Detection of inspiratory resistive loads in double-lung transplant recipients

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Zhao, Weiyiing, A. Daniel Martin, and Paul W. Davenport. Detection of inspiratory resistive loads in double-lung transplant recipients. J Appl Physiol 93: 1779–1785, 2002.—The afferent pathways mediating respiratory load perception are still largely unknown. To assess the role of lung vagal afferents in respiratory sensation, detection of inspiratory resistive loads was compared between 10 double-lung transplant (DLT) recipients with normal lung function and 12 healthy control (Nor) subjects. Despite a similar unloaded and loaded breathing pattern, the DLT group had a significantly higher detection threshold (2.91 ± 0.5 vs. 1.55 ± 0.3 cmH2O·l−1·s−1) and Weber fraction (0.50 ± 0.1 vs. 0.30 ± 0.1) compared with the Nor group. These results suggest that inspiratory resistive load detection occurs in the absence of vagal afferent feedback from the lung but that lung vagal afferents contribute to inspiratory resistive load detection response in humans. Lung vagal afferents are not essential to the regulation of resting breathing and load compensation responses.

respiratory sensation; load compensation; psychophysics; inspiratory load

Sensations associated with breathing against external mechanical loads have been studied by using psychological methods (12, 21, 22, 36, 38). Load detection is one of the two perceptual processes of respiratory mechanosensation (13). It has been shown that the threshold for detection of resistive loads was a constant fraction of the baseline resistance (36), which is known as the Weber fraction.

The role of afferent feedback from the lung and lower airways, which is one of the sensory systems that may be involved in load perception, remains controversial. Two strategies have been adopted to determine the role of pulmonary receptors in respiratory load perception: either the principal afferent nerve (vagus nerve) is selectively blocked (17); or alternatively, all other possible sources are eliminated leaving only the vagi intact (4). High-level quadriplegic subjects with a tracheostomy provide indirect evidence about the role of pulmonary afferents in respiratory sensation, because both respiratory muscle afferents and upper airway receptors are bypassed, leaving only the pulmonary receptors intact (4). It was reported that these patients could reliably detect changes in tidal volume as little as 100 ml, which was comparable to that of normal subjects (4). These data suggest that pulmonary stretch receptors can provide conscious perception of volume, at least in the absence of all other signals. In contrast, other studies reported that the detection threshold of mechanical loads was not affected either by bilateral block vagus nerve (17) or after upper and lower airway anesthesia (7, 9, 10) in normal subjects. However, it is possible that some pulmonary stretch receptors may escape anesthesia because the anaesthetic agents could not penetrate to the smooth muscle.

Lung transplantation recipients provide a good model to study the role of lung and lower airway receptors in respiratory sensation because the afferent information from receptors located distal to the surgical anastomosis are interrupted. Tapper et al. (34) compared the detection threshold of inspiratory resistive load in heart-lung transplant recipients and normal subjects, and they found no significant difference in Weber fraction between lung transplant recipients and healthy control subjects. On the other hand, Peiffer et al. (27) found that the slope of the linear relationship between the Borg scores and peak inspiratory mouth pressure (Pm) associated with breathing against resistive loads was significantly lower in lung transplant recipients. However, magnitude estimation and load detection are two different perceptual processes, and they may involve different neural mechanisms.

The objective of this study was to investigate the role of afferent input from lung and lower airways in detection of external inspiratory resistive loads by recruiting double-lung transplant (DLT) recipients as a lung denervation model. Unloaded and loaded breathing patterns were also compared between DLT recipients and matched normal (Nor) subjects. We hypothesized that the absence of pulmonary afferents in those DLT recipients would result in a higher detection threshold and Weber fraction compared with those in Nor subjects.
METHODS

Subjects. A total of 10 DLT patients and 12 Nor subjects were recruited in this study. All subjects were Caucasian. The DLT subjects were recruited from the University of Florida Medical Center. None of the DLT subjects had any evidence of current respiratory or neurological disease. The time since the DLT patients received transplant surgery varied from 1.5 to 5.5 yr. None of these patients had evidence of rejection when they participated in this study. All the DLT subjects were on immunosuppressive (Imuran, Prograf, etc.) and steroid medications (prednisone, etc.) when they participated in the study. Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1) were tested for each subject. Subjects with a FVC or FEV1 <70% of predicted values were excluded from this study. Only one DLT subject was excluded from this study because of abnormal lung function (FVC: 60.8% of predicted value; FEV1: 41.9% of predicted value). The Institutional Review Board of University of Florida reviewed and approved this study. All participants provided informed consent before participating in this study.

Procedures. Subjects were asked to refrain from strenuous physical activity, large meals, and caffeine for at least 4 h before the test. All subjects performed pulmonary function testing in the sitting position. Standard instructions according to American Thoracic Society Standards for spirometry testing were given to each subject. All subjects performed a FVC maneuver. Each test was repeated two to four times with at least a 1-min rest between each repetition. Values were also contrasted with age- and gender-predicted values.

Background respiratory resistance was measured by using the forced oscillation method. The subject was seated in front of the apparatus and breathed “normally” through the mouthpiece, with his or her cheeks supported by both hands. Approximately 10 tidal breaths were collected continuously to analyze the resistance by computer (Jaeger Toennies, Medizintechnikum System, version 4.5). The test was repeated at least three times for each subject with a 1-min rest between repetitions. The average of three measures was used as the subject’s respiratory system resistance.

Inspiratory muscle strength was measured as the maximal inspiratory pressure (MIP). Subjects were in a standing position when they performed the test. After exhaling to residual volume, subjects were instructed to place their lips around the mouthpiece and inspire as forcefully as possible with their nose clamped. The test was repeated until three measurements within 10% variation were obtained. There was at least a 1-min rest between repetitions. The maximal value obtained was recorded as the subject’s MIP.

During the detection experiment, the subject was seated in a lounge chair in a sound isolated chamber, separated from the experimenter and the experimental apparatus. The subjects were instructed to breathe normally through a mouthpiece connected to a non-rebreathing valve (2600 series, Hans Rudolph) with their nose clamped. The inspiratory port of the valve was connected to the resistive loading manifold (model 4813, Hans Rudolph). Pm was measured at the center of the non-rebreathing valve and recorded on a polygraph. Inspiratory airflow was measured by a differential pressure transducer (model MP45, Validyne) and signal conditioner (model CD316, Validyne) connected to a pneumotachograph. Inspired volume (Vi) was obtained by electrical integration of the airflow signal. Pm, inspiratory airflow, and Vi were recorded on a polygraph (model 7, Grass Instruments) and stored and analyzed on a computer (Chart, Powerlab AD Instrument). The resistive loads were sintered bronze disks placed in series in the loading manifold and separated by stopped ports. The load was applied for the entire inspiration and then removed. The subject was asked to press the signal button held in their dominant hand as soon as they sensed the presence of a load. A series of test loads were presented in a practice session to familiarize the subject with the load sensation and the range of loads. During the subsequent experimental session, the subject listened to music of their choice to mask experiment sounds. A series of resistive loads (0.2, 0.8, 1.24, 1.64, 2.48, 3.26, 6.95, and 11.46 cmH2O·1−1·s) were presented in a randomized block design, with each loaded breath separated by two to six unloaded breaths. A total of 10 presentations of each load magnitude were presented in two experimental trials with a 5-min break between trials. The subject was monitored by video camera throughout the experiment.

Data analysis. The number of detections for each load was summed and divided by the number of total presentations to obtain the detection percent for each load. The detection percent was plotted against the magnitude of added load. The detection threshold (ΔR50) was determined as the magnitude of load corresponding to a detection percent of 50%. The Weber fraction (ΔR50/R0) for each load was computed by dividing ΔR50 by the sum of the subject’s respiratory background resistance (Rb) and the resistance of the apparatus. The resistance of the apparatus was 1.6 cmH2O·1−1·s. Detection latency for each load was also computed by measuring the time from the start of inspiratory flow to the onset of the detection signal. A two-tailed t-test was used to compare ΔR50 and Weber fraction between the DLT and the Nor groups. A two-way repeated-measures ANOVA was performed to study the effects of group and load on detection percent and detection latency. Contrast analysis was performed to compare the effect of different loads. The P value for each contrast test was corrected by dividing 0.05 by the total number of contrasts.

Peak Pm, Vi, peak inspiratory airflow, inspiratory duration (Ti), expiratory duration (Te), time to peak airflow (TP), breathing frequency (f), and minute ventilation (Ve) were recorded for each loaded breath and the preceding unloaded breath, which was the control breath. The breathing pattern was compared by using two-way repeated-measures ANOVA to study the effects of group and load. Contrast analysis was performed to compare the effects of different resistive loads. The P value for each contrast test was corrected by dividing 0.05 by the total number of contrasts.

The descriptive statistics of all the variables were calculated and expressed as means ± SE. Significance level was set at 0.05, unless multiple contrast analysis was used.

RESULTS

The group mean demographic characteristics and pulmonary functions of all the subjects who participated in this study are shown in Table 1. The DLT and the Nor group were comparable in age, height, and weight. Background respiratory resistance and MIP between the two groups were not significantly different. Both FVC and FEV1 were significantly lower in the DLT group than in the Nor group (97.0 ± 4.9% of predictive value vs. 119.5 ± 5.0, and 83.8 ± 6.1 vs. 108.3 ± 3.7% of predictive value, respectively). However, both FVC and FEV1 were still within normal range in the DLT group. Furthermore, FEV1/FVC ratio was not significantly different between the two groups (88.1 ± 6.0% of predicted value for DLT vs. 93.9 ± 2.8% of predicted value for Nor; P = 0.371).
During the load detection experiment, all the subjects were asked to breathe “normally.” There was no cue or airflow targeting. Two-way repeated-measures ANOVA found no significant group, load, and interaction effects on $P_m$, $V_t$, peak inspiratory airflow, $T_i$, $T_e$, $T_p$, $f$, and $V_e$ during control breathing. During loaded breathing, group and interaction effects were also not significant, except for the interaction effect of $V_e$. The main effects of load were significant for all the above breathing pattern measures. As the magnitude of the resistive loads increased, $P_m$, $T_i$, and $T_p$ increased, whereas $V_t$, airflow, $T_e$, $f$, and $V_e$ decreased. The $P_m$, peak inspiratory airflow, and $T_i$ response during control breathing and loaded breathing are shown in Fig. 1.

The total number of false-positive responses during the detection experiment were compared between the DLT and Nor groups, and no significant difference was found ($4.8 \pm 2.7$ for DLT vs. $4.2 \pm 1.1$ for Nor; $P = 0.621$). The detection threshold ($\Delta R_{de}$) was significantly higher for the DLT subjects ($2.91 \pm 0.5$ cmH$_2$O·L$^{-1}$·s$^{-1}$ for DLT vs. $1.55 \pm 0.3$ cmH$_2$O·L$^{-1}$·s$^{-1}$ for Nor; $P < 0.05$). The Weber fraction was also significantly elevated in the DLT group ($0.502 \pm 0.1$ in DLT vs. $0.295 \pm 0.05$ in Nor; $P < 0.05$). The results of detection threshold and Weber fraction are shown in Figs. 2 and 3, respectively.

Detection latency and detection percent in response to different level of resistive loads are shown in Figs. 4 and 5, respectively. Detection latency decreased and detection percent increased as the magnitude of the resistive load increased. Two-way repeated-measures ANOVA found significant group ($P < 0.05$), load ($P < 0.001$), and interaction effects ($P < 0.05$) on detection percent. The DLT group had significantly lower detection percent than the Nor group. Detection latency did not display significant effects of group ($P = 0.67$) or load ($P = 0.084$), nor was there significant interaction ($P = 0.422$) between the two factors.

**DISCUSSION**

The results of the present study showed that both loaded and control breathing patterns were similar in the DLT group and the Nor group during breathing against inspiratory resistive loads. Despite a similar ventilatory response to resistive loads, detection threshold and Weber fraction were significantly elevated in the DLT group compared with the Nor group. These results suggest that the lung vagal afferent inputs are not essential to the regulation of resting breathing pattern and load compensation response. In
addition, resistive load detection occurs in the absence of lung vagal afferents, yet these afferents contribute to load detection.

The absence of pulmonary vagal afferents in many mammals has been found to be associated with an increased tidal volume and reduced f (11, 28), which were believed to be due to abolition of pulmonary stretch receptor input to the Hering-Breuer inflation reflex. However, the inflation reflex is relatively weak in humans compared with animals and is demonstrable only with large inflations. It would thus be expected that the vagal influence on breathing pattern might be substantially less in humans. Vagal blockade (18) and airway anesthesia (37) experiments have failed to show any significant effects of pulmonary afferents on resting breathing patterns in humans. However, the results from those studies may be confounded by the technical limitation on the completeness of lung deafferentation.

Lung transplantation interrupts afferent traffic from receptors located distal to the surgical anastomosis, thus providing a model to investigate the role of lung vagal afferents in regulation of breathing in human. In this study, the breathing pattern recorded for both loaded breathing and the breathing cycle before each load (control breath) was used to evaluate the subjects’ spontaneous resting breathing pattern and load compensation response. All subjects were instructed to breathe normally throughout the experiment, and no visual or auditory cue was provided to indicate the loaded inspiration. We found that there were no significant differences in peak Pm, VI, peak inspiratory airflow, Ti, Te, TP, f, and V˙E during both control breathing and loaded breathing between DLT subjects and Nor subjects. Our results suggest that lung vagal afferents

Fig. 2. Detection thresholds of the DLT group and the Nor group. *DLT group had a significantly higher detection threshold than the Nor group (P < 0.05).

Fig. 3. Scatterplot of Weber fraction in the DLT group and the Nor group. *Group mean value of Weber fraction for the DLT group was significantly higher than that of the Nor group (P < 0.05).

Fig. 4. Detection latency of the DLT group and the Nor group at different levels of resistive loads during loaded breathing. Values are as means ± SE; n, no. of subjects.

Fig. 5. Detection percent of the DLT group and the Nor group at different levels of resistive loads during loaded breathing. Values are means ± SE; n, no. of subjects. *DLT group had a significantly higher detection percent than the Nor group (P < 0.05).
are not essential to the regulation of resting breathing pattern and load compensation responses in humans.

Our findings were consistent with other lung transplant studies (23, 33). Shea and co-workers (33) compared resting breathing pattern in heart-lung transplant patients, heart transplant patients, as well as normal subjects. They found no difference in ventilation, tidal volume, f, Ti, and Te among all three groups during wakefulness and sleep. Kimoff et al. (23) also failed to find any major differences in ventilatory level or pattern between heart-lung transplant patients and normal subjects. In contrast, other studies have reported elevated f and reduced Ti in lung transplant recipients (24, 32). However, the lung transplant subjects in those studies had a restrictive spirometric pattern. A relationship between increased lung elastance and increased f has been reported by Renzi and colleagues (29, 31). Furthermore, Sanders et al. (31) observed that the heart-lung transplant patients recipients with a restricted spirometric pattern had a higher f during wakefulness and sleep compared with those recipients without a restrictive pattern. Therefore, the reduced Ti and increased f are probably related to the presence of underlying pulmonary restriction rather than to lung-lower airway deafferentation. In the present study, none of the DLT subjects displayed a restrictive spirometric pattern. The difference in our result and those of Kinnear et al. (24) and Sanders et al. (32) is probably due to the difference in the pulmonary function of lung transplant patients recruited.

The ventilatory response to added mechanical loads can be regarded as the sum of two components: one representing the effect of the passive respiratory system and one representing the effect of neural load-compensating mechanisms (2). The load-compensating component represents the action of neural mechanisms that modify the pressure developed by loaded respiratory muscles. Receptors in lung and lower airway could potentially contribute to these neural adjustments. However, our results showed no significant group difference. For both the DLT and Nor subjects, as the magnitude of the resistive load increased, Pm, Ti, and TP increased, whereas Vi, airflow, Te, f, and Ve decreased. These results indicate that load compensation can occur in the absence of vagal afferent input, as long as the remaining afferent pathways are intact. Our results were similar to those of Forster et al. (15), who reported that first-breath load compensation remained after pulmonary vagal denervation in ponies. Load compensation response in lung transplant patients was also studied by Peiffer et al. (27). In contrast to our findings, they reported that the lung transplant recipients produced higher peak Pm and inspiratory flow rate. However, in their protocol, the load was applied after a short vocal cue. Subjects’ breathing patterns might change in response to the cue. It is not known whether there is a difference in their reaction to the cue between DLT and the Nor groups, but it is likely that the cue allowed the subject to prepare for the load, thus adding a voluntary component to the load compensation response. Moreover, the inspiratory resistive loads were presented for the duration of two consecutive inspiratory breaths according to their methods. It is not known whether their data came from the first or the second loaded breath. Load compensation responses will be different for a first-breath response compared with a second-breath response. Finally, muscle strength was not compared between their lung transplant recipients and controls. Lung transplant recipients usually have weakened respiratory muscles because of the use of steroid medications and deconditioning after surgery. Most studies demonstrated a close relationship between weak muscle strength and increased respiratory drive (1, 16). The changes in loaded breathing pattern might be a result of changes in respiratory drive and respiratory muscle force in those patients (5, 35). The present study did not show any evidence of muscle weakness in the DLT patients. Therefore, the impact of respiratory muscle strength and drive on load compensation would be minimal in this study.

An important assumption of this study is that DLT recipients are, and remain, lung vagally denervated after surgery. The results of several investigations performed in animals found reappearance of a weak Hering-Breuer inflation reflex as early as 5 mo after pulmonary autotransplantation (14, 25). However, reinnervation would be less likely in the context of human allotransplantation than with simple reimplantation of an excised lung as in the canine model because no attempt is made to approximate nerves in DLT patients (23). In a study investigating the integrity of the cough reflex, which is mediated mostly by pulmonary receptors, after lung transplant, Higenbottom and co-workers (19) observed a significantly diminished cough response to ultrasonically nebulized distilled water for up to 3 yr after lung transplant. More compelling evidence for persistent lung denervation after human lung transplant has been provided by Iber et al. (20). They recently reported persistently absent expiratory prolongation after passive lung inflation during sleep in bilateral lung transplant recipients for a period of 49 mo after surgery. In contrast to the above findings, Butler et al. (8) recently reported early respiratory events (cough or apnea) and noxious sensations evoked by injections of lobeline (>30 μg/kg) occurred in a few bilateral lung transplantation subjects who were studied more than 1 yr after transplantation. Their results suggested that there might be functional reinnervation of the lungs after bilateral lung transplantation. However, changes in nonpulmonary receptors may have occurred over time to recover the sensitivity to lobeline in those patients. In this study, the time since the patients received DLT surgery varied from 1.5 to 5.5 yr, with an average of 3.45 yr. Although we did not test the reinnervation in our patients, it seems unlikely that reinnervation had occurred on the basis of previous findings (19, 20).

Although the detection of inspiratory loads has been studied extensively, the site at which such detection occurs is still not known. There are a variety of mechanoreceptors located in lung and lower airway that are
innervated by the vagus nerves. Afferent information from those receptors related to respiratory mechanical changes during loaded breathing may contribute to the detection of external loads. The present study showed that despite a similar loaded breathing pattern, the DLT group had a significantly higher detection threshold (2.91 \pm 0.5 \text{ cmH}_2\text{O} \cdot 1^{-1} \cdot \text{s}; P < 0.05) and Weber fraction (0.50 \pm 0.1; P < 0.05) than the Nor group. The group effect on detection percent was significant, with a lower detection percent found in the DLT group. These results suggest that pulmonary vagal afferents may contribute to load detection.

Two strategies have been adopted to determine the role of pulmonary receptors in respiratory load perception: either their principle afferent nerve (vagus nerve) is selectively blocked, or alternatively, all other possible sources are eliminated, leaving only the vagal input intact. High-level quadriplegic subjects with tracheostomies provide indirect evidence about the role of pulmonary afferents in respiratory sensation, because both respiratory muscle afferents and upper airway receptors are bypassed, leaving only the pulmonary receptors intact. It has been reported that these patients could reliably detect changes in tidal volume as little as 100 ml, which was comparable to that of normal subjects (4). Similarly, other studies (26, 39) also found that detection of external loads did not appear to be impaired in quadriplegic patients in whom afferent pathways from the chest wall are disrupted. These finding suggest the possibility that pulmonary receptors may contribute to load detection.

Contradictory results on role of lung vagal afferents in load detection were reported by Guz et al. (17). They studied the effect of bilateral block of the vagus and glossopharyngeal nerves in two healthy subjects. The difference threshold for elastic load detection was not affected by the nerve block. Furthermore, there was also no change in the sensation associated with a high resistive load in one subject. Burki (6) and Chaudhary and Burki (9, 10) showed that upper and lower airway anesthesia in normal subjects did not alter the detection thresholds of either resistive or elastic loads. Nonetheless, it is possible that some pulmonary stretch receptors may escape topical anesthesia because the anaesthetic could not penetrate to the smooth muscle or because the drug was carried away rapidly by the rich blood flow (3). Moreover, because both upper and lower airway receptors were blunted in their methods, it is not possible to make a conclusion about the specific role of lung and lower airway afferents in load detection.

Lung transplantation, through an interruption of afferent nerve fibers from the lung and lower airways, provides an opportunity to study the contribution of neural feedback from lung and lower airways to respiratory sensation. Besides the present study, there is only one another investigation of resistive load detection in lung transplant recipients by Tapper and his co-workers (34). They compared the detection threshold of inspiratory resistive loads in heart-lung transplant recipients, heart transplant recipients, and normal subjects, and they found no significant difference in the Weber fraction associated with a 50% probability of load detection between the heart-lung transplant recipients and the normal group. Therefore, they concluded that lower respiratory tract afferents did not play a significant role in the perception of respiratory resistive loads. The difference between the present study and the study of Tapper et al. might be due to the difference in lung transplant patients and the method applied to determine detection threshold. The DLT subjects in the present study are older than the heart-lung transplant patients in the study of Tapper et al. (46.5 \pm 4.4 \text{ yr}, 33.7 \pm 1.5 \text{ yr}, \text{ respectively}). The effect of age on resistive load detection ability is unclear. Tapper et al. used a tracking procedure to determine the detection threshold. The tracking procedure causes more false-positive responses. The relationship between false-positive response rate and detection threshold is not known. Moreover, the imposition of resistance or shams was signaled by an audible cue during the preceding exhalation. It is reasonable to believe that a cue would improve a person’s detection performance, which might result in the lower Weber fraction found in their heart-lung transplant patients.

In the present study, the DLT patients’ FEV1 and FVC values were significantly lower than those of the Nor subjects (83.8 \pm 6.1 \text{ vs.} 108.3 \pm 3.7\% \text{ of predictive values}, and 97.0 \pm 4.9 \text{ vs.} 119.5 \pm 5.0\% \text{ of predictive values, respectively}). However, both FEV1 and FVC are well within normal limits. It is unlikely that an increased detection threshold found in the DLT group was due to their lung function. The Weber fraction, which controlled for the effect of background resistance, was significantly higher in the DLT group than the Nor group. Furthermore, no significant correlation has been found between either FVC or FEV1 and Weber fraction in both DLT and Nor subjects (P > 0.05).

Two DLT subjects had higher detection threshold and Weber fraction values compared with the remainder of the DLT group (Figs. 2 and 3). Their pulmonary function and demographics were similar to those of other DLT subjects. The two subjects were 3.5 and 2.0 yr posttransplantation. There was no significant correlation between time postsurgery and detection threshold for the DLT group. The cause of their high detection threshold was not clear either. It should also be noted that in the present study, all DLT subjects were on immunosuppressive agents and steroid medications. It is possible that the present results could be affected by the medications taken by the DLT recipients. Further studies are necessary to investigate the effect of these medications on resistive load detection.

CONCLUSIONS

In summary, we found that both unloaded and loaded breathing patterns were similar in the DLT group and the Nor group, suggesting that lung vagal afferents are not essential to the regulation of resting breathing pattern and load compensation response.
Furthermore, resistive load detection did occur in DLT patients. This means that nonvagal afferents are activated during breathing against resistive loads and do elicit a load detection response. However, the DLT recipients had a significantly higher detection threshold and Weber fraction than the Nor group. The impaired detection capability is likely due to the loss of lung vagal afferent inputs in those lung-denervated patients. The results of this study suggest that vagal afferents play a role in resistive load detection. The detection threshold is increased with the loss of vagal afferents. However, the effect of DLT medications on load detection cannot be ruled out.

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