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MODULATION OF THE POSTPRANDIAL ACID AND BILE POCKETS AT THE GASTRO-OESOPHAGEAL JUNCTION BY DRUGS THAT AFFECT GASTRIC MOTILITY

Pharmacological influences on acid and bile pockets

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Running head: acid pocket and motility drugs

ABSTRACT

Objectives: Previous studies have established the presence of a postprandial acid pocket at the gastro-oesophageal junction (GOJ). We investigated whether altering gastric motility would affect the location and the extent of postprandial acid pockets in healthy volunteers. In addition, we studied the presence of bile in this pocket.

Methods: 16 healthy volunteers underwent pH and Bilitec® probe stepwise pull-through to measure regional differences in pH and bile absorbance before and 10, 30 and 50 min after a liquid meal. At the start of the meal, saline, erythromycin or sumatriptan was administered.

Results: After saline, ingestion of a meal induced an acid pocket, with a mean pH drop of 2.26 (compared to 0.25 before the meal, $p < 0.05$) and a nadir pH of 2.71. The acid pocket persisted 30 and 50 minutes postprandially (pH drops 1.59 and 1.68 and nadir pH 3.17 and 2.52 respectively). Compared to saline, erythromycin significantly suppressed the pH drop and nadir pH (on average 0.66 and 3.4 at 10 min; comparable patterns at 30 and 50 min). After sumatriptan a significantly lower nadir pH was observed at 30 and 50 min (respectively 2.02 and 1.74, $p < 0.05$ compared to saline). A postprandial bile pocket was noted in 50% of pull-throughs after saline, compared to 78% after erythromycin and 31% after sumatriptan.

Conclusions: The presence of a bile pocket in a subset of the subjects confirms the heterogeneity of postprandial intragastric contents. Erythromycin disrupts the acid pocket but increases the presence of bile, while sumatriptan has opposite effects.

INTRODUCTION

Gastro-oesophageal reflux disease (GORD) is present when the reflux of gastric contents causes typical lesions or symptoms such as heartburn, regurgitation and noncardiac chest pain (1). GERD is one of the most common disorders affecting the gastrointestinal tract (2). Although acid reflux is classically accepted to be the cause of these symptoms, the relationship between acid and heartburn is far from clear (3). While many GORD patients achieve symptom relief and healing of endoscopic lesions with proton pump inhibitors (PPIs), others have persistent reflux symptoms despite PPI therapy (3-5). These remaining symptoms are due to either acid or nonacid gastro-oesophageal reflux episodes or are not related to reflux at all (6,7).

Simultaneous pH and Bilitec® monitoring is a valuable tool for characterizing the composition of the refluxate (5,8). In previous studies combined pH and Bilitec® monitoring has been used to establish that reflux often consists of a mixture of acid and bile (8-10). In addition, duodenogastric reflux, also referred to as bile reflux, has been implicated in the pathogenesis of oesophageal lesions in GORD (10) and in the generation of symptoms that persist in spite of PPI therapy (5).

Fletcher et al described a postprandial highly acidic unbuffered gastric juice or acid pocket at the gastro-oesophageal junction (GOJ) (11). Postprandial exposure of the most distal oesophagus to acid has been implicated in the pathogenesis of GERD lesions in the distal esophagus (11,12). More recent imaging studies have confirmed the presence of a liquid acid pocket, near the junction, and have shown its position relative to the squamocolumnar junction represents a major risk factor for the occurrence of acidic reflux into the distal oesophagus in healthy subjects and GORD patients (13,14). Conversely, changing the extent or the position of the acid pocket relative to the gastro-oesophageal junction might be relevant for GORD treatment. As outlined above, duodenogastric reflux is an additional important factor in the pathogenesis of GORD (5,8-10), but whether bile is also present in the acid pocket just distal to the gastro-oesophageal squamocolumnar junction still remains unclear.

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2 The aims of this study therefore were to investigate 1) whether the postprandial acid
3 pocket is associated with a bile pocket in healthy volunteers and 2) whether altering
4 gastric motility would affect the location and the extent of postprandial acid and bile
5 pockets in healthy volunteers.
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For Peer Review

MATERIALS AND METHODS

Subjects

The study was performed in 16 healthy volunteers (HV) (9 women and 7 men, mean age 27.8 (range 21-39)). All HV were free of any gastrointestinal symptoms and none of them had a history of reflux disease or surgery to the upper gastrointestinal tract or thorax. Volunteers were asked not to smoke on the morning of the test and not to eat at least 12 hours prior to the test. None of them took any medication. Exclusion criteria were peptic ulcer disease; previous oesophageal, gastric or biliary surgery; previous abdominal or thoracic radiotherapy; active gastrointestinal bleeding; presence of oesophageal or fundic varices; oesophageal or upper small intestinal Crohn's disease; infectious oesophagitis or active neoplastic disease. All volunteers gave written informed consent for the study, which was approved by the Ethical Committee of the hospital.

Study protocol

The volunteers underwent acid and bile pocket studies on 3 different occasions, at least one week apart. Probes for assessing acid and duodeno-gastro-oesophageal reflux were introduced via the mouth into the oesophagus 5cm proximal to the lower oesophageal sphincter (LOS), defined by preceding pull-through manometry. Combined pH and Bilitec® monitoring was performed by stepwise pull-through before and 10, 30 and 50 minutes after a liquid meal (200 ml Nutridrink®, 300 kcal). At the start of the meal, placebo (saline 100 ml IV), the gastrokinetic erythromycin (Abbott, 200 mg IV in 100 ml of saline) or the gastric relaxant sumatriptan (GlaxoSmithKline, 6 mg SC) was administered (15-18) in a random fashion, with the sequence determined by drawing envelopes. The setup of the study protocol is shown in Figure 1.

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pH and Bilitec® monitoring

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2 A combined pH and Bilitec® monitoring was performed using a two-channel
3 antimony pH catheter with separate skin reference electrode attached to a fiberoptic
4 spectrophotometer Bilitec® 2000 (Synectics Medical, Stockholm, Sweden). Before
5 each study, the pH probe was calibrated in buffer solutions of pH 1.0 and 7.0 while
6 Bilitec® probe was calibrated in water (5,9,10). A stepwise pull-through was
7 performed pulling back 1cm every minute from 10 cm below the LOS until 5 cm
8 above the LOS. Pull-through was performed pre- and postprandially (10, 30 and 50
9 minutes after drinking a liquid meal of 200 ml, ie 300 kcal) in semi-recumbent
10 position.
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16 Data analysis

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20 Analysis was performed on average preprandial and postprandial pH and bilirubin
21 absorbances in the gastric body. In the range of 10 cm below to 5 cm above the
22 LOS, we studied the presence and persistence of an acid pocket at 10, 30 and 50
23 minutes postprandially.
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26 Intragastric pH was defined as the mean pH between 10 cm below and 5 cm above
27 the LOS. Delta pH was defined as the difference between the intragastric pH and the
28 nadir pH, with nadir pH being the lowest pH between 2 cm below and 2 cm above the
29 LOS (ie the lowest pH at the level of LOS). The presence of an acid pocket was
30 defined as a segmentary (≥ 4 cm) drop in pH of more than one unit compared to
31 intragastric pH. The extent of the acid pocket was defined as the distance between
32 the upper and lower limit of the acid pocket, and the latter were expressed as the
33 distance from the LOS where the presence of the pH-drop started and ended. We
34 also studied the presence of a "bile pocket" defined as a segmentary rise (≥ 4 cm) in
35 Bilitec® absorbance ≥ 0.14 . This cut-off level is the one used in esophageal bilirubin
36 absorbance studies and has also been applied to intragastric measurements (11,15).
37 Although in vitro studies showed that fiberoptic bilirubin measurements are influenced
38 by an acidic pH, the impact in in vivo measurements seems negligible (19,20).
39 Statistical analysis was performed using Student's *t* test.
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RESULTS

Acid pocket

In the placebo studies, preprandial (T0) intragastric pH was significantly lower than the oesophageal pH (1.62 ± 0.39 versus 4.85 ± 0.59 , $p < 0.0001$). Ingestion of a meal caused a buffering of intra-gastric pH at 10, 30 and 50 min (Table 1 and 2). Ingestion of the meal was also associated with the appearance of an acid pocket at 10, 30 and 50 minutes postprandially (Figure 2). The extent of the acid pocket is summarized in Table 3 and was generally between 2 cm below and 3 cm above the GOJ.

Significant ($p < 0.05$) differences in delta pH (mean 2.26 versus 0.25 before meal) and nadir pH (mean 2.71 versus 1.26 before meal) were present between LOS -2 and +2cm, 10 minutes after the meal. A similar pattern was found after 30 and 50 minutes with respectively mean delta pH 1.59 and 1.68 and nadir pH 3.17 and 2.52 (Figure 2).

“Bile” pocket

Preprandially the averaged Bilitec® pull-throughs did not show a significant variance in the proximal stomach and bilirubin absorbance values were low. Postprandially, the intragastric bile content was significantly elevated compared to preprandially (Table 4). An example of a bile pocket is shown in Figure 3. A postprandial bile pocket was noted in 50% of pull-throughs in the saline group after 50 minutes (Figure 4).

Influence of erythromycin

The intragastric pH at 50 minutes was significantly higher after erythromycin compared to saline (4.19 ± 2.01 (SEM 0.50) versus 5.40 ± 1.15 (SEM 0.30), $p < 0.05$). Compared to saline, the pH at the LOS after the meal was significantly higher after erythromycin. No obvious postprandial acid pocket was observed in most of the subjects at the LOS after erythromycin. There was a significant suppression of the

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2 delta pH (mean 0.66) and nadir pH (mean 3.43) after 10 minutes, and comparable
3 patterns at 30 and 50 minutes (Figure 2). These values indicate suppression or
4 elimination of the acid pocket in most of the patients, but when still present, the
5 extent of the acid pocket after erythromycin did not seem to differ significantly (Table
6 3).
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10 After erythromycin, peak bilirubin absorbance was significantly higher after 30 and 50
11 minutes in comparison to placebo (Table 4). Analyses of individual postprandial
12 tracings on 45 successful Bilitec® pull-throughs after erythromycin showed the
13 presence of a bile pocket in 11 subjects (73,3%) after 10 and 30 minutes and in 13
14 subjects (86,7%) after 50 minutes. A postprandial bile pocket was present in 77,8%
15 of pull-throughs after erythromycin, compared to 45,2% in the saline group ($p < 0.005$).
16 (Figure 4). The majority of the bile pockets (55%) coincided with the acid pocket, 27%
17 were situated immediately proximal to the acid pocket and 18% were situated
18 immediately distal to the acid pocket.
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27 **Influence of sumatriptan**

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29 The intragastric pH did not differ significantly after administration of sumatriptan, and
30 the delta pH and nadir pH after 10 minutes was not altered (Tables 1 and 2).
31 However, after 30 and 50 min, nadir pH (respectively 2.02 and 1.74 versus 3.17 and
32 2.52 after saline) was lower compared to saline (Table 2). In addition, sumatriptan
33 tended to increase the extent of the acid pocket at 30 and 50 minutes postprandially
34 (Figure 2), both for the intragastric extent as for the extent above the LES (Table 3).
35 A postprandial bile pocket occurred in 31% of the subjects (Figure 4).
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Discussion

Gastro-oesophageal reflux occurs mainly during the postprandial period through increased triggering of transient lower oesophageal sphincter relaxations (TLOS) (21) and is most pronounced in the distal oesophagus (22). Ingestion of a meal is expected to increase the intragastric pH by the meal's buffering capacity, but it was previously established that a pocket of unbuffered, highly acidic juice is present at the gastro-oesophageal junction (GOJ) after intake of a solid meal in healthy volunteers, a so-called acid pocket (11-14). On the other hand, intragastric Bilitec® studies have shown that duodeno-gastric bile reflux is a physiological postprandial event, which occurs in every healthy volunteer, raising the possibility that bile may also be present at the GOJ in some subjects (23), thereby potentially contributing to the pathogenesis of GORD-related lesions and symptoms. A close correlation exists between acid and duodenogastric reflux exposure of the distal esophagus, and both acid and duodenogastric reflux occurred simultaneously in the majority of the reflux episodes in patients with GORD who were not treated with acid-suppressive therapy, suggesting that both are simultaneously present at the GOJ (8,9,230).

Factors that determine the location and the extent of the acid and bile pockets have not been identified yet, but gastric motility is likely to play a role, as it is a strong determinant of movement and location of gastric contents. This is further supported by observations that gastric motility may play a role in symptom generation in some non-erosive reflux disease (NERD) patients (24). The fiberoptic spectrophotometric probe Bilitec® 2000 was developed to quantify duodeno-gastro-oesophageal reflux by evaluation of bilirubin concentration (25). Owing to its construction, the Bilitec® probe is prone to solid food impaction causing absorbance and measurement artifacts (25). Previously, we have shown that a liquid meal (Nutridrink® 200ml, 300Kcal) does not interfere with Bilitec® measurements (26). The present study established that this meal induces an acid pocket in the stomach in all subjects and a bile pocket in a subset of subjects. After saline administration, a significant drop in pH, indicative of the acid pocket, was present at the gastro-oesophageal junction at all time points studied (10, 30 and 50 minutes after the meal) in spite of the relatively low volume and low caloric contents of the liquid meal we used.

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2 Previously we have shown that the gastrokinetic erythromycin is a strong stimulant of
3 gastric contractility (15,16,27,28). Erythromycin induces faster clearance of
4 duodeno-gastric reflux from the stomach, which depends on enhanced antral
5 contractile activity rather than a premature phase III (23). Erythromycin increases
6 lower oesophageal sphincter pressure in man in the fasting and fed state, but has no
7 effect on oesophageal body peristalsis (29). In the present study, erythromycin
8 rapidly suppressed and eliminated the acid pocket, which was shown numerically as
9 a significant suppression of the delta pH and nadir pH at all time points. Furthermore,
10 the postprandial intragastric pH was significantly elevated and was more rapidly
11 neutralized after administration of erythromycin, which may reflect a better mixing of
12 the gastric acid contents and a more rapid emptying. The lack of simultaneous
13 measurement of gastric contractility in the proximal stomach is a clear limitation of
14 the present experimental set-up, which should be addressed in follow-up studies.
15 However, motility of the proximal stomach cannot be reliably assessed by manometry
16 catheters, and barostat measurements would interfere with the physiological location
17 of the acid pocket. It is conceivable that magnetic resonance imaging may provide
18 the most suitable tool for such studies (13). Acid suppression by erythromycin as a
19 contributing mechanism cannot entirely be excluded, and the effect of erythromycin
20 on gastric acid secretion in man has not been well studied. However, the increased
21 presence of bile at the gastro-oesophageal junction after erythromycin is supportive
22 of a mixing effect rather than an acid suppressive action. A

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34 The 5HT₁ agonist sumatriptan on the other hand, is a strong inhibitor of gastric
35 contractility which reduces fasting fundic tone, prolongs the meal-induced fundic
36 relaxation and significantly delays gastric emptying (17,18,30). In a previous study
37 by our group, we demonstrated that sumatriptan prevented the decay in rate of
38 transient LES relaxations that occurs after a meal and favored the occurrence of
39 gastroesophageal reflux despite increasing LES pressure (31). The sustained
40 postprandial high rate of transient LES relaxations after sumatriptan may be a
41 consequence of a prolonged fundus relaxation and retention of meal in the proximal
42 stomach (17,18). In the current study, a significantly lower nadir pH was observed 30
43 and 50 minutes after sumatriptan administration, suggestive of a longer persistence of
44 the acid pocket.

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2 In addition to confirming the presence of an acid pocket after a liquid meal and its
3 modulation by motility-modifying drugs, we also demonstrated the presence of a bile
4 pocket in a subset of the subjects (Figure 3). Besides confirming the heterogeneous
5 nature of intragastric contents shortly after the meal, this observation is also
6 supportive of potential synergistic actions of acid and bile in the pathogenesis of
7 GORD related distal oesophageal lesions. It remains to be investigated whether this
8 bile pocket is different in patients with severe reflux oesophagitis or Barrett's
9 oesophagus (7,14). The current study is limited in that respect, as only healthy
10 volunteers were included, and future studies should address whether these findings
11 can be confirmed in GERD patients too.

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18 In general, altering the gastric motility affects the location and the extent of
19 postprandial acid and bile pockets in healthy volunteers. We have demonstrated in
20 the present study that enhanced motility by erythromycin disrupt the acid pocket but
21 increases the presence of a bile pocket at the GOJ, while motor inhibition by
22 sumatriptan has opposite effects. These studies confirm that gastric motility
23 modifying drugs could be a target for the treatment of GORD, through their actions on
24 the presence and extent of caustic material at the GOJ.
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32 **ACKNOWLEDGMENTS AND DISCLOSURES**

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37 Leuven to Prof. J. Tack. DD and RV performed the research. JT designed the study.
38
39 VB, KB, RB, DD, JT and RV analysed the data. VB, KB and JT wrote the paper.
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43 Competing Interests: the authors have no competing interests
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For Peer Review

TABLES

Table 1. Mean intra-gastric pH values, before and after the meal, with administration of saline, sumatriptan or erythromycin. * $p < 0.05$ compared to saline. † $p < 0.05$ for sumatriptan compared to erythromycin.

Table 2. Mean intra-oesophageal pH, mean nadir pH in LOS and mean nadir pH in the acid pocket, before and after the meal, with administration of saline, sumatriptan or erythromycin. * $p < 0.05$ compared to saline. † $p < 0.05$ compared to erythromycin.

Table 3. Extent of the acid pocket, after the meal, with administration of saline, sumatriptan or erythromycin. *: only 2 subjects with an acid pocket; **:only 1 subject with an acid pocket

Table 4. Mean bilirubin absorbance in the stomach, the esophagus and the acid pocket, before and after the meal, with administration of saline, sumatriptan or erythromycin. * $p < 0.05$ compared to saline. † $p < 0.05$ compared to erythromycin.

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Figure legends

Figure 1: Design of the study. Probes for assessing acid and duodeno-gastro-oesophageal reflux were introduced via the mouth. The lower oesophageal sphincter (LOS) was defined by preceding pull-through manometry. Dual pH and Bilitec® monitoring was performed by stepwise pull-through before and 10, 30 and 50 minutes after a liquid meal (200 ml Nutridrink®, 300 kcal). At the start of the meal, saline 100 ml, erythromycin 200 mg IV or sumatriptan 6 mg SC was administered.

Figure 2: pH monitoring. Average pH in stomach, LOS and oesophagus is shown in a time dependent fashion (pre-prandial, 10, 30 and 50 minutes postprandial). The X-axis represents the distance (from -10 cm intragastric to 5 cm in the oesophagus), while pH values are depicted on the Y-axis. No differences were noticed pre-prandially. An acid pocket (segmentary $\text{pH} \leq 4$) can be observed at all time points after saline administration (arrows). The acid pocket is absent after erythromycin administration but more pronounced after sumatriptan administration.

Figure 3: Example of a bile pocket. Pull-through profiles with a pH and a Bilitec probe before (upper panel) and after (lower panel) a liquid meal. Note the neutralisation and pH drop (acid pocket, black arrow) at the level of the lower esophageal sphincter in the lower panel pH trace, and the presence of a rise in bilirubin absorbance and an absorbance peak (grey arrow) below the level of the lower esophageal sphincter in the lower panel.

Figure 4: Bilitec monitoring. In healthy volunteers a bile pocket is present at all time points. The number is increased after erythromycin administration but decreased after sumatriptan administration.

TABLES

Table 1. Mean intra-gastric pH values, before and after the meal, with administration of saline, sumatriptan or erythromycin. *p<0.05 compared to saline. ‡ p<0.05 compared to erythromycin. †p<0.05 compared to postprandial values

	mean intra-gastric pH			
	preprandial	10 min	30 min	50 min
Saline	1.51†	4.97	4.77	4.19
Erythromycin	1.81†	4.09	5.17	5.40*
Sumatriptan	1.45†	4.20	3.65‡	3.34‡

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Table 2. Mean intra-oesophageal pH, mean nadir pH in LOS and mean nadir pH in the acid pocket, before and after the meal, with administration of saline, sumatriptan or erythromycin. *p<0.05 compared to saline. ‡ p<0.05 compared to erythromycin.

	mean intra-oesophageal pH			
	preprandial	10 min	30 min	50 min
Saline	4.73	4.61	4.57	4.05
Erythromycin	4.89	4.82	5.22	5.32*
Sumatriptan	5.31	3.94	3.93‡	4.20‡

	mean nadir pH in LOS			
	preprandial	10 min	30 min	50 min
Saline	1.26	2.71	3.17	2.52
Erythromycin	1.58	3.43*	4.20*	4.13*
Sumatriptan	1.54	2.16‡	2.02‡	1.74‡

	mean nadir pH in pocket			
	preprandial	10 min	30 min	50 min
Saline	/	2.09	3.43	1.89
Erythromycin	0.96	3.66*	4.24	2.84
Sumatriptan	0.96	2.06‡	1.53*‡	1.76

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Table 3. Extent of the acid pocket, after the meal, with administration of saline, sumatriptan or erythromycin. *: only 2 subjects with an acid pocket; **:only 1 subject with an acid pocket

		Average pH AP	Upper limit (cm)	Lower limit (cm)	Average extent (cm)
Saline	10 min	3,2	3,2	-2,4	5,6
	30 min	3,5	2,8	-1,4	4,2
	50 min	3,4	3,6	-2,4	6,0
Erythromycin	10 min	-	-	-	-
	30 min*	4,9	2,0	-3,5	5,5
	50 min**	4,6	3,0	-3,0	6,0
Sumatriptan	10 min	2,8	3,5	-3,3	6,8
	30 min	2,7	2,3	-3,3	5,5
	50 min	3,1	4,0	-2,3	6,3

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Table 4. Mean bilirubin absorbance in the stomach, the esophagus and the acid pocket, before and after the meal, with administration of saline, sumatriptan or erythromycin. *p<0.05 compared to saline. † p<0.05 compared to erythromycin.

mean intra-gastric bilirubin absorbance				
	preprandial	10 min	30 min	50 min
Saline	0.13	0.17	0.11	0.25
Erythromycin	0.08	0.10	0.16	0.28*
Sumatriptan	0.04	0.06†	0.13	0.11†

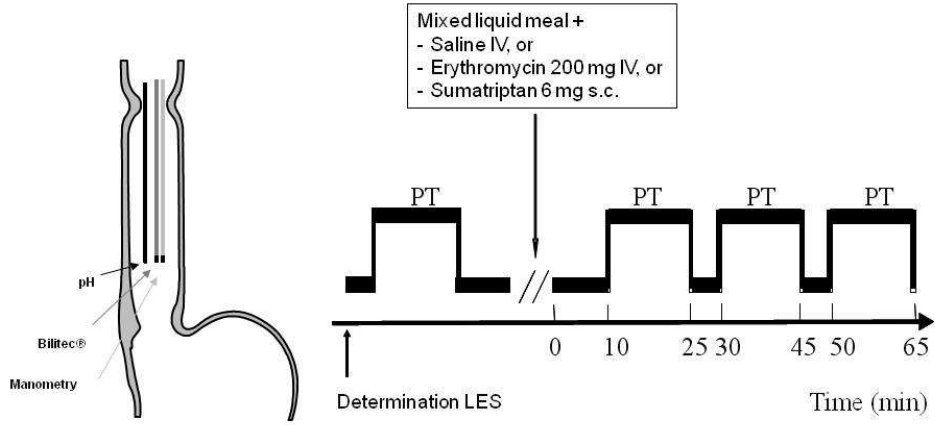
mean intra-oesophageal bilirubin absorbance				
	preprandial	10 min	30 min	50 min
Saline	0.09	0.13	0.14	0.15
Erythromycin	0.09	0.16	0.19	0.19*
Sumatriptan	0.06	0.10†	0.12†	0.14

peak bilirubin absorbance in pocket				
	preprandial	10 min	30 min	50 min
Saline	0.31	0.22	0.19	0.21
Erythromycin	/	0.25	0.30*	0.37*
Sumatriptan	/	0.18†	0.19†	0.19†

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Figure 1

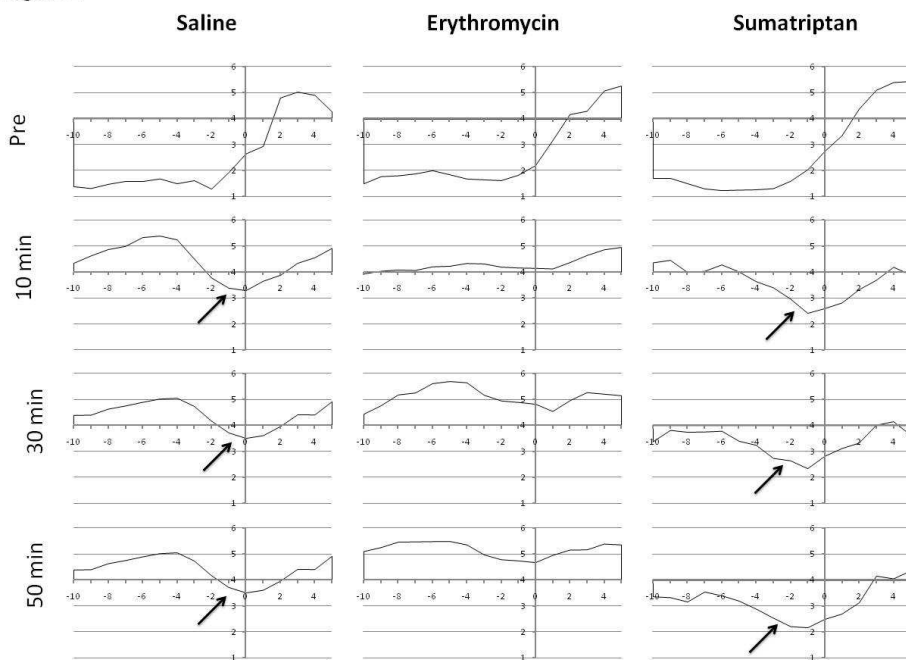


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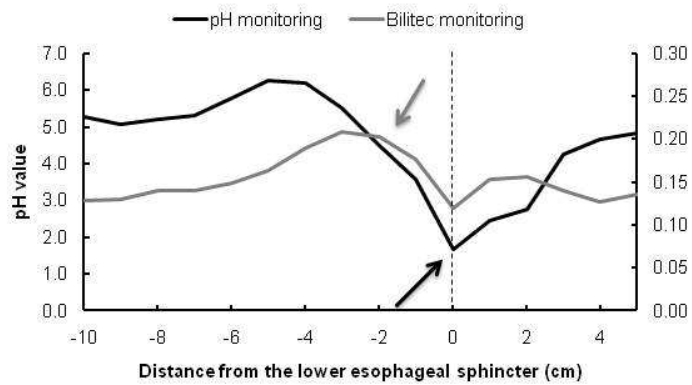
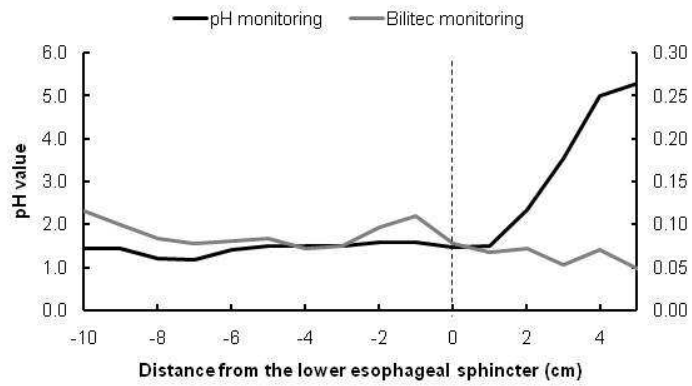
Figure 2



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Figure 3

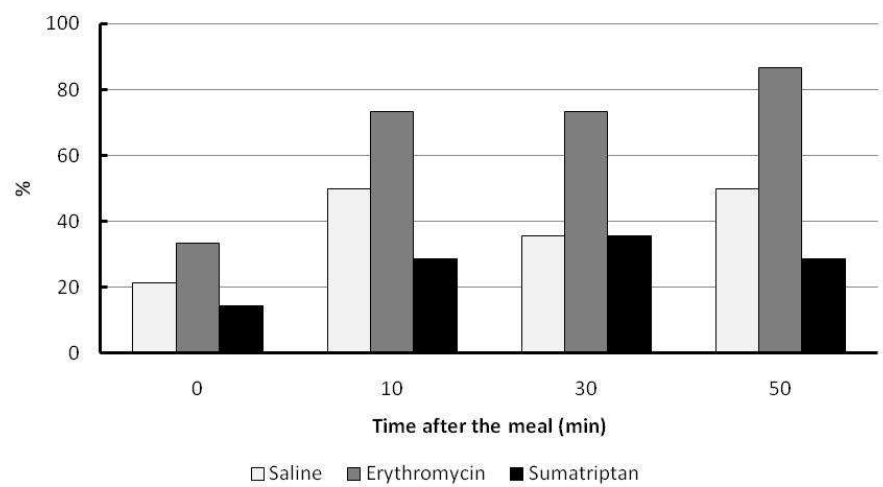


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Figure 4



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Review

REVIEWERS' COMMENTS TO AUTHOR:

Reviewer: 1

Comments for Transmission to the Authors I am unclear if the order of drugs/placebo was randomized (and by what method of randomization)? Or if not, how was this done in an unbiased fashion?

The order of the drugs and placebo was randomized, by drawing envelopes. This has been added in the method section of the manuscript, with some additional references.

The cut-off to define a bile acid pocket seems arbitrary - what was this based upon?

The cut-of value is based on previous literature evaluating both intra-esophageal and intra-gastric bilirubin monitoring. Although Bilitec® monitoring has been reported to underestimate bilirubin concentrations in an acid environment, this seems limited in an in vivo setting. This has been added to the methods section. The anatomical location was similar to the one for the acid pocket.

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Reviewer: 2

Comments for Transmission to the Authors Boecxstaens et al. assess the effects of erythromycin, a prokinetic, and sumatriptan, an antikinetic, on the composition of the "acid / bile pocket" present at the GOJ in healthy volunteers. The acid / bile pocket almost certainly represents the layer of gastric secretions that collects on the surface of the meal.¹ Anatomical changes and / or increased secretion in reflux patients has been shown to increase the size and acidity of this "pocket" and this is thought to promote reflux in this group.²⁻⁴

The paper is well written and concise. The inclusion only of healthy volunteers impacts on the potential relevance of this work.

Studies regarding the postprandial acid pocket in patients with gastro-oesophageal reflux disease are currently ongoing in our centre to assess the characteristics of the acid pocket in these patient group.

The method focuses exclusively on the acid/bile pocket and does not attempt to explain the mechanism of effect.

This study is a proof-of-concept study to establish that altering the location and extent of the acid pocket can be achieved by stimulating or inhibiting gastric motility. We think the findings confirm that such an effect was observed. We agree that we did not simultaneously assess gastric motility, and that this is a limitation. On the other hand, the effects of erythromycin and of sumatriptan on gastric motility have been extensively documented in the literature and are remarkably reproducible. This has been added to the manuscript, with some additional references, as suggested by the reviewer..

This study shows that the prokinetic increases gastric emptying and mixing of the acid layer with the meal and duodenal secretions so neutralising the acid. Conversely the antikinetic slows emptying and decreases gastric mixing with the opposite effect. These are novel observations; however the proposed mechanism of effect is not documented.

This study is a proof-of-concept study to establish that altering the location and extent of the acid pocket can be achieved by stimulating or inhibiting gastric motility. We think the findings confirm that such an effect was observed. We agree that we did not simultaneously assess gastric motility, and that this is a limitation. On the other hand, the effects of erythromycin and of sumatriptan on gastric motility have been extensively documented in the literature and are remarkably reproducible. This has been added to the manuscript, with some additional references, as suggested by the reviewer.

In the absence of patient data the clinical relevance of these findings is difficult to assess.

Studies regarding the postprandial acid pocket in patients with gastro-oesophageal reflux disease are currently ongoing in our centre to assess the characteristics of the acid pocket in these patient group. We have acknowledged this limitation in the discussion section.

1. Goetze O, Treier R, Fox M, Steingoetter A, Fried M, Boesiger P, et al. The effect of gastric secretion on gastric physiology and emptying in the fasted and fed state assessed by magnetic resonance imaging. *Neurogastroenterol Motil* 2009;21(7):725-42.

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3 Added to article.
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5 2. Fletcher J, Wirz A, Young J, Vallance R, McColl KE. Unbuffered highly
6 acidic gastric juice exists at the gastroesophageal junction after a meal.
7 *Gastroenterology* 2001;121(4):775-83.
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9 3. Fletcher J, Wirz A, Henry E, McColl KE. Studies of acid exposure
10 immediately above the gastro-oesophageal squamocolumnar junction: evidence of
11 short segment reflux. *Gut* 2004;53(2):168-73.
12

13 Added to article.
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15 4. Beaumont H, Bennink RJ, de Jong J, Boeckstaens GE. The position of the
16 acid pocket as a major risk factor for acidic reflux in healthy subjects and
17 patients with GORD. *Gut* 2010;59(4):441-51.
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Reviewer: 3
Comments for Transmission to the Authors: none

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