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A study of pathophysiological factors associated with gastro-esophageal reflux disease in twins discordant for gastro-esophageal reflux symptoms

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Abstract

Background *Differences in lower esophageal sphincter (LES) and peristaltic function and in transient LES relaxations (TLESR) have been described in patients with gastro-esophageal reflux disease (GERD). However, some of these differences may be the result of chronic GERD rather than being an underlying contributory factor.* ***Methods*** *Twins discordant for GERD symptoms, i.e., only one twin had GERD symptoms, underwent standard LES and esophageal body manometry, and then using a sleeve sensor prolonged LES and pH monitoring, 30 min before and 60 min after a 250 mL 1200 kcal lipid meal.* ***Key Results*** *Eight monozygotic and 24 dizygotic female twins were studied. Although there was no difference in preprandial LES pressure (symptomatic 13.2 ± 7.1 mmHg vs asymptomatic 15.1 ± 6.2 mmHg, P = 0.4), LES pressure fell further postprandially in symptomatic twins (LES pressure area under the curve 465 ± 126 vs 331 ± 141 mmHg h, P < 0.01). 12/37 (32%) of acid reflux episodes in symptomatic twins occurred due to low LES pressure or deep inspiration/strain and 0/17 in asymptomatic twins (P = 0.01). There was no difference between symptomatic and asymptomatic twins in: peristaltic amplitude, ineffective esophageal body motility, hiatus hernia prevalence, or LES length. There was also no difference in TLESR frequency preprandially (symptomatic median 1(range 0–2)* vs *asymptomatic 0(0–2), P = 0.08) or postprandially (2.5(1–8)* vs *3(1–6), P = 0.81).* ***Conclusions & Inferences*** *Twins with GERD symptoms had lower postprandial LES pressure and given the close genetic link between the twins, it is possible that such differences are caused by GERD. Acid reflux episodes associated with a hypotensive LES were seen in symptomatic, but not in asymptomatic twins.*

Keywords *esophagus, gastro-esophageal reflux, lower esophageal sphincter, transient lower esophageal sphincter relaxations, twins.*

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INTRODUCTION

Gastro-esophageal reflux disease (GERD) develops when the reflux of gastric contents into the esophagus causes troublesome symptoms or complications.[1] The pathophysiology of GERD is multifactorial, but all patients with GERD have an increased frequency of episodes of acid reflux and transient lower esophageal sphincter relaxation (TLESR) is the most frequent mechanism associated with gastro-esophageal reflux episodes and essentially the only operant mechanism during periods of normal LES pressure (>10 mmHg).[2,3] Following acid reflux, its potential to damage the esophagus depends on how quickly the refluxate is cleared back into the stomach. Esophageal acid clearance in GERD is delayed in the presence of peristaltic dysfunction of the esophageal body[4] and of a hiatus hernia.[5,6]

However, it is not clear whether these pathophysiological factors cause or perpetuate GERD or are a consequence acid gastro-esophageal reflux. For example, esophageal shortening has been demonstrated in animal experiments in response to acid instillation into the esophagus and hiatus hernia may therefore potentially be the result of chronic gastro-esophageal

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reflux rather than an initiating cause.[7] Furthermore, following acid instillation into the esophagus in cats, basal LES pressure falls and peristalsis is impaired, suggesting that acid reflux may potentially also be the cause of these putative pathophysiological factors.[8]

Recent evidence suggests genetic factors may account for a considerable part of the variation in liability to GERD. Families with multiple members affected by symptomatic, endoscopic or complicated GERD have been described.[9,10] Two case-control studies have revealed aggregation of GERD symptoms in the families of patients with GERD.[11,12] Finally, a study examining GERD symptoms in twins suggested that up to 43% of the variation in liability to GERD symptoms related to genetic factors.[13]

The study of twin pairs, in which only one twin is affected by a particular condition (i.e., discordant twins), is a powerful tool for detecting risks of small individual effect with small samples.[14] As monozygotic (MZ) twins are genetically identical and dizygotic (DZ) twins share ~50% of their segregating genes, if the pathophysiological abnormalities associated with GERD contribute to its development, affected and unaffected twins should exhibit them. If the pathophysiological abnormalities are caused by GERD, only the affected twins will exhibit them. Pathophysiological factors associated with GERD were therefore studied in MZ and DZ twin pairs discordant for GERD symptoms.

METHODS

Subjects

Monozygotic and DZ twin pairs who were discordant for GERD symptoms in our original study of 4480 twin pairs[13] were asked 3 years later to complete a short questionnaire to ascertain whether they were still discordant for GERD symptoms. The zygosity of the twins was established using a standardized questionnaire and genotyping in cases of uncertainty.[15]

GERD symptoms questionnaire

A questionnaire assessed GERD symptoms within the previous 12 months. The questionnaire is a shortened version of a questionnaire utilized in a community study of GERD symptoms[16] and includes eight questions on heartburn, acid regurgitation and antacid consumption, their frequencies and current drug consumption. It has been validated in 100 community subjects with good validity for a diagnosis of GERD symptoms against an interview (κ 0.68) and a good reliability on re-test 4 weeks later in the 60 subjects who completed a second questionnaire (κ 0.61). GERD was defined as at least weekly symptoms of heartburn or acid regurgitation. Twin pairs were considered discordant if one twin had at least weekly symptoms of heartburn or acid regurgitation and the co-twin had no GERD symptoms and had not taken antacid or other medications for GERD in the last 12 months.

Esophageal manometry and pH recordings

To aid tolerability, a miniaturized, multi-lumen, perfused assembly was utilized, comprising a 6-cm reverse perfused sleeve sensor, a gastric port 2 cm distal to the sleeve, and esophageal body ports 6, 11 and 16 cm proximal to the sleeve, with an outer diameter of 2.5 mm (Dentsleeve, Adelaide, Australia). All lumens were perfused with distilled water, at 0.5 mL min^{-1} using flow rate resistors, by a low-compliance pneumohydraulic capillary perfusion pump (Arndorfer Medical Specialties, Greendale, WI, USA). Manometric signals were received by external pressure transducers (Dentsleeve), which were calibrated prior to each study using a sphygmomanometer. Esophageal pH was monitored using a monocrystant antimony pH catheter (Synectics, Stockholm, Sweden), which was calibrated, prior to and after each study, with buffers of pH 1 and pH 7. The external diameter of the pH catheter was 2.3 mm. A submental electromyogram (EMG) was recorded using two bipolar cutaneous electrodes positioned under the chin.

Electromyogram signals, esophageal body, LES, and gastric pressures and pH signals were transmitted via a seven channel MPR-2 recorder (Gaeltec, Isle of Skye, UK) to a personal computer and recorded at 8 HZ using dedicated software (Gaeltec).

Protocol

Subjects who remained discordant on the second questionnaire were invited by post to take part in the study. They were asked to discontinue all drugs known to effect gastrointestinal function. Proton pump inhibitors were discontinued for 7 days and H_2-receptor antagonists and prokinetics agents for 48 h before the study. Antacids were allowed up to 12 h before the study. Subjects were asked to refrain from smoking and alcohol for 12 h prior to the study. Subjects attended the Oesophageal Laboratory at St Thomas' Hospital after a 4-hour fast. Following informed consent, the nasal and oropharyngeal mucosa were anaesthetized with 1% xylocaine spray (Astra Pharmaceuticals, King's Langley, UK) to facilitate intubation. The manometric catheter was then passed into the stomach via the nose with the subject sitting upright. While the subjects lay supine, the distance from the nares to the upper border of the LES was measured using the station pull-through technique.[17,18] The perfused sleeve assembly was then placed so that the midpoint of the sleeve sensor lay 1 cm below the upper border of the LES. Following accommodation to the catheter, 10 swallows of 5 mL of water were then used to assess peristaltic function. Subjects were then asked to sit upright in a chair. Finally, the pH catheter was inserted to lie 5 cm proximal to the upper border of the LES.

Preprandial manometric and pH recordings were undertaken for 30 min. Participants were then asked to drink a 1200 kcal, 250 mL long-chain triglyceride meal at 37 °C (Calogen, Scientific Hospital Supplies, Liverpool, UK) over 5 min. Manometric and pH recordings were continued for a further 60 min.

Data analysis

All data analysis was undertaken by two of the investigators (PI and NT), who were blinded to the symptom status of the twins until after the analysis had been completed. All TLESR and acid reflux events were reviewed and a consensus reached on whether

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the TLESR met study criteria and what the mechanism associated with the acid reflux event was.

Peristaltic function Ten wet swallows were analyzed for each subject. The mean proximal, mid and distal esophageal peristaltic amplitude and the percentage of subjects with ineffective esophageal motility [IOM; $\geq$ 30% low-amplitude (<30 mmHg) or non-transmitted contractions in the distal esophagus during 10 wet swallows][19] were calculated. A swallow was defined for TLESR and reflux mechanism analysis as an EMG complex of at least 0.05 mV followed by a sequentially propagated esophageal pressure wave of at least 10 mmHg amplitude.

LES pressure End-expiratory sleeve pressures were analyzed during pre- and postprandial periods. Recordings in the 30 s following a swallow and in the 15 s before and after a gastric pressure rise to more than 30 mmHg [such as is normally seen with late phase 2 and phase 3 of the migrating motor complex[20]] were excluded from the analysis. All end-expiratory sleeve readings were referenced to gastric pressure and mean values for each 10-minute study period were used to assess changes in LES pressure following the meal. LES pressure area under the curve (AUC) was subsequently calculated for the 60 min postprandial period.

Intra-gastric pressure End-expiratory intra-gastric pressures were measured for 1 min during the preprandial period, in the absence of gastric pressure rises due to the migrating motor complex as described above, and at five end-expiratory time points during the postprandial period and analyzed to produce mean values for pre- and post-prandial periods. As gastric pressure values were recorded in the upright rather than supine position during the study, they were higher than published gastric pressure values.

Hiatus hernia A hiatus hernia was diagnosed when the characteristic double high pressure zone, due to the separation of pressure zones produced by the diaphragm and LES, was demonstrated on station pull-through.[21]

TLESR Transient lower esophageal sphincter relaxations were defined as a fall of at least 3 mmHg in LES pressure to within 2 mmHg of gastric pressure, at a rate of at least 1 mmHg s^{-1}, reaching a nadir within 10 s of onset, with no EMG signal 4 s before and 2 s after the onset of relaxation.[22] Swallow-associated prolonged LES relaxations lasting at least 10 s in the absence of multiple swallows were also considered to be TLESR.[22] Transient lower esophageal sphincter relaxations were also analyzed for acid reflux and an esophageal common cavity. An esophageal common cavity was defined as an abrupt increase in esophageal pressure to gastric pressure in at least two distal esophageal recording sites.[23]

Acid reflux episodes Acid reflux episodes were defined as an abrupt fall of at least one pH unit to less than pH 4 for at least 4 s, or, if the pH was already <4, by a further abrupt fall of >1 pH unit for at least 4 s.

Mechanisms of acid reflux were defined by their association with characteristic manometric patterns as follows: (i) TLESR-related—reflux during a TLESR; (ii) hypotensive LES—reflux associated with persistently low LES pressure or slow drifts in LES pressure to 3 mm Hg or less that failed to reach the criteria for TLESR; (iii) swallow-related—reflux during the LES relaxation associated with a propagated or failed primary peristaltic sequence or multiple swallows; and (iv) strain/deep inspiration-related—reflux occurring during straining defined as a sharp increase in esophageal and gastric pressure, the latter more than twice the normal increase in gastric pressure with respiration, or during deep inspiration defined as a sharp fall in esophageal pressure and sharp increase in gastric pressure more than twice the normal respiratory increase in gastric pressure.

Ethics approval

The study was approved by St Thomas' Hospital Research Ethics Committee (ref EC03/056).

Sample size

Based on the data from previous studies, which evaluated patients with GERD and healthy controls, to find a difference between symptomatic and asymptomatic twins in LES pressure and frequency of TLESR with acid reflux, using a two-sided test with a significance level of 5% and a power of 80%, it was estimated that a minimum of 20 pairs of twin volunteers would be required.[4,24–26]

Statistical analysis

Results are expressed as mean and SD (M $\pm$ SD), unless otherwise indicated. Parametric and non-parametric (Mann–Whitney U, Wilcoxon signed ranks test and Spearman rank correlation) tests were used when appropriate.

Significance was expressed at $P < 0.05$ level. The SPSS software package for Windows (release 12.0; SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

RESULTS

Subjects

Eighty-three percent (137/166) of MZ twins and 68% (247/362) of DZ twins, who were discordant for GERD symptoms in our original study, responded. In 50, (13 MZ and 37 DZ) only one twin of a pair responded and they were excluded. Forty-seven MZ twin pairs and 60 DZ twin pairs were no longer sufficiently discordant for the purpose of this study and were therefore also excluded. Fifteen MZ and 45 DZ pairs were eligible and were invited to take part. Four MZ (27%) and 12 DZ (27%) pairs agreed to participate. The demographic characteristics of the study population are described in Table 1, including non-responders to both the questionnaire and to the invitation for the pathophysiological study and those studied. Non-responders were younger than those included in the study. The characteristics of the study population are shown in Table 2. Symptomatic twins had had heartburn (94%) and/or acid regurgitation (94%) and had taken antacids (75%) or other medications for GERD (31% PPI, 19% H2 antagonist) several times a week in the previous 12 months. The

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Table 1 The demographic details of the study population and non-responders

	Non-responders (n = 144)	Declined to participate (n = 88)	Included in study (n = 32)	Included *vs* non-responders (P value)	Included *vs* declined (P value)
MZ:DZ	29:115	22:66	8:24	0.005	0.25
Age (M ± SD)	52(13)	58(13)	59(9)	0.003	0.14
Female (%)	89	93	100	0.08	1.0

Table 2 The characteristics of the study population

Factor	Symptomatic twins	Asymptomatic co-twins	P value
BMI (kg m^{-2}; mean ± SD)	30.1 ± 5.0	26.9 ± 5.2	0.04
Weight (kg; mean ± SD)	76.2 ± 15.1	69.3 ± 15.4	0.05
Ever smoked (%)	33	20	0.3
Alcohol consumed (%)	80	80	0.8
Pregnancies (median (range))	2 (0–6)	4 (0–6)	0.19

symptomatic twins were heavier and had significantly higher body mass index (BMI) values than the asymptomatic twins.

The primary care physicians of the symptomatic twins were contacted to ascertain if the twins had undergone upper gastrointestinal endoscopy. Seven of sixteen (44%) had undergone endoscopy. Two subjects had endoscopic esophagitis and one had a short segment of Barrett's esophagus. The other four had no reported endoscopic signs of GERD and none were reported as having a hiatus hernia.

Esophageal body motility

There was no difference in proximal, mid or distal esophageal peristaltic amplitude or in the percentage of symptomatic and asymptomatic twins with ineffective esophageal motility (Table 3).

Table 3 Esophageal motility, intra-gastric pressure and LES length in symptomatic and asymptomatic twins

	Symptomatic twins	Asymptomatic co-twins	P value
Proximal peristaltic amplitude (mmHg)	43.9 ± 18.7	56.9 ± 22.4	0.09
Mid peristaltic amplitude (mmHg)	71.3 ± 33.1	75.6 ± 35.9	0.72
Distal peristaltic amplitude (mmHg)	80.8 ± 41.0	78.2 ± 37.7	0.85
Ineffective esophageal motility (%)	19	13	1.0
Preprandial intra-gastric pressure (mmHg)	29.4 ± 6.2	29.0 ± 4.9	0.8
Postprandial intra-gastric pressure (mmHg)	28.4 ± 6.9	28.1 ± 5.10	0.9
Total LES length (cm)	3.73 ± 0.99	4.17 ± 0.48	0.12
Intra-abdominal LES length (cm)	1.96 ± 0.79	2.14 ± 0.53	0.46

LES, lower esophageal sphincter.

Intra-gastric pressure

There were no differences in pre- and post-prandial intra-gastric pressure between the twins (Table 3). Pre- and post-prandial intra-gastric pressure significantly correlated with BMI [Spearman's ρ = 0.49 (P = 0.005) and Spearman's ρ = 0.58 (P = 0.001), respectively].

Lower esophageal sphincter length

There was no difference in fasting total or intra-abdominal LES length between symptomatic and asymptomatic twins. One symptomatic twin and one asymptomatic twin had manometric evidence of hiatus hernia

Basal lower esophageal sphincter pressure

Mean values for basal LES pressure during each 10-minute study period in the pre- and post-prandial periods are shown in Fig. 1. Mean preprandial LES pressure was not different in symptomatic and asymptomatic twins (13.2 ± 7.1 mmHg *vs* 15.1 ± 6.2 mmHg, ANOVA P = 0.41). During the postprandial period, symptomatic twins had significantly lower basal LES pressure

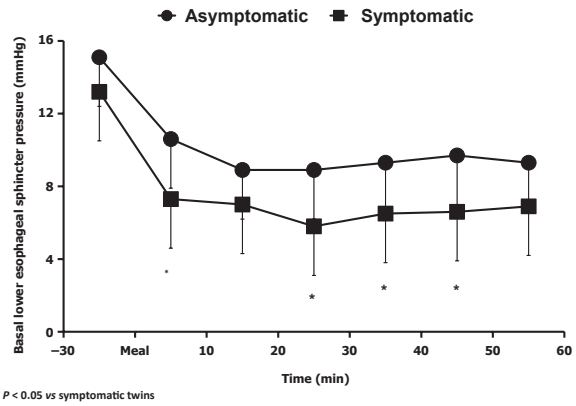

Figure 1 Basal lower esophageal sphincter pressure in the pre- and post-prandial periods in the symptomatic and asymptomatic twins.

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at nearly all time points (Fig. 1). The nadir LES pressures in symptomatic twins were significantly lower than in asymptomatic twins (4.6 ± 2.6 mmHg *vs* 7.1 ± 2.3 mmHg, $P < 0.01$). Postprandial LES pressure area under curve (AUC) in symptomatic twins was also greater (465 ± 126 mmHg h *vs* 331 ± 141 mmHg h, ANOVA $P < 0.01$). There was no correlation between increasing BMI and nadir LES pressure [Spearman's $\rho = -0.13$ ($P = 0.47$)] or LES pressure AUC [Spearman's $\rho = -0.04$ ($P = 0.81$)].

Acid reflux episodes

Acid reflux episodes significantly increased postprandially in both symptomatic and asymptomatic twins [median 0(range 0–1) to 1(0–9) ($P < 0.01$) and 0(0) to 0.5 (0–7) ($P = 0.01$), respectively], but there was no significant difference between the two groups (preprandial $P = 0.32$, postprandial $P = 0.31$). Total acid exposure times were higher in the symptomatic twins, but this just failed to reach statistical significance (symptomatic 103.4 ± 177.3 s *vs* asymptomatic twins 17.8 ± 35.1 s, $P = 0.07$). The length of acid reflux episodes was 30.9 ± 54.1 s in symptomatic twins and 7.4 ± 10.6 s in asymptomatic twins, but this difference was also not statistically significant ($P = 0.10$). The mechanisms associated with episodes of acid reflux were significantly different between the symptomatic and asymptomatic twins (Chi-square 12.45, $P = 0.01$; Table 4). Transient lower esophageal sphincter relaxation was the predominant mechanism in both symptomatic and asymptomatic twins, but hypotensive LES and strain/deep inspiration as mechanisms associated with acid reflux were seen in 7/16 (44%) of the symptomatic twins and in none of the asymptomatic twins.

TLESR

The numbers of TLESR in the pre- and postprandial periods are shown in Fig. 2. In both groups, TLESR significantly increased [symptomatic preprandial median 1(range 0–2) to postprandial 2.5(1–8) ($P = 0.001$); asymptomatic twins 0(0–2) to 3(1–6) ($P = 0.001$)]. However, there were no significant differences in the number of pre-prandial and postprandial TLESR between the groups ($P = 0.08$ and 0.81, respectively).

The number of TLESR associated with acid reflux or with an esophageal common cavity increased but this was not statistically significant both in asymptomatic and symptomatic twins (Fig. 2). In the symptomatic twins, the percentage of TLESR associated with acid reflux was 8% preprandially and 28% postprandially ($P = 0.13$) and the percentage of TLESR associated with a common cavity was 54% and 72% ($P = 0.2$). In the asymptomatic twins, the percentage of TLESR associated with acid reflux was 0% preprandially and 20% postprandially ($P = 0.22$) and the percentage of TLESR associated with a common cavity was 67% and 61% ($P = 0.8$). There was no significant difference between symptomatic and asymptomatic twins in the percentage of TLESR associated with acid reflux (preprandial $P = 0.49$; postprandial $P = 0.38$) or in the percentage of TLESR associated with a common cavity (preprandial $P = 0.60$, postprandial $P = 0.24$).

DISCUSSION

This study is a unique opportunity to examine a number of pathophysiological factors associated with GERD in a case-control study in which the cases and controls are as closely genetically related as is likely to be feasible. Given this close genetic link between the twins, pathophysiological differences between those with and without GERD symptoms are likely to have been caused by their GERD, rather than to have initiated it. Where there is no difference between symptomatic and asymptomatic twins, the situation is less clear. The pathophysiological factor may be an underlying genetically determined contributory factor, if both sets of twins differ from values for asymptomatic control subjects in previous studies. If the values for both sets of twins are similar to asymptomatic controls in previous studies, this would suggest that these factors do not contribute to the development of GERD or the study design or power are insufficient to detect a difference in a subset of the twins that would result from the development of GERD.

Table 4 The mechanisms associated with episodes of acid gastro-esophageal reflux in the symptomatic and asymptomatic twins

Study group	Acid reflux episodes	TLESR	Hypotensive LES	Swallow-related	Strain/deep inspiration	Undetermined
Asymptomatic twins	17	10	0	5	0	2
Symptomatic twins	37	17	8	8	4	0

TLESR, transient lower esophageal sphincter relaxation; LES, lower esophageal sphincter.

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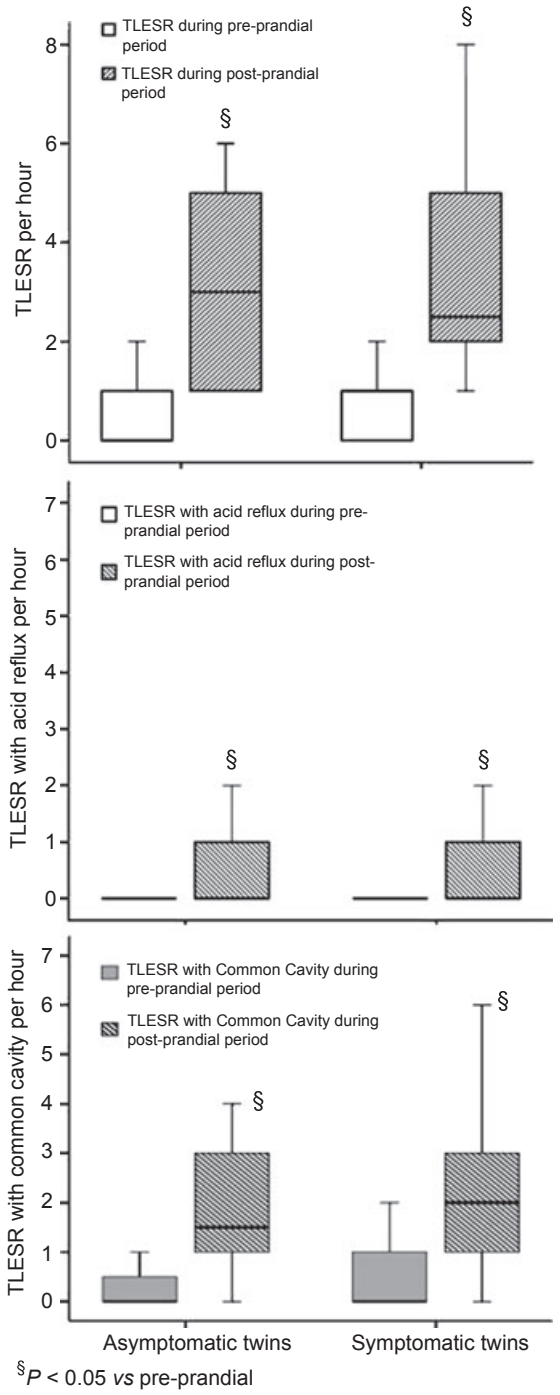

Figure 2 The number of transient lower esophageal sphincter relaxations, those with acid reflux and those with common cavities in the pre- and post-prandial periods in the symptomatic and asymptomatic twins (median, inter-quartile range, range).

In this study, LES pressure fell more after the meal in the symptomatic twins. It has been recognized for some time that fasting LES hypotension is associated with GERD induced esophagitis and that it correlates with the severity of mucosal injury.[24] Animal studies reveal that esophageal acid instillation causes LES hypotension.[8] However, healing of esophagitis does not significantly improve LES pressure.[27] This is the first report describing lower postprandial but not fasting LES pressure and a greater fall in postprandial LES pressure in GERD than control subjects. This observation may have relevance in the pathophysiology of GERD as 44% of symptomatic twins had acid reflux episodes associated with low LES pressure or deep inspiration or straining overcoming a low pressure LES, whereas no asymptomatic twins had acid reflux episodes associated with these mechanisms. Static studies of the mechanisms associated with acid reflux have emphasized that the majority of these episodes are associated with TLESR, as they were in this study.[22] Although ambulatory studies of the mechanisms associated with acid reflux in asymptomatic subjects confirmed the primacy of TLESR as the mechanism associated with nearly all acid reflux,[28] in the studies of patients with GERD a third of acid reflux episodes were associated with persistently low LES pressure, particularly in the presence of a hiatus hernia.[29,30] The greater fall in postprandial LES pressure and more frequent acid reflux episodes due to mechanisms associated with low LES pressure in the symptomatic twins suggest that they may be caused by GERD or develop during its natural history, rather than being the original cause of GERD developing. This is consistent with a commonly held but unproven theory that GERD develops from more frequent TLESR or a higher percentage of TLESR being associated with acid reflux and esophagitis, leading to LES hypotension, esophageal peristaltic dysfunction and esophageal shortening.[31] It is suggested that this in turn leads to hiatus hernia and more acid reflux, perpetuating the situation. This study raises the possibility that postprandial LES hypotension may potentially be an important pathophysiological factor perpetuating GERD that develops early during the natural history of GERD before fasting LES hypotension.

The symptomatic twins were significantly heavier and had higher BMI values than the asymptomatic twins, despite the relatively small number of subjects studied. Increasing BMI was also a key determinant of the presence of GERD symptoms in our original twin study.[13] Obesity is thought to contribute to gastro-esophageal reflux by increasing intra-gastric pressure and augmenting the pressure gradient across the gastro-esophageal junction, thereby promoting gastro-esophageal reflux during TLESR or periods of very low LES pressure.[32] The higher BMI values of the

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symptomatic twins might therefore explain the increased frequency of reflux events associated postprandially with low LES pressure compared with the asymptomatic twins. However, although, as expected, increasing BMI correlated with intra-gastric pressure in this study, there was no evidence of a difference in intra-gastric pressure between the symptomatic and asymptomatic twins. Furthermore, although increasing BMI promotes reflux during TLESR or periods of very low LES pressure, it does not seem to be responsible for the lower postprandial LES pressures among the symptomatic twins.

A clear finding from this study is that it seems very unlikely that differences in TLESR frequency are a common contributory factor to the development of GERD. There was clearly no difference in TLESR frequency between the two groups of twins. Initial data suggested that TLESR were more common in subjects with GERD[25,] but subsequent experience has refuted this.[18] Instead of an increased frequency of TLESR, subjects with GERD appeared to be more prone to reflux of acid during a TLESR.[18] There are a number of possible explanations for the increased prevalence of acid reflux during TLESR in GERD patients, including a difference in compliance of the esophago-gastric junction (EGJ),[33] a higher pressure gradient across the EGJ[34] and differences in the meal distribution or the localization of an acid pocket on top of the meal.[35] A recent study using impedance planimetry demonstrated that GERD patients have a more distensible EGJ when compared with controls.[36] Excessive compliance of the EGJ decreases the resistance to flow across the EGJ and allows greater volumes of gastric content and in particular more liquid rather than gas to reflux into the esophagus.[37] In this study, we were unable to show a clear difference in the number of TLESR associated with acid reflux between the two groups of twins and did not have the benefit of impedance monitoring to look for differences in the prevalence of liquid reflux during TLESR.

This study has a number of important limitations. We were unfortunately unable to recruit sufficient pairs of twins to reach the 20 pairs suggested to be necessary by the power calculation. However, although we were unable to demonstrate a difference in the proportion of TLESR with acid reflux between the symptomatic and asymptomatic twins, possibly as a consequence, we were able to show a difference in postprandial LES pressure, as previously discussed. The study relied on a symptomatic diagnosis of GERD, as endoscopy or 24 h pH monitoring was not feasible in the whole study population. Only half of the symptomatic twins had undergone endoscopy and only three had unequivocal endoscopic evidence of GERD. Despite the fact that 50% of the symptomatic twins took prescription therapy for GERD, it is conceivable that the symptomatic group was diluted with subjects with very minor GERD or esophageal hypersensitivity without excess esophageal acid exposure, limiting the ability of the study to detect pathophysiological differences between the groups. Although the lipid meal induced TLESR and acid reflux episodes in the majority of subjects, there is data to suggest that a mixed liquid and solid meal produces significantly more acid reflux episodes than a liquid meal in GERD subjects compared with control subjects and this may have obscured differences between the two groups of twins.[38] At the time the studies were undertaken, impedance monitoring was unfortunately not available to us. As noted previously, impedance monitoring would have allowed assessment of the height and therefore by extrapolating the volume of the refluxate. This may in turn have explained the borderline differences in acid exposure noted between the groups and potentially the genesis of GERD symptoms, as these are more common with larger volume reflux events that reach further up the esophagus[39] Finally, a recent comparison of the sleeve sensor and high-resolution manometry for the detection of TLESR, suggested that the sleeve sensor missed a number of TLESR.[40,41] It is possible that some of the acid reflux episodes in this study that were classified as associated with low-LES pressure might be due to TLESR that fail to reach the criteria for TLESR with the sleeve sensor but might be recognized as TLESR if high resolution manometry had been available at the time of the study.

In conclusion, twins with GERD symptoms had lower postprandial but not fasting LES pressures than asymptomatic twins. It is possible that this is a consequence of GERD, given the close genetic link between the twins. Low postprandial LES pressures were responsible for acid reflux episodes in nearly half of the symptomatic twins and none of the asymptomatic twins.

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CONFLICT OF INTEREST

The authors have no competing interests.

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AUTHOR CONTRIBUTION

Conception, planning, study design, collection and interpretation of data, statistical analysis and drafting of the article: Paola Iovino and Nigel Trudgill; conception, planning and study design, laboratory testing, collection, and interpretation of data: Imtiyaz Mohammed; laboratory testing and collection of data Angela Anggiansah, and Roy Anggiansah; conception, planning and study design Lynn F Cherkas and Tim D Spector.

REFERENCES

1 Vakil N, van Zanten SV, Kahrilas P, Dent J, Jones R. Global Consensus GroupThe Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. *Am J Gastroenterol* 2006; **101**: 1900–20.

2 Dent J, Holloway RH, Toouli J, Dodds WJ. Mechanisms of lower oesophageal sphincter incompetence in patients with symptomatic gastrooesophageal reflux. *Gut* 1988; **29**: 1020–8.

3 Mittal RK, Holloway RH, Penagini R, Blackshaw LA, Dent J. Transient lower esophageal sphincter relaxation. *Gastroenterology* 1995; **109**: 601–10.

4 Kahrilas PJ, Dodds WJ, Hogan WJ, Kern M, Arndorfer RC, Reece A. Esophageal peristaltic. Dysfunction in peptic esophagitis. *Gastroenterology* 1986; **91**: 897–904.

5 Mittal RK, Lange RC, McCallum RW. Identification and mechanism of delayed esophageal acid clearance in subjects with hiatus hernia. *Gastroenterology* 1987; **92**: 130–5.

6 Sloan S, Kahrilas PJ. Impairment of esophageal emptying with hiatal hernia. *Gastroenterology* 1991; **100**: 596–605.

7 Paterson WG, Kolyn DM. Esophageal shortening induced by short-term intraluminal acid perfusion in opossum: a cause for hiatus hernia? *Gastroenterology* 1994; **107**: 1736–40.

8 Eastwood GL, Castell DO, Higgs RH. Experimental esophagitis in cats impairs lower esophageal sphincter pressure. *Gastroenterology* 1975; **69**: 146–53.

9 Jochem VJ, Fuerst PA, Fromkes JJ. Familial Barrett's esophagus associated with adenocarcinoma. *Gastroenterology* 1992; **102**: 1400–2.

10 Eng C, Spechler SJ, Ruben R *et al.* Familial Barrett esophagus and adenocarcinoma of the gastroesophageal junction. *Cancer Epidemiol Biomarkers Prev* 1993; **2**: 397–9.

11 Trudgill NJ, Kapur KC, Riley SA. Familial clustering of reflux symptoms. *Am J Gastroenterol* 1999; **94**: 1172–8.

12 Romero Y, Cameron AJ, Locke GR *et al.* Familial aggregation of gastroesophageal reflux in patients with Barrett's esophagus and esophageal adenocarcinoma. *Gastroenterology* 1997; **113**: 1449–56.

13 Mohammed I, Cherkas LF, Spector TD, Trudgill NJ. Genetic influences in gastro-oesophageal reflux disease: a twin study. *Gut* 2003; **52**: 1085–9.

14 Bell JT, Spector TD. A twin approach to unraveling epigenetics. *Trends Genet* 2011; **27**: 116–25.

15 Martin NG, Martin PG. The inheritance of scholastic abilities in a sample of twins. Ascertainments of the sample and diagnosis of zygosity. *Ann Hum Genet* 1975; **39**: 213–8.

16 Mohammed I, Nightingale P, Trudgill NJ. Risk factors for gastro-oesophageal reflux disease symptoms: a community study. *Aliment Pharmacol Ther* 2005; **21**: 821–7.

17 Kraus BB, Wu WC, Castell DO. Comparison of lower esophageal sphincter manometrics and gastroesophageal reflux measured by 24-hour pH rcording. *Am J Gastroenterol* 1990; **85**: 692–6.

18 Trudgill NJ, Riley SA. Transient lower esophageal sphincter relaxations are no more frequent in patients with gastroesophageal reflux disease than in asymptomatic volunteers. *Am J Gastroenterol* 2001; **96**: 2569–74.

19 Fouad YM, Katz PO, Hatlebakk JG, Castell DO. Ineffective esophageal motility: the most common motility abnormality in patients with GERD-associated respiratory symptoms. *Am J Gastroenterol* 1999; **94**: 1464–7.

20 Diamant NE, Akin AN. Effect of gastric contractions on the lower esophageal sphincter. *Gastroenterology* 1972; **63**: 38–44.

21 Agrawal A, Tutuian R, Hila A, Freeman J, Castell DO. Identification of hiatal hernia by esophageal manometry: is it reliable? *Dis Esophagus* 2005; **18**: 316–9.

22 Holloway RH, Penagini R, Ireland AC. Criteria for objective definition of transient lower esophageal sphincter relaxation. *Am J Physiol* 1995; **268** (Gastrointest. Liver Physiol. 31): G128–33.

23 Wyman JB, Dent J, Heddle R, Dodds WJ, Toouli J, Downton J. Control of belching by the lower oesophageal sphincter. *Gut* 1990; **31**: 639–46.

24 Zaninotto G, DeMeester TR, Schwizer W, Johansson KE, Cheng SC. The lower esophageal sphincter in health and disease. *Am J Surg* 1988; **155**: 104–11.

25 Holloway RH, Kocyan P, Dent J. Provocation of transient lower esophageal sphincter relaxations by meals in patients with symptomatic gastr-esophageal reflux. *Dig Dis Sci* 1991; **36**: 1034–9.

26 Hirschowitz BI. A critical analysis, with appropriate controls, of gastric acid and pepsin secretion in clinical esophagitis. *Gastroenterology* 1991; **101**: 1149–58.

27 Allen ML, McIntosh DL, Robinson MG. Healing or amelioration of esophagitis does not result in increased lower esophageal sphincter or esophageal contractile pressure. *Am J Gastroenterol* 1990; **85**: 1331–4.

28 Schoeman MN, Tippett MD, Akkermans LMA, Dent J, Holloway RH. Mechanisms of gastroesophageal reflux in ambulant healthy human subjects. *Gastroenterology* 1995; **108**: 83–91.

29 Penagini R, Schoeman MN, Dent J, Tippett MD, Holloway RH. Motor events underlying gastro-oesophageal reflux in ambulant patients with reflux oesophagitis. *Neurogastroenterol Mot* 1996; **8**: 131–41.

30 Van Herwaarden MA, Samsom M, Smout AJPM. Excess gastroesophageal reflux in patients with hiatus hernia is caused by mechanisms other than transient LES relaxations. *Gastroenterology* 2000; **119**: 1439–46.

31 Mittal RK, Balaban DH. The esophagogastric junction. *N Engl J Med* 1997; **336**: 924–32.

32 de Vries DR, van Herwaarden MA, Smout AJ, Samsom M. Gastroesophageal pressure gradients in gastroesophageal reflux disease: relations with hiatal hernia, body mass index,

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and esophageal acid exposure. *Am J Gastroenterol* 2008; **103**: 1349–54.
33 Pandolfino JE, Shi G, Trueworthy B, Kahrilas PJ. Esophagogastric junction opening during relaxation distinguishes nonhernia reflux patients, hernia patients, and normal subjects. *Gastroenterology* 2003; **125**: 1018–24.
34 Scheffer RC, Gooszen HG, Hebbard GS, Samsom M. The role of transsphincteric pressure and proximal gastric volume in acid reflux before and after fundoplication. *Gastroenterology* 2005; **129**: 1900–9.
35 Fletcher J, Wirz A, Young J, Vallance R, McColl KE. Unbuffered highly acidic gastric juice exists at the gastroesophageal junction after a meal. *Gastroenterology* 2001; **121**: 775–83.
36 Kwiatek MA, Pandolfino JE, Hirano I, Kahrilas PJ. Esophagogastric junction distensibility assessed with an endoscopic functional luminal imaging probe (EndoFLIP). *Gastrointest Endosc* 2010; **72**: 272–8.
37 Simren M, Silny J, Holloway R *et al.* Relevance of ineffective oesophageal motility during oesophageal acid clearance. *Gut* 2003; **52**: 784–90.
38 Sifrim D, Holloway R, Silny J *et al.* Patients with gastroesophageal reflux disease have a selective increase of acid reflux when they have their habitual meals during 24-hr ambulatory pH-impedance recordings. *Gastroenterology* 2002; **122**: A417–8.
39 Bredenoord AJ, Weusten BLAM, Curvers WL, Timmer R, Smout AJPM. Determinants of perception of heartburn and regurgitation. *Gut* 2006; **55**: 313–8.
40 Rohof WO, Boeckxstaens GE, Hirsch DP. High-resolution esophageal pressure topography is superior to conventional sleeve manometry for the detection of transient lower esophageal sphincter relaxations associated with a reflux event. *Neurogastroenterol Motil* 2011; **23**: 427–32, e173.
41 Roman S, Zerbib F, Belhocine K, des Varannes Bruley, Mion F. High resolution manometry to detect transient lower oesophageal sphincter relaxations: diagnostic accuracy compared with perfused-sleeve manometry, and the definition of new detection criteria. *Aliment PharmacolTher* 2011; **34**: 384–93.

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