Asynchrony Injures Lung and Diaphragm in Acute Respiratory Distress Syndrome

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OBJECTIVES: Patient-ventilator asynchrony is often observed during mechanical ventilation and is associated with higher mortality. We hypothesized that patient-ventilator asynchrony causes lung and diaphragm injury and dysfunction.

DESIGN: Prospective randomized animal study.

SETTING: University research laboratory.

SUBJECTS: Eighteen New Zealand White rabbits.

INTERVENTIONS: Acute respiratory distress syndrome (ARDS) model was established by depleting surfactants. Each group (assist control, breath stacking, and reverse triggering) was simulated by phrenic nerve stimulation. The effects of each group on lung function, lung injury (wet-to-dry lung weight ratio, total protein, and interleukin-6 in bronchoalveolar lavage), diaphragm function (diaphragm force generation curve), and diaphragm injury (cross-sectional area of diaphragm muscle fibers, histology) were measured. Diaphragm RNA sequencing was performed using breath stacking and assist control ($n = 2$ each).

MEASUREMENTS AND MAIN RESULTS: Inspiratory effort generated by phrenic nerve stimulation was small and similar among groups (esophageal pressure swing ≈ −2.5 cm H_2O). Breath stacking resulted in the largest tidal volume (>10 mL/kg) and highest inspiratory transpulmonary pressure, leading to worse oxygenation, worse lung compliance, and lung injury. Reverse triggering did not cause lung injury. No asynchrony events were observed in assist control, whereas eccentric contractions occurred in breath stacking and reverse triggering, but more frequently in breath stacking. Breath stacking and reverse triggering significantly reduced diaphragm force generation. Diaphragmatic histology revealed that the area fraction of abnormal muscle was ×2.5 higher in breath stacking (vs assist control) and ×2.1 higher in reverse triggering (vs assist control). Diaphragm RNA sequencing analysis revealed that genes associated with muscle differentiation and contraction were suppressed, whereas cytokine- and chemokine-mediated proinflammatory responses were activated in breath stacking versus assist control.

CONCLUSIONS: Breath stacking caused lung and diaphragm injury, whereas reverse triggering caused diaphragm injury. Thus, careful monitoring and management of patient-ventilator asynchrony may be important to minimize lung and diaphragm injury from spontaneous breathing in ARDS.

KEY WORDS: acute respiratory distress syndrome; asynchrony; diaphragm injury; lung injury; mechanical ventilation; spontaneous breathing

Breathing by contracting one's own respiratory muscles, that is, spontaneous breathing, is physiologically natural and brings various benefits, such as better gas exchange and avoidance of diaphragm atrophy and has thus been facilitated during mechanical ventilation in the ICU. As spontaneous breathing has become central in ventilatory management, physicians have recognized that spontaneous breathing could also injure the lungs (termed

DOI: 10.1097/CCM.0000000000005988

KEY POINTS

Question: Does patient-ventilator asynchrony cause lung and diaphragm injury and dysfunction?

Findings: An experimental study using lung-injured rabbits found that breath stacking caused lung and diaphragm injury, whereas reverse triggering caused diaphragmatic injury.

Meanings: Careful monitoring and management of patient-ventilator asynchrony may be important in minimizing lung and diaphragm injury from spontaneous breathing in acute respiratory distress syndrome.

effort-dependent lung injury) in acute respiratory distress syndrome (ARDS) through the mechanisms such as increased lung stress and strain, the pendelluft phenomenon, and increased perfusion (1).

Patient-ventilator asynchrony is common when facilitating spontaneous breathing during mechanical ventilation in the ICU (2–4) and assumed to be one of the mechanisms of effort-dependent lung injury (1, 5, 6). Clinical observational studies indicate that patient-ventilator asynchrony is associated with poor outcomes, such as higher ICU, hospital mortality, and longer duration of mechanical ventilation (2–4, 7, 8), arising the hypothesis that patient-ventilator asynchrony might potentially cause lung and diaphragm injury and hence worsen clinical outcomes. Despite this plausible hypothesis, there is no direct causal evidence to support the deleterious effects of patient-ventilator asynchrony on the lungs and diaphragm.

Therefore, we tested the hypothesis that patient-ventilator asynchrony injures the lungs and diaphragm using an established experimental model of ARDS. We simulated each type of asynchrony, that is, "breath stacking" and "reverse triggering" by bilateral phrenic nerve stimulation in lung-injured rabbits. We also simulated assisted breath using bilateral phrenic nerve stimulation and allocated an "assist control" as control group.

MATERIALS AND METHODS

This study was approved by the Laboratory Investigation Committee, Osaka University Medical School (no. 02033000) on June 25, 2020. The animals were cared for in accordance with the hospital's standards for the care and use of laboratory animals. Detailed Materials and Methods are described in the **Supplemental Data** (http://links.lww.com/CCM/H374).

Animal Preparation

Eighteen New Zealand White rabbits (adult, male; 3.6 ± 0.1 kg) were anesthetized and tracheostomized. This study included only males to minimize data variability. Esophageal and gastric balloons (CareFusion, San Diego, CA) were inserted to measure the esophageal pressure (P_{es}) and gastric pressure.

Phrenic Nerve Stimulation

The bilateral phrenic nerves were identified and exposed. Bilateral phrenic nerve stimulation was identical among three groups at 1.33 Hz (i.e., rate 80/min), for 0.3 seconds and as the minimum possible voltage to obtain negative deflection in P_{es} between –2 and –3 cm H_2O.

Experimental Protocol

Lung injury was induced by repeated lung lavage (9) until Pao_2/Fio_2 < 150 mm Hg. The animals were then randomly assigned to one of three groups (n = 6 each).

- Assist control group;
- Breath stacking group;
- Reverse triggering group.

All animals were deeply sedated to prevent spontaneous activity of the diaphragm (i.e., no negative deflection in the P_{es} unless the phrenic nerves were stimulated). Phrenic nerve stimulation activated the diaphragmatic contraction, inducing each type of ventilation as follows. Randomization was performed using a bag of coded letters.

Assist Control Group

Animals were ventilated under volume-controlled ventilation (VCV) mode with V_T 6–8 mL/kg, rate 40/min (far below stimulation frequency), inspiratory time 0.35 seconds, flow trigger, and positive end-expiratory pressure (PEEP) 2 cm H_2O. Thus, diaphragmatic contraction caused by bilateral phrenic nerve stimulation

triggered mechanical breath at respiratory rate of 80/min for 4 hours (**Fig. 1**). This group is assumed to be a control group.

Breath-Stacking Group

Breath stacking was defined as two consecutive breaths occurring in close proximity that appeared to represent a single inspiratory effort generated by bilateral phrenic nerve stimulation (10). Animals were ventilated with VCV mode with V_T 6–8 mL/kg, rate 60/min, inspiratory time 0.1 seconds (far below stimulation duration), minimum flow trigger, and PEEP 2 cm H_2O. Because phrenic nerve stimulation (0.3 s) exceeded the preset inspiratory time (0.1 s), diaphragmatic contraction triggered two consecutive mechanical breaths with very short exhalation, simulating breath stacking in every breath for 4 hours (Fig. 1).

Reverse-Triggering Group

Reverse triggering was defined as an inspiratory spontaneous effort occurring after a ventilator-initiated breathing and during the inspiratory phase without evidence of animal-initiated assisted breathing (11). Animals were ventilated with VCV mode with V_T 6–8 mL/kg, rate 95/min, inspiratory time 0.30 seconds and PEEP 2 cm H_2O. To avoid breath stacking, followed by reverse triggering, the flow trigger was adjusted to be less sensitive. Because preset mechanical breath (95/min) exceeded the phrenic nerve stimulation rate (80/min), diaphragmatic contraction occurred randomly during the inspiratory phase after ventilator-initiated breathing. Such random interaction resulted in a variety of phenotypes of reverse triggering with different time point of initiation and termination (Fig. 1).

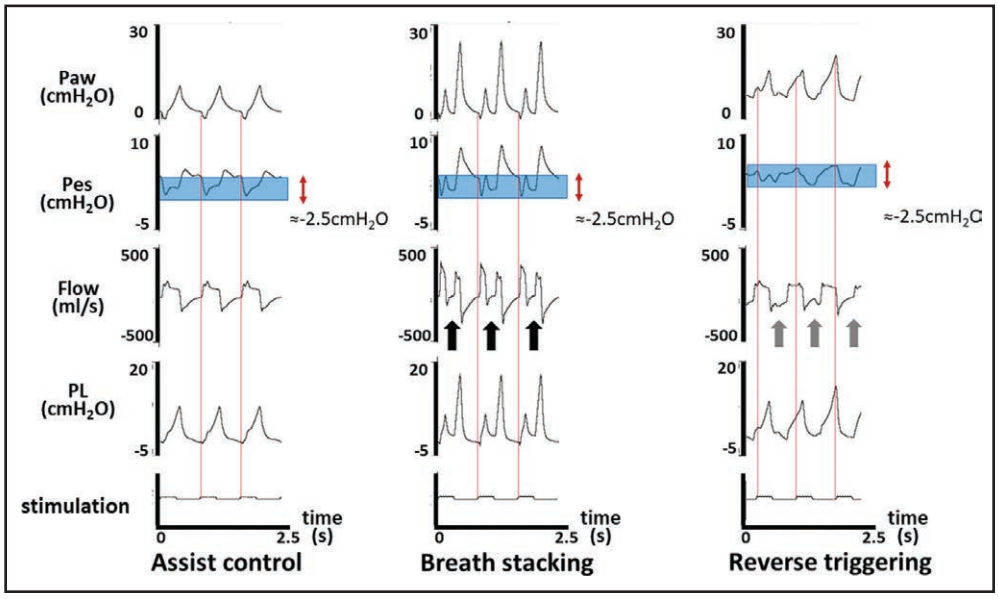

Figure 1. Assist control, breath stacking, and reverse triggering simulated by bilateral phrenic nerve stimulation. The traces illustrate each group as follows: assist control (*left*), breath stacking (*middle*), and reverse triggering (*right*). Each group underwent bilateral phrenic nerve stimulation with the same setting: 1.33 Hz (i.e., rate 80/min), duration 0.3 s. The *red dotted lines* indicate the start of stimulation. In "assist control," diaphragmatic contraction caused by bilateral phrenic nerve stimulation triggered mechanical breath, as evident from negative deflection of airway pressure and esophageal pressure (P_{es}). In "breath stacking," duration of phrenic nerve stimulation (0.3 s) exceeded preset inspiratory time (0.1 s) so that diaphragmatic contraction caused two consecutive mechanical breaths with a very short exhalation (*black arrow*), resulting highest inspiratory transpulmonary pressure (P_L). In "reverse triggering," mechanical breath rate (95/min) exceeded phrenic nerve stimulation rate (80/min) under less sensitive flow trigger so that diaphragmatic contraction occurred during inspiratory phase after ventilator-initiated breath without breath stacking (*gray arrow*). During the 4-hr protocol, the voltage of the stimulation was adjusted to obtain a similar negative deflection in the P_{es} (*blue-colored area*). Paw = airway pressure.

Asynchrony Events

Asynchrony events were quantified using an asynchrony index (modified from a previously reported method [2]) defined as the number of asynchrony events divided by the respiratory rate. The asynchrony index was calculated by averaging the values recorded for 10 minutes at each time point.

Diaphragm Force-Frequency Curve

The force-frequency curve, that is, transdiaphragmatic pressure (Pdi) measurements at each frequency, was evaluated at baseline (before the induction of lung injury) and at the end of the protocol. Pdi was measured against an occluded airway at end expiration during bilateral supramaximal

phrenic nerve stimulation (12) at frequencies of 10, 30, 50, 80, and 100 Hz. All measurements were obtained using PEEP with 2 cm H_2O.

Lung Inflammation and Injury Assessments

The upper lobe of the right lung was used to determine the wet-to-dry lung weight ratio. The left lung was lavaged to measure the total protein and interleukin-6 (IL-6). The lower lobe of the right lung was used for histopathologic analysis.

Diaphragm Injury Assessment

Ventral diaphragm specimens were quickly frozen and stained with hematoxylin and eosin (HE) and nicotinamide adenine dinucleotide dehydrogenase-tetrazolium reductase (NADH-TR). Cross-sections stained with HE were used to assess the diaphragmatic injury score. This was assessed using the point-counting technique as previously described (13). These analyses were performed by an investigator blinded to group allocation (A.M.F.F.). Cross-sections stained with NADH-TR were used to classify muscle fibers as either type I or II (14).

RNA Sequencing of Diaphragm

RNA extraction and sequencing were performed by Macrogen (Tokyo, Japan). The same asynchrony event, that is, eccentric contraction, was observed in breath stacking and reverse triggering, but with different frequencies (more in breath stacking and less in reverse triggering). Thus, RNA sequencing was performed for breath stacking versus assist controls to investigate the biological significance of transcriptional changes in diaphragms suffering from eccentric contraction. Two samples in which the area fraction of abnormal muscle showed the average value for each group were chosen as representatives. Expression profiles were represented as read counts and normalization values based on fragments per kilobase of exon per million mapped reads. K-means clustering was performed using the iDEP online software (version 0.96; Department of Mathematics and Statistics, South Dakota State University, Brookings, SD) (15). Gene ontology (GO) analysis was performed using Enricher online software (Computational Systems Biology, Icahn School of Medicine at Mount Sinai, New York, NY) (16).

Statistical Analysis

Statistical analysis was performed using standard software (SPSS 24 Advanced Statistics, IBM, NY; JMP pro15.0, SAS Institute, Cary, NC). Results are expressed as mean ± SD. Two-way analysis of variance (ANOVA) for repeated measurements was used to evaluate the effects of time and group. One-way ANOVA for repeated measurements was used to compare force-frequency curve and respiratory parameters. All tests were two-tailed, and differences were considered significant when p value of less than 0.05.

RESULTS

Respiratory Variables

Oxygenation (Pao_2/Fio_2) improved over time in "assist control" and "reverse triggering" and was greater versus "breath stacking" after 2 hours (**Appendix 5, Table 1**, http://links.lww.com/CCM/H374). The values of V_T (6–8 mL/kg) were similar in "assist control" and "reverse triggering" throughout the protocol, but "breath stacking" had largest V_T (>10 mL/kg) and highest respiratory rate for 4-hour protocol, resulting in lower values of $PaCo_2$ and higher values of pH at 1 and 2 hours (Appendix 5, Table 1, http://links.lww.com/CCM/H374). "Breath stacking" observed highest values of peak P_L and peak ΔP_L throughout the protocol, resulting in the worst dynamic respiratory system and dynamic lung compliance after 2 hours of the protocol (Appendix 5, Table 1, http://links.lww.com/CCM/H374). ΔP_{es}, that is, spontaneous inspiratory effort generated by bilateral phrenic nerve stimulation was similar in all groups throughout the protocol (≈ –2.5 cm H_2O; Appendix 5, Table 1, http://links.lww.com/CCM/H374; and Fig. 1). Eccentric contractions were observed during both breath stacking and reverse triggering (Fig. 1); however, the asynchrony index was much higher during breath stacking than during reverse triggering (**s-Table 2**, http://links.lww.com/CCM/H374).

Diaphragm Contractile Properties

Pdi at baseline was similar among the groups at all stimulation frequencies (s-Table 2, http://links.lww.com/CCM/H374). In "assist control," ΔPdi (except at 10 Hz) did not differ significantly between at baseline time and at the end of protocol (s-Table 2, http://links.lww.com/CCM/H374). In "breath stacking,"

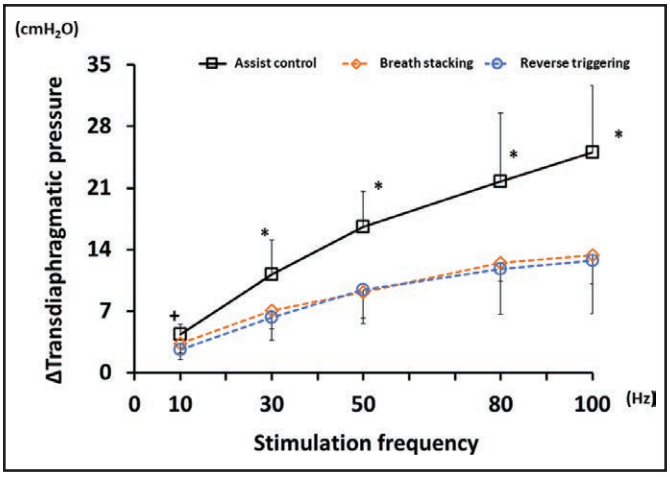

Figure 2. Force-frequency curve of the diaphragm. Diaphragmatic force generation was evaluated at the end of the protocol as change in transdiaphragmatic pressure at each stimulation frequency. **"**Breath stacking" resulted in less diaphragm force generation at all frequencies except 10 Hz than "assist control. " "Reverse triggering" resulted in less diaphragm force generation at all frequencies than "assist control." $^{*}p < 0.05$ vs all, ^{+}p value of less than 0.05 vs "reverse triggering."

ΔPdi at the end of protocol was significantly reduced (vs "assist control") at all stimulation frequencies except 10 Hz (**Fig. 2**) and was significantly reduced (vs "baseline") at all stimulation frequencies except 30 Hz (s-Table 2, http://links.lww.com/CCM/H374). In "reverse triggering," ΔPdi at the end of protocol was significantly reduced (vs "assist control") at all stimulation frequencies (Fig. 2) and was significantly reduced (vs "baseline") at all stimulation frequencies except 50, 80 Hz (s-Table 2, http://links.lww.com/CCM/H374).

Lung Injury

Lung injury was greatest in "breath stacking," in terms of wet-to-dry lung weight ratio (**s-Fig. 1*A***, http://links.lww.com/CCM/H374), protein concentration (**s-Fig. 1*B***, http://links.lww.com/CCM/H374), and IL-6 concentration in bronchoalveolar fluid (**s-Fig. 1*C***, http://links.lww.com/CCM/H374). "Breath stacking" observed highest wet-to-dry lung weight ratio (6.4 ± 1.3; $p < 0.01$ vs all), highest concentration of total protein (493 ± 338 mg/dL; $p < 0.01$ vs "assist control," $p < 0.05$ vs "reverse triggering") and highest concentration of IL-6 (2.9 ± 2.4 pg/mL; $p < 0.05$ vs "assist control") in bronchoalveolar fluid. Histological lung injury in each group is presented in illustrative sections (**Fig. 3*A***).

Diaphragm Injury

The histological diaphragmatic injury in each group is presented in illustrative sections (**Fig. 3*B***). Histograms show the distribution of the cross-sectional area of diaphragm muscle fibers (total type I and type II muscle fibers, type I muscle fibers, and type II muscle fibers) in each group (**s-Fig. 2**, http://links.lww.com/CCM/H374). Overall, diaphragm muscle fibers were most enlarged in "breath stacking": total muscle fibers (4,446 ± 684 μm²) were ≈ 70% larger versus "assist control" (2,636 ± 625 μm²; $p < 0.01$) and ≈ 30% larger versus "reverse triggering" (3,415 ± 516 μm²; $p < 0.05$). Type I muscle fibers in "breath stacking" (3,606 ± 696 μm²) were larger than in "assist control" (2,359 ± 538 μm²; $p < 0.01$); type II muscle fibers in "breath stacking" (5,275 ± 775 μm²) were larger than both in "assist control" (2,925 ± 757 μm²; $p < 0.01$) and "reverse triggering" (4,006 ± 563 μm²; $p < 0.01$). In "reverse triggering," overall diaphragm muscle fibers (3,415 ± 516 μm²) were ≈ 30% more enlarged than in "assist control" (2,636 ± 625 μm²; $p < 0.05$): type I muscle fibers did not differ significantly from "assist control," but type II muscle fibers (4,006 ± 563 μm²) was larger than in "assist control" (2,925 ± 757 μm²; $p < 0.05$) (s-Fig. 2, http://links.lww.com/CCM/H374). The cross-sectional areas of the diaphragm muscle fibers stained with NADH-TR in each group are presented with illustrative sections (**Fig. 3*C***).

Normal muscle, abnormal muscle, and connective tissue of the ventral diaphragm were compared among the three groups using the point-counting technique (**Fig. 4**). In "breath stacking," the area fraction of abnormal muscle (65% ± 10%) was ×2.5 higher (vs "assist control" 26% ± 8%; $p < 0.01$) and the area fraction of connective tissue was highest (vs all; $p < 0.01$), resulting in the lowest area fraction of normal muscle (vs all; $p < 0.05$). In "reverse triggering," the area fraction of abnormal muscle (53% ± 19%) was ×2.1 higher (vs "assist control" 26% ± 8%; $p < 0.01$) and the area fraction of normal muscle was lower (vs "assist control"; $p < 0.01$).

For RNA sequencing of the diaphragm, the top 2,000 most variable genes, determined by SD, were subjected to K-means clustering and divided into three clusters: cluster A ($n = 406$), cluster B ($n = 377$), and cluster C ($n = 1,217$) (**s-Fig. 3**, http://links.lww.com/CCM/H374). Clusters A and B showed a group of genes whose expression was down-regulated and up-regulated, respectively,

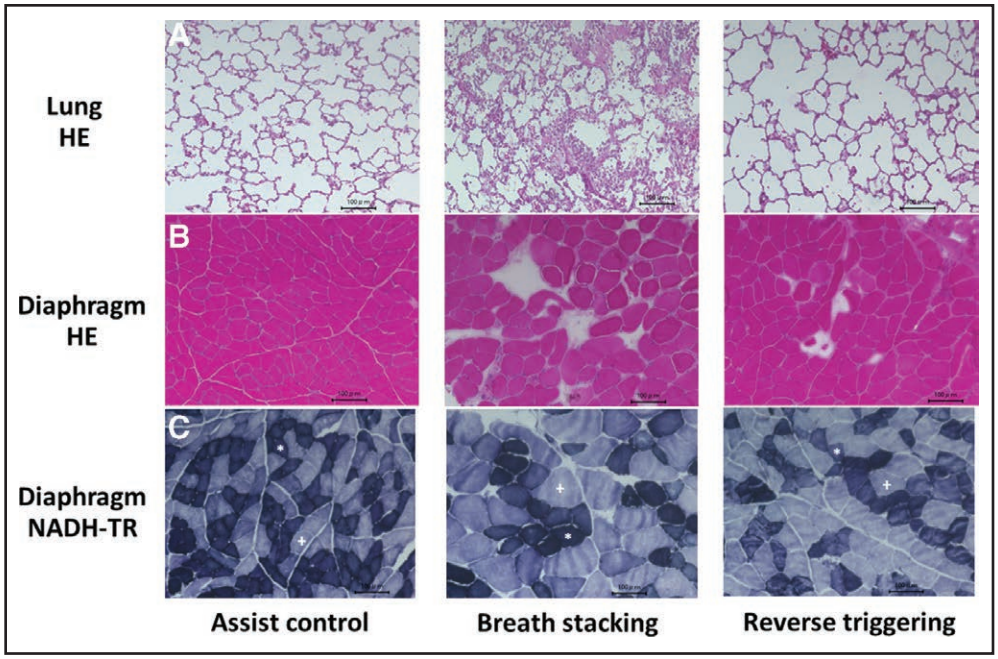

Figure 3. Representative images of the lung and diaphragm. Representative images (original magnification ×200) are shown (**A**: dependent lung, hematoxylin and eosin [HE]; **B**: diaphragm, HE; **C**: diaphragm, nicotinamide adenine dinucleotide dehydrogenase-tetrazolium reductase [NADH-TR]) in "assist control" (*left*), "breath stacking" (*middle*), and "reverse triggering" (*right*). **A**, "Breath stacking" had severe alveolar damage with hyaline membrane formation and neutrophil infiltration. **B**, Both in "breath stacking" and "reverse triggering," diaphragm muscle fibers were injured, characterized by necrosis and influx of inflammatory cells. **C**, Type 1 muscle fibers appear dark (*) and type II muscle fibers appear light (+). "Breath stacking" enlarged both type I and type II muscle fibers while reverse triggering enlarged type II muscle fibers only.

during breath stacking. GO analysis in clusters A and B found that genes associated with biological processes of "positive regulation of striated muscle cell differentiation," "muscle contraction," and "muscle organ development" were down-regulated (**Fig. 5*A***) in breath stacking, whereas genes associated with biological processes of "cytokine-mediated signaling pathway," "response to unfolded protein," and "cellular response to cytokine stimulus" were up-regulated in breath stacking (**Fig. 5*B***). Up-regulation of genes associated with molecular function of "cytokine activity" and "chemokine activity" were also observed in breath stacking (Fig. 5*B*).

DISCUSSION

Current experimental data suggest that careful monitoring and management of patient-ventilator asynchrony may be important for minimizing lung and diaphragm injury from spontaneous breathing in ARDS. This is because breath stacking injures the lungs by doubling the V_T and inspiratory P_L, and breath stacking and reverse triggering (without breath stacking) cause diaphragm injury and dysfunction, probably because of eccentric diaphragm contraction.

Asynchrony and Lung Injury

This study showed that breath stacking injured the lungs, as evidenced by worse oxygenation, worse lung compliance, higher concentrations of total protein and IL-6 in the bronchoalveolar fluid, and a higher wet-to-dry lung weight ratio (Appendix 5, Table 1, http://links.lww.com/CCM/H374; Fig. 1). These findings were confirmed by histological analysis of the lungs (Fig. 3). A key mechanism whereby breath stacking increased lung injury is overdistension of the alveoli because of larger V_T (≈ ×1.5 larger than targeted) and higher inspiratory P_L, increasing the risk of ventilator-induced lung injury (5, 10). Thus, physicians need to be aware of circumstances in which breath stacking occurs more frequently, such as higher respiratory drive, severe lung injury, more restricted V_T during lung-protective ventilation, and shorter inspiratory time (2, 10).

Our data showed that reverse triggering did not increase lung injury (vs "breath stacking" or "assist control") (Appendix 5, Table 1 and s-Fig. 1, http://links.lww.com/CCM/H374). This finding seems unexpected, considering previous observations (17, 18). Several explanations have been proposed for this.

First, global V_T and inspiratory P_L did not increase with "reverse triggering" (vs "assist control"). This is because breath stacking followed by reverse triggering was avoided by changing the flow sensitivity less, as per the protocol, and VCV guaranteed the same global V_T as the preset value during reverse triggering. Notably, reverse triggering increases global V_T when

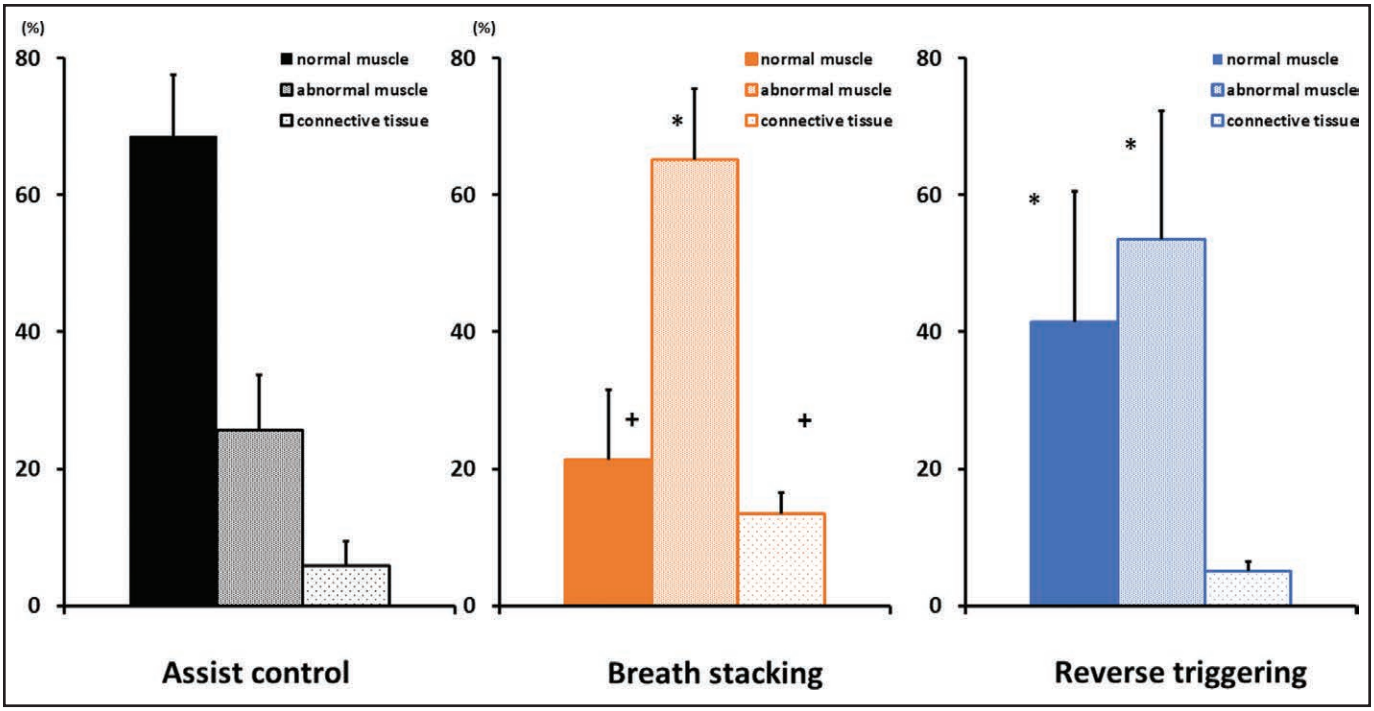

Figure 4. Comparison of normal muscle, abnormal muscle, and connective tissue of the ventral diaphragm. The distributions of normal muscle, abnormal muscle and connective tissue are shown in "assist control" (*left*), "breath stacking" (*middle*), and "reverse triggering" (*right*). *Bar plots* illustrate the quantitative point scoring of muscle injuries in all groups. "Assist control" had highest number of normal muscle fibers (≈ 70%). "Breath stacking" had significantly higher area fraction of abnormal muscle than "assist control" and highest area fraction of connective tissue, resulting in lowest area fraction of normal muscle. "Reverse triggering" had significantly higher area fraction of abnormal muscle and significantly lower area fraction of normal muscle than "assist control." *p value of less than 0.05 vs "assist control," +p value of less than 0.05 vs "all."

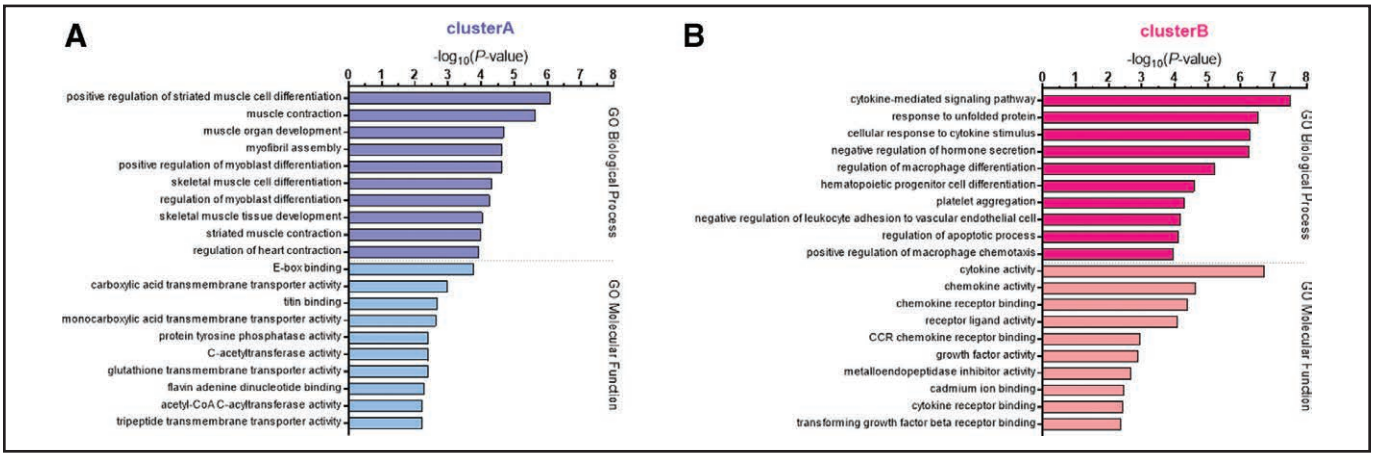

Figure 5. RNA sequencing of the diaphragm. RNA sequencing analysis of the diaphragms from the assist control (AC) and breath-stacking (BS) groups was performed ($n = 2$, each). **A**, *Blue bars* in cluster A show the group of genes whose expression was down-regulated in BS vs AC group. **B**, *Red bars* in cluster B show the group of genes whose expression was up-regulated in BS vs. AC groups. Gene ontology (GO) enrichment analysis of the biological process and molecular function terms was performed using the genes of clusters A and B, respectively. GO biological process and GO molecular function terms were ranked by p value, and the top 10 terms are listed. The *bars* show $-\log_{10}$ (p value). CCR = CC chemokine receptors.

it occurs during the pressure-controlled or pressure-regulated volume control mode accompanying stacked breath (18). In such cases, reverse triggering can be potentially injurious to lungs. Second, our previous study using electrical impedance tomography found that reverse triggering increased dependent lung stretch, accompanied by pendelluft, despite a constant V_T during VCV (17). Also the magnitude of the dependent

lung stretch was proportional to the strength of the diaphragmatic contraction during reverse triggering (17). In this study, a weak diaphragmatic contraction by bilateral phrenic nerve stimulation, that is, negative deflection in P_{es} of ≈ –2.5 cm H_2O might be insufficient for causing overstretch in dependent lung regions. Reverse triggering caused higher (but not significant) concentration of total protein (×1.8 vs "assist control") and IL-6 (×1.5 vs "assist control") in bronchoalveolar fluid. Thus, the impact of reverse triggering (whether injurious or not) on the lungs may depend on how much global V_T is increased with reverse triggering and/or how strongly the diaphragm contracts during reverse triggering.

Asynchrony and Diaphragm Injury

This study revealed that both breath stacking and reverse triggering injured the diaphragm, thus causing diaphragm muscle weakness (Figs. 2–5; and **s-Fig. 2,** http://links.lww.com/CCM/H374). First, the magnitude of diaphragmatic contraction, that is, negative deflection in P_{es} was relatively weak (≈ –2.5 cm H_2O) and similar among all groups; diaphragm injury was also observed in "breath stacking" and "reverse triggering," but not in "assist control." This suggests that the mechanism of diaphragmatic injury in our study was not vigorous inspiratory effort, that is, under-assisted myotrauma (19). Second, breath stacking and reverse triggering generate diaphragmatic contractions during the expiration of a mechanical breath (Fig. 1). Thus, breath stacking and reverse triggering cause eccentric contraction of the diaphragm muscles, resulting in diaphragm injury, that is, eccentric myotrauma (19). Notably, eccentric contractions are much more likely to injure muscles than concentric contractions, where force is generated during muscle shortening (19–21). Third, fast-twitch fibers (type II) are more susceptible to eccentric muscle injury than slow-twitch (type I) fibers (22, 23). In our study, eccentric contraction was observed more frequently in breath stacking than reverse triggering, evident from the asynchrony index (Appendix 5, Table 1, http://links.lww.com/CCM/H374), possibly explaining the different patterns of muscle fiber damage in breath stacking versus reverse triggering (s-Fig. 2, http://links.lww.com/CCM/H374). Fourth, the diaphragm muscles suffering from eccentric contraction were highly involved in cytokine- and chemokine-mediated proinflammatory responses, supporting our histological diaphragm analyses. Interestingly, muscle differentiation and generation in the diaphragm were suppressed at the gene expression level. This suggests that eccentric diaphragmatic injury may cause difficult weaning from mechanical ventilation by suppressing muscle regeneration after diaphragmatic injury, thus prolonging diaphragmatic muscle weakness.

Study Limitation

There are several limitations to this study. First, the model (rabbits, surfactant depletion model) was short-term and did not accurately reflect the far longer usual time course of clinical ARDS. To enhance the impact of asynchrony on the lungs and diaphragm, we artificially simulated it by stimulating the bilateral phrenic nerves with a higher asynchrony index than that reported in clinical studies (2, 3). Thus, caution is necessary when extrapolating our results to a clinical context. Second, phrenic nerve stimulation per se might affect diaphragmatic function because of a potential refractory period, stimulation potentiation, and direct damage. To minimize such risks, minimal stimulus intensity to generate a weak inspiratory effort, that is, negative deflection of P_{es} of –2.5 cm H_2O was used throughout the 4-hour protocol while protecting phrenic nerves with cold saline. Force generation from the diaphragm was similar at baseline and the protocol end in the assist control group, indicating no or minimal damage of phrenic nerves by artificial stimulation. Third, short inspiratory time in the breath-stacking group resulted in higher peak flow (and pressure), which might affect the difference in lung injury (24). Fourth, the difference in frequency between a preset mechanical breath (95/min) and paced breath (80/min) enables reverse triggering. This causes random interactions, resulting in a variety of reverse-triggering phenotypes at different time points (initiation and termination) (Fig. 1), reflecting the clinical scenario of reverse triggering (18). In our model of reverse triggering, reverse triggering and ineffective effort was observed and its proportion to a respiratory rate was 47.8% ± 2% and 8.2% ± 2%, respectively. Therefore, our model is robust in studying reverse triggering. Spontaneous inspiratory effort occurring during the expiratory phase (not the inspiratory phase) was defined as ineffective effort (4) and excluded when calculating the asynchrony index.

CONCLUSIONS

Breath stacking worsened lung injury by increasing the tidal volume and inspiratory lung stress. Breath stacking and reverse triggering (without breath stacking) caused diaphragm injury and dysfunction, probably because of eccentric diaphragm contraction. Thus, careful monitoring and management of patient-ventilator asynchrony may be important to minimize lung and diaphragm injury from spontaneous breathing in ARDS.

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (http://journals.lww.com/ccmjournal).

Dr. Hashimoto participated in study design, conducted the study, analyzed the data, and wrote the article. Dr. Yoshida designed the study, analyzed the data, wrote and revised the article, and supervised the study. Dr. Firstiogusran performed histological data analyses. Dr. Nukiwa performed RNA sequencing data analyses. Drs. Taenaka and Koyama conducted the experiments. Drs. Uchiyama and Fujino interpreted data.

Grant-in-Aid for Scientific Research(B) (no. 20H03780), the Ministry of Education, Culture, Sports, Science and Technology, Japan, Grant for the Promotion of Joint Research, Fukuda Foundation for Medical Technology.

The authors have disclosed that they do not have any potential conflicts of interest.

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This study was approved by the Laboratory Investigation Committee, Osaka University Medical School (no. 02033000).

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