Lung mechanics in individuals with spinal cord injury: effects of injury level and posture

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Baydur, Ahmet, Rodney H. Adkins, and Joseph Milic-Emili. Lung mechanics in individuals with spinal cord injury: effects of injury level and posture. J Appl Physiol 90: 405–411, 2001.—Individuals with spinal cord injury (SCI) exhibit reduced lung volumes and flow rates as a result of respiratory muscle weakness. These features have not, however, been investigated in relation to the combined effects of injury level and posture. Changes in forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), and inspiratory capacity (IC) and expiratory reserve volume (ERV) were assessed by injury level in the seated and supine positions in 74 individuals with SCI. The main findings were 1) FVC, FEV1, and IC increased with descending SCI level down to T10, below which they tended to level off; 2) supine values of FVC and FEV1 tended to be larger in the supine compared with the seated posture down to injury level T10, caudal to which they were less than when seated; 3) IC increased proportionately more down to injury level L1, below which it declined slightly and plateaued; 4) ERV was measurable even at high cervical injuries, was generally smaller in the supine position, reached peak values in both positions at T10, injury level, and then rapidly declined at lower levels; 5) when subjects were separated according to current, former, and never smokers, only formerly smoking paraplegic individuals demonstrated spirometric values significantly less than paraplegic individuals who never smoked. Changes in spirometric measurements in SCI are dependent on injury level and posture. These findings support the concept that the increase in vital capacity in supine position is related to the effect of gravity on abdominal contents and increase in IC.

MATERIALS AND METHODS

Seventy-four individuals with chronic SCI were consecutively recruited as outpatients between January 1994 to May 1995 from a larger study investigating the long-term medical and physical impairments of SCI individuals. All subjects were free of acute cardiorespiratory illnesses at the time of the study. Of the 74 subjects, 62 (84%) were men and 12 (16%) women; 31 (42%) were tetraplegic (injury level C3–C7) while the remaining 43 (58%) were paraplegic (injury level: T1–L4). Mean duration of injury was 157 ± 104 (SD) mo (range 8–521 mo).

Smoking history was available in 63 subjects: 24 (38%) were smokers (11 tetraplegic individuals, 13 paraplegic individuals), and 39 (62%) were nonsmokers. Eighteen of the 31 (58%) tetraplegic individuals showed complete injury (i.e., detailed neurological examination showed no detectable motor or sensory function below the level of injury), whereas 27 (63%) of the paraplegic individuals had complete injury. None of those with tetraplegia required the use of a cervical orthosis or body jacket for bony stability at the time of testing. When the studies were conducted, all patients were confined to wheelchairs, had chest roentgenograms within normal limits, and had chest physical examinations showing no signs of respiratory or cardiac disease. Subjects with clinical signs of cardiorespiratory illness or any infection were excluded. Approval was provided by the institutional review board of Rancho Los Amigos National Rehabilitation Center before the study was initiated.

Subjects underwent spirometric testing using a rolling seal spirometer (PFT Horizon or System 2200, Sensormedics, 8750-7587/01 $5.00 Copyright © 2001 the American Physiological Society

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Anaheim, CA). Forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC) were measured according to recommendations by the American Thoracic Society (3). To achieve accurate “time zero” and ensure a maximal effort curve, only volume-time profiles were analyzed in which the extrapolated volume was <5% of the FVC or 0.100 liter, whichever was greater. Values of inspiratory capacity (IC), expiratory reserve volume (ERV), and forced expiratory flow at 50% VC (FEF₅₀) were derived by comparing the maximum expiratory flow-volume curve with its immediately preceding tidal flow-volume curve. IC was computed by subtracting ERV from FVC. To avoid errors in such computations, the breathing circuit was checked for leaks before and after each testing session. Predicted values of FVC, FEV₁, FEV₁/FVC, and FEF₅₀ were those of Morris et al. (26). Predicted values of IC and ERV were derived from differences between corresponding predicted values of TLC and FRC, and between TLC and FVC, respectively (5).

Each subject was tested in random order in the seated and supine posture, the latter with the back of their wheelchair lowered to horizontal position or with the individual transferred to a flat, cushioned examining bench. Sighs and irregular breaths were discarded. This method of analysis ensured that any change of end-expiratory volume really represented a physiological and not a voluntary response of our subjects. All tracings during which leaks were detected were eliminated. In one tetraplegic subject, IC and ERV were considered a physiological and not a voluntary response of our subjects. All tracings during which leaks were detected were eliminated. In one tetraplegic subject, IC and ERV could not be recorded because of a system leak.

Statistical analysis. Group means for each variable of spirometry were compared between seated and supine positions and tested for significance by Wilcoxon’s rank-sum test (20). Differences in spirometric variables among levels were determined by ANOVA and Tukey’s post hoc comparisons in current smokers, former smokers, and never smokers. Relationships between FVC and injury level were assessed using regression analysis by assigning a corresponding numerical value between 1 and 24 to neurological levels C1 through L4.

RESULTS

Mean seated FVC values in the paraplegic and tetraplegic groups were, respectively, 86.1 ± 17.6 (SD) and 57.2 ± 18.7% predicted (P < 0.001). When each group was divided into complete and incomplete injuries, mean predicted FVC was 84 ± 19 (SD) and 90 ± 16%, respectively, for the paraplegic individuals and 54 ± 21 and 61 ± 15%, respectively for the tetraplegic individuals. Differences in FVC between the complete and incomplete injuries within each group were not statistically significant (ANOVA). Table 1 lists FVC, FEV₁, FEF₅₀, IC, and ERV values (as percent predicted) for the paraplegic and tetraplegic subjects in seated posture. It shows that percent predicted values in the tetraplegic individuals were 34, 32, 28%, 13, and 48% less, respectively, than in the paraplegic individuals (P < 0.001, P < 0.001, P < 0.01, respectively, Wilcoxon’s rank-sum test). Figure 1 shows that values for FVC, FEV₁, IC, and ERV in general increased with descending injury levels in seated individuals. ERV also increased with descending injury level down to level T₁₂, below which it fluctuated between 39 and 67% predicted.

Effect of smoking on lung function. Smoking history was available in 63 (85%) of the individuals. Mean FVC in 52 seated nonsmokers (of whom 35 never smoked) and 11 currently smoking subjects was 75 ± 25 (SD) predicted and 78 ± 25% predicted, respectively (Table 2). Former smokers, in particular, demonstrated significantly lower FVC, FEV₁, FEF₅₀, and IC values than never smokers [by 14% (P < 0.05), 15% (P < 0.05), 21% (P < 0.01) and 19% (P < 0.01), respectively]. Table 2 and Fig. 2 also show that, whereas tetraplegic subjects demonstrated lower overall values of FVC, FEV₁, FEF₅₀, IC, and ERV, only formerly smoking paraplegic subjects showed significantly lower values for FVC, FEV₁, IC, and ERV than paraplegic individuals who never smoked.

Effect of posture on lung function. Table 3 shows mean (± SD) values of FVC, FEV₁, FEF₅₀, IC and ERV for tetraplegic and paraplegic subjects in the seated and supine postures. Differences in FVC and FEV₁ between the two postures were not statistically different, whereas FEF₅₀ decreased by 7.5% [not significant (NS)] and 6.7% (P < 0.05) in tetraplegic individuals and paraplegic individuals, respectively, in the supine position. Overall, IC increased by 16% (P < 0.01) and 3.2% (NS), and ERV decreased by 35% (P < 0.01) and 21% (P < 0.01) in tetraplegic individuals and paraplegic individuals, respectively, in the supine posture. Figure 3 shows FVC, FEV₁, IC, and ERV in the seated and supine postures at each injury level. It shows that, whereas FVC and FEV₁ were not significantly different between the positions, values of IC were higher in the supine position by as much as 28% at injury levels above T₆, whereas they were approximately the same in both postures at T₈ and below. By contrast, ERV was almost uniformly less in supine posture (by as much as 30%) throughout all injury levels. As in Fig. 1, all four variables increased with descent of injury level down to level T₁₀, at which point they declined somewhat, with ERV decreasing by as much as 60% at L₁.

Table 1. Pulmonary function in seated individuals with spinal cord injury

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>FVC, liters</th>
<th>FEV₁, liters</th>
<th>FEF₅₀, l/s</th>
<th>IC, liters</th>
<th>ERV, liters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetraplegic</td>
<td>31</td>
<td>39 ± 11</td>
<td>174 ± 8</td>
<td>71 ± 15</td>
<td>57 ± 19</td>
<td>65 ± 20</td>
<td>68 ± 23</td>
<td>74 ± 23</td>
<td>33 ± 18</td>
</tr>
<tr>
<td>Paraplegic</td>
<td>41</td>
<td>41 ± 13</td>
<td>174 ± 11</td>
<td>76 ± 18</td>
<td>86 ± 18*</td>
<td>95 ± 18*</td>
<td>95 ± 27*</td>
<td>108 ± 29†</td>
<td>63 ± 24*</td>
</tr>
<tr>
<td>Combined</td>
<td>74</td>
<td>40 ± 12</td>
<td>175 ± 11</td>
<td>76 ± 18</td>
<td>74 ± 23</td>
<td>82 ± 24</td>
<td>83 ± 29</td>
<td>94 ± 31</td>
<td>50 ± 27</td>
</tr>
</tbody>
</table>

Anthropometric values are means ± SD, and spirometric values are means ± SD %predicted. FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; FEF₅₀, forced expiratory flow at 50% vital capacity; IC, inspiratory capacity; ERV, expiratory reserve volume. *Significant difference compared with tetraplegic individuals, P < 0.001 (Wilcoxon’s rank-sum test). †Significant difference compared with tetraplegic individuals, P < 0.001 (Wilcoxon’s rank-sum test).
DISCUSSION

To our knowledge, this is the first study in patients with SCI that has assessed respiratory function by injury level in both the seated and supine postures. The main findings of our study are that 1) FVC, FEV<sub>1</sub>, and IC, expressed as percent predicted, increased with descending spinal injury levels down to level T<sub>10</sub>, below which they tended to level off; 2) supine values for IC were, in general, larger in the supine compared with the seated posture down to injury level L<sub>1</sub>, below which they declined slightly and plateaued; 3) ERV was almost consistently smaller in the supine position, reaching peak values in both positions at injury level T<sub>10</sub> and then rapidly declining at lower levels; 4) at most injury levels, values for FVC, FEV<sub>1</sub>, and FEV<sub>1</sub>/FVC in non-smoking patients exceeded those of (current and former) smokers by 5–20%.

Comparison of SCI individuals with normal subjects in the seated position: effect of injury. Individuals with traumatic transection of the lower cervical cord have paralysis of the intercostal and abdominal muscles, whereas the diaphragm is intact. In the chronic stage of the injury, both lung and chest wall compliances are reduced; measurements with respiratory magnetometers have shown that the reduced chest wall compliance is due to abnormal stiffness of the rib cage (9, 13).

Table 2. Effects of smoking on lung function in seated individuals with spinal cord injury

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>FVC, liters</th>
<th>FEV&lt;sub&gt;1&lt;/sub&gt;, liters</th>
<th>FEF&lt;sub&gt;50&lt;/sub&gt;, l/s</th>
<th>IC, liters</th>
<th>ERV, liters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetraplegic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smokers</td>
<td>11</td>
<td>53 ± 21</td>
<td>61 ± 22</td>
<td>68 ± 30</td>
<td>70 ± 25</td>
<td>29 ± 14</td>
</tr>
<tr>
<td>Former smokers</td>
<td>9</td>
<td>60 ± 13</td>
<td>67 ± 12</td>
<td>63 ± 12</td>
<td>77 ± 17†</td>
<td>36 ± 8</td>
</tr>
<tr>
<td>Current smokers</td>
<td>4</td>
<td>56 ± 29</td>
<td>66 ± 33</td>
<td>69 ± 41</td>
<td>63 ± 11</td>
<td>41 ± 37</td>
</tr>
<tr>
<td>Paraplegic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smokers</td>
<td>24</td>
<td>91 ± 16</td>
<td>100 ± 16</td>
<td>102 ± 24</td>
<td>114 ± 27</td>
<td>67 ± 20</td>
</tr>
<tr>
<td>Former smokers</td>
<td>8</td>
<td>77 ± 21*</td>
<td>85 ± 20&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>83 ± 27</td>
<td>87 ± 27&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>69 ± 32‡</td>
</tr>
<tr>
<td>Current smokers</td>
<td>7</td>
<td>90 ± 13</td>
<td>94 ± 16</td>
<td>94 ± 31</td>
<td>123 ± 30</td>
<td>47 ± 22</td>
</tr>
</tbody>
</table>

Values are means ± SD %predicted values. P values are based on overall analysis of variance performed for never, former, and current smokers. Significant differences are based on Tukey's method of multiple-group comparisons. *P < 0.05, former vs. never smokers. †P < 0.05, former vs. current smokers. ‡P < 0.01, former vs. current smokers.
The abdominal component of the chest wall is abnormally compliant because of abdominal muscle paralysis (9, 21). As a result of the muscle paralysis and reduced distensibility of the lung and rib cage, VC in tetraplegic individuals is decreased to 50–80% of predicted values (2, 4, 8, 9, 15, 23, 25). These findings are similar to those of our study in which FVC averaged 57% in all seated tetraplegic individuals.

Of interest was the finding that ERV was a measurable finite volume in tetraplegic subjects. Subjects with traumatic tetraplegia of the lower cervical cord develop severely compromised expiratory muscle (i.e., anterolateral wall of the abdomen, the expiratory intercostals, and the triangularis sterni) function. Patients with traumatic tetraplegia, however, can still empty their lungs actively, and De Troyer et al. (7) and Estenne et al. (11, 12) recently established that this phenomenon results primarily from the action of the clavicular portion of the pectoralis major. Our mean values of IC [2.2 liters (74% predicted)] and ERV [0.49 liter (33% predicted)] are very similar to those reported by Estenne and De Troyer (10) in seated tetraplegic individuals [2.1 liters (69% predicted) and 0.44 liters (31% predicted), respectively] and other reports with respect to IC (4, 18) and ERV (4, 15, 18).

Relationship between pulmonary function and injury level. An objective of this study was to determine the relationship between lung function and the level of the lesion. Significant correlations were observed between all variables and injury levels, with the exception of FEV1/FVC in smokers, in whom the lack of correlation was because of the small number of subjects and scatter of values. Even in nonsmokers, it is of note that the relationship between FEV1/FVC and injury level was a reciprocal one. This finding may be explained by a greater elastic recoil at low lung volumes caused by stiffer lungs (8, 9, 13, 15) and rib cage (9, 13, 21) observed with tetraplegic subjects compared with paraplegic subjects. The correlation coefficients between FVC and FEV1 and injury levels ranged between 0.73 and 0.89, respectively, when expressed in liters, and between 0.60 and 0.79, respectively, when expressed as percent predicted. Bluechardt et al. (6) found a similar correlation between FVC and injury level (r = 0.72) in tetraplegic individuals 10 mo after injury. Correlation coefficients were slightly less for IC and ERV, although still highly significant statistically over the entire

Table 3. Pulmonary function in patients with spinal cord injury in seated and supine positions

<table>
<thead>
<tr>
<th>Subjects</th>
<th>FVC, liters</th>
<th>FEV1, liters</th>
<th>FEF50, l/s</th>
<th>IC, liters</th>
<th>ERV, liters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetraplegic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>31</td>
<td>31</td>
<td>31</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Seated</td>
<td>2.70 ± 0.91*</td>
<td>2.36 ± 0.74*</td>
<td>3.31 ± 1.18*</td>
<td>2.21 ± 0.70*</td>
<td>0.49 ± 0.26*</td>
</tr>
<tr>
<td>Supine</td>
<td>2.87 ± 0.82*</td>
<td>2.41 ± 0.67*</td>
<td>3.06 ± 1.12*</td>
<td>2.56 ± 0.70*</td>
<td>0.32 ± 0.16*</td>
</tr>
<tr>
<td>Paraplegic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>43</td>
<td>43</td>
<td>43</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>Seated</td>
<td>4.09 ± 0.96</td>
<td>3.40 ± 0.75</td>
<td>4.49 ± 1.17</td>
<td>3.16 ± 0.83</td>
<td>0.91 ± 0.38</td>
</tr>
<tr>
<td>Supine</td>
<td>3.98 ± 0.86</td>
<td>3.28 ± 0.67</td>
<td>4.19 ± 1.10</td>
<td>3.26 ± 0.78</td>
<td>0.72 ± 0.31*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of subjects. *Significant difference compared with corresponding paraplegic individuals, P < 0.01 (Wilcoxon's rank-sum test). †Significant difference compared with seated, P < 0.05 (Student's t-test). ‡Significant difference compared with seated, P < 0.01 (Student's t-test).
range of neurological levels. This is in keeping with other authors who have similarly found increases in IC and ERV with descending injury level (4, 10).

A sharp increase in FVC and IC was noted at lower thoracic injury levels. Figure 1 shows that IC and FVC reached maximum values of 150 and 112% predicted, respectively, at L1. The higher values of IC were related to the decrease in ERV between injury levels T11 and L2 in the absence of a proportional fall in FVC. These findings are similar to those reported by Bluechardt et al. (6), who also found that FVC and FEV1 reached peak values at midthoracic injury levels before declining slightly at lower thoracic levels. The explanation for this “rise and dip” in the IC values at these injury levels can be explained on the basis of the pattern of innervation of the abdominal muscles. The rectus abdominis, internal and external obliques, and transversus abdominis muscles are innervated sequentially from the lower six thoracic nerves and also from the first lumbar nerve for the internal oblique and transversus. The quadratus lumborum, on the other hand, is innervated by the 12th thoracic and first three or four lumber spinal nerves (28). It contracts synergistically with the diaphragm, exerting a downward force on the 12th rib and preventing any tendency for the vertebral part of the diaphragm to pull the 12th rib upward. Thus this muscle and the vertebral part of the diaphragm may operate as a single functional unit. If the quadratus lumborum is selectively paralyzed, as in T11–L2 injuries, the diaphragm should be displaced more cephalad, resulting in a decrease in ERV and increase in IC.

Effects of posture on lung function. In normal subjects, changing from seated to supine posture results in only a 7% reduction in VC, whereas ERV decreases by 65% and IC increases reciprocally by ~55% (1). Most of the changes are attributed to fluid shifts in and out of lung tissues.
the thorax and support the view that the intrathoracic blood volume is larger in the supine than in the upright posture. Subjects with traumatic tetraplegia behave differently. They demonstrate an increase in FVC and IC when assuming the supine posture (10, 16, 18, 19, 24), as did our subjects (Table 3, Fig. 4). One concept of mechanism suggests that the diaphragm increases its inspiratory excursion in supine position because its muscle fibers are longer at end expiration and operate on a more favorable portion of their length-tension curve, resulting in an increase in IC (18, 19, 22).

Estenne and De Troyer (10) found that the increase in VC observed in 14 tetraplegic individuals adopting the supine position is due to a reduction in residual volume, which is not dependent on an abnormal increase in intrathoracic blood volume as in normal subjects (1) but on the effect of gravity on the abdominal contents. Tetraplegic subjects with paralyzed abdominal muscles can only use the clavicular portion of the pectoralis major and some other muscles of the shoulder girdle (7). As a result, the expired volume in such subjects is only contributed by the upper portion of the rib cage.

That FEV₁ increases to the same degree as FVC in Table 3 indicates a close correlation between the two variables ($r = 0.97$ in our seated subjects) and is consistent with the findings of Roth et al. (29), who demonstrated a close correlation ($r = 0.88$) between these two measurements in 52 seated individuals with SCI.

Effect of smoking on lung function. The overall values of FVC, FEV₁, and FEF₅₀ for tetraplegic individuals (combined for never, former, and current smokers) listed in Table 2 are similar to those reported in other studies (2, 4, 23), a sizable proportion (39–45%) of which had subjects who were described as smokers but who were free of clinically apparent respiratory disease. We demonstrated significantly larger values of FVC, FEV₁, FEF₅₀, and IC in never smokers only in our paraplegic subjects (Table 2 and Fig. 2, A and B). The only other study we were able to find that separated smokers from nonsmokers was that of Almenoff et al. (2), who assessed only tetraplegic individuals. As in our study, they found no differences in FVC, FEV₁, and FEV₁/FVC between tetraplegic smokers and nonsmokers. The lack of difference in spirometric values between smoking and nonsmoking tetraplegic subjects may be because of already markedly reduced lung volumes and the effects of microatelectasis (8). Many subjects with cervical cord injury have an obstructive impairment, characterized by an increase in resting airway tone, as well as a restrictive defect (2). In addition it is possible that currently smoking paraplegic individuals retain spirometric values close to those of never smokers by periodic stretching of their airways while smoking, thereby relaxing bronchial smooth muscle and reversing microatelectasis. Deep inspirations are known to reverse airway obstruction induced in healthy or asthmatic subjects (14, 27).

Recently, Fredberg et al. (17) showed that the mere act of tidal breathing dynamically maintains the relaxation of, and an increase in the hysteresivity of airway smooth muscle in bovine trachea. Because tetraplegic individuals breathe more shallowly than paraplegic individuals, such stretching and relaxation of airway smooth muscle is less likely to occur than in paraplegic individuals. Thus physiological differences between smokers and never smokers are less apparent in tetraplegic individuals than in paraplegic individuals, particularly if the latter group is better able to take deep breaths (whether during smoking or otherwise).

In summary, we have shown in SCI subjects an injury level-associated increase in lung volumes. Most variables attained peak values at lower thoracic injury levels before declining slightly at lower levels. Even subjects with high cervical cord injuries demonstrated measurable values of ERV, most likely because of the action of the clavicular portion of the pectoralis major and possibly other rib cage muscles. When subjects were separated according to smoking history, only formerly smoking paraplegic subjects demonstrated spirometric values significantly less than paraplegic individuals who never smoked. We did not observe differences in spirometric values between smoking and nonsmoking tetraplegic subjects. Assumption of the supine posture resulted in increases in spirometric measurements, with the largest change observed in the IC. These findings are in keeping with previous studies demonstrating that the increase in IC and VC with assumption of the supine position is related to the effect of gravity on the abdominal contents and a concomitant reduction in residual volume (10).

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REFERENCES