Published in final edited form as:

J Pediatr. 2006 July ; 149(1): 77–82. doi:10.1016/j.jpeds.2006.02.041.

Effect of Maturation of the Magnitude of Mechanosensitive and Chemosensitive Reflexes in the Premature Human Esophagus

Sudarshan Rao Jadcherla, MD, Raymond G. Hoffmann, PhD, and Reza Shaker, MD

Sections of Neonatology and Pediatric Gastroenterology and Nutrition at the Columbus Children's Hospital, Department of Pediatrics, the Ohio State University School of Medicine and Public Health, Columbus, Ohio; the Department of Biostatistics, Medical College of Wisconsin, Milwaukee, Wisconsin; and the Division of Gastroenterology and Hepatology, Department of Internal Medicine, and the Dysphagia Institute, Medical College of Wisconsin, Milwaukee, Wisconsin

Abstract

Objectives—To investigate the effect of esophageal mechanosensitive and chemosensitive stimulation on the magnitude and recruitment of peristaltic reflexes and upper esophageal sphincter (UES)-contractile reflex in premature infants.

Study design—Esophageal manometry and provocation testing were performed in the same 18 neonates at 33 and 36 weeks postmenstrual age (PMA). Mechanoreceptor and chemoreceptor stimulation were performed using graded volumes of air, water, and apple juice (pH 3.7), respectively. The frequency and magnitude of the resulting esophago-deglutition response (EDR) or secondary peristalsis (SP), and esophago-UES-contractile reflex (EUCR) were quantified.

Results—Threshold volumes to evoke EDR, SP, or EUCR were similar. The recruitment and magnitude of SP and EUCR increased with volume increments of air and water in either study ($P < .05$). However, apple juice infusions resulted in increased recruitment of EDR in the 33 weeks group ($P < .05$), and SP in the 36 weeks group ($P < .05$). The magnitude of EUCR was also volume responsive (all media, $P < .05$), and significant differences between media were noted ($P < .05$). At maximal stimulation (1 mL, all media), sensory-motor characteristics of peristaltic and EUCR reflexes were different ($P < .05$) between media and groups.

Conclusions—Mechano- and chemosensitive stimuli evoke volume-dependent specific peristaltic and UES reflexes at 33 and 36 weeks PMA. The recruitment and magnitude of these reflexes are dependent on the physicochemical properties of the stimuli in healthy premature infants.

Gastroesophageal reflux (GER) is frequent in neonates and infants, and its role in the cause of or association with neonatal morbidity including dysphagia, chronic lung disease, or apparent life-threatening events is not understood.^{1,2,3} The mechanisms of GER disease are as well defined in neonates as in adults.^{4,5} Transient lower esophageal sphincter (LES)

relaxation is the most common cause, but hypotonic LES and poor esophageal peristalsis are also described.^{6,7} The refluxate (gas, liquid, or mixed contents) provokes esophageal distention or acidification, and it may trigger luminal clearance to provide aerodigestive safety. Clearance mechanisms including primary peristalsis (PP), secondary peristalsis (SP) and esophago-upper esophageal sphincter (UES)-contractile reflexes (EUCR) that may prevent the entry of refluxate into the pharynx or larynx are well recognized in healthy adults and those with GER disease.⁸⁻¹¹ Immaturity, rapid development, and changing feeding behavior distinctly separate evolving pathophysiology in neonates from that of older subjects.

Aerodigestive symptoms may result from delayed clearance, airway aspiration, or chemoreceptor stimulation.¹²⁻¹⁵ We described methods to evaluate the esophageal body and UES motor responses resulting from esophageal provocation.¹⁶⁻¹⁸ In our previous studies, we validated the technique of esophageal afferent provocation using different media (air or liquids) and characterized the occurrence of esophago-deglutition response (EDR), SP, and EUCR. The aim of the current study was to advance the investigation of (1) the impact of maturation on mechanosensitivity and chemosensitivity of esophageal afferents in the recruitment and magnitude of peristaltic reflexes and UES-contractile pressure; and (2) to compare the sensory-motor responses at maximal stimulus. We tested the working hypothesis that maturation modifies the esophageal sensory-motor responses to provocation by using different modes of stimuli and by applying multiple comparisons using multiple logistic regression models.

Methods

Study Participants

The study was designed to evaluate the premature neonates at two distinct time intervals. Participants had to satisfy all the inclusion and exclusion criteria for each study visit. The inclusion criteria were (a) 26 weeks postmenstrual age (PMA) or greater at study entry; (b) stable vital signs; (c) absence of contraindication to gavage feeding; (d) absence of prokinetics or acid-suppressive therapies; and (e) the upper limit of gestation was 32.0 weeks. Subjects with congenital birth defects, neurological abnormalities, perinatal asphyxia, and chromosomal disorders were excluded. Neonates who had respiratory distress syndrome in the first week of life were eligible.

The initial study was performed at an average age of 33 weeks when subjects were stable and tolerating enteral gavage feeds. The second study was performed at an average age of 36 weeks when nipple feeding was successful. The Human Research Review Committee of the Medical College of Wisconsin and the Human Rights Review Board of Children's Hospital of Wisconsin approved the study protocol. Written informed consent was obtained from parents.

Protocol for Manometric Study and Mid-esophageal Infusions

We described the manometric methods previously.¹⁷ Briefly, a specially designed manometry catheter with UES sleeve, a mid-esophageal infusion port, and five side-holes

and a distal gastric recording site was passed. The catheter was connected to a minimally compliant micromanometric water perfusion system (Dentsleeve Pty. Ltd., Adelaide, Australia), Abbott Transpac transducers (Abbott Laboratories, Chicago, IL), and Grass amplifiers and recording system (Grass Instruments, Quincy, Mass). Water was perfused at 0.02 mL/minute/port, and the response rate was 160 mm Hg/second.

The esophageal provocation protocol was described by us before in neonates and adults.^{17,19} Briefly, all studies were done in supine position using an infant-sized appropriate manometry catheter. After the infant was allowed to adapt to the catheter systems, the volume escalation protocol was performed in the same order for both studies: (1) air; (2) sterile water; and (3) apple juice. Stimuli were given via the midesophageal infusion port. Infusions of air (0.1, 0.5, 1.0, and 2.0 mL), water (0.1, 0.5, and 1.0 mL), and apple juice (0.1, 0.5, and 1.0 mL) were administered three times during esophageal quiescence. Every effort was made to give the infusions abruptly; however, because of varying physical characteristics of the media or the resistance in the catheter to fluid movement, the infusion was given over 0.5 to 2 seconds.

Data Analysis

Recordings were analyzed as described previously.¹⁷ In brief; esophageal provocation was recognized by the presence of common cavity pressure change (Figure 1) measured as an intra-esophageal pressure increase, infusion port pressure increase, and event marker.^{7,17} Upon esophageal provocation, (a) SP was identified by the presence of esophageal pressure waves independent of pharyngeal swallow within 8 seconds; (b) EDR was identified by the occurrence of pharyngeal swallow–initiated primary esophageal peristalsis within 5 seconds; and (c) EUCR as a rise in UES tone of >4mm Hg (Figure 1). EDR was distinct from spontaneous PP in that the former occurred in response to esophageal infusions. Resting UES pressure was measured as the mean of five readings taken at end-expiration before each infusion. Change in UES pressure was calculated as the difference between resting upper esophageal sphincter pressure (UESP) and maximum pressure increase after the infusion and expressed as a percentage.

Upon stimulation with any infusion there were two possibilities: (1) absence or (2) presence of a response. A response can occur either as an isolated reflex (SP or EDR or EUCR) or a combination of these responses. A combination of responses was considered as a composite response. Threshold volume was measured as the least infusion volume that resulted in a response at least 50% of the times infusions were given. Mean group threshold volume for each infusion type for each reflex (SP, EDR, and EUCR) was calculated. Response time was measured as the time taken for a reflex to occur from the point of maximal infusion signal recorded from the infusion port.

Statistical Analysis

Mean values for each reflex were calculated as mean $\pm$ SD, unless stated otherwise. Data derived at either PMA of study were grouped separately and compared using paired *t* tests. Multiple logistic regression models were used to test the relationships between continuous variables (dose of each stimulus) with binary outcomes (nominal responses) for each reflex

(e.g., air-SP, air-EUCR, air-EDR, and likewise for water- and apple juice-mediated effects) for either study. To examine the effect of maturation on the dose response relationship, a generalized estimating equation (GEE) approach was used to test for differences in volume relationships. The odds ratios, 95% confidence intervals, and *P* values were determined for each group to predict the likelihood of the specific response. Analysis of variance and Fisher's protected least significant differences test were used to compare between the categories (media or volume or study). To evaluate the relationship between dose volume and magnitude of EUCR, repeated measures analysis of variance was used. *P* < .05 was considered significant. All tests were performed using SAS software and StatView for Windows 5.0.1 (SAS Institute, Cary, NC) and were based on accepted statistical applications.^{17,20,21}

Results

Demographics

Eighteen healthy premature infants (12 males, 6 females; 26 to 33.7 weeks gestational age) were studied longitudinally. The mean PMAs at initial study were 33.4 weeks (median 33.6 weeks, range 29.1-36.0 weeks) and at later study were 35.7 weeks (median 35.8 weeks, range 32.4-38.9 weeks). Growth was appropriate at birth, for the initial study, and for the later study. Thirteen infants required respiratory assistance for respiratory distress syndrome (6.6 ± 3.6 ventilation days). None of the infants had chronic lung disease (oxygen requirement at 36 weeks PMA) or neurological abnormalities (intraventricular bleeds or periventricular leukomalacia) at discharge. At the 33 weeks study, only one infant (6%) had been started oral feeds, and at the 36 weeks study, 15 infants (83%) were orally fed ($\chi^2 = 19$, *P* < .001). All infants attained full nipple feeds at discharge.

There were no differences in vital signs before or after esophageal stimulation at either 33 weeks or 36 weeks. No adverse events were noted at and between each of the study visits.

Sensory Thresholds and Motor Responses to Graded Stimuli

Threshold Volumes—The success rate of evoking a composite reflex response (EDR, SP or EUCR) to graded esophageal stimulations was 81% at 33 weeks and 80% at 36 weeks. There was a volume-dependent increase in recruitment of reflexes in both studies with all three infusion media (*P* < .05, Figure 2). Over all, at 36 weeks, the volume sensitivities for the three reflexes were similar (*P* = NS), but at 33 weeks, they were different (*P* < .05, Table I).

Relationship between Recruitment of Esophageal Reflexes versus Graded Stimuli—To evaluate the effect of dose volume, medium, and maturation on the recruitment of reflexes, we used a multiple logistic regression model for the pooled data, and comparisons were made between the maturational stages using GEE methods (Table II). The likelihood of enhanced recruitment of SP and EUCR with increment in infusion volumes of air and water was evident (*P* < .05) in both studies.

In contrast, with increase in volumes of apple juice: (a) at 33 weeks, the likelihood of EDR is 5.3 times, EUCR is 1.8 times, and SP is 0.9 times, in that order of magnitude; (b) at 36

weeks, the likelihood of EDR is 1.5 times, EUCR is 3.2 times, and SP is 5.2 times, in that order of magnitude. EDR is the chief clearance mechanism at 33 weeks and SP at 36 weeks ($P < .05$, GEE).

Magnitude of EUCR with GRADED Esophageal Stimuli—We compared the change in UES pressure with graded stimulus modes. At either study, a graded volume-response relationship was evident for each stimulus ($P < .05$, Figure 3). Comparisons between the studies were significant with all infusion media ($P < .05$).

Sensory Thresholds versus Motor Responses at Maximal Stimulus (1mL)

Common cavity Pressure Change—The common cavity pressure change was compared between the 33 weeks and 36 weeks studies: air (9.6 ± 3 mm Hg vs 8.1 ± 4.6 mm Hg, $P = \text{NS}$), water (13 ± 6.2 mm Hg vs 8.8 ± 6.3 mm Hg, $P < .04$), and apple juice (12 ± 6.6 mm Hg vs 10 ± 7 mm Hg, $P = \text{NS}$).

Response Time to the Onset of Motor Response—The response time (seconds) to evoke SP between the 33 weeks and 36 weeks studies were: for air (4.2 ± 1.8 v. 2.8 ± 1.2 , $P = \text{NS}$), water (2.7 ± 1.6 vs 3.5 ± 1.2 , $P = \text{NS}$), and apple juice (3.3 ± 1.8 vs 3.1 ± 1.9 , $P = \text{NS}$). The response time (seconds) to evoke EDR in the 33 weeks and 36 weeks groups were: for air (2.3 ± 0.9 vs 3.1 ± 1.9 , $P = \text{NS}$), water (2.4 ± 1.7 vs 3 ± 2.7 , $P = \text{NS}$), and apple juice (1.9 ± 0.8 vs 1.3 ± 0.3 , $P = \text{NS}$). We also compared the response times for both SP and EDR between air versus water and between water versus apple juice at either study (both comparisons, $P = \text{NS}$).

Frequency of Esophageal Peristaltic Reflexes (EDR or SP)—Frequency of responses between the 33 weeks and 36 weeks groups within the infusion media did not reveal any differences in distribution of reflexes with air or water stimuli at either study ($P = \text{NS}$). However, with apple juice, the percentage distribution of peristaltic reflexes (EDR: SP: None) at 33 weeks was 29:45:26 and at 36 weeks was 8:69:23 ($\chi^2 = 17$, $P < .001$). Frequency of SP was greater and EDR lesser at 36 weeks.

Comparison between the infusion media at identical maturational stages revealed similarly distributed responses ($P = \text{NS}$) between air versus water in both studies. No differences were seen between water versus apple juice in the 33 weeks study. In contrast, in the 36 weeks study, the percentage distribution of peristaltic reflexes (EDR: SP: None) with water was 21:56:23, and with apple juice was 8:69:23 ($\chi^2 = 7$, $P < .03$). Frequency of SP was greater and EDR lesser with apple juice compared with water stimuli.

Magnitude of EUCR—The magnitude of EUCR (% change in UESP over baseline) at the 33 weeks versus 36 weeks study was similar (air stimulus, 86 ± 48 vs 124 ± 81 ; water stimulus, 130 ± 87 vs 159 ± 114 ; and apple juice 128 ± 71 vs 136 ± 83 ; $P = \text{NS}$ for all media). However, water infusions generated higher UES magnitude at 36 weeks (vs apple juice, $P < .015$) but not at 33 weeks.

Discussion

Using novel experimental and analytical methods, we investigated the vago-vagal reflex arc during maturation in human infants by evaluating the sensory-motor responses to graded stimuli and maximal stimuli. The choice of stimuli permitted systematic and safe evaluation of mechanosensitive and chemosensitive receptor evoked reflexes such as may happen during esophageal distention or acidification. At inception, our working hypothesis was that maturation modifies the esophageal sensory-motor responses to provocation. We characterized the differences and similarities in these responses with maturation.

Specific maturational differences include (1) lower thresholds for mechano- and chemostimulation to evoke SP in the 33 week study; (2) with acidic medium, the principal peristaltic reflex was EDR in the 33 weeks study, and SP in the 36 weeks study; (3) in the 36 weeks study, water infusions generated higher UES magnitude than did apple juice in contrast to the 33 weeks study; and (4) changes in UES magnitude with volume increments were higher between the studies for all media.

In both studies, important similarities were observed: (1) all media induced similar mechano-distention at the maximal volume tested; (2) similar latent intervals occurred between stimulation and evoked motor responses for all media; (3) recruitment of SP and EUCR can be predictable with graded increment in air or water infusions; and (4) volume-dependent increase in UES magnitude occurred with all media.

The rationale for measuring the common cavity pressure change was to test if there was a difference in pressure generated (at a constant infusion volume) that resulted in a reflex response. The average common cavity pressures with all the three media at 1 mL volumes were less at 36 weeks, and with water infusion this difference was statistically significant. The physiological significance of this isolated finding is uncertain, but one may hypothesize that with maturation the esophageal compliance (volume-pressure relationship) increases, as a result of esophageal growth.

In the 36 weeks study, threshold volumes of different media were similar, thus implying similar receptor sensitivities. In contrast, such similarities were not evident at the 33 weeks study. The comparable response times suggest that the activation of afferent-efferent pathways in eliciting motor phenomenon, during maturation, and between the media is analogous between the groups. With a greater age gap between the studies, one may see differences in these variables.

Application of multiple logistic regression and GEE methods allowed us to characterize the complex relationship between the independent variables (33 weeks and 36 weeks groups, three media, and graded volumes) and the dependent variables (binary outcome of the three specific reflexes).^{17,20,21} The outcome of these results suggests that similar mechano-receptor thresholds trigger SP or EUCR at higher volumes of air or water by 33 and 36 weeks PMA. However, inter-group differences with apple juice infusions support that SP is the chief clearance mechanism at older PMA in contrast to EDR at younger PMA. This mechanism may be because of differences in pH sensitive chemoreceptor function across

maturation, and it may be one of the reasons for frequent swallowing noted in younger infants such as may happen in GER.²²

With advanced maturation, at a maximal stimulus, apple juice favored enhanced recruitment of SP (contrasting water). These findings confirm that the chemoreceptor function is sensed differently at advanced maturation in that SP remains the dominant reflex response. The proportions of SP or EDR between air versus water at either study were similar, thus suggesting similar mechanoreceptor activity with these media.

Sensory-motor differences were also observed in response to mechano- and chemostimulation of the esophagus in human adults,^{23,24} and in animal models.^{25,25} Stimulus-response function to intraluminal graded distention resulted in a linear increase in vagal and spinal afferent nerve activity, along with an increase in magnitude at maximal stimulus^{25,26}; correspondingly, the linear dose-dependent response noted in our study may be explained by the same mechanism in *in vivo* human premature infants.

Magnitude of EUCR was volume-dependent at both studies, and this may reflect differences in sensory-motor recruitment with dose increments. This finding may be because of increase in muscle mass and therefore stronger contraction or enhanced nerve-muscle interaction. An increase in UES tone has been noted in children and adults during GER events^{8,15,27}; and from our study, an increased level of aerodigestive tract protection is evident with volume increments. Mechanisms such as EUCR may provide airway protection against retrograde aspiration. However, effect of media (neutral pH vs acidic pH) at a maximal stimulus evoked greater magnitude with water. An absence of chloride ions (water) or stimulation of osmoreceptors may be responsible for this difference in UES magnitude.^{13,14} Thach et al made similar observations in response to pharyngeal water stimuli, but not saline, causing obstructive apnea in neonatal human or animal models.^{13,14} In our study, EUCR may protect the airway by increasing the cricopharyngeal tone to maintain aerodigestive clearance.

In summary, we identified the ontogeny of aerodigestive reflexes at 33 weeks and 36 weeks PMA in healthy human premature neonates who achieved appropriate feeding milestones and were asymptomatic at discharge. Our findings are reassuring in that esophageal provocation in healthy preterm neonates such as may happen during GER may result in mechano- or chemostimulation-mediated reflexes that facilitate clearance and airway protection. Basal esophageal motility has been described by others and us.^{5,16,28} In this study we evaluated the adaptive motor responses evoked by esophageal stimulation. We propose that the sensory-motor characteristics of the evoked reflexes may be responsible for the esophageal motility, rather than the reflux *per se*. Esophageal mechano- and chemosensitive stimuli are associated with appropriate aerodigestive defense responses during normal health and maturation, and an ability to provide esophageal clearance mechanisms. Volume and nature of stimuli and development can modify the esophageal motor response. Future studies must evaluate these neuromotor characteristics to specifically identify the sensory-motor deficits in diseases affecting the neonatal aerodigestive tract.

Acknowledgments

We gratefully acknowledge the excellent technical support from Hao Q. Duong, BS, and Peri Ann Meyers, RN, for nursing observations during studies.

Supported in part by NIH (NIDDK) 7 RO3 61502-02 and RO1 DK 068158.

References

1. Jadcherla SR, Rudolph CD. Gastroesophageal reflux in the preterm neonate. *Neo Reviews*. 2005; 6:e87–e98.
2. Orenstein SR. Gastroesophageal reflux. *Curr Prob Pediatrics*. 1991; 21:193–241.
3. Sondheimer JM. Gastroesophageal reflux: update on pathogenesis and diagnosis. *Pediatr Clin North Am*. 1998; 35:103–16. [PubMed: 3277128]
4. Werlin SL, Dodds WJ, Hogan WJ, Arndorfer RC. Mechanisms of gastroesophageal reflux in children. *J Pediatr*. 1980; 7:244–9. [PubMed: 7400890]
5. Omari TI, Barnett C, Snel A, Goldsworthy W, Haslam R, Davidson G, et al. Mechanisms of gastroesophageal reflux in healthy premature infants. *J Pediatr*. 1998; 133:650–4. [PubMed: 9821423]
6. Orlando RC. Overview of the mechanisms of gastroesophageal reflux. *Am J Med*. 2001; 111:174S–177S. [PubMed: 11749946]
7. Mittal RK, Chiareli C, Liu J, Shaker R. Characteristics of lower esophageal sphincter relaxation induced by pharyngeal stimulation with minute amounts of water. *Gastroenterology*. 1996; 111:378–84. [PubMed: 8690202]
8. Torrico S, Kern M, Aslam M, Narayanan S, Kannappan A, Ren J, et al. Upper esophageal sphincter function during gastroesophageal reflux events revisited. *Am J Physiol Gastrointest Liver Physiol*. 2000; 279:G262–G267. [PubMed: 10915633]
9. Kahrilas PJ, Dodds WJ, Dent J, Logemann JA, Shaker R. UES function during deglutition. *Gastroenterology*. 1988; 96:52–62. [PubMed: 3371625]
10. Schoeman MN, Holloway RH. Stimulation and characteristics of secondary esophageal peristalsis in normal subjects. *Gut*. 1994; 35:152–8. [PubMed: 8307463]
11. Shaker R, Lang IM. Reflex mediated airway protective mechanisms against retrograde aspiration. *Am J Medicine*. 1997; 103:64S–73S.
12. Menon AP, Schefft GL, Thach BT. Airway protective and abdominal expulsive mechanisms in infantile regurgitation. *J Appl Physiol*. 1985; 59:716–21. [PubMed: 4055562]
13. Thach BT. Maturation and transformation of reflexes that protect the laryngeal airway from liquid aspiration from fetal to adult life. *Am J Medicine*. 2001; 111:69S–77S.
14. Pickens DL, Schefft GL, Thach BT. Pharyngeal fluid clearance and aspiration preventive mechanisms in sleeping infants. *J Appl Physiol*. 1989; 66:1164–71. [PubMed: 2708242]
15. Sondheimer JM. Upper esophageal sphincter and pharyngoesophageal motor function in infants with and without gastroesophageal reflux. *Gastroenterology*. 1983; 85:301–5. [PubMed: 6862154]
16. Jadcherla SR, Shaker R. Esophageal and UES motor function in babies. *Am J Medicine*. 2001; 111:64–68.
17. Jadcherla SR, Duong HQ, Hoffman R, Shaker R. Esophageal body and UES motor responses due to abrupt esophageal provocation in premature infants. *J Pediatr*. 2003; 143:31–8. [PubMed: 12915821]
18. Jadcherla SR. Manometric evaluation of esophageal-protective reflexes in infants and children. *Am J Medicine*. 2003; 115:157–60.
19. Uluap SO, Toohill RJ, Gu C, Shaker R. Loss of secondary peristalsis is not a contributory pathogenetic factor in posterior laryngitis. *Ann Otol Rhinol Laryngo*. 2001; 110:152–7.
20. Millikan, GA.; Johnson, DE. Simultaneous inference procedures and multiple comparisons. In: Millikan, GA., editor. *Analysis of Messy Data, Volume I: Designed Experiments*. Boca Raton, FL: Chapman and Hall/CRC; 1992. p. 29-46.

21. Hsu, J. Multiple Comparisons: Theory and Methods. Boca Raton, FL: Chapman and Hall/CRC Press; 1996.
22. Jeffrey HE, Ius D, Page M. The role of swallowing in active sleep in the clearance of reflux in term and preterm infants. *J Pediatr.* 2000; 137:545–8. [PubMed: 11035836]
23. Fass R, Naliboff B, Higa L, Johnson C, Kodner A, Munakata J, et al. Differential effect of long-term esophageal acid exposure on mechanosensitivity and chemosensitivity in humans. *Gastroenterology.* 1998; 115:1363–73. [PubMed: 9834263]
24. Kern MK, Birn RM, Jaradeh S, Jesmanowicz A, Cox RW, Hyde JS, et al. Identification and characterization of cerebral cortical response to esophageal mucosal acid exposure and distention. *Gastroenterology.* 1998; 115:1353–62. [PubMed: 9834262]
25. Sengupta JN. An overview of esophageal sensory receptors. *Am J Med.* 2000; 108:87S–89S. [PubMed: 10718458]
26. Sengupta JN, Saha JK, Goyal RK. Stimulus-response function studies of esophageal mechanosensitive nociceptors in sympathetic afferents of opossum. *J Neurophysiol.* 1990; 64:796–812. [PubMed: 2230925]
27. Willing J, Davidson GP, Dent J, Cook I. Effect of gastro-oesophageal reflux on upper oesophageal sphincter motility in children. *Gut.* 1993; 34:904–10. [PubMed: 8344577]
28. Jadcherla SR, Duong HQ, Hofmann C, Hoffmann RG, Shaker R. Characteristics of upper esophageal sphincter and esophageal body during maturation in healthy human neonates compared with adults. *Neurogastro-enterol Motil.* 2005; 17:663–70.

Glossary

EDR	Esphago-deglutition response
EUCR	Esophago-USE-contractile reflex
GEE	Generalized estimating equation
GER	G astroesophageal reflux
LES	Lower esophageal sphincter
PMA	Postmenstrual age
PP	Primary peristalsis
SP	Secondary peristalsis
UES	Upper esophageal sphincter
UESP	Upper esophageal sphincter pressure

Figure 1.

A mid-esophageal stimulus is given using the specially built manometric catheter, and is identified by the common cavity (CC) pressure change and the upstroke on the infusion port. Responses to apple juice at 33 weeks are esophag-deglutition response (EDR), and at 36 weeks were secondary peristalsis (SP) and esophago-UES-contractile response (EUCR).

Figure 2.

Recruitment of composite responses with graded increments of infusion media. Volume-dependent recruitment of reflex responses was observed in both studies with all media (* $P < .05$). In both studies, composite responses were noted nearly 90% of the times at an infusion volume of 1mL.

Figure 3.

Magnitudes of esophago-UES-contractile reflex (EUCR) in response to the three stimulus modes. Infusion volume-dependent change in UES pressure is evident for all media in both studies (*P < .05)

Table I
Threshold volume of infusion media (mL, mean $\pm$ SD) vs specific reflexes

Stimulus-PMA	SP	EUCR	EDR
Air - 33 wk	$0.5 \pm 0.6^*$	0.9 ± 0.6	0.7 ± 0.8
Water - 33 wk	0.4 ± 0.4	0.6 ± 0.4	0.6 ± 0.3
Apple Juice - 33 wk	$0.3 \pm 0.3^\dagger$	$0.4 \pm 0.4^*$	0.7 ± 0.5
Air - 36 wk	0.5 ± 0.7	0.8 ± 0.7	0.6 ± 0.6
Water - 36 wk	0.5 ± 0.4	0.6 ± 0.4	0.5 ± 0.4
Apple Juice - 36 wk	0.5 ± 0.4	0.5 ± 0.4	0.7 ± 0.4

Multiple comparisons were made within and between the media and maturational ages.

* $P < .05$ vs EUCR air, 33 weeks;

$^\dagger P < .05$ vs EDR apple juice, 33 weeks.

Table II

Graded stimulus-response relationship across maturation

Infusion	Response	33 weeks		36 weeks	
		Odds Ratio (95%CI)	P	Odds Ratio (95%CI)	P
Air	SP	1.9 (1.1–3.0)	<.01	1.6 (1.0–2.6)	<.05
	EUCR	3.2 (1.9–5.5)	<.001	1.7 (1.1–2.7)	<.02
	EDR	1.3 (0.7–2.4)	NS	1.1 (0.7–2.0)	NS
Water	SP	3.0 (1.0–8.7)	<.04	5.2 (1.8–15.0)	<.002
	EUCR	3.7 (1.2–11)	<.02	5.3 (1.8–15.4)	<.002
Apple Juice	EDR	2.4 (0.7–8.1)	NS	1.7 (0.5–6.2)	NS
	SP*	0.9 (0.3–2.6)	NS	5.2 (1.8–15.1)	<.005
	EUCR	1.8 (0.6–5.3)	NS	3.2 (1.2–9)	<.02
	EDR	5.3 (1.1–24.7)	<.03	1.5 (0.3–8.3)	NS

To characterize the relationship between the independent variables (33 weeks and 36 weeks, the three media, and graded volumes) and the dependent variables (binary outcome of the three specific reflexes, i.e., SP, EUCR, EDR), we applied multiple logistic regression and GEE methods. Significant values denote a positive correlation between volume increments and the predictive reflex. For example, with a unit increase in dose volume of apple juice, the occurrence of SP at 33 weeks was 0.9 times vs 5.2 times at 36 weeks, and that SP is more frequent with advanced maturation

* ($P < .05$, GEE).