Motor unit involvement of diaphragm and intercostal muscles in eupnea tachypnea and bradypnea of young female sedentary adults

Saha P.B, D, E, Pathak R. B, Chatterjee P. C, D, Bandyopadhyay A.* A, C, F

Department of Physiology, Serampore College, Serampore, Hooghly, West Bengal, India

ABSTRACT

Purpose: The involvement of motor units during their activities in primary and secondary muscles rapidly becomes prominent as a diagnostic tool for pathological conditions. But no research has been published so far indicating the involvement of motor units during physiological circumstances like Eupnea, Bradypnea, and Tachypnea. These findings would help to determine the neuromuscular mechanism of respiratory mechanics in physiological situations and to compare it with clinical conditions related to respiratory muscles.

Aim of the study: The experimental findings would help to determine the neuromuscular mechanism of respiratory mechanics in physiological situations and to compare it with clinical conditions related to respiratory muscles.

Materials and methods: Thirty healthy sedentary adult females participated in this investigation. The Tachypnea and Bradypnea were deliberately achieved by asking them to hyperventilate with shallow breaths and to hold their breath for a period in a supine posture accordingly. The participation of motor units and force generated in the diaphragm and intercostal muscles were assessed by surface electromyography (sEMG).

Results: The motor unit activities for three various situations such as silent breathing reduced breathing, and fast breathing rates significantly vary with each other in both the primary respiratory muscles (p<0.05). But there was insignificant variation in motor unit discharges between Eupnea and bradypnea of both muscles.

Conclusions: In supine posture during Tachypnea, the quick and enhanced expansion of the thoracic cavity requires more motor unit activation in the diaphragm and intercostal muscle thus suggesting higher involvement. In Eupnea and Bradypnea virtually equal participation of both muscles was discovered. The modest increase in intercostal motor unit discharge in bradypnea than Eupnea might be owing to the larger extension of the intercostal muscle during bradypnea than the diaphragm does.

Keywords: Electromyography, Diaphragm, Respiratory Muscle, Respiratory Rate

DOI:

*Corresponding Author:
Dr. Anupam Bandyopadhyay, Associate Professor
Department of Physiology, Serampore College, Serampore, Hooghly, West Bengal, India
Contact No: +919051741084
Email ID: baneranupam@gmail.com

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INTRODUCTION

The Coronavirus disease 2019 (Covid-19) pandemic boosts the demand for distinct respiratory improvements at all levels. Varied position of the body, techniques of respiration, and different exercises how far to improve the gaseous exchange is the key worry. The alveolus, the functional and structural unit of the lungs, is a key concern for increasing the quality of respiration. There are several aspects that aid to expose more alveoli at a time and are only achievable when the chest cavity is increased. The growth of the chest cavity is controlled by several respiratory muscles of the body. So, keeping in mind better respiration and lack of knowledge regarding the neuromuscular activities of two key respiratory muscles this work aims to emphasize the motor unit involvement of two major respiratory muscles in different physiological states.

In order to inhale oxygen and exhale carbon dioxide, the intercostal and diaphragmatic muscles contract and relax, respectively. Motor units, which are responsible for of these muscles' activation during respiration, regulate this process. To fully understand the underlying processes of breathing control, it is crucial to know how the motor units in the diaphragm and intercostal muscles are involved during various breathing patterns, such as eupnea, tachypnea, and bradypnea. Additionally, investigating the role of the motor unit in breathing in particular populations, such as young, sedentary adult females, can offer insightful information about how physical fitness and health condition may affect breathing patterns.

Both mechanical and nonmechanical complicated components are involved in regular breathing and are altered by many factors such as age, lifestyle, diseases, and changes in posture [1,2]. Different postures including forwarding head positions and kyphosis have been demonstrated to change the breathing mechanism and mobility of the diaphragm [3–7]. It has been observed that deficits arise in normal breathing mechanics by diminishing diaphragm mobility and strength when cervicothoracic mobility is disrupted [8]. It has been revealed by the researchers that forward head movement and rotated neck postures impact the breathing pattern which leads to breathing disorders. Neck issues are often encountered in an aged person and disrupt normal breathing [9]. In torticollis, musculoskeletal difficulties occur in the shoulder, neck, and back which leads to twisting of the neck on one side and interferes with the mobility of the diaphragm [10,11]. Most of the recorded research is based on lung function testing. Only a few studies have been published on the enlargement of the chest by involving the motor unit in respiratory muscles. Some research reflects the effect of varied postures on respiratory function including electromyographic studies. But there has been no research so far that focus on the importance of the neuromuscular participation of two primary muscles in diverse physiological states like Eupnea, Bradypnea, and Tachypnea.

An estimated 3.2 million people die each year as a result of insufficient physical activity, which affects about 31% of the world's population under 15 years old [12]. Living a sedentary lifestyle is characterized as spending most of your time sitting, lying down, or watching TV with little to no physical activity [13]. Sedentary behaviour is prevalent around the world, and related non-communicable diseases are becoming more common. It is common knowledge that a lack of physical activity, or physical inactivity, is bad for your health. With 4-5 million deaths each year caused by it, physical inactivity is the fourth-leading risk factor for mortality worldwide [14].

This investigation is hypothesized because of limited reports of the diaphragm and other respiratory muscles' neural activity in healthy sedentary young adult females. Once the brain control of different muscles involved in respiratory phases is properly understood, it would be straightforward to devise different exercises or approaches for enhancing respiration.

This study aims to investigate the neuromuscular involvement of different muscles during supine postures and different phases of respiratory cycles including normal, lower, and higher respiratory rates in sedentary healthy young adult females. The results of this research may have significant effects on how respiratory function is evaluated and how people with respiratory disorders train their respiratory muscles.

MATERIALS AND METHODS

Selection of the participants

Adult healthy young female college students, age ranges 18-25 years (average 21.367±1.586 years) were engaged in this study. Thirty (30) sedentary healthy female college students with no history of pulmonary or neuromuscular disease voluntarily cooperated in this investigation.

This observational study was carried out at the Sports and Exercise Physiology Laboratory, Serampore College, affiliated to the University of Calcutta, West Bengal, India. The volunteers were allowed to take rest in the supine position for 10 minutes in the laboratory at 25 degrees centigrade room temperature and the average relative humidity was documented as 56 % - 68 % for consecutive seven days during the tests by a digital hygrometer.

Inclusion criteria

Before experimenting, the purpose of the study was thoroughly explained in detail to the
participants. Those who agreed to participate voluntarily (age ranges 18-25 years) having no prior respiratory disease and were double Vaccinated (COVID) as well as showing no symptoms of COVID like high body temperature, cold, cough, sneezing, etc. were included in the study.

Exclusion criteria
Participants with ages <18 years and >25 years were excluded from the study. The participants who had respiratory diseases and engaged in yoga or any breathing exercise regularly were also not included in this study. Moreover, those who are not fully vaccinated with at least two COVID vaccines and showed any COVID symptoms like high body temperature, cold and cough, sneezing, etc. were also not participated. Ethically who did not agree to participate voluntarily were also excluded.

Ethics Approval
The study was approved by the Human Ethical Committee (HEC, Serampore College, affiliated to the University of Calcutta, Serampore, Hooghly, West Bengal, India, numbered SC/HEC/2022/ P1B) according to the Helsinki Declaration of 1975.

MEASUREMENTS

Anthropometric Measurements
- **Stature**: Stature (cm) was the perpendicular distance between the transverse planes of the Vertex and the inferior aspect of the feet and it was measured by SECA 213 Portable Stadiometer (range of measurement 60cm to 220cm). The stature was measured by the “Stretch Stature Method” [15,16].
- **Weight, Lean body mass & Fat%**: Weight, Lean Body Mass, and Fat% were measured with BIA (Bioimpedance analysis). Body composition analysis was determined by the bio-impedance analyzer. The segmental body composition monitor Tanita BC-601 has the BIA method of measuring body composition with scientifically proven accuracy by sending low and safe electrical scales to measure the muscle and fat in combination with total body measurement [17]. The measurements were collected using the standard setting after manually imputing the measured height, weight, gender, and age of the subject.
- **Body Mass Index**: The BMI was measured, using BMI Calculator by putting an individual’s height and weight. BMI = kg/m² [18].

Electromyographic Recording
EMG recording kit (iWorx) was set up and prepped for recording. The EMG recording was done on two separate respiratory muscles such as the diaphragm and the intercostal muscle. The muscles were isolated and surface button electrodes were put applying the gel to the skin [19]. The ultrasonic gel was applied to the sections where electrodes have been positioned. The ultrasound gel improves the coupling between the electrode and the skin, cancelling the air pockets present in the stratum corneum of the dermis, which boosts the conductivity of the signal. The surface EMG electrodes were attached to the physiologic amplifier device. To do the recording the participant was requested to lie down. To effectuate the collection of surface signals from the diaphragm and intercostal muscle, pairs of surface electrodes were positioned according to the following scheme:
- For the diaphragm, one set of electrodes was implanted in the lowest intercostal spaces on the right side of the body, near the midclavicular line.
- One pair for the external intercostal muscles, at the fifth intercostal space at the posterior axillary line.
- A ground electrode was placed on the sternum. The distance between a pair of electrodes was minimum, not more than 2 cm, and care was taken to insert the electrodes in the same orientation as the muscle fibers.

The subjects were requested to exhibit 3 types of breathing patterns as per the criteria of the study. These 3 artificial breathing patterns, were assigned to 3 different respiratory conditions - Eupnea, Tachypnea, and Bradypnea. For Eupnea, the subjects participated in the usual respiration. The breathing rate for an adult at rest is 12 to 20 breaths per minute. A respiratory rate under 10 or over 25 breaths per minute is considered Bradypnea and Tachypnea correspondingly. For Tachypnea, participants were instructed to take rapid shallow breathing, a breathing rate of more than 20 breaths per minute [20]. Bradypnea was achieved by enabling the participant to undergo breath-holding with shallow inhalation. Breathing rate is below 10 breaths per minute for more than 2 minutes [21]. The EMG recordings of the diaphragm and intercostal muscles were done in these two circumstances.

Statistical Analysis
Statistical analysis of all data done by IBM Statistical Package for Social sciences (SPSS) version 25. The data were found to be not normally distributed by the Shapiro-Wilk normality assumption test. Kruskal Wallis non-parametric analysis of variance (ANOVA) was performed to find out whether any significant difference in the variables among the groups exists or not followed by Mann-Whitney U multiple comparison test for the significant variables. Mann-Whitney U test was used to figure out the inter-group differences of the
variables. For all situations, \( p < 0.05 \) was considered a statistically significant outcome.

**RESULTS**

**General characteristics**

The general characteristics of the participants are shown in Table 1. The average age of the participants (18-25 years) is 21.367±1.586 years. The average fat percentage of young females is very high (32.65±9.48%) though the BMI indicates (23.73±3.83 kg/m²) their healthy body weight. The average respiratory rate (RR) is also within the normal rate (17.50±2.162 breaths/minutes) though the different respiratory conditions were deployed individually.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>21.367±1.586</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>154.69±6.56</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>56.99±10.46</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.73±3.83</td>
</tr>
<tr>
<td>Lean Body Mass</td>
<td>37.49±4.63</td>
</tr>
<tr>
<td>Fat %</td>
<td>32.65±9.48</td>
</tr>
<tr>
<td>Respiratory Rate (per min)</td>
<td>17.50±2.162</td>
</tr>
</tbody>
</table>

SD = Standard Deviation

**Electromyographic variables**

The analysis of electromyographic variables in the three different breathing conditions was shown along with the Kruskal-Wallis nonparametric ANOVA in Table 2. Three different conditions are Eupnic (RR 17.5±2.162 breaths/min), Tachypnic (RR 68.6±9.637 breaths/min), and Bradypnic (7.93±2.033 breaths/min). Significant differences were found in mean values of root mean square (RMS) and maximum voluntary contraction (MVC) in Diaphragm (Fig. 1) and Intercostal (Fig. 2) muscles during Eupnea, Bradypnea, and Tachypnea (\( p < 0.05 \)).

Since the RMS and MVC values of the Diaphragm and Intercostal muscles were found to be significant, Mann-Whitney \( U \) multiple comparison tests were performed to find out the inter-group differences. Both the RMS and MVC of the Diaphragm and Intercostal muscles were found to be significantly higher in the Tachypnic condition than in both the Eupnic and Bradypnic conditions (Fig. 1 and Fig. 2), while no inter-group significant difference was found between Eupnic and Bradypnic conditions (Table 3).

**Table 1.** Mean and standard deviation values of Age, Height, Weight, BMI, Lean Body Mass, and Fat % of young healthy adult females (n=30)

<table>
<thead>
<tr>
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<td>Respiratory Rate (per min)</td>
<td>17.50±2.162</td>
</tr>
</tbody>
</table>

SD = Standard Deviation

**Table 2.** Kruskal-Wallis nonparametric ANOVA test values and level of significance (\( p \) values) of RMS and MVC in Eupnea, Bradypnea, and Tachypnea between Diaphragm and Intercostal muscles of young healthy adult females

<table>
<thead>
<tr>
<th>Variables</th>
<th>Respiratory Conditions according to Respiratory Rate (RR) (Means ±SD)</th>
<th>Kruskal-Wallis H value</th>
<th>Level of significance (( p ) values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diaphragm Root Mean Square (RMS) (mV)</td>
<td>Eupnic (n=28) (10-20 per minute) (17.5±2.162 breaths/min)</td>
<td>0.027±0.034</td>
<td>0.029±0.038</td>
</tr>
<tr>
<td>Diaphragm Maximum Voluntary Contraction (MVC) (mV)</td>
<td>Tachypnic (n=28) (&gt;20 per minute) (68.6±9.637 breaths/min)</td>
<td>0.136±0.057</td>
<td>0.230±0.196</td>
</tr>
<tr>
<td>Intercoastal Root Mean Square (RMS) (mV)</td>
<td>Bradypnic (n=28) (&lt;10 per minute) (7.93±2.033 breaths/min)</td>
<td>0.028±0.022</td>
<td>0.127±0.166</td>
</tr>
<tr>
<td>Intercoastal Maximum Voluntary Contraction (MVC) (mV)</td>
<td>Eupnic (n=28) (10-20 per minute) (17.5±2.162 breaths/min)</td>
<td>0.093±0.058</td>
<td>0.207±0.095</td>
</tr>
</tbody>
</table>

(n=30) ; *\( p \) value<0.05 is statistically significant
Table 3. Mann-Whitney U multiple comparison test values and level of significance (p-value) of RMS and MVC of Diaphragm and Intercostal muscles in Eupnea, Bradypnea, and Tachypnea

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mann -Whitney U multiple comparison test (U, p values)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eupnea vs Tachypnea</td>
</tr>
<tr>
<td>Diaphragm Root Mean Square (RMS) (mV)</td>
<td>77.000 (p=0.000*)</td>
</tr>
<tr>
<td>Diaphragm Maximum Voluntary Contraction (MVC) (mV)</td>
<td>170.500 (p=0.000*)</td>
</tr>
<tr>
<td>Intercostal Root Mean Square (RMS) (mV)</td>
<td>25.500 (p=0.000*)</td>
</tr>
<tr>
<td>Intercostal Maximum Voluntary Contraction (MVC) (mV)</td>
<td>121.000 (p=0.000*)</td>
</tr>
</tbody>
</table>

*p value<0.05 is statistically significant, ns= not statistically significant

Figure 1. Graphical representations of the Electromyographic variables (RMS and MVC) of the Diaphragm muscle in different respiratory conditions i.e., Eupnic, Tachypnic, and Bradypnic (n=30, ns- Not statistically significant, *p<0.05)

Figure 2. Graphical representations of the Electromyographic variables (RMS and MVC) of the Intercostal muscle in different respiratory conditions i.e., Eupnic, Tachypnic, and Bradypnic (n=30, ns- Not statistically significant, *p<0.05)
DISCUSSION

Respiratory mechanics is the interplay of the lung, chest wall, and respiratory muscles. The role of respiratory muscles is well understood when the motor actions of different respiratory muscles can be investigated. This study reveals the involvement of the motor units’ activities of two basic muscles of respiration - the diaphragm and intercostal muscles. Surface electromyography is employed for this purpose for enlightening the neuromuscular activities in physiological circumstances like Eupnea, Tachypnea, and Bradynea. This study solely included young healthy female college students for learning the role of the motor units under physiological situations, not any pathological ones.

The higher body weight and fat content influence the reduction in lung volumes and this is more in males than women [22]. The high-fat content alters the thoracoabdominal kinematics in the supine posture, with an increase of the abdominal and a decreased rib cage contribution to ventilation, suggesting that in supine posture hypoventilation can occur in the lung. Without altering the alterations in the chest cavity lung volumes could not be increased or decreased. It can be linked to increased adiposity which disturbs the appropriate actions of respiratory muscles during the expansion and deflation of the thoracic cavity. The average fat percentage of young ladies is found very high (32.65±9.48%) and their BMI suggests (23.73±3.83 kg/m²) prevalence of overweight according to the WHO’s recommendation. Females have higher fat deposition in subcutaneous and hip regions which would not impede respiration.

Tachypnea created artificially by vigorous hyperventilation involves additional motor units for creating maximum contraction and thereby modifying the maximum capacity of the thoracic cavity. In Eupnea, the modest expansion in the chest cavity is related to the reduced involvement of motor units in respiratory muscles in supine posture. Bradynea due to breath-holding intermittently for a few seconds involves fewer motor unit actions than Eupnea.

In supine posture during Tachypnea, the diaphragm and intercostal muscles create a maximal force of contraction due to more motor units participating in the young female. Even in the supine posture, the increase in the thoracic cavity by the diaphragm and intercostal muscles is sufficient to include more motor unit recruitment, and hence the force of contraction increases. In Bradynea and Eupnea, no alterations in motor unit involvement of the diaphragm and intercostal muscle and thus no increase in muscle force have been detected showing changes in the thoracic cavity practically the same in supine posture. The elevated mean values of RMS and MVC of the intercostal muscle in Bradynea indicate during supine posture it might be involved.

CONCLUSION

This study is restricted to healthy young females in various physiological conditions like quiet breathing, hyperventilation, and breath-holding for finding out the involvement of the motor unit in two major muscles of respiration. To understand the neuromuscular activities in respiratory mechanics in physiological conditions and compare them with the clinical condition this study provides enormous information. It would be also helpful to identify the clinical disorders that happen in the respiratory muscles associated with respiratory diseases where the reduction of air inhalation and exhalation occur. Once the disabilities of respiratory muscles are known, the process of rectifications can be improved by improvising different respiratory exercises for those concerned muscles. Besides, motor unit discharging patterns can be properly analysed in the pathology of respiration. More research is required for emphasizing the involvement of motor units in physiological conditions for comparison with pathological situations.

Due to the small sample size of only n=30, one of the major limitations of this research is that it may not be possible to generalise its findings to a larger population. As a result of the study’s small sample size, it is possible that the results do not correctly reflect the true motor unit involvement of young, sedentary adult females during various breathing patterns in their diaphragm and intercostal muscles. The limited sample size may also make it more difficult to identify subtle but clinically important differences in the involvement of the motor unit in various breathing patterns. A small sample size may also reduce the study’s statistical power, increasing the risk of type II errors, which occur when differences between groups or conditions are overlooked due to inadequate power. The study’s internal validity may be impacted by bias and confounding, which may be more likely with only 30 subjects. The results of this research should therefore be interpreted cautiously due to the small sample size, even though it may offer some insights into the motor unit involvement of the diaphragm and intercostal muscles during breathing.

ORCID

Prognya Saha
https://orcid.org/0000-0002-2765-4595

Rajdeep Pathak
https://orcid.org/0000-0002-0704-4343

Priyam Chatterjee
https://orcid.org/0000-0002-8303-2395
Anupam Bandyopadhyay  
https://orcid.org/0000-0001-7678-1913

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