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# Esophageal secondary peristalsis following acid infusion and chemical clearance correlate with mucosal integrity and acid sensitivity in GERD patients

Ming-Wun Wong, Jui-Sheng Hung, Wei-Yi Lei, Tso-Tsai Liu, Chih-Hsun Yi, Shu-Wei Liang, Chandra Prakash Gyawali, Jen-Hung Wang and Chien-Lin Chen 

## Abstract

**Background:** Acid sensitivity can be altered in patients with gastroesophageal reflux disease (GERD). Secondary peristalsis helps clear gastro-esophageal refluxate and residual ingested food bolus.

**Objectives:** The aim of this study was to investigate the associations among acid sensitivity, esophageal mucosal integrity, chemical clearance, and secondary peristalsis before and after esophageal acid infusion.

**Design:** This was an investigator-initiated, prospective, cross-sectional study.

**Methods:** Adult reflux patients underwent high resolution manometry and 24 h impedance-pH monitoring off acid suppression to identify GERD phenotypes, including non-erosive reflux disease (NERD), reflux hypersensitivity (RH), and functional heartburn (FH). Secondary peristalsis was assessed using five rapid 20 mL air injections into the esophagus before and after infusion of hydrochloric acid (0.1 N) into the mid-esophagus. Conventional acid infusion parameters recorded included lag time, intensity rating, and sensitivity score. Chemical clearance was evaluated using the post-reflux swallow-induced peristaltic wave (PSPW), and mucosal integrity was assessed by the mean nocturnal baseline impedance (MNBI) derived from impedance-pH monitoring.

**Results:** A total of 88 patients (age 21–64 years, 62.5% women) completed the study including 12 patients with NERD, 45 with RH, and 31 with FH. There was no significant difference in acid infusion parameters between patients with NERD, RH, and FH. Upon acid infusion, patients who exhibited successful secondary peristalsis had longer lag time, higher MNBI, and shorter bolus contact time than those without secondary peristalsis. Meanwhile, patients with intact PSPW demonstrated significantly higher intensity ratings in response to acid perfusion and higher MNBI than those with impaired PSPW. The lag time correlated positively with MNBI ( $r=0.285$ ;  $p=0.007$ ).

**Conclusion:** In conclusion, the protective effect of esophageal secondary peristalsis and chemical clearance on esophageal mucosal integrity was demonstrated. Concerning acid sensitivity, longer lag time in patients with intact secondary peristalsis may be attributed to better esophageal mucosal integrity, while stronger intensity ratings may have a greater tendency to induce PSPW and protect esophageal mucosal integrity.

**Keywords:** acid sensitivity, esophageal peristalsis, gastroesophageal reflux disease, mucosa integrity

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## Introduction

Gastroesophageal reflux disease (GERD) consists of symptoms and esophageal mucosal injury resulting from the retrograde movement of gastric content through the esophagogastric junction into the esophagus and beyond.<sup>1</sup> Despite the identification of clinical GERD phenotypes with varying response to acid suppression,<sup>2</sup> the majority of patients with GERD symptoms have normal endoscopy.<sup>3</sup> Infusion of acid into the esophagus in patients with GERD may induce heartburn,<sup>4,5</sup> believed to result from stimulation of acid-sensitive nerves endings in the mucosa and submucosa of the esophagus.<sup>6</sup> Esophageal acid sensitivity has been shown to positively correlate with GERD symptom severity and negatively correlate with mucosal integrity.<sup>7-9</sup> The esophageal hypervigilance and anxiety scale (EHAS) is a validated tool used to evaluate cognitive-affective aspects of centrally mediated esophageal symptom perception.<sup>10</sup> Previous studies have demonstrated that GERD symptom severity positively correlates with EHAS levels rather than traditional reflux metrics.<sup>11,12</sup> Subsequent research has provided additional evidence to suggest that the perception of laryngopharyngeal reflux symptoms is more significantly associated with EHAS than reflux burden.<sup>13</sup> However, the variability in esophageal acid sensitivity across different GERD phenotypes, as well as its connection with EHAS, remain inconclusive.<sup>14-16</sup>

The primary and secondary peristalsis mechanisms are responsible for removing refluxate, with the latter playing a more significant role in volume clearance due to the activation of local mechanoreceptors.<sup>17,18</sup> Acid reflux burden is more profound in patients with absent contractility and those lacking a secondary peristaltic response to esophageal air distension.<sup>19</sup> Chemical clearance, which is triggered by the vagal esophago-salivary reflex and salivary swallow, increases the esophageal pH, and acts as a crucial defense mechanism against the hazardous component of the refluxate. The post-reflux swallow-induced peristaltic wave (PSPW) has been identified as a novel impedance-pH variable that characterizes the esophageal chemical clearance mechanism.<sup>20</sup> Studies have shown that lower PSPW index values are linked to erosive GERD in comparison to non-erosive GERD, and very low PSPW index values can predict neoplastic progression in short-segment Barrett's esophagus.<sup>20,21</sup> Although esophageal acid sensitivity is

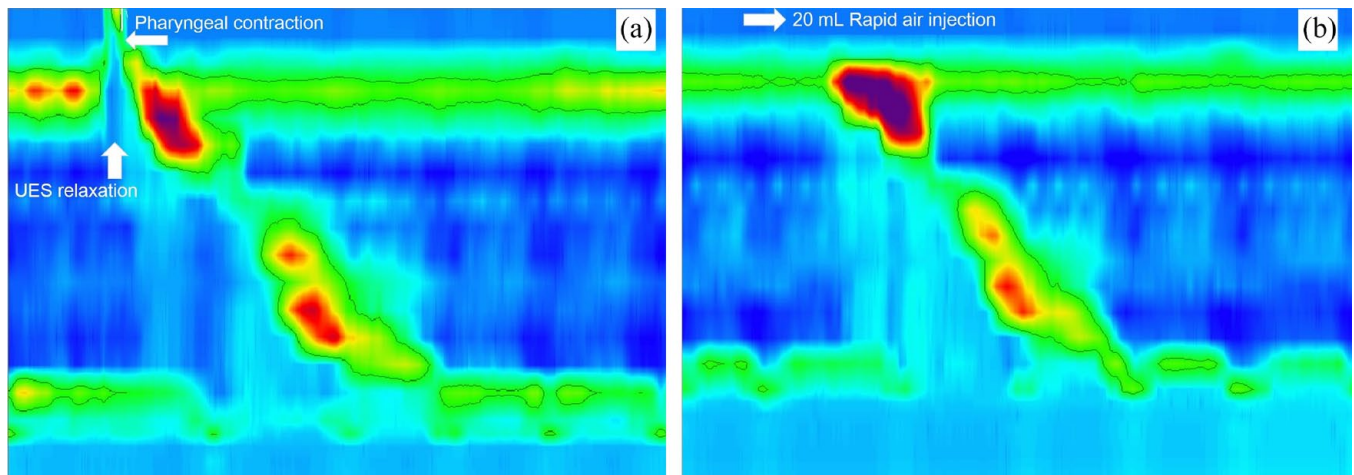
similar in GERD patients with and without ineffective esophageal motility,<sup>9</sup> the correlation between esophageal acid sensitivity and secondary peristalsis as well as PSPW index has not been studied.

In this study, we hypothesized that esophageal acid sensitivity varies depending on presence or absence of secondary peristalsis. Specifically, we hypothesized that secondary peristalsis clears luminal acid, resulting in intact esophageal mucosal integrity, and consequently, less esophageal acid sensitivity, whereas impaired secondary peristaltic responses would be associated with impaired esophageal mucosal integrity and higher esophageal acid sensitivity. We tested this hypothesis by evaluating secondary peristalsis on esophageal high resolution manometry (HRM) before and after acid infusion in patients with chronic GERD symptoms, who also underwent multi-channel intraluminal impedance-pH (MII-pH) monitoring off acid suppression. Interrelationships between esophageal acid sensitivity parameters, secondary peristaltic responses, PSPW index, and esophageal mucosal integrity were thereby assessed.

## Methods

### Subjects

This is an investigator-initiated, prospective, cross-sectional study, which was conducted and reported according to the Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) Guidelines.<sup>22</sup> Consecutive patients aged 20–65 years with typical GERD symptoms (heartburn and/or acid regurgitation) for at least 6 months were prospectively enrolled from the gastroenterology outpatient department of Hualien Tzu Chi Hospital between 2020 and 2022. All participants underwent esophagogastroduodenoscopy (EGD) to exclude erosive esophagitis, Barrett's esophagus, eosinophilic esophagitis, esophageal stricture, or esophageal cancer. Eligible patients underwent MII-pH monitoring off antisecretory therapy for phenotyping GERD symptoms to non-erosive reflux disease (NERD), reflux hypersensitivity (RH), and functional heartburn (FH). Participants were excluded if they were treated with proton-pump inhibitor (PPI), histamine-2 receptor antagonists, aspirin, or nonsteroidal anti-inflammatory drug in the 2 weeks preceding the investigation, and if



**Figure 1.** (a) This figure depicts a high-resolution manometry plot from a representative participant, illustrating the pattern of primary peristalsis. The arrows indicate the timing of swallowing, including upper esophageal sphincter relaxation and pharyngeal contraction. (b) This figure demonstrates the pattern of secondary peristalsis via air injection in a high-resolution manometry plot from a representative participant. The arrow indicates the timing of the stimulus (20 mL air injection), and the static upper esophageal pressure confirms that subsequent esophageal contraction was not triggered by swallowing.

they had any major organ disease or cancer. All study participants underwent an evaluation using the EHAS. The summation of individual item scores from the EHAS generates a total EHAS score between 0 and 60, whereby higher scores are indicative of heightened levels of esophageal hypervigilance and symptom-specific anxiety.<sup>10</sup> Written informed consent was obtained from each participant. The study was performed in accordance with the principles of the Declaration of Helsinki, and the study protocol was approved by the Ethical Committee of Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation.

#### *Esophagogastroduodenoscopy*

Reflux esophagitis was characterized according to the Los Angeles Classification into four grades.<sup>23</sup> Barrett's esophagus was diagnosed when metaplastic changes of specialized columnar epithelium was found on targeted biopsy or salmon colored mucosa in the distal esophagus.<sup>24</sup> Patients were excluded if there was evidence of erosive reflux esophagitis (Los Angeles Classification A to D), Barrett's esophagus, esophageal tumor, stricture, or infectious esophagitis on EGD.

#### *High resolution manometry*

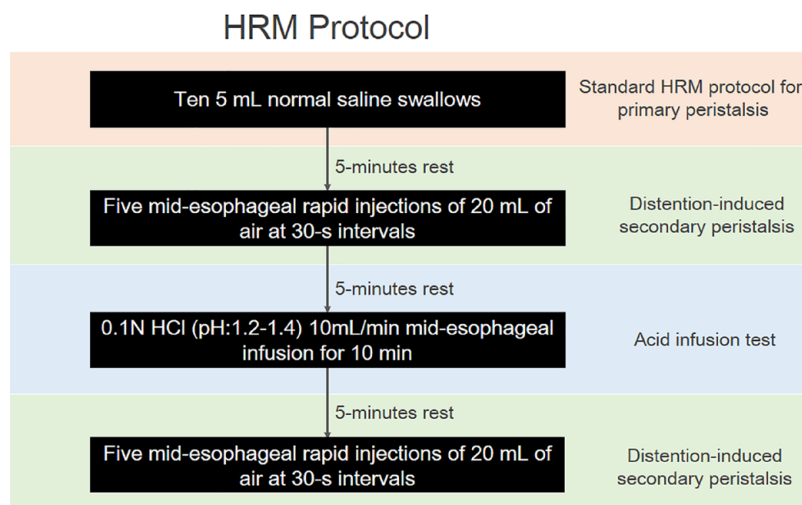
HRM was performed in the supine position after an overnight fast, using a catheter with 22 unidirectional pressure sensors located at

2 cm intervals (Laborie/Medical Measurement Systems, Enschede, The Netherlands). The catheter was passed transnasally into the esophagus, and positioned with at least three distal sensors in the stomach, thereby allowing data recording from the hypopharynx to the stomach using external pressure transducers (Argon Medical Devices, Plano, TX, USA), and stored on a personal computer.

Primary peristalsis was evaluated using 10 supine swallows of 5 mL water at 30 s intervals in each subject (Figure 1(a)). Before and after the acid infusion test, secondary peristalsis was evaluated using five mid-esophageal rapid injections of 20 mL of air at 30 s intervals in each patient (Figure 1(b)).<sup>25</sup> Upon completion of each sequence of air injections, the participants were instructed to perform a dry swallow to eliminate any residual air. To validate a successful case of secondary peristalsis, a characteristic contraction pattern of the esophagus needed to be observed after air injection without any signs of relaxation in the upper sphincter. Participants had absence of secondary peristalsis if no typical esophageal peristaltic contraction pattern was identified during 10 mid-esophageal rapid air injections.

#### *Modified acid infusion test*

All subjects underwent a modified acid perfusion test,<sup>26</sup> where 0.1 N hydrochloric acid solution was



**Figure 2.** The figure outlines a HRM protocol that encompasses several procedures, including the standard HRM protocol for primary peristalsis, distention-induced secondary peristalsis before acid infusion, the acid infusion test, and distention-induced secondary peristalsis after acid infusion. HRM, high-resolution manometry.

infused at a rate of 10 mL/min for 10 min (or till symptoms developed, if <10 min) through the side hole of the HRM catheter positioned in the mid-esophagus. All patients were requested to report whenever they had typical heartburn sensation. All of the patients were allowed to continue free swallowing during each infusion. Three parameters were utilized to evaluate the response to acid infusion, including the lag time, sensory intensity rating, and acid perfusion sensitivity score. Lag time was defined as the time in seconds (s) from the start of acid perfusion to the initial typical symptom perception, and the sensory intensity rating was assessed at the end of acid perfusion using a validated verbal descriptor scale that ranged from no sensation (0) to extremely intense (20). The acid perfusion sensitivity score was obtained by multiplying the duration of typical symptom perception, expressed in seconds (s), by the sensory intensity rating at the end of acid perfusion, and then dividing the product by 100.<sup>27</sup> The complete HRM protocol is summarized in Figure 2.

#### *MII-pH monitoring*

Study patients with normal endoscopy underwent MII-pH monitoring as per standard clinical protocol. A MII-pH catheter (Medical Measurement Systems, Enschede, The Netherlands) was placed transnasally, with positioning based on HRM

identification of the upper margin of the lower esophageal sphincter (LES). The catheter consisted of six impedance sensors positioned 3, 5, 7, 9, 15, and 17 cm from the LES and pH sensors positioned 5 cm above and 10 cm below the LES. MII-pH testing was used to assess acid exposure time (AET), symptom association probability (SAP), and mean nocturnal baseline impedance (MNBI) as part of this study.

Total AET, defined as the proportion of time the distal esophagus is exposed to a pH < 4.0, was extracted from pH-impedance studies and considered pathologic when  $\geq 4\%$ . A positive SAP was defined as  $> 95\%$ , corresponding to  $p < 0.05$ . Based on AET and SAP, patients were stratified into NERD (AET  $\geq 4\%$ ), RH (AET < 4%, positive SAP), and FH (AET < 4%, negative SAP). A value of total AET of  $\geq 4\%$  is defined as an abnormal finding in Asian adults. This definition is based on the Lyon Consensus and has been further adapted for the Asian population according to the 2020 Asian GERD Consensus Conference, also known as the Seoul Consensus.<sup>3,28</sup>

MNBI was calculated by extracting and averaging baseline impedance values at stable nocturnal 10 min periods at 1:00 a.m., 2:00 a.m., and 3:00 a.m. at the 5 cm positions.<sup>29</sup> The term PSPW denotes a swallow event that transpires within a time frame of 30 s after the termination

of a reflux episode. The PSPW, in turn, triggers an antegrade augmentation of impedance levels by 50%, relative to the preswallow baseline. This increase commences at the most proximal impedance site and extends to all the distal impedance sites. To calculate the PSPW index for a given 24 h MII-pH tracing, the count of reflux episodes immediately followed by a PSPW within the specified time frame is divided by the total number of reflux episodes observed.<sup>20,30</sup> According to the latest research results, we employed two cut-off points, PSPW indices of <53% and <61%, to indicate impaired esophageal chemical clearance.<sup>31</sup>

The duration of bolus contact time in each reflux episode was determined by calculating the interval between the first instance of impedance drop below 50% of the baseline, indicating the onset of reflux, and the subsequent return to 50% of the baseline impedance level in the distal impedance channel, signifying the conclusion of the reflux event.

### Statistical analyses

All data are reported as means  $\pm$  standard error of mean. Amongst GERD phenotypes, continuous data were compared using a one-way analysis of variance, and categorical data were compared using the chi-squared test. For comparison of patients with and without secondary peristalsis, independent sample *t*-test was used to analyze continuous variables and chi-squared test was used to compare categorical variables. Correlations between acid infusion parameters and pH-impedance metrics as well as EHAS were assessed using Pearson's correlation. Statistical significance required *p* value <0.05. Statistical analyses were performed with SPSS 22.0 for Windows (SPSS, Inc., Chicago, IL, USA).

### Sample size calculation

The sample size for Pearson's correlation analysis was determined utilizing G\*Power version 3.1.9.2,<sup>32</sup> consistent with the research objectives pursued. The related parameters were configured as follows: a medium effect size of 0.3 for correlation coefficient ( $\rho$ ), a type I error rate of 0.05, and a statistical power of 0.80. Based on these specifications, the calculated sample size for the study was determined to be at least 84 subjects.

## Results

### Clinical characteristics of study patients

Over the study period, 100 consecutive patients were screened, of whom 12 patients were excluded (10 due to erosive esophagitis and 2 due to Barrett's esophagus on EGD). Consequently, 88 patients (mean age 43.1 years, range 21–64 years, 62.5% female) were enrolled, which included 12 patients with NERD (9 with pH > 6, 3 with pH between 4 and 6), 45 with RH, and 31 with FH (Table 1). Patients with NERD had higher total, upright, and supine AET than those with RH or FH ( $p < 0.05$ ). No significant differences were observed between GERD phenotypes in terms of age, sex, body mass index, MNBI, PSPW index, bolus contact times, EHAS, acid infusion parameters, successful secondary peristalsis generation rate at baseline, or after acid infusion ( $p = \text{NS}$ ).

### Acid infusion parameters and MNBI between patients with and without secondary peristalsis

At baseline, 65 patients exhibited successful secondary peristalsis at least once, while 21 patients did not show any secondary peristalsis upon air injection. Patients with successful secondary peristalsis had shorter bolus contact time than those without secondary peristalsis. (8.2 s *versus* 11.1 s,  $p < 0.001$ ) There were no significant group differences in lag time, intensity rating, acid sensitivity score, or MNBI between patients with and without secondary peristalsis at baseline ( $p = \text{NS}$ , Table 2).

After acid infusion, 66 patients had at least one successful secondary peristalsis generated, while 22 patients had no secondary peristalsis. Patients with successful secondary peristalsis had longer lag time, higher MNBI, and shorter bolus contact time than those without secondary peristalsis (lag time: 87.4 s *versus* 46.6 s,  $p = 0.002$ , Figure 3(a); MNBI: 2690.2  $\Omega$  *versus* 2138.9  $\Omega$ ,  $p = 0.02$ , Figure 3(b); 8.8 s *versus* 11.5 s,  $p < 0.001$ ). There were no significant group differences in intensity rating or acid sensitivity score between patients with and without secondary peristalsis after acid infusion ( $p = \text{NS}$ , Table 2).

### Acid infusion parameters and MNBI between patients intact and impaired PSPW

At a PSPW index cut-off of 53%, 52 patients demonstrated intact PSPW, while 36 patients had impaired PSPW. Patients with intact PSPW showed significantly higher intensity ratings in

**Table 1.** Clinical characteristics among GERD phenotypes.

Characteristics\Phenotypes	NERD <sup>a</sup> (N= 12)	RH (N= 45)	FH (N= 31)	p-Value
Age	45.7 (4.5)	41.6 (1.7)	44.4 (2.2)	0.462
Female, n (%)	75.0% (9)	62.2% (28)	58.1 (18)	0.588
BMI	27.0 (0.9)	24.2 (0.6)	23.9 (0.7)	0.065
AET (total)	6.3 (0.7)	1.5 (0.6)	0.8 (0.2)	<0.001
AET (upright)	8.5 (1.2)	1.9 (0.6)	1.3 (0.3)	<0.001
AET (supine)	2.9 (1.0)	0.2 (0.1)	0.2 (0.1)	<0.001
Lag time (s)	44.0 (2.2)	65.8 (8.7)	106.7 (22.6)	0.049
Intensity rating	14.0 (0.9)	13.5 (0.8)	13.3 (0.9)	0.918
Acid sensitivity score	28.6 (10.1)	27.5 (3.8)	36.3 (5.6)	0.417
Secondary peristalsis (baseline) (%)	75.0% (9)	68.2% (30)	86.7% (26)	0.192
Secondary peristalsis (after acid infusion) (%)	58.3% (7)	71.1% (32)	87.1% (27)	0.102
MNBI (Ω)	2373.2 (206.9)	2432.8 (111.6)	2795.3 (115)	0.063
PSPW index (%)	48.6 (8.5)	55.0 (4.3)	56.3 (4.9)	0.716
Bolus contact times (s)	10.6(0.7)	9.6 (0.5)	8.9 (0.4)	0.193
EHAS	30.0 (4.4)	28.4 (1.8)	31.5 (2.5)	0.602

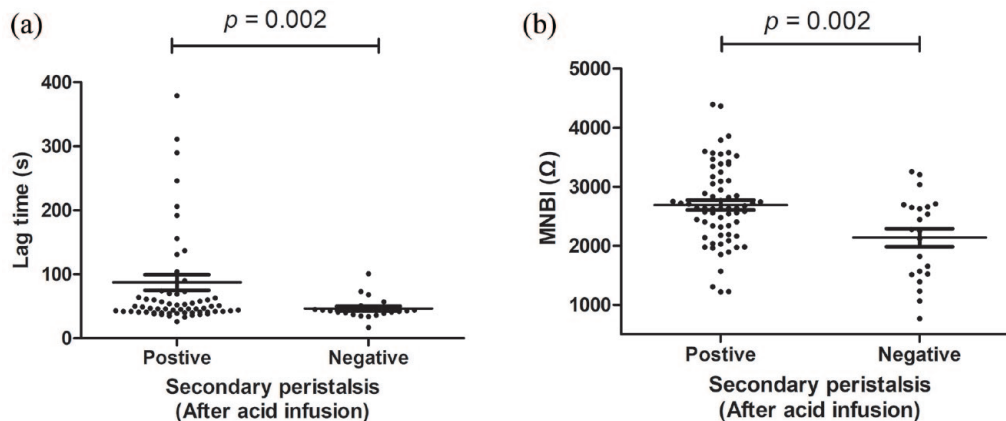
<sup>a</sup>Among patients with NERD, nine patients had a pH value greater than 6, while the remaining three patients had a pH value between 4 and 6. AET, acid exposure time; BMI, body mass index; EHAS, esophageal hypervigilance and anxiety scale; FH, functional heartburn; GERD, gastroesophageal reflux disease; MNBI, mean nocturnal baseline impedance; NERD, non-erosive reflux disease; PSPW, post-reflux swallow-induced peristaltic wave; RH, reflux hypersensitivity.

**Table 2.** Acid infusion parameters and MNBI between patients with and without secondary peristalsis at the baseline and after acid infusion.

Secondary peristalsis	Secondary peristalsis (baseline)		p-Value	Secondary peristalsis (after acid infusion)		p-Value
	Positive (N= 65)	Negative (N= 21)		Positive (N= 66)	Negative (N= 22)	
Lag time (s)	81.0 (10.7)	67.2 (21.3)	0.542	87.4 (12.2)	46.6 (3.6)	0.002
Intensity rating	13.5 (0.5)	12.8 (1.3)	0.612	13.4 (0.6)	14.0 (1.2)	0.622
Acid sensitivity score	31.5 (3.4)	26.1 (6.1)	0.438	33.4 (3.6)	22.7 (5.8)	0.132
MNBI (Ω)	2603(88.2)	2338.3 (171.8)	0.244	2690.2 (83.3)	2138.9 (152.0)	0.002
Bolus contact time (s)	8.2 (0.2)	11.1 (0.9)	0.027	8.8 (0.3)	11.5 (0.8)	<0.001

MNBI, mean nocturnal baseline impedance.





**Figure 3.** (a) Lag time between patients with and without secondary peristalsis after acid infusion. (b) MNBI between patients with and without secondary peristalsis after acid infusion.

**Table 3.** Acid infusion parameters and MNBI between patients intact and impaired PSPW.

PSPW	PSPW index (53%)		<i>p</i> -Value	PSPW index (61%)		<i>p</i> -Value
	Positive (N=52)	Negative (N=36)		Positive (N=40)	Negative (N=48)	
Lag time (s)	82.4 (12.9)	69.7 (13.5)	0.508	90.4 (16.5)	66.2 (10.3)	0.200
Intensity rating	14.7 (0.7)	12.2 (0.8)	0.024	14.6 (0.7)	12.6 (0.7)	0.047
Acid sensitivity score	32.4 (4.3)	28.3 (4.3)	0.517	33.5 (4.7)	28.5 (4.0)	0.417
MNBI (Ω)	2861.8 (84.7)	2105.5 (106.5)	<0.001	2939.6 (104.5)	2229.7 (88.0)	<0.001
Bolus contact time (s)	9.8 (0.5)	9.0 (0.3)	0.172	9.2 (0.5)	9.7 (0.4)	0.514

MNBI, mean nocturnal baseline impedance; PSPW, post-reflux swallow-induced peristaltic wave.

response to acid perfusion and higher MNBI values than those with impaired PSPW (intensity rating: 14.7 *versus* 12.2,  $p=0.024$ ; MNBI: 2861.8Ω *versus* 2105.5Ω,  $p<0.001$ ). However, no significant differences were observed between patients with intact and impaired PSPW in terms of lag time, acid sensitivity score, or bolus contact time ( $p=NS$ , Table 3).

At a PSPW index cut-off of 61%, 40 patients demonstrated intact PSPW, while 48 patients had impaired PSPW. Patients with intact PSPW showed significantly higher intensity ratings in response to acid perfusion and higher MNBI values than those with impaired PSPW (intensity rating: 14.6 *versus* 12.6,  $p=0.047$ ; MNBI:

2939.6Ω *versus* 2229.7Ω,  $p<0.001$ ). However, no significant differences were observed between patients with intact and impaired PSPW in terms of lag time, acid sensitivity score, or bolus contact time ( $p=NS$ , Table 3).

#### *Correlations between acid infusion parameters and reflux parameters on MII-pH, as well as the EHAS*

Lag time during acid infusion correlated positively with MNBI ( $r=0.285$ ;  $p=0.007$ ). No other significant correlations were found between other acid infusion parameters and pH-impedance characteristics, as well as the EHAS ( $p=NS$ ), as described in Table 4.

**Table 4.** Correlations between acid infusion parameters and reflux parameters on MII-pH.

Acid infusion parameters	Lag time (s)	Intensity rating	Acid sensitivity score
AET (total)	-0.149	-0.030	-0.115
AET (upright)	-0.164	-0.025	-0.072
AET (supine)	-0.119	-0.047	-0.104
MNBI ( $\Omega$ )	0.285*	0.077	0.029
EHAS	0.031	0.171	-0.089

\* $p < 0.05$ .

AET, acid exposure time; EHAS, esophageal hypervigilance and anxiety scale; MII-pH, multichannel intraluminal impedance-pH; MNBI, mean nocturnal baseline impedance.

### Discussion

In this study evaluating relationships between acid sensitivity, mucosal integrity, and secondary peristalsis in patients with typical GERD symptoms, we demonstrate that patients with intact secondary peristalsis after acid infusion had higher distal MNBI (indicating intact mucosal integrity) and longer lag time to initial heartburn perception (indicating lesser acid sensitivity) compared to patients with impaired secondary peristalsis. In addition, lag time to initial heartburn perception positively correlated with MNBI, but not associated with AET. However, esophageal acid sensitivity and successful secondary peristalsis generation rates were similar across NERD, RH, and FH. We conclude that presence of secondary peristalsis leads to intact mucosal integrity, and consequently, lesser acid sensitivity.

Current Rome IV criteria identify three distinct phenotypes of heartburn patients based on reflux monitoring when endoscopy is normal: NERD (with abnormal esophageal acid exposure), RH (normal esophageal acid exposure, but abnormal symptom reflux association), and FH (normal esophageal acid exposure and negative symptom reflux association).<sup>33</sup> Esophageal pain perception and acid infusion findings are heterogenous within these phenotypes in the available literature. Yang *et al.*<sup>14</sup> demonstrated that FH is associated with lower pain perception thresholds on either esophageal balloon distention or electrical stimulation compared with patients with NERD, while Weijenberg *et al.*<sup>16</sup> identified more acid sensitivity in terms of shorter lag time to acid perfusion with NERD

than with FH. In contrast, Woodland *et al.*<sup>15</sup> reported no significant differences in acid sensitivity between NERD and FH. Our results also indicate no difference in acid perfusion parameters between patients with NERD, RH, and FH. The heterogeneity of these findings may relate to different study designs or types of stimulation used; alternatively, factors other than GERD phenotypes may be responsible for acid sensitivity. In addition, similar to previous research, psychological distress may be present across GERD phenotypes, and EHAS levels appear to be comparable among the different GERD phenotypes in the present study.<sup>11,12</sup> Furthermore, EHAS involves central signal modulation, while acid sensitivity is a function of the peripheral sensory system. Based on our current results, there appears to be no significant correlation between the severity of the two.

Esophageal mucosal integrity functions as a barrier that prevents the nerve endings in the submucosal layer from exposure to noxious refluxed gastric content.<sup>34,35</sup> Sensitivity to acid has been shown to be enhanced in patients with GERD compared to healthy controls, and positively correlates with impaired distal esophageal mucosal integrity.<sup>36</sup> Furthermore, patients with slow recovery of esophageal impedance following acid infusion had lower distal baseline impedance and more frequent acid sensitivity than those with fast recovery.<sup>15</sup> Our results demonstrate that lag time during the acid perfusion test correlated positively with MNBI, which supports the reported association of acid sensitivity with altered mucosal permeability and abnormal integrity in the distal esophagus.<sup>7</sup>

Secondary esophageal peristalsis may play an important role in esophageal clearance during sleep when the effectiveness of primary peristalsis is significantly suppressed.<sup>37</sup> In addition, intact secondary peristalsis probably plays an important role in esophageal acid clearance, which may directly impact nocturnal acid reflux burden.<sup>19</sup> In the present study, regarding acid perfusion responses, patients with intact secondary peristalsis have demonstrated lower acid sensitivity, as evidenced by a longer lag time, compared to those with impaired secondary peristalsis. However, patients with intact PSPW function have shown greater intensity ratings in response to acid. In terms of esophageal mucosal integrity, both intact secondary peristalsis and PSPW function are associated with higher values of MNBI. This observation suggests a potential significance in esophageal physiology, as impaired secondary peristalsis after acid infusion may contribute to increased esophageal acid sensitivity by impairing mucosal integrity. Meanwhile, stronger acid sensitivity symptoms may have a greater propensity to trigger PSPW, which may act as a protective mechanism against gastric acid reflux on the esophageal mucosa. Furthermore, in patients with impaired secondary peristalsis, it has been observed that bolus contact times are significantly prolonged. However, the presence or absence of PSPW impairment is not related to bolus contact times. This may be attributed to the fact that secondary peristalsis is primarily responsible for mechanical clearance of reflux, whereas PSPW is involved in chemical clearance.

Our study has several important clinical implications, particularly for patients with typical GERD symptoms and normal endoscopy findings. By focusing on this group, which excluded those with erosive esophagitis and Barrett's esophagus, we aim to better understand the pathophysiology of esophageal acid sensitivity in patients without any visible esophageal mucosal injury. Both triggered secondary peristalsis after acid infusion and PSPW appear to play a protective role in esophageal mucosal integrity as indicated by their positive association with MNBI. However, their roles in acid perfusion responses are distinct, with intact secondary peristalsis associated with lower acid sensitivity as indicated by a longer lag time, and intact PSPW function associated with greater intensity ratings in response to acid. Our findings also highlight the lack of correlation

between central signal modulation in EHAS and the function of the peripheral sensory system in acid sensitivity. Taken together, incorporating secondary peristalsis, acid sensitivity testing, and EHAS evaluation into pH impedance studies (AET, MNBI, PSPW index) may provide a more comprehensive understanding of the pathophysiology of GERD and guide the development of more effective and personalized treatment approaches for patients.

There are some limitations in this study that needs to be addressed. First, our sample size was low for the individual GERD phenotypes, especially patients with NERD, which potentially risks type 2 error and may have suppressed differences between acid infusion parameters and MNBI among GERD phenotypes. Second, the notion that lag time during acid infusion has a weak correlation with MNBI in our study suggests that other factors such as the extent of sensory neural distribution may impact our findings.<sup>38</sup> Third, the study only measured acid sensitivity in patients with esophageal hypersensitivity, and did not assess symptom sensitivity to distension. Esophageal distension can potentially reproduce reflux symptoms, especially in patients with impaired volume clearance due to impaired secondary peristalsis. Thus, future studies assessing symptom sensitivity to distension in addition to acid sensitivity would have provided a more comprehensive understanding of the underlying mechanisms contributing to esophageal hypersensitivity in these patients. Fourth, our findings may not be generalizable to all GERD patients due to the exclusion of patients with erosive esophagitis and Barrett's esophagus. Future research should investigate secondary peristalsis, mucosal integrity, and acid sensitivity in those patients for more comprehensive understanding of GERD pathophysiology. Finally, outcome data from therapy driven by esophageal testing was not collected, especially response to PPI therapy. Further investigation is warranted to evaluate whether acid sensitivity could be a factor in predicting the need for long-term PPI therapy *versus* PPI discontinuation. Despite these limitations, our work proposes a pathophysiological model of secondary peristalsis – mucosal integrity – acid sensitivity axis in symptomatic patients with normal endoscopy.

### Conclusion

In conclusion, both competent esophageal secondary peristalsis and chemical clearance are protective for intact esophageal mucosal integrity. With regards to acid sensitivity, longer lag time in patients with competent secondary peristalsis may contribute to better esophageal mucosal integrity, while stronger intensity ratings may facilitate triggering PSPW and consequently protect esophageal mucosal integrity. Our study highlights the relationship between esophageal motility and sensory systems, which will aid in further exploration of esophageal physiology.

### Declarations

#### *Ethics approval and consent to participate*

The study was performed in accordance with the principles of the Declaration of Helsinki. This study protocol was approved by the Research Ethical Committee of Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (IRB109-011-A). Written informed consent was obtained from each participant.

#### *Consent for publication*

Not applicable.

#### *Author contributions*

**Ming-Wun Wong:** Conceptualization; Data curation; Formal analysis; Methodology; Writing – original draft; Writing – review & editing.

**Jui-Sheng Hung:** Data curation; Formal analysis; Writing – review & editing.

**Wei-Yi Lei:** Data curation; Formal analysis; Writing – review & editing.

**Tso-Tsai Liu:** Data curation; Writing – review & editing.

**Chih-Hsun Yi:** Data curation; Writing – review & editing.

**Shu-Wei Liang:** Data curation; Writing – review & editing.

**Chandra Prakash Gyawali:** Writing – review & editing.

**Jen-Hung Wang:** Data curation; Formal analysis; Writing – review & editing.

**Chien-Lin Chen:** Conceptualization; Formal analysis; Methodology; Project administration; Resources; Supervision; Writing – review & editing.

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### Competing interests

The authors declare that there is no conflict of interest.

### Availability of data and materials

Not applicable.

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### Supplemental material

Supplemental material for this article is available online.

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