Physiology of esophageal sensorimotor malfunctions in neonatal neurological illness

Sudarshan R. Jadcherla,1,2 Chin Yee Chan,2 Rebecca Moore,2 Soledad Fernandez,3 and Reza Shaker4

1Sections of Neonatology, Pediatric Gastroenterology and Nutrition, 2The Neonatal and Infant Feeding Disorders Program, Center for Perinatal Research, Department of Pediatrics, and 3Center for Biostatistics, The Ohio State University College of Medicine, The Research Institute at Nationwide Children’s Hospital, Columbus, Ohio; 4Division of Gastroenterology, Department of Internal Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin

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Jadcherla SR, Chan CY, Moore R, Fernandez S, Shaker R. Physiology of esophageal sensorimotor malfunctions in neonatal neurological illness. Am J Physiol Gastrointest Liver Physiol 304: G574–G582, 2013. First published January 17, 2013; doi:10.1152/ajpgi.00404.2012.—We aimed to define the sensorimotor characteristics of aero-digestive reflexes evoked upon midesophageal provocations in neuropathology infants. Provocative esophageal motility testing was performed in 20 neuropathology infants and 10 controls at 42.3 ± 0.6 and 38.9 ± 0.9 wk postmenstrual age. Data from 1,073 infusions were examined for the sensory thresholds, response frequencies, response magnitude of upper esophageal sphincter (UES) contractile reflexes, lower esophageal sphincter (LES) relaxation reflexes, and peristaltic reflexes using mixed statistical models. Threshold volumes for air and liquid in neuropathology and control infants were similar for all reflexes. Graded air- and liquid volume-dependent UES contractile reflex, LES relaxation reflex, and peristaltic reflex frequency recruitment were present in neuropathology and control subjects for the media (P < 0.0001) and the reflexes (P < 0.0001). In neuropathology infants (vs. controls), UES contractile magnitude is higher (P < 0.0001); LES relaxation reflex occurred earlier (P = 0.008); LES nadir duration lasted longer (P = 0.006); secondary peristalsis is the chief method of esophageal clearance (P < 0.0001); pharyngeal swallows and deglution apneas are less frequent (P = 0.001); proximal, midesophageal waveform magnitudes and duration are exaggerated (P < 0.008). UES contractile reflex was longer with liquid than air in both groups (P = 0.03). We concluded that 1) perception to midesophageal provocation remains preserved in neuropathology neonates; 2) sustained and exaggerated myogenic response from afferent activation is evident by increased excitatory efferent outputs to the UES and esophageal body and increased inhibitory efferent outputs to the LES; 3) dysfunctional regulation of pharyngeal swallowing and ineffective deglutition responses indicate the possibility of impaired descending modulation and central malfunctions of brainstem and vagal nuclei; dysphagia, gastroesophageal reflux (GER) disease, and life-threatening choking events (26, 29). Despite the prevalence of such problems, the mechanisms of malfunction of aero-digestive reflexes are unclear.

The integrity, coordination, and functions of upper esophageal sphincter (UES), lower esophageal sphincter (LES), and esophageal body are essential to maintain swallowing, esophageal peristalsis, and airway protection (2, 23, 24, 28). During health, the sequence of aero-digestive reflexes evoked upon esophageal provocation facilitates peristaltic clearance and prevents the stimulus spread into the vicinity of airway (18).

We have recently developed and validated multimodal esophageal provocative methods to examine the functions of vagal afferent and efferent pathways by defining sensorimotor characteristics of esophageal body, UES contractile reflexes, and LES relaxation reflexes in healthy human neonates (7, 8, 11, 12, 24). Specifically, mechanosensitive, osmosensitive, and chemosensitive provocation of the midesophagus was undertaken using air, water (pH 7.0), and apple juice (pH 3.7), respectively, and the effects on upstream (UES) and downstream (LES) reflexes and esophageal peristalsis were evaluated. By using these media in graded incremental volumes, we defined the responses to varying physical and chemical characteristics of stimulus, media, and volume (12, 25). During GER events, physical and chemical characteristics of the re-fluxate provoke the esophagus. By using different media to provoke the esophagus, we tested the individual effects of mechanosensitive, osmosensitive, and chemosensitive stimulation. The effect of esophageal provocation in neonatal neurological illness is unclear and therefore contributed to the rationale for this study.

The rationale for the present study has the following scientific basis: we have previously validated and tested methods to define esophageal provocation-induced vago-vagal reflexes in neonates, such as those induced by esophageal mechanosensitive and chemosensitive stimulation during maturation. Similar work has been accomplished in human adults and animal models (6, 20). The latter work clearly establishes the role of the vagus nucleus and vagal innervations and networks modulating the esophageal and sphincteric sensorimotor activity and the role of brain stem. Provocative manometry model of investigating vago-vagal reflexes in the present pathological setting of neonatal neurological illness has not been studied before. Therefore, we undertook the aims to test the hypothesis that infants with neonatal neurological illness have malfunctions of vago-vagal esophageal and sphincteric reflexes. Specifically, we defined and differentiated the sensorimotor characteristics of esophageal mechanosensitive, osmosensitive, and intracranial hemorrhage

NEONATAL NEUROLOGICAL CONSEQUENCES are important antecedents for aero-digestive and developmental morbidities (3, 15, 16). Specifically, intracranial hemorrhage and perinatal asphyxia constitute the most frequent neonatal neurological illness, with an estimated risk of developmental disability in 25–50% and cerebral palsy in 25% (30, 31). Clinical prototype of aero-digestive concern include excessive pooling of oral secretions, inability to swallow secretions, aspiration pneumo-
chemosensitive stimulation-induced peristaltic reflexes, UES contractile reflexes, and LES relaxation reflexes. We also examined the esophageal sensitivity by measuring sensory stimulus threshold volume, response latency, and response duration. We examined the motor characteristics by defining the reflex-recruitment frequency, stimulus-response relationships, and response magnitude.

MATERIALS AND METHODS

Participants

Twenty neurologically impaired neonates (6 males; 36.0 ± 1.3 wk gestational age, GA) were evaluated at 42.3 ± 0.6 wk postmenstrual age (PMA) when they had oral feeding problems and were therefore receiving nasogastric feedings. Generally, by full-term PMA, most healthy infants are on full oral feeds (16) regardless of GA at birth. Because full-term healthy infants are a difficult group to recruit and are generally discharged within 48 h; to compare data, in this study 10 healthy control infants that had independent oral feeding skills (4 male; 32.7 ± 1.5 wk GA) were studied at 38.9 ± 0.9 wk PMA. The controls were admitted initially for transitional respiratory distress and received routine neonatal care. These subjects were feeding and thriving appropriately during the hospital course and did not have any neuropathology or other gastrointestinal problems either at admission or at evaluation. All subjects were evaluated by the principal investigator (S. Jadherva) and the attending neonatologist and were deemed appropriate at study. None of the subjects had a presumed or proven clinical diagnosis of GER, and none were receiving prokinetics, acid suppressive therapy, or xanthines at the time of study or discharge. Infants with congenital gastrointestinal abnormalities, birth defects, chromosomal disorders, and congenital brain abnormalities were excluded.

The study procedures were approved by the ethics committee at the Institutional Research Review Board at the Nationwide Children’s Hospital Research Institute, Columbus, Ohio. The study protocol conforms to the standards set by the latest revision of the Declaration of Helsinki, and the methods and procedures were approved by Nationwide Children’s Hospital Research Institute constituted Institutional Research Review Board and were in accordance with the Health Insurance Portability and Accountably Act. Written, informed consents were obtained from parents before study.

Manometry Methods

The use of esophageal manometry methods and multimodal provocation techniques in neonates was described by our group (8, 9, 11). Briefly, the catheter assembly (Dentsleeve International; Mui Scientific, Mississauga, Ontario, Canada) was connected to the pneumohydraulic micromanometric water perfusion system via the resistors, pressure transducers (tumor necrosis factor receptor disposable pressure transducers), and amplifiers (modalpures, Solar 2; MMS Medical Instruments, Dover, NH). The esophageal manometry catheter assembly with dual sleeves (recording from UES and LES) and four side ports recording from pharynx, proximal, middle, and distal esophageal loci and a terminal gastric recording port was used. The catheter also includes a dedicated sensorimotor infusion channel to provide esophageal stimulus. The water perfusion rate was 0.02 ml/min per port for esophageal ports, 0.01 ml/min per port for the pharyngeal port, and 0.04 ml/min per port for the sleeves. The catheter was passed nasally in the unsedated supine lying neonate, and all studies were done in the same manner, with the transducers at the level of the subject’s esophagus (midaxillary line). Vital signs were monitored for safety during the manometry study.

Manometric Experimental Protocol

Continuous data acquisition and analysis were performed based on manometric waveform characteristics (8, 9, 11). Middle esophageal provocations with air, water, and apple juice (pH = 3.70) were performed to test the effects of mechanosensitive, osmosensitive, and chemosensitive stimulation, respectively. During catheter placement and pull through, the UES and LES sleeves were positioned such that they straddled the UES and LES high-pressure zones, respectively, and were identified by the presence of a consistent increase in pressure >5.0 mmHg above the baseline for at least 15 s, in addition to the changes in pressure with respiration. After neonates were allowed to adapt for about 15 min, we evaluated responses to midesophageal provocation.

Manometry Data Analysis

The manometric waveforms related to UES, LES, and esophageal reflex characteristics were analyzed as defined before (7–12, 24). Briefly, esophageo-deglutition response (EDR) was defined as a deglutition response to esophageal stimulation, which begins with onset of the pharyngeal waveform associated with UES relaxation, propagates into the proximal, middle, and distal esophageal segments, and is accompanied by LES relaxation. Secondary peristalsis (SP) occurs in response to sensorimotor provocation and is defined as the propagation of waveforms distally from the proximal, middle, and distal esophageal segments in the absence of pharyngeal waveform and UES relaxation. The onset of proximal esophageal upstroke from the stimulus onset defines the response latency for SP. Response onset to peristaltic reflex was taken from onset of the stimulus to the onset of EDR or SP. Deglutition apnea and changes in respiratory rhythm related to peristaltic reflexes were defined using respiratory inducance plethysmography.

Analysis Related to UES and LES Characteristics

Resting UES pressure was measured as an average of five UES pressure measurements at the end of expiration observed before stimulus. Response latency to UES contractile reflex is defined as the time taken from the onset of stimulus for an increase in UES pressure of at least 4 mmHg above baseline. Maximum UES contraction pressure was taken at the maximum pressure reached after onset of UES contraction. UES contractile magnitude is defined as the pressure differences between the Maximum UES contraction pressure and the resting UES pressure. UES contractile reflex duration is measured from the onset of UES contraction to the peak of UES contractile reflex. Resting LES pressure was measured as an average of five LES pressure measurements observed before stimulus. All pressures were taken at the end of expiration and in relation to gastric pressure.

Statistical Methods

Subject characteristics, manometric measurements, and outcome variables were compared between the neuropathology and control groups. Multinomial mixed models and linear mixed models with compound symmetry matrix were used to analyze the repeated-measures data. Statistical tests were adjusted for multiple comparisons using the Tukey-Kramer method. These models were fit using PROC GENMOD for categorical responses and PROC MIXED for continuous responses in SAS (SAS v9.2; SAS Institute, Cary, NC). To evaluate the effect of graded volume and media on the recruitment of reflexes, we used logistic regression models and compared between the groups (Table 2). Descriptive data are reported as least-square means ± SE, percentages, or as range unless stated otherwise.

RESULTS

Subject and Disease Characteristics

Ten control infants were studied at 38.9 ± 0.9 wk PMA when they had independent oral feeding skills, contrasting 20 neuropathology infants that were dysphagic and dependent on nasogastric feeding tubes at evaluation, 42.3 ± 0.6 wk PMA.
In the neuropathology vs. control groups, respectively, the median (27) APGAR scores at 1 min were 2 (0–9) vs. 6 (2–8) \((P = 0.04)\); at 5 min they were 4 (0–9) vs. 8 (4–9) \((P = 0.03)\).

At evaluation for the present study, the study group composition was heterogeneous and primarily had the following brain insults: perinatal asphyxia \((N = 12)\), intracranial hemorrhage and ventriculomegaly \((N = 6)\), neonatal encephalopathy, and seizures \((N = 2)\). During the admission and before our evaluation of these dysphagic infants, other associated specific recognizable brain insults in some of them included severe intraventricular hemorrhage (grades 3 and 4), posthemorrhagic hydrocephalus \((N = 4)\), and meningitis \((N = 2)\).

**Effects of Esophageal Provocation on Upstream and Downstream Motility**

A total of 357 esophageal infusions (graded volumes of 143 air, 109 water, and 105 apple juice) were given in the control group, and a total of 716 esophageal infusions (289 air, 219 water, and 208 apple juice) were given in the neuropathology group. Esophageal provocation-induced UES contractile reflex, LES relaxation reflex, and peristaltic reflexes (Fig. 1) were analyzed for sensory-motor characteristics.

**Sensorimotor characteristics of UES contractile reflex.** Comparing neuropathology vs. controls, for UES contractile reflex characteristics, we found that the threshold volumes to evoke UES contractile reflex were similar, response latencies \((s)\) were similar \((3.5 \pm 0.3 vs. 3.3 \pm 0.2, P = 0.6)\), the response durations \((s)\) were similar \((2.8 \pm 0.2 vs. 3.0 \pm 0.2, P = 0.1)\), and the frequency recruitments were also similar \((49.9\% vs. 48.8\%, P = 0.7)\).

Significantly, the neuropathology group had higher resting UES pressures, greater maximal UES contractile pressures, and greater change in UES contractile magnitudes (Fig. 2).

**Sensorimotor characteristics of LES relaxation reflex.** In neuropathology vs. controls, for LES relaxation reflex characteristics, the threshold volumes were similar, response latencies \((s)\) were similar \((3.8 \pm 0.2 vs. 3.5 \pm 0.2, P = 0.3)\), and frequency recruitment of LES relaxation reflex was similar \((47.0\% vs. 50.4\%, P = 0.3)\).

Significantly, the neuropathology group had quicker LES relaxation and for a prolonged nadir duration compared with

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**Fig. 1. Esophageal manometry describing secondary peristalsis during midesophageal provocation.** An example of upper esophageal sphincter (UES) contractile reflex, lower esophageal sphincter (LES) relaxation reflex, and peristaltic reflex characteristics for neuropathy (A) and control group (B). Higher resting UES pressure and longer LES nadir duration are noted in neuropathy group.

**Fig. 2.** The resting UES pressure (A), maximum UES pressure (B), and UES contractile magnitude (C) upon esophageal provocation in the control vs. neuropathology group are shown. *\(P = 0.002\); **\(P < 0.0001\).
the control group (Fig. 3). No other significant differences between the neuropathology and control groups were noted with respect to resting LES pressures (17.4 ± 0.9 mmHg vs. 18.3 ± 1.2 mmHg, \( P = 0.5 \)), LES nadir pressures (2.0 ± 0.6 mmHg vs. 3.7 ± 0.8 mmHg, \( P = 0.09 \)), or the LES relaxation magnitudes (22.3 ± 0.8 mmHg vs. 21.4 ± 1.1 mmHg, \( P = 0.5 \)).

To further clarify whether the LES relaxation characteristics were dependent on the type of peristaltic reflex, i.e., during deglutition or secondary peristalsis, individual comparisons were made (Table 1). Specifically, the frequency recruitment, response latency, response duration, and magnitude of LES relaxation were similar with EDR between neurologically impaired subjects vs. controls. On the other hand, LES relaxation characteristics during SP were similarly distributed except for the isolated findings that LES relaxation period was shorter and nadir duration longer in neurologically impaired subjects vs. controls.

**Sensorimotor characteristics of peristaltic reflex.** Mean threshold volumes to evoke peristaltic reflexes were similar between the groups. Significantly, however, the mean response latencies (in s) to onset of peristaltic reflexes were shorter in the neuropathology group compared with the control group (3.7 ± 0.1 vs. 4.5 ± 0.2, \( P < 0.0001 \)). In addition, the characteristics of esophageal body waveform propagation upon provocation, including amplitude and duration, were significantly greater and prolonged in the neuropathology group (Table 2). Although, midesophageal provocations resulted in similar frequency recruitment of peristaltic reflexes (62.2% vs. 55.2%, neuropathology vs. control group, \( P = NS \)), the characteristic distribution of peristaltic reflex type, i.e., EDR and SP were distinct. Comparisons between neuropathology (20.2% EDR, 79.8% SP) vs. controls (43.9% EDR, 56.1% SP) were different (\( P < 0.0001 \)). Furthermore, the neuropathology group had lower frequency of deglutition apnea vs. control (19.3% vs. 30.0%, \( P = 0.001 \)) and lower frequency of respiratory rate changes (vs. control, 29.9% vs. 48.0%, \( P < 0.0001 \)).

**Effect of Infusion Media and Group on the Response Latency and Response Duration of UES Contractile, Peristaltic, and LES Relaxation Reflexes**

We compared the response latency to the onset of the above adaptive reflexes between the neuropathy vs. control groups (Fig. 4). Stimulations with the liquids (water and apple juice) did not yield any differences on any variables of interest; therefore, we combined them into liquid category, and data are presented as such. Comparing air vs. liquid stimuli, liquid stimuli resulted in significantly longer response latencies to UES contractile reflexes as well as LES relaxation reflexes in both neuropathy and control groups. However, the response latencies to peristaltic reflexes were significantly longer with liquid stimulations vs. air in neuropathy group, but this was not so in the control subjects.

**Table 1. The characteristics of LES during esophageal provocation-induced esophago-deglutition response and secondary peristalsis**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>Neuropathy</th>
<th>( P ) Value</th>
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<tbody>
<tr>
<td><strong>Esophago-Deglutition Response</strong></td>
<td></td>
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<tr>
<td>Frequency of LES relaxation reflex, %</td>
<td>75.3</td>
<td>73.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Resting LES pressure, mmHg</td>
<td>19.3 ± 1.7</td>
<td>21.3 ± 1.7</td>
<td>0.4</td>
</tr>
<tr>
<td>LES nadir pressure, mmHg</td>
<td>1.0 ± 1.0</td>
<td>1.8 ± 1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Response time to LES relaxation reflex, s</td>
<td>3.9 ± 0.3</td>
<td>4.0 ± 0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>LES relaxation time, s</td>
<td>3.0 ± 0.5</td>
<td>4.1 ± 0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>LES nadir duration, s</td>
<td>6.9 ± 0.9</td>
<td>4.8 ± 0.9</td>
<td>0.09</td>
</tr>
<tr>
<td>LES relaxation pressure drop, mmHg</td>
<td>22.2 ± 1.5</td>
<td>23.2 ± 1.6</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Secondary Peristalsis</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Frequency of LES relaxation reflex, %</td>
<td>57.6</td>
<td>55.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Resting LES pressure, mmHg</td>
<td>17.7 ± 1.1</td>
<td>17.3 ± 1.6</td>
<td>0.8</td>
</tr>
<tr>
<td>LES nadir pressure, mmHg</td>
<td>2.0 ± 0.6</td>
<td>3.6 ± 1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Response time to LES relaxation reflex, s</td>
<td>3.7 ± 0.2</td>
<td>3.3 ± 0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>LES relaxation time, s</td>
<td>2.2 ± 0.2</td>
<td>3.2 ± 0.4</td>
<td>0.02</td>
</tr>
<tr>
<td>LES nadir duration, s</td>
<td>4.8 ± 0.3</td>
<td>3.6 ± 0.5</td>
<td>0.04</td>
</tr>
<tr>
<td>LES relaxation pressure drop, mmHg</td>
<td>22.6 ± 1.0</td>
<td>20.3 ± 1.6</td>
<td>0.2</td>
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</tbody>
</table>

LES, lower esophageal sphincter.
**Table 2. The characteristics of esophageal waveform propagation upon provocation between neuropathology and control subjects**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>Neuropathology</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude of proximal esophagus, mmHg</td>
<td>36.3 ± 2.8</td>
<td>45.3 ± 2.0</td>
<td>0.008</td>
</tr>
<tr>
<td>Duration of proximal esophagus propagation, s</td>
<td>3.8 ± 0.4</td>
<td>5.3 ± 0.3</td>
<td>0.002</td>
</tr>
<tr>
<td>Amplitude of middle esophagus, mmHg</td>
<td>52.2 ± 3.2</td>
<td>72.8 ± 2.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Duration of middle esophagus propagation, s</td>
<td>3.8 ± 0.4</td>
<td>5.7 ± 0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Amplitude of distal esophagus, mmHg</td>
<td>44.9 ± 3.9</td>
<td>60.3 ± 2.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Duration of distal esophagus propagation, s</td>
<td>4.3 ± 0.5</td>
<td>5.5 ± 0.3</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Between the group comparisons, neuropathology group responded sooner (P < 0.05) than controls for UES contractile reflexes with air, but not with liquid, stimulation. On the other hand, response latencies to peristaltic reflexes were quicker in the neuropathology group with both air and liquid stimulations (vs. controls). No group differences for response latency were noted for LES relaxation reflex.

The durations of UES contractile reflexes (in s) were similar between neuropathology vs. control groups, respectively, with air stimulation (2.7 ± 0.4 vs. 2.7 ± 0.4, P = NS) and liquid stimulation (3.3 ± 0.2 vs. 2.9 ± 0.3, P = NS). In contrast, between the neuropathology vs. controls, respectively, the magnitude of UES contractile reflexes (mmHg) was significantly higher with air stimulation (31.3 ± 2.0 vs. 20.8 ± 2.9, P = 0.02) and with liquid stimulation (31.8 ± 1.8 vs. 21.4 ± 2.4, P = 0.003). On the other hand, the durations of LES relaxation reflexes (in s) were similar between groups with air stimulation (neuropathology vs. control, 5.0 ± 0.5 vs. 4.6 ± 0.8, P = 0.8) but significantly longer in the neuropathology subjects with liquid stimulations (5.6 ± 0.5 vs. 4.2 ± 0.6, P = 0.03). The mean magnitudes of LES relaxation reflexes (mmHg) were similar between the neuropathology and control groups for both air (21.0 ± 1.2 vs. 22.0 ± 1.9, P = 1.0) and liquid stimulations (23.4 ± 1.1 vs. 21.1 ± 1.4, P = 0.6).

**Stimulus-Response Relationships Between Neuropathology vs. Controls**

The effect of physico-chemical characteristics of media (air vs. liquids-water and apple juice), graded volumes, and group (neuropathology vs. control) on the frequency recruitment of adaptive reflexes of interest were investigated using multiple logistic regression model. The likelihood of enhanced recruitment of UES contractile reflexes, LES relaxation reflexes, and peristaltic responses with increment in infusion volumes of air, water, and apple juice was evident in both groups (Fig. 5 and Table 3). Between the groups, differences for dose-response relationships were not evident.

**DISCUSSION**

Progressive brain maturation of feeding skills and aero-digestive reflexes is time dependent and modified by comorbidities (8, 11, 13, 14). Of relevance, neonatal neurological illness is associated with aero-digestive abnormalities, including dysphagia, aspiration, gastroesophageal reflux, and life-threatening airway events. The underlying mechanisms of malfunction of aero-digestive protective reflexes are unclear. In this study, we investigated the effects of esophageal provocation on the physiology of UES, LES, and esophageal body reflexes. We summarize and explain the findings, discuss interpretation and implications below.

**Significant Findings Distinguishing the Neuropathology Group**

With middle esophageal provocation-induced aero-digestive reflexes, the neuropathology group (contrasting controls) was
distinct with the following salient differences: 1) UES resting tone, maximal tone, and the magnitude of contractile response were all greater; 2) LES relaxation reflexes occurred earlier, and LES relaxation nadir durations were longer; 3) higher frequency recruitment of peristaltic reflexes, in that SP and polymorphic waveforms dominated; 4) there were less frequent deglutition response and less deglutition apnea. The physico-chemical characteristics of media (air or liquids) did not influence the frequency recruitment of these reflexes; and 5) esophageal body waveform amplitudes and peristaltic waveform durations were greater at all proximal, middle, and distal esophageal loci. These distinctive findings characterize the effects of global neonatal brain injury on aero-digestive reflex mechanisms.

Table 3. Graded dose-response relationship across media and groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>P Value</th>
<th>Media</th>
<th>Odds Ratio (95% CI)</th>
<th>P Value</th>
<th>Media</th>
<th>Odds Ratio (95% CI)</th>
<th>P Value</th>
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<tbody>
<tr>
<td><strong>UES Contractile Reflex</strong></td>
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<tr>
<td>Air</td>
<td>1.7 (1.3–2.4)</td>
<td>0.0008</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.5 (1.1–2.0)</td>
<td>0.01</td>
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<tr>
<td>Water</td>
<td>2.6 (1.6–4.4)</td>
<td>0.0002</td>
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<td></td>
<td></td>
<td></td>
<td>3.4 (2.3–4.9)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Apple Juice</td>
<td>2.9 (1.6–5.0)</td>
<td>0.0002</td>
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<td></td>
<td>3.8 (2.0–6.9)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Liquids</td>
<td>2.7 (1.9–3.9)</td>
<td>&lt;0.0001</td>
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<td></td>
<td>2.7 (1.9–3.9)</td>
<td>&lt;0.0001</td>
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<td><strong>LES Relaxation Reflex</strong></td>
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<tr>
<td>Air</td>
<td>1.3 (1.0–1.5)</td>
<td>0.02</td>
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<td></td>
<td></td>
<td></td>
<td>1.3 (1.1–1.7)</td>
<td>0.01</td>
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<tr>
<td>Water</td>
<td>3.2 (1.7–6.2)</td>
<td>0.0005</td>
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<td></td>
<td></td>
<td></td>
<td>3.9 (2.5–6.3)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Apple Juice</td>
<td>2.9 (1.4–6.2)</td>
<td>0.006</td>
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<td>4.3 (2.6–7.2)</td>
<td>&lt;0.0001</td>
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<td>Liquids</td>
<td>3.0 (1.7–5.0)</td>
<td>&lt;0.0001</td>
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<td></td>
<td>4.1 (2.7–6.3)</td>
<td>&lt;0.0001</td>
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<td><strong>Peristaltic Reflex</strong></td>
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<tr>
<td>Air</td>
<td>1.3 (1.1–1.5)</td>
<td>0.0007</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.9 (0.5–17.1)</td>
<td>0.3</td>
</tr>
<tr>
<td>Water</td>
<td>23.2 (5.9–91.6)</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25.8 (7.2–91.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Apple Juice</td>
<td>5.2 (1.4–19.8)</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45.1 (7.5–272.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Liquids</td>
<td>1.8 (1.3–2.6)</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.6 (2.4–8.7)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Logistic regression and generalized estimating equation methods were applied to evaluate the relationship between the response (binary outcome of the 3 specific reflexes, i.e., UES contractile reflex, LES relaxation reflex, peristaltic reflex) and the independent variables (media and graded volume). Significant odds ratio indicates a positive correlation between volume increments and the predictive reflex. For example, with a unit increase in dose volume of air, the occurrence of upper esophageal sphincter (UES) contractile reflex was 1.7 in control group.

Fig. 5. Graded dose-response relationship of UES contractile reflex (A), LES relaxation reflex (B), and peristaltic reflex (C) in control and neuropathology groups is shown. $P < 0.05$ for all media in both groups except for frequency of LES relaxation reflex with air in control group.
The upregulation of UES tone and reflex gain and increased inhibition of LES tone can be explained by a variety of mechanisms, including changes in the central modulation of brainstem motor centers, changes in descending inputs (loss due to neuropathy, white matter injury, damage to basal ganglia, etc.), or neuropathy of brainstem (reticular, premotor inputs to swallowing group motoneurons, etc.). More detailed interrogation of neural pathways is needed to elucidate basic science mechanisms.

**Important Similarities Between the Controls vs. Neuropathology Groups**

Important similarities were 1) with UES contractile responses, both groups were able to distinguish response sensitivity between air vs. liquid stimuli, in that reaction with air was earlier than liquid. Stimulus dose-UES contractile reflex relationships were significant with air, water and apple juice. Response latencies and response durations were similar. 2) Response latencies to LES relaxation reflexes were prolonged with liquid than with air in both groups. The frequencies of occurrence of LES relaxation reflexes were volume dependent and were significantly different for air, water, and apple juice. Resting LES tones and magnitudes of LES relaxations were similar. 3) The frequencies of occurrence of peristaltic reflexes were volume dependent and were significantly different for air, water, and apple juice. 4) LES relaxation characteristics during EDR or SP were similar for most characteristics, and isolated differences may be related to volume effects.

The lack of differences is also an important finding, as neonatal period is a phase of rapid growth and development, and neurological recovery is possible. These similarities in aero-digestive reflexes in the neuropathology group may constitute a potential mechanism for underlying neuroplasticity and recovery. Longitudinal studies are needed to ascertain sustained improvement and restoration of changes from the baseline.

**Interpretation of Findings from Neuropathology Perspective**

The sensory and motor characteristics of UES contractile reflexes are distinct in the neuropathology group. Although threshold volumes with various stimulus modes were similar, quicker response latencies to UES contractile reflexes are suggestive of afferent neuronal hyperexcitability. Greater UES contractile properties are suggestive of increased efferent neuronal hyperexcitability. Greater UES contractile properties were suggestive of increased efferent neuronal activation and therefore muscular contraction, such as may happen in spasticity. Indeed, the resting LES pressures were also greater in the neuropathology group. Furthermore, the sensory and motor characteristics of peristaltic reflexes were also distinct. A quicker response latency to air and liquid is suggestive of neuronal hyperexcitability and hypervigilant state. In addition, the discrepant proportion of greater SP suggests that peripheral reflexes are maintained and central reflexes (deglutition reflex) are impaired. Thus swallowing failure and airway provocation can happen in the presence of excessive secretions or feedings or more proximal GER events. Greater amplitudes and durations of panesophageal waveforms are suggestive of neuromuscular hyperexcitability and hypertonic contractility. Under such conditions, esophageal peristaltic clearance can take longer. These effects may be modulated by both peripheral and central vagal afferent-efferent pathways (4, 5, 19). LES relaxation reflex is facilitated by increased inhibitory neuronal activity to the LES, resulting in relaxation (6, 17). Changes with quicker and sustained fall in LES pressure from the resting tone are suggestive of increased inhibitory neuronal stimulation, and this mechanism is evident by the prolonged LES nadir and shorter relaxation duration. Collectively, these findings imply sustained and exaggerated increase in afferent outputs, as well as increased excitatory and inhibitory efferent outputs.

Middle esophageal stimulation-induced response is mainly SP in adults, contrasting with occurrence of both EDR (defined as occurrence of deglutition with esophageal stimulation) and SP in healthy neonates (occurring in almost equal proportions). This pattern undergoes further normal maturation with growth and age, including LES relaxation characteristics during SP and EDR (7, 11, 24). In the present study, the frequency recruitment of SP was significantly \( P < 0.0001 \) higher in neuropathology group (79.8%) compared with control group (56.1%), implicating central brain stem effects, in that the discrepant proportion of greater SP suggests that peripheral reflexes are maintained and central reflexes (deglutition reflex) are impaired. Despite these differences, LES relaxation characteristics during EDR or SP were similar in many respects between controls and neurologically impaired subjects. These observations implicate that inhibitory pathways governing LES relaxation were similarly stimulated whether by peripherally mediated (as in SP) or centrally mediated (as in EDR) mechanisms. These similarities aside, isolated differences in LES relaxation may be related to 1) the effects of stimulus volumes, 2) heterogeneity of neurological defects, 3) heterogeneity within the biological model studied, or 4) ongoing developmental influences regulating restoration of normality.

**Pathophysiological Implications of this Study**

Paucity of central swallowing mechanisms such as primary peristaltic reflexes or EDR is a marker of vulnerability of proximal aero-digestive tract (8, 11, 13, 14). Indeed, deglutition response is a chief response even in premature infants. Methods to improve sucking and swallowing skills need further investigation in neurologically impaired subjects (1). Hypertonicity of UES and proximal esophageal body contractile waveforms suggest skeletal muscle dysfunction. In the presence of both paucity of swallowing and exaggerated skeletal muscle tonicity, oral contents cannot undergo anterograde clearance and pose a risky state for airway aspiration. Furthermore, the presence of increased inhibitory LES tone suggests that GER can occur (20–22), and, at the same time, distal esophageal clearance can occur because LES can relax faster and for a longer period of time. Additionally, the presence of SP as a dominant esophageal clearance mechanism also supports the notion that reflux clearance can still occur by this mechanism.

Heterogeneity within the neurologically impaired neonate likely explains some similarities (with control infant) and may be due to developmental influences regulating restoration of normality. These factors may be governed by neuroplasticity mechanisms that may aim to restore normalization. Because neonatal period is a dynamic growth phase and is confounded by maturation, longitudinal studies are needed to evaluate changes in sensorimotor characteristics and effects of neuro-
plasticity. The role of sensory experiences and therapies in modulation and acceleration of safe feeding skills during the period of dynamic somatic and brain growth in infants can be explored. Such studies will help in further understanding of necessary neural pathways that may have restored safe swallowing skills. Such studies will further clarify persistence or amelioration of dysfunctional neuromotor markers, which may then serve as a prelude to the development of translational clinical research protocols.

**Significance and Future Directions**

Significantly, the provocative methodological approaches used to define the neuromotor markers may be helpful in localizing deficits where dysphagia is a dominant problem. Because neuropathic subjects commonly present with dysphagia and its consequences, we specifically studied this group. The present study can also be the basis for testing the integrity of peristalsis and gastroesophageal junction function. We speculate that such approaches when performed before and after the chronic medical or surgical therapies may help clarify the therapeutic effects so as to help formulate scientific basis for such interventions. As development of neurological disease is not a static but dynamic problem, our approaches can also be useful to study maturational changes in neuropathic subjects.

The next level of investigation in these neurologically impaired infants must investigate longitudinal changes in specific neurological lesions so as to understand the connectivity of networks pertinent to dysphagia and mechanisms of adaptation upon esophageal provocation. This approach will then help identify the therapeutic targets at the level of UES, LES or esophageal musculature in relation to reflexes, regulation of tone, regulation of coordination, restoration of esophageal quiescence, as well as the mechanisms of symptom generation. Therapeutic strategies will then be possible, in addition to defining the physiological basis for success or failure of therapies, which will in turn have significant implications. Larger studies are needed to discern the effects of potential confounders (gestational and postnatal maturity, pathological basis of lesion, sex, associated deficits) on adaptive reflexes. Conversely, the effects of prevailing therapeutic strategies on aero-digestive adaptive reflexes in neuropathic infants can be investigated to clarify mechanisms of amelioration or lack thereof, given that the present study serves as a foundation for potential neuromotor markers.

In summary, this is the first study to interrogate the aero-digestive tract in neurologically vulnerable neonates. We clarified the sensorimotor pathophysiology of vago-vagal reflexes involved with esophageal clearance, airway protection, and swallowing. Dysmotility mechanisms underlie in UES, esophageal body, and LES contractile and relaxation properties. Although, perception to midesophageal provocation remains preserved in neurologically impaired neonates, sustained and exaggerated myogenic response from afferent activation is evident by 1) increased excitatory efferent outputs to the UES and esophageal body and 2) increased inhibitory efferent outputs to the LES. Dysfunctional regulation of pharyngeal swallowing and infrequent deglutition responses indicate central malfunctions within the brain stem and vagal nuclei.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


