal and Chief Cell Density:

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 4). The degree of reduction was similar for the two cell types.

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. 3). 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. 3).

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].

Intragastric Acidity and Histology in CagA Positive *H.pylori* Subjects

Seventeen of the *H.pylori* positives were CagA seropositive and fourteen CagA seronegative. The associations with reduced intragastric acidity in comparison with *H.pylori* negatives was more apparent for the CagA positives being significant for five of the six intragastric sites both fasting and after the meal in the CagA positives but in only two of the six intragastric sites for the CagA negatives and only under fasting conditions (Table 1 – supplement). There was a statistically significant difference between the CagA negative and

CagA positives for only two of the six sites during fasting and one of the six sites after the meal.

The CagA positives had a significantly greater combined inflammatory cell infiltrate evident at three of the eleven biopsy locations (6mm and 18mm distal SCJ, and distal body greater curve), compared to the CagA negatives (Table 2 – supplement). The reduction in parietal and chief cell density was significant at each intragastric location for both CagA positive and negative subjects with no apparent difference between these two groups.

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.[13,14,15] This phenomenon has been called the acid pocket and is thought to be important in GERD induced oesophageal damage after a meal when reflux is most common.

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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 [16] and this was slightly increased close to the GEJ and also 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 gastroesophageal 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 TLESRs are most frequent. [17]

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.[18] We did not measure the secretion of pepsin and other digestive enzymes produced by the chief cells but

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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.[19] 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. [20]

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). [21-24] 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. [25]

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. [26] 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.[27] 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.[28] In 1998, our own group reported a reduced acid secretory response to gastrin stimulation in 20

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H.pylori positive versus 24 *H.pylori* negative healthy volunteers.[29] Several studies in the Japanese population have reported reduced gastric secretory function in *H.pylori* positive healthy volunteers.[30,31]

Our current study differs from previously published studies in a number of 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 gastroesophageal acid reflux.[32] In the light of our current study, the observed increase in gastroesophageal 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.[33] 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.[34] 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.

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FIGURE LEGENDS

Fig 1. Schematic diagram of the relative positions of the 12 sensor pH catheter, 36 sensor manometer and SCJ (identified by attached metal clip)

Fig 2. Median pH for 0-30 minute period after meal relative to LES and SCJ in *H.pylori* positive (HP+) and negative (HP-) subjects

Fig 3. 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; **JL2**: 6mm distal SCJ; **JL3**: 12mm distal SCJ; **JL4**: 18mm distal SCJ; **BG3**: Fundus; **BL**: mid-body lesser curve; **BG2**: mid-body greater curve; **BG1**: distal body greater curve; **IA**: incisura angularis; **Ant**: antrum.

Supplement Fig 1. Flow diagram showing progress of study participants through each stage

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AUTHOR DISCLOSURES:
None to declare.

AUTHOR CONTRIBUTIONS:

DRM: Clinical investigations, manometry, pHmetry, data analysis and drafting manuscript.

MHD: Histological assessment, biopsy orientation, data analysis and drafting manuscript.

AAW: Recruitment of volunteers and assisting clinical investigations.

CO: Technical assistance in histology & scanning of histological slides.

SAB: Radiological assessment.

JJG: Histological assessment and drafting manuscript.

KELM: Conception of original idea, drafting manuscript and overall supervision.

Main Title

THE GASTRIC ACID POCKET IS ATTENUATED IN
H. PYLORI INFECTED SUBJECTS

Short Title:

Acid Pocket and *H. pylori*

David R Mitchell[1], Mohammad H Derakhshan[1], Angela A Wirz[1], Clare Orange[2],

Stuart A Ballantyne[3], James J Going[4], Kenneth E.L. McColl[1]

[1]Section of Gastroenterology, Institute of Cardiovascular & Medical Sciences, University of Glasgow, Glasgow, UK

[2]University Department of Pathology, School of Medicine, University of Glasgow, Glasgow, UK

[3]Department of Clinical Radiology, NHS Greater Glasgow & Clyde, Glasgow, UK

[4]Institute of Cancer Sciences, University of Glasgow, Glasgow, UK

CORRESPONDING AUTHOR:

Prof Kenneth E.L. McColl,
Institute of Cardiovascular & Medical Sciences,
University of Glasgow,
126 University Place, Glasgow, G12 8TA, UK
Email: kenneth.mccoll@glasgow,ac.uk

<u>**ABBREVIATIONS**</u>:

GEJ: Gastroesophageal Junction, **SCJ:** Squamocolumnar Junction, **LES:** Lower oesophageal sphincter, ***H.pylori:*** *Helicobacter pylori,* **GERD:** gastroesophageal reflux disease, **TLESRs:** Transient lower esophageal sphincter relaxations, **PMN:** polymorphonuclear cells, **MN:** mononuclear cells, **IM:** Intestinal metaplasia, **NSAID:** Non-steroidal anti-inflammatory drug; **PPI:** Proton pump inhibitor; **BMI**: Body mass index.

Keywords:

Acid pocket, *Helicobacter pylori*, gastric secretion, gastroesophageal junction, atrophic gastritis.

Word count: 4485

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ABSTRACT

Objective: Gastric acid secretory capacity in different anatomical regions, including the postprandial acid pocket, was assessed in *H. pylori* positive and negative volunteers in a Western population.

Design: We studied 31 *H.pylori* positive and 28 *H.pylori* negative volunteers, matched for age, gender and BMI. Jumbo biopsies were taken at 11 pre-determined locations from the gastroesophageal junction and stomach. Combined high resolution pHmetry (12 sensors) and manometry (36 sensors) was performed for 20 minutes fasted and 90 minutes 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.

Results: Under fasting conditions, the *H.pylori* positives had less intragastric acidity compared to negatives at all sensors >1.1cm distal to the peak lower oesophageal sphincter (LES) pressure ($p<0.01$). Postprandially, intragastric acidity was less in *H.pylori* positives at sensors 2.2, 3.3 and 4.4cm distal to the peak LES pressure ($p<0.05$), but there was 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 to *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.

Conclusion: In population volunteers, *H.pylori* positives have reduced intragastric acidity which most markedly affects the postprandial acid pocket.

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SUMMARY BOX

WHAT IS ALREADY KNOWN?

1. There is a negative association between *H. pylori* infection and both gastroesophageal reflux disease and oesophageal adenocarcinoma.

2. The mechanism of this negative association is unclear but might be related to *H. pylori* reducing gastric acidity.

3. The gastric acid which refluxes into the oesophagus originates from the proximal gastric acid pocket.

NOVEL FINDINGS:

1. In population volunteers, intragastric acidity was less in those with *H. pylori* infection and this was most marked in the proximal stomach close to the gastroesophageal junction.

2. The density of parietal cells and chief cells was reduced in *H. pylori* positives compared to negatives.

3. The reduction in intragastric acidity and severity of inflammation were more marked in CagA positive versus CagA negative *H. pylori* infected subjects.

CLINICAL IMPLICATIONS:

The reduced intragastric acidity close to the gastroesophageal junction in the *H. pylori* infected subjects provides a mechanism for the negative association between the infection and reflux disease and its complications.

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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. [1]

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.[2] 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.[3]

An unexplained observation regarding the infection is its negative association with gastroesophageal reflux disease (GERD) and its complications of Barrett's oesophagus and oesophageal adenocarcinoma, with these disorders being less than half as common in infected subjects. [4, 5] 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.[6] 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

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infection on gastric secretory function in the 90% of infected subjects without these complications.[7]   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 gastroesophageal junction (GEJ), referred to as the acid pocket, which refluxes and causes oesophageal damage.[8] 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.[9]   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.

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

METHODS & MATERIALS

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.

Study design

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 [10] 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).

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).

Biopsy specimen processing

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.[11] 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.

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 (LES), 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 is shown in

Fig **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.

Equipment

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).

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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).

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.

Data analysis

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.

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 (TLESRs). 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 LES pressure was taken as

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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.

Histopathological Assessment

A. Studies using Conventional H&E:

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 [12].   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.

B. 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.

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Quantification of Secretory Cells:

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.

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.

Ethics

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

RESULTS

Of the 137 subjects assessed for eligibility for the study, 49 were excluded due to current or recent use of proton pump inhibitor (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). 88 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 body mass index (BMI) (Fig. S1).

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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/m^2; *p*=0.72). There were 7 current smokers in the *H.pylori* positive group compared to 1 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 to 0 (range 0-3) for the *H.pylori* negative subjects (*p*=0.002).   17/31 (54.8%) of the *H.pylori* positives were taking no medication compared to 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, 4 *H.pylori* positive subjects had a hiatus hernia (2-4cm in length), 1 subject had LA Grade A reflux esophagitis, and one subject had 3cm of Barrett's mucosa.   None of the *H.pylori* negatives had a hiatus hernia, although two subjects had reflux esophagitis (LA grade A and B).

Gastroesophageal Acidity

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 LES pressure (Table 1). 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 LES 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 SCJ.

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 LES but no significant difference was detected by the more distal sensors placed at 5.5 and 6.6cm distal to this reference point (Table 1).   These three sensors detecting a

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significant difference in gastric acidity between the two groups were those closest to the GEJ with the most proximal of them being only 0.6cm distal to the SCJ (Fig. 2).

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 3cm distal to the peak LES pressure, at the peak LES pressure and also extending 1.1cm above the peak LES pressure (Table 2)**.**

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Table 1. Median (IQR) pH values at sensors relative to peak LES pressure comparing *H.pylori* negative (n=28) and positive (n=31) groups during 20 minute fasting period and three 30 minute postprandial periods..

Sensor location	Fasting				0-30 minutes				30-60 minutes				60-90 minutes		
	HP-	HP+	p value		HP-	HP+	p value		HP-	HP+	p value		HP-	HP+	p value
5cm proximal	7.20 (0.70)	7.19 (0.74)	0.933		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	7.33 (0.78)	7.37 (0.62)	0.525		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 LES pressure	7.34 (0.79)	7.28 (0.51)	0.499		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	7.06 (1.63)	7.13 (0.51)	0.213		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	5.79 (4.26)	6.94 (1.38)	0.004		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	2.27 (2.58)	6.13 (5.06)	<0.001		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	1.70 (1.16)	4.11 (4.95)	<0.001		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	1.68 (0.66)	2.88 (3.66)	<0.001		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	1.62 (3.66)	2.39 (3.06)	0.003		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

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Table 2: Median (IQR) percentage time pH<4 at sensors relative to peak LES pressure comparing *H.pylori* negative (n=28) and positive (n=31) groups during 20 minute fasting period and three 30 minute postprandial periods.

Sensor location	Fasting				0-30 minutes				30-60 minutes				60-90 minutes		
	HP-	*HP+*	*p* value		*HP-*	*HP+*	*p* value		*HP-*	*HP+*	*p* value		*HP-*	*HP+*	*p* value
5cm proximal	0.0 (0.0)	0.0 (0.0)	0.271		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	1.1 (2.8)	0.0 (0.1)	0.004		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 LES pressure	1.7 (4.9)	0.0 (1.0)	0.001		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	6.6 (30.6)	1.0 (7.3)	0.008		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	32.1 (65.4)	2.8 (21.7)	0.004		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	75.6 (48.2)	13.5 (75.6)	0.003		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	93.0 (42.3)	42.4 (42.3)	<0.001		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	97.6 (14.0)	60.4 (62.1)	0.001		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	99.5 (4.8)	84.8 (61.6)	0.011		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

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Gastric Histopathology

A.   Conventional H&E Staining

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 3).   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 also 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.   There was minimal evidence of PMN cell infiltrate at any location in the *H.pylori* negatives.

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 also in the biopsies close to the SCJ.   In a further 3 it was limited to the region close to the SCJ.   In 1 subjects 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.

Gastric Gland Height

The height 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 (Table 4).

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Table 3: 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*	

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Table 4. Median (IQR) of glandular thickness and densities of parietal and chief cells at each biopsy location comparing *H.pylori* negatives (n=28) and positives (n=31).

Biopsy location	Glandular Thickness (mm)			Parietal cell density (cells/mm^2)			Chief cell density (cells/mm^2)		
	H.pylori -	*H.pylori* +	*P* value	*H.pylori* -	*H.pylori* +	*P* value	*H.pylori* -	*H.pylori* +	*P* value
Across SCJ, Greater curve	0.30 (0.20–0.30)	0.25 (0.20–0.30)	0.515	67 (0-162)	17 (10-39)	0.185	94 (0-156)	22 (3-52)	0.150
Across SCJ, Lesser curve	0.28 (0.0–0.30)	0.20 (0.10–0.30)	0.461	50 (14-127)	9 (0-51)	0.012	89 (17-139)	22 (0-62)	0.017
6mm distal SCJ	0.35 (0.30–0.40)	0.30 (0.20–0.30)	0.006	231 (175-286)	144 (59-190)	<0.001	245 (203-272)	129 (52-190)	<0.001
12mm distal SCJ	0.40 (0.40–0.45)	0.30 (0.30–0.35)	<0.001	317 (300-362)	193 (137-250)	<0.001	379 (312-404)	206 (125-299)	<0.001
18mm distal SCJ	0.45 (0.40–0.50)	0.35 (0.30–0.40)	<0.001	357 (334-383)	241 (201-283)	<0.001	404 (374-421)	273 (194-353)	<0.001
Fundus	0.43 (0.40–0.45)	0.40 (0.35–0.40)	0.008	347 (285-401)	258 (220-292)	<0.001	421 (384-451)	310 (255-389)	<0.001
Mid-body, Lesser curve	0.40 (0.40–0.45)	0.35 (0.30–0.40)	<0.001	361 (316-381)	235 (166-290)	<0.001	401 (367-419)	285 (206-367)	<0.001
Mid-body, Greater curve	0.45 (0.40–0.45)	0.35 (0.30–0.40)	<0.001	356 (318-398)	250 (201-297)	<0.001	420 (372-441)	305 (243-354)	<0.001
Distal body, Greater curve	0.40 (0.35–0.49)	0.30 (0.25–0.35)	<0.001	322 (293-349)	107 (25-263)	<0.001	365 (296-398)	136 (17-292)	<0.001
Incisura Angularis	0.33 (0.30–0.40)	0.25 (0.20–0.30)	<0.001	203 (124-250)	12 (0-87)	<0.001	215 (98-296)	7 (0-99)	<0.001
Antrum	0.20 (0.13–0.30)	0.20 (0.0–0.20)	0.041	40 (6-67)	7 (0-18)	0.002	22 (1-85)	0 (0-5)	<0.001

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B.   Immunohistochemistry

Parietal and Chief Cell Density:

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 4). The degree of reduction was similar for the two cell types.

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. 3).   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. 3).

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].

Intragastric Acidity and Histology in CagA Positive *H.pylori* Subjects

Seventeen of the *H.pylori* positives were CagA seropositive and fourteen CagA seronegative. The associations with reduced intragastric acidity in comparison with *H.pylori* negatives was more apparent for the CagA positives being significant for five of the six intragastric sites both fasting and after the meal in the CagA positives but in only two of the six intragastric sites for the CagA negatives and only under fasting conditions (Table 1 – supplement). There was a statistically significant difference between the CagA negative and

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CagA positives for only two of the six sites during fasting and one of the six sites after the meal.

The CagA positives had a significantly greater combined inflammatory cell infiltrate evident at three of the eleven biopsy locations (6mm and 18mm distal SCJ, and distal body greater curve), compared to the CagA negatives (Table 2 – supplement). The reduction in parietal and chief cell density was significant at each intragastric location for both CagA positive and negative subjects with no apparent difference between these two groups.

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.[13,14,15] This phenomenon has been called the acid pocket and is thought to be important in GERD induced oesophageal damage after a meal when reflux is most common.

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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 [16] and this was slightly increased close to the GEJ and also 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 gastroesophageal 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 TLESRs are most frequent. [17]

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.[18] We did not measure the secretion of pepsin and other digestive enzymes produced by the chief cells but

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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.[19] 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. [20]

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). [21-24] 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. [25]

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. [26] 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.[27] 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.[28] In 1998, our own group reported a reduced acid secretory response to gastrin stimulation in 20

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H.pylori positive versus 24 *H.pylori* negative healthy volunteers.[29]   Several studies in the Japanese population have reported reduced gastric secretory function in *H.pylori* positive healthy volunteers.[30,31]

Our current study differs from previously published studies in a number of 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 gastroesophageal acid reflux.[32]   In the light of our current study, the observed increase in gastroesophageal 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.[33] 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.[34]   This would suggest that our findings of reduced acid secretory function is representative of what is happening in Western countries.

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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.

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FIGURE LEGENDS

Fig 1. Schematic diagram of the relative positions of the 12 sensor pH catheter, 36 sensor manometer and SCJ (identified by attached metal clip)

Fig 2. Median pH for 0-30 minute period after meal relative to LES and SCJ in *H.pylori* positive (HP+) and negative (HP-) subjects

Fig 3. 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; **JL2**: 6mm distal SCJ; **JL3**: 12mm distal SCJ; **JL4**: 18mm distal SCJ; **BG3**: Fundus; **BL**: mid-body lesser curve; **BG2**: mid-body greater curve; **BG1**: distal body greater curve; **IA**: incisura angularis; **Ant**: antrum.

Supplement Fig 1. Flow diagram showing progress of study participants through each stage

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AUTHOR DISCLOSURES:
None to declare.

AUTHOR CONTRIBUTIONS:

DRM: Clinical investigations, manometry, pHmetry, data analysis and drafting manuscript.

MHD: Histological assessment, biopsy orientation, data analysis and drafting manuscript.

AAW: Recruitment of volunteers and assisting clinical investigations.

CO: Technical assistance in histology & scanning of histological slides.

SAB: Radiological assessment.

JJG: Histological assessment and drafting manuscript.

KELM: Conception of original idea, drafting manuscript and overall supervision.

Fig 1. Schematic diagram of the relative positions of the 12 sensor pH catheter, 36 sensor manometer and SCJ (identified by attached metal clip)

175x224mm (300 x 300 DPI)

Fig 2. Median pH for 0-30 minute period after meal relative to LES and SCJ in H.pylori positive (HP+) and negative (HP-) subjects

228x176mm (300 x 300 DPI)

Fig 3. 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; JL2: 6mm distal SCJ; JL3: 12mm distal SCJ; JL4: 18mm distal SCJ; BG3: Fundus; BL: mid-body lesser curve; BG2: mid-body greater curve; BG1: distal body greater curve; IA: incisura angularis; Ant: antrum.

254x190mm (300 x 300 DPI)

Table S1: 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 LES 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)

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Table S2: 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	