# Stabilizing function of the diaphragm: dynamic MRI and synchronized spirometric assessment

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## ABSTRACT

The aim was to describe diaphragmatic behavior during postural limb activities and examine the ventilatory and stabilizing functions of the diaphragm.

Thirty healthy subjects were examined in the supine position using a dynamic MRI system assessed simultaneously with specialized spirometric readings. The diaphragmatic excursions (DEs) were measured at 3 diaphragmatic points in the sagittal plane; the diaphragm positions (DPs) as related to a reference horizontal baseline were determined. Measurements were taken during tidal breathing (TB) and isometric flexion of upper or lower extremities against external resistance together with TB.

Mean DE in both upper and lower postural limb activities was greater compared to the TB condition (*P*'s<.05), with the effect greater for lower limb activities. Inspiratory DPs in the upper and lower extremity activities were lower compared to TB alone ($P<0.01$). Expiratory DP was lower only for lower extremity activities ($P<0.01$). DP was most affected at the apex of the crescent and crural (posterior) portion of the diaphragm. DEs correlated strongly with tidal volume ($V_T$) in all conditions. Changes in DEs relative to the initial value were minimal for upper and lower extremities, but were related to lower values of $V_T$ ($P<0.03$).

Significant involvement of the diaphragm in the limb postural activities was found. Resulting DEs and DPs differed from the TB conditions, especially in lower extremity activities. The differences between the percent changes of DEs versus $V_T$ found for lower extremity activities were confirmed by both ventilatory and postural diaphragm recruitment in response to postural demands.



## KEY WORDS

Diaphragm; Stabilizing function; Dynamic Magnetic Resonance Imaging; Lung function; Thorax

## INTRODUCTION

The stabilizing function of the diaphragm has been studied by several authors who have demonstrated that diaphragmatic activity can assist with mechanical stabilization of the trunk along with concurrent maintenance of ventilation (2, 7, 9, 13-15, 29-31). The diaphragm contributes to postural control during trunk stabilization (9, 10) and voluntary limb movement (11). The diaphragm and abdominal muscles together create a hydraulic effect in the abdominal cavity, which assists spinal stabilization (6, 22, 27) by stiffening the lumbar spine through increased intra-abdominal pressure (10). Therefore, poor coordination of the diaphragm and abdominal muscles may result in compromised stability and dysfunction of the lumbar spine (25). The stability of spine, shoulder girdle and pelvic girdle is established prior to execution of a postural task by a central mechanism of anticipatory postural adjustments (11), which occur independently from the respiratory activity of the diaphragm. Proper stabilization is critical for all dynamic activities ranging from simple functional tasks to skilled athletic maneuvers (32). Moreover, some studies suggest that co-activation between the diaphragm, abdominal muscles and pelvic floor musculature is necessary to create the sensory-motor control which is of great clinical importance and is often lacking in conditions such as vertebrogenic disorders (12, 22).

Since 1995 advanced MRI technology (8) has been utilized to gain a better understanding of dynamic diaphragm function, specifically the relationship between the ventilatory and postural tasks of the diaphragm (3-5,18,21-23, 27, 33, 35, 36). EMG (7, 9-11, 13, 15, 29, 30) and ultrasound imaging (17) have also provided significant data concerning the functional components necessary for optimal stabilization of the spine.

To our knowledge, previous studies have not clearly defined the stabilizing postural function of the diaphragm using dynamic MRI in combination with simultaneous spirometric recordings. Visualizing the diaphragm during tidal breathing alone and together with isometric contraction of upper and lower extremities (independent of respiration) can provide information concerning the diaphragmatic contributions to posture and respiration during different activities. Therefore, the purpose of this study was to perform a detailed analysis of normal diaphragmatic excursions in healthy subjects during postural loading created by isometric upper and lower limb contractions in the supine position. In addition, differentiating the respiratory from the postural functions of the diaphragm was conducted through measurement of simultaneous spirographic changes. Utilizing dynamic MRI and synchronized spirography simultaneously may contribute to a better understanding of the complex function of the diaphragm and its influence on spinal stability.

## MATERIAL AND METHODS

### SUBJECTS

Thirty healthy subjects participated in this study, 5 males (17%) and 25 females (83%), with a mean age of 29.3 (range: 22.2 – 56.2) years. The subjects did not have a history of pulmonary disease or any other chronic disease that would affect their respiratory function. Pulmonary function tests (PFT) performed were normal for all subjects: FEV1=105.3±9.8% predicted, FVC=110.0±12.1% predicted, FEV1/FVC=99.0±8.4% predicted. Average BMI of the subjects was 22.5±2.6 $kg.m^{-2}$.

### METHODS

This study was approved by the institutional ethical committee. All subjects were questioned to ensure that they met the inclusion criteria of the study. All testing procedures were thoroughly explained to the participants with a detailed description of the the dynamic MRI and spirometery assessments. All subjects reported that they understood the test procedures and gave informed consent.

All subjects were evaluated by dynamic MRI with simultaneous spirometric recordings. All subjects fasted at least 4 hours before each assessment procedure. Diaphragm activity, measured by movement of the diaphragm, was evaluated by dynamic MRI with subjects in the supine position with their heads supported 5cm above the MRI plinth. Volumetric changes during the breathing cycle were recorded with a specially designed spirometer and specialized computer software. The subjects wore noseclips to prevent any air exchanage through the nostrils. A mouthpiece connected to a pneumotachograph was placed in the subject's mouth and the subjects were allowed to practice normal breathing through the mouthpiece. After the subjects were trained in normal breathing with the mouthpiece for 2 minutes, measurements were taken during tidal breathing (TB) at rest and again with isometric limb contractions of the upper and lower extremities. To ensure consistency during the testing procedures, the same physiotherapist performed all assessments. Data collection time was 20 seconds in each condition per subject to record standard MRI measurements together with the spirometric readings.

Diaphragm activity was assessed under the following conditions:

1. Tidal breathing (TB): The subject was in the supine position with the extremities relaxed along the torso. The subject was instructed to breathe normally. After the initial synchronization between spirometric and MRI recordings (see below), simultaneous synchronized spirometric and MRI recordings were taken.

2. Isometric flexion of upper extremities (UE): The starting postion of the subject was supine with arms and legs relaxed with their arms resting along the torso. The subject was instructed to continue to breathe normally thoughout the assessment. The physiotherapist placed their hands on the dorsal surface of the subject‘s forearms while the subject’s arms remained at rest. The subject was then instructed to keep their elbows strainght and push with both arms upward against therapist´s resistance applied distally on the subject’s forearms performing an isometric contraction. The force production generated by the subjects corresponded to a grade 4 manual muscle test (19).Measurements of diaphgram movement and spirometry readings were recorded throughout the 20 second data collection period.

3. Isometric flexion of lower extremities (LE): The starting postion of the subject was supine with arms and legs relaxed with their arms resting along the torso. The subject was instructed to continue to breathe normally thoughout the assessment. The physiotherapist placed their hands on the anterior surface of the subject’s thighs while the subject remained at rest. The subject was instructed to push upward with both lower extremities against the therapist´s resistance applied on the anterior aspect of the subject’s thighs performing an isometric contraction. The force production generated by the subjects corresponded to a grade 4 manual muscle test (19). Measurements of diaphgram movement and spirometry readings were recorded throughout the 20 seconds data collection period.

**MRI assessments**

MRI scans were conducted in an open 0.23 T Siemens MRI scanner and processed with software version NUMARIS/4 syngo MR 2004A. The diaphragm was imaged in the sagittal plane with the subjects supine using a body coil – size L. The imaging plane was placed sagittally in the axial topogram directed paravertebrally to the right, mid-way between the vertebral body center and the edge of the thoracic wall. Slice thickness was 33 mm. The sequence was configured as follows; 1NSA (number of scan acquisitions), image matrix was 240 x 256 pixels, TR = 4.48 ms, TE = 2.24 ms, FA = 90°, TSE 1, FOV = 328 mm. Sequence duration was 20 seconds, with 77 images acquired at regular intervals; one image every 260 ms. Each subject had 4 markers (10 ml syringes of water) affixed to the skin surface and placed as follows:

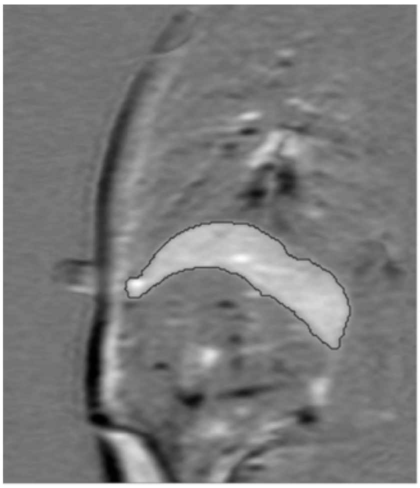
1. mid-clavicular line at the level of the jugular opening
2. inferior ventral costal margin, mid clavicular line
3. umbilicus
4. thoracolumbar junction in the dorsal axillar line.

**MRI Analysis of Diaphragm Movement**

The MR image files were converted to Analyze format with MRIcro software. In each 20 sec sequence, for tidal breathing and postural activity conditions, the baseline position of the diaphragm was determined. The most caudal baseline position of the diaphragm was subtracted from the position of the other images in the sequence to determine the position changes of the diaphragm throughout the 20-second collection period. Fig. 1a demonstrates the "crescent" shaped image of diaphragm excursion (DE) contrasting the most caudal and cranial diaphragm positions (DP) during tidal breathing.

The DE images were converted to binary images to calculate its area in pixels. The bottom edge of the DE represents the most caudal baseline DP during inspiration. The top edge of the DE represents the diaphragm in its most cranial position during expiration. Successive images with the next highest pixel count were analyzed in order as the excursion of the diaphragm changed during the breathing cycle.

The next analysis was completed on the subtracted maximal "crescent" area of each image where the horizontal, anterior-to-posterior (AP) alignment was calculated between the front and back markers placed on each subject's body (Fig. 1b the total AP distance was linked with the dotted line from point A to point E). The total horizontal distance was divided into six equal sections, demarcating five equidistant points with C marking the mid point of the line from points A to E (Fig 1b). The upper and lower edges of DE were determined at each of the three points B, C and D. The distance at each point from the horizontal baseline was calculated to determine the difference in inspiratory position compared to the expiratory position of the diaphragm in mm ($B_{1;}$ $B_2$ and $C_{1;}$ $C_{2\ e}$ etc., respectively - see Fig. 1b). For statistical analysis on the acquired data, see the section on Statistical Analysis.



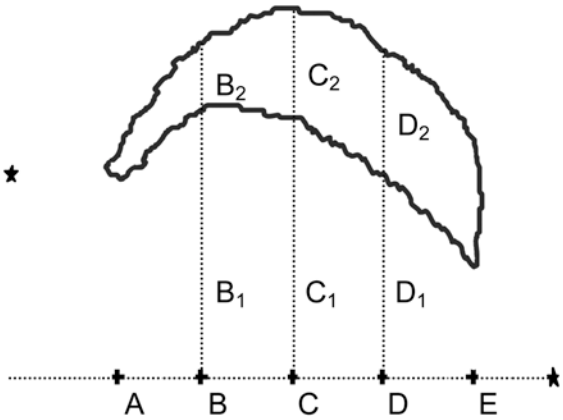



Fig.1a Fig.1b

**Fig. 1a**
Subtracted image of the diaphragm excursions (DEs) in its most caudal (inspiratory) and cranial (expiratory) diaphragm positions (DP) during tidal breathing.

**Fig. 1b**
Schematic description of three diaphragmatic points (B, C and D) used for DE calculations. The following 6 distances [mm] were obtained by measuring the distance between the horizontal baseline in both expiratory and inspiratory DP. DE points: B1 - D1 points were derived from the inspiratory DP obtained from MRI images; B2 - D2 points were derived from expiratory DP obtained from respective MRI images. The inspiratory DP is designated by points B1, C1 and D1. The expiratory DP diaphragm is designated by points B1 + B2; C1 + C2 etc., Total DE is designated by B2, C2 etc.

**Synchronized spirometric recording**

Spirometric measurements were obtained using the MasterScope Jaeger spirometer (version 4.67, Jaeger, VIASYS, Wuerzburg, Germany). Tidal volumes were recorded by a specially designed pneumotachograph with a plastic isoresistive membrane (Jaeger pneumotach, with guaranteed linearity of flow from 0.2 to 12 $l.s^{-1}$). The isoresistive membrane allows for precise two-way measurements of airflow throughout the breathing cycle. The trans-membrane pressure changes that occur during breathing were introduced into the spirometer by two 230 cm-long teflon tubes (i.d. 1.3mm) with very low compliance. This allowed safe and reliable spirometric recording while in a strong magnetic field. A specialized reading and recording BreathRecorder software (J. Volejník, Kurka-Jaeger Servis, Ltd., Czech Republic), was developed for the purposes of this study. The flow signal measuring trace volume was converted and digitally integrated using an AD converter and saved on hard disk. Prior to spirometric measurement every subject was familiarized to the mouthpiece in a supine position for a 2-min period; no recordings were performed during that time. The recording system was calibrated to each subject using a 1-liter calibration pump prior to data collection.

**Processing of synchronized spirometric recordings**

The spirometric data was processed using Software Grapher (J. Volejník, Kurka-Jaeger Servis, Ltd., Czech Republic). From the 20 seconds of recorded data in each condition, 4 to 7 respiratory cycles were used to calculate the tidal volume ($V_T$). Correction for the body surface area (BSA) was made in the adjusted $V_T$ measurements. The correlation between VT and DEs was then calculated.

**Synchronization of spirometric recordings and MRI sequence**

The spirometric recordings were synchronized at the beginning of the 20-second MRI sequence within the initial 200-300 msec by an electronic marker imprinted simultaneously on both recordings. The individually marked spirometric recordings were converted to DICOM format and synchronized with the dynamic MRI sequence of diaphragm movement images. The synchronized progression of the trace volume-time spirometric curve and the corresponding diaphragm movement were monitored using DICOM Scanview software.

**Pulmonary function tests**

Spirometric recordings of pulmonary function tests (PFTs) were performed on the same day for all subjects with a MasterScope Jaeger spirometer (version 4.5, Jaeger, VIASYS, Wuerzburg, Germany) with a special module for the assessment of repiratory muscles. All subjects were properly instructed and coached by an experienced technician during all PFTs. Proper procedures for quality assurance based on the criteria of the American Thoracic Society (1) were used for these measurements. The following PFT parameters were measured: FEV1, FVC, FEV1/FVC. PFT results are presented as percentages of the reference values.

**Statistical analysis**

The following statistical analysis was performed using Commercial software SPSS, ver. 15 (SPSS Inc. Headquarters, Chicago, IL, USA): A general linear model with repeated meausures was used with absolute inspiratory or expiratory positions of diaphragm as dependent variables.

Two within subjects factors were considered

Factor 1: Condition 3 levels: TB, UE, LE

Factor 2: Point 3 levels: B,C,D

F test with Greenhouse-Geisser correction for lack of sphericity for tests of within-subjects effects and subsequently conventional tests of specific within-subjects contrasts were done. Furthermore Kolmogorov-Smirnov test for normality, Paired t-test and assessment of Pearson corelation coefficient for DEs derived from MRI and tidal volumes were used. Two-tailed P value of less than .017 was considered significant for tests of three coefficients based on the Bonferroni correction in which the P value of .05 was divided by the number of tests.

## RESULTS

### A. Diaphragmatic excursions (DEs)

DE measurements during tidal breathing (TB) with simultaneous postural activity of upper extremities (UE condition) were larger compared to the DE excusions during TB alone without postural activity. The mean±SD in the UE condition was 5270±1935 $mm^2$ vs. 4487±1485 $mm^2$ for the TB condition ($P<0.01$). DEs during the LE condition were also greater compared to the TB condition, 5373±2593 $mm^2$ vs. 4487±1485 $mm^2$ ($P<0.02$) (Fig. 2, Fig. 3).

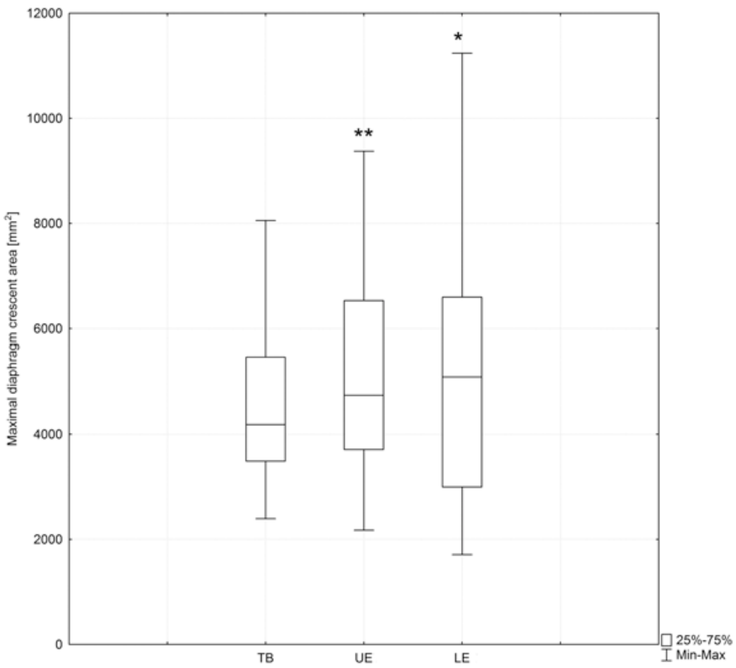



**Fig.2**
Diaphragm excursions in three different conditions
Mean values of DEs in 30 healthy subjects are shown; DEs measured during tidal breathing with simultaneous postural activity of upper extremities (UE condition) is more prominent compared to TB condition without postural activity (TB), i.e., 5270±1935 (mean±SD) $mm^2$ versus 4487±1485 $mm^2$ ($P<0.01$). DEs during tidal breathing with simultaneous postural activity of lower extremities (LE) is also higher compared to TB condition, i.e., 5373±2593 (mean±SD) versus 4487±1485 $mm^2$ ($P<0.02$).
* P<0.02 vs. TB
** P<0.01 vs. TB

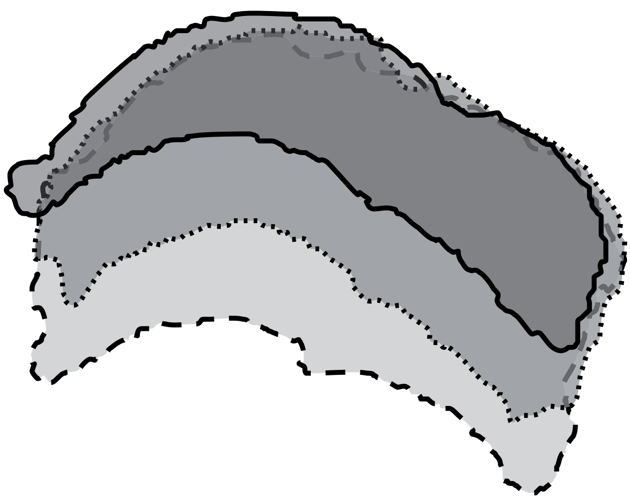


**Fig.3**

Inspiratory and expiratory DP are presented for a representative subject during the following conditions: the solid outline represents TB; the dotted outlines represent the UE condition and the dashed outline represents the LE condition. Diaphragm excursions during UE and LE are greater compared to TB.

**B. Diaphragm positions (DP)**

**Inspiratory diaphragm position**

We have demonstrated a significant difference in the inspiratory position of the diaphragm between TB alone vs both the UE and the LE conditions (see Fig. 4 and Tab. 1). We have also found significant differences between the UE and LE conditions comparing TB during UE contractions and TB during LE contractions among points B vs C, C vs D as well as B vs D (see Tab. 2).

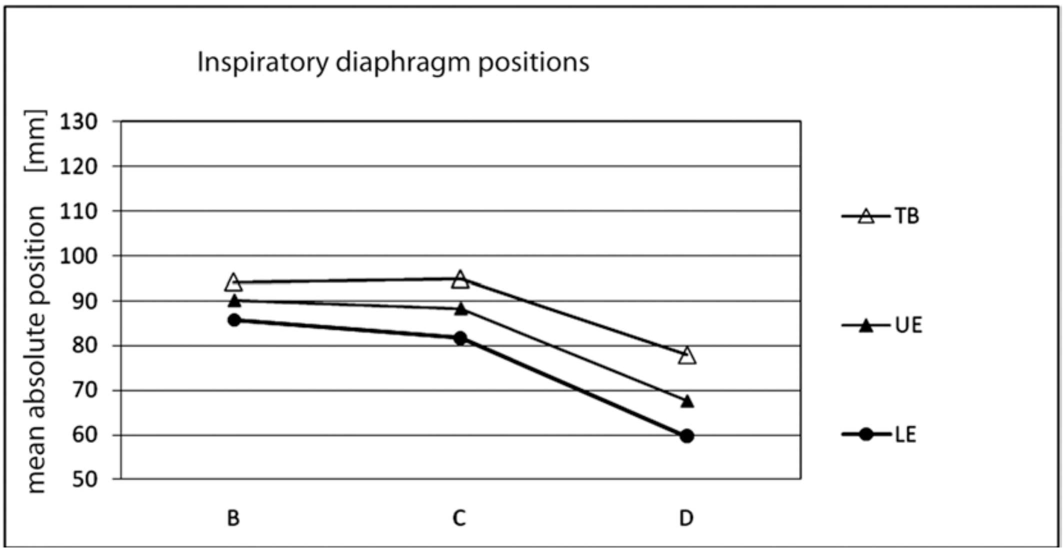



**Fig. 4**

Comparison of inspiratory DP during the following conditions: The upper solid curve (with open triangles) represents the inspiratory DP during tidal breathing (TB); the middle solid curve (with full triangles) represents inspiratory DP during UE condition; the lower solid curve (with full circles) represents inspiratory DP during LE condition.

| | Inspiratory position of diaphragm | | | | | |
|---|---|---|---|---|---|---|
| | TB | UE | LE | UE-TB | LE-TB | LE-UE |
| B | 94.15 | 90.14 | 85.71 | 4.01 | 8.44 | 4.43 |
| C | 94.81 | 88.30 | 81.70 | 6.51 | 13.11 | 6.60 |
| D | 77.93 | 67.61 | 59.73 | 10.33 | 18.20 | 7.87 |
| | | | P < | 0.005 | 0.0005 | 0.02 |

**Tab. 1**
Comparison of inspiratory DP (points B,C and D) during TB, UE and LE conditions and related differences among positions.

| conditions | comparisons between points | P < |
|---|---|---|
| UE vs. TB | C vs. B | 0.01 |
| | D vs. B | 0.002 |
| | D vs. C | 0.002 |
| LE vs. TB | C vs. B | 0.003 |
| | D vs. B | 0.002 |
| | D vs. C | 0.003 |
| LE vs. UE | C vs. B | NS |
| | D vs. B | NS |
| | D vs. C | NS |

**Tab. 2**
Detailed comparisons of contributions of particular points B, C and D to the diaphragm position (for details – see Methods)

**Expiratory diaphragm position**

We did not find a significant difference in the expriatory diaphragm position between TB alone and the UE condition among points B, C or D (Tab. 3). However, we did find a significant difference in the expiratory position of the diaphragm between TB alone and the LE condition among points B, C and D. Marginal differences between the LE condition and the UE condition were also found among points B vs C and B vs D (see Fig. 5, Tab. 3) while no difference was found for C vs D (see Fig. 5, Tab. 3, Tab. 4).

| | Expiratory position of diaphragm | | | | | |
|---|---|---|---|---|---|---|
| | TB | UE | LE | UE-TB | LE-TB | LE-UE |
| B | 119.14 | 119.80 | 116.54 | -0.66 | 2.59 | 3.25 |
| C | 127.76 | 127.57 | 122.06 | 0.19 | 5.70 | 5.51 |
| D | 118.19 | 116.16 | 108.99 | 2.03 | 9.19 | 7.17 |
| | | | P < | NS | 0.01 | 0.005 |

**Tab. 3**
Comparison of expiratory DP (points B,C and D) during TB, UE and LE conditions and related differences among positions.

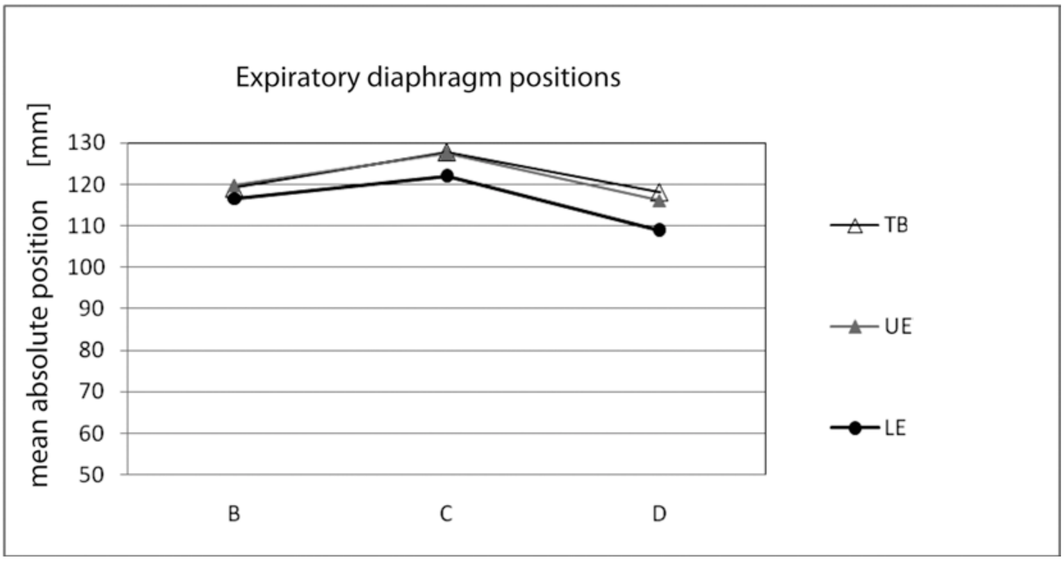



**Fig. 5**
Comparison of expiratory DP during the following conditions: The upper solid curve (with open triangles) represents expiratory DP during tidal breathing (TB); the middle solid curve (with full triangles) represents expiratory DP during UE condition; the lower solid curve (with full circles) represents expiratory DP during LE condition.

| conditions | comparisons between points | P < |
|---|---|---|
| UE vs. TB | C vs. B | NS |
| | D vs. B | NS |
| | D vs. C | NS |
| LE vs. TB | C vs. B | 0.002 |
| | D vs. B | 0.002 |
| | D vs. C | 0.01 |
| LE vs. UE | C vs. B | 0.05 |
| | D vs. B | 0.03 |
| | D vs. C | NS |

**Tab. 4**
Detailed comparisons of contributions of particular points B, C and D to the DP (for details – see Methods)

**C. Relationships between DEs and tidal volumes**

We did not find differences between DEs and tidal volumes in TB alone as well as the UE conditions. However, we found significantly lower values of tidal volume compared to DEs in the LE conditions (see Fig. 6 and Fig.7).

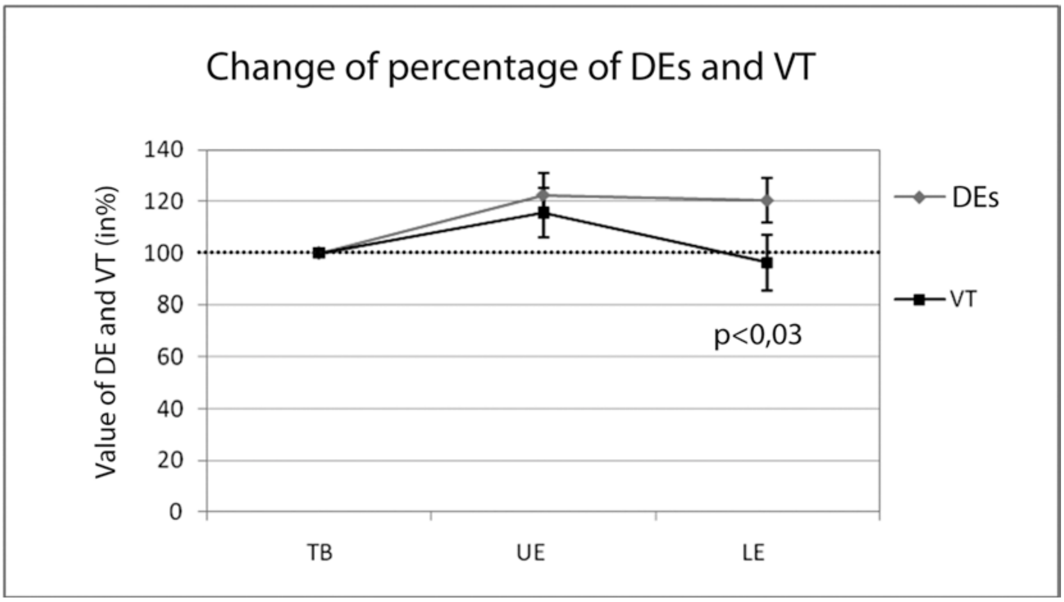



**Fig. 6**
Increase in percentage of DEs and tidal volumes in three following conditions: TB, UE and LE. While both parameters change insignificantly in the UE condition, both parameters differ significantly in the LE condition.

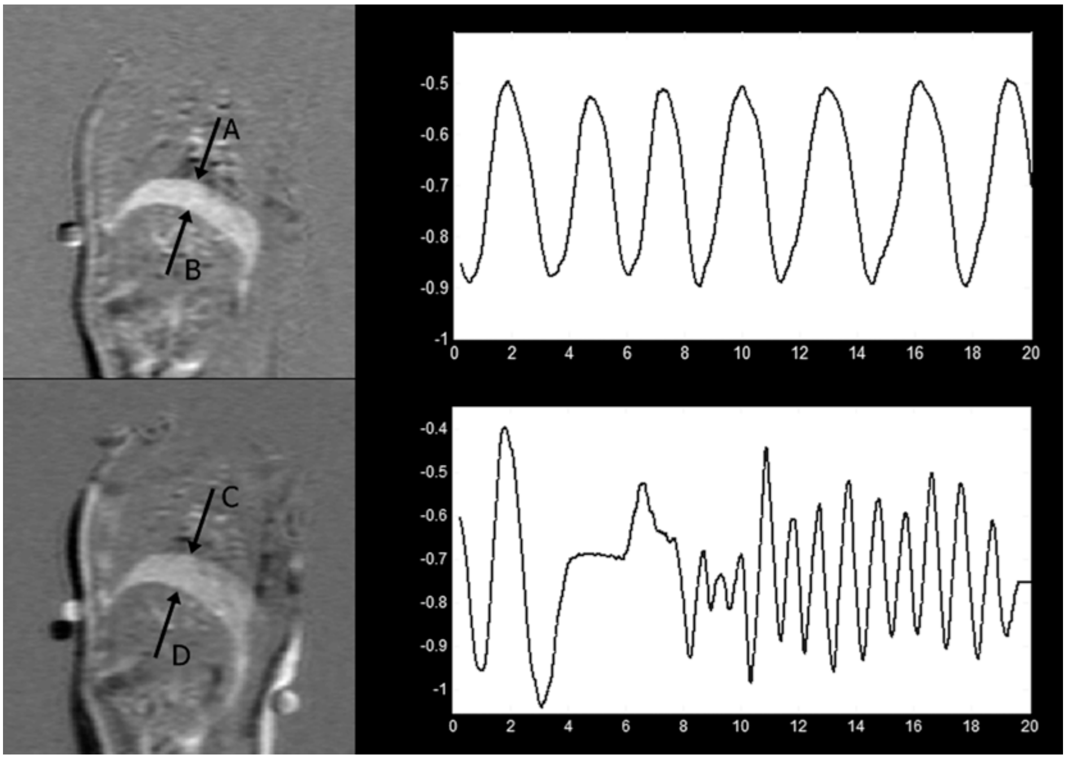



**Fig. 7**
Comparisons of DEs and spirographic records (tidal volumes) in TB (upper graph) and LE conditions (lower graph) in a representative subject.

**Correlations between DEs and tidal volumes**

The following correlations among DEs [$mm^2$] (derived from MRI) and spirometric values [mL] were found (see Tab. 5).

| condition | | r | P < |
|---|---|---|---|
| TB | DE/BSA vs. VT/BSA | 0.61 | 0.001 |
| UE | DE/BSA vs. VT/BSA | 0.58 | 0.001 |
| LE | DE/BSA vs. VT/BSA | 0.57 | 0.001 |

**Tab. 5**
Correlations among DEs corrected for body surface area, BSA and tidal volumes corrected for BSA (for details – see Methods).

**DISCUSSION**

The role of the abdominal muscles and the diaphragm in trunk stabilization has been under investigation for more than 50 years. However, the specific role of the diaphragm still remains poorly understood. As early as 1951, Wade and Gilson obtained dynamic imaging of diaphragmatic excursions under fluoroscopy simultaneously with spirometry readings. They initially concluded that the resting level of the diaphragm and the pattern of its movement must have been determined by the pressure differences between the abdominal and thoracic cavities (37).

Since 1997, when Hodges and colleagues (9) pointed to the importance of the postural function of the diaphragm, many authors have focused on the non-ventilatory contributions of the diaphragm. Our study involved a comprehensive analysis of diaphragmatic function in order to provide findings with possible application to a variety of clinical conditions, such as subjects with severe vertebrogenic disorders (12, 22).

In the present paper, we found that the diaphragmatic excursions (DE) in postural upper and lower limb activities enlarged significantly, and that the changes appeared to occur simultaneously in the upper and lower extremities, although the changes seemed more pronounced the lower extremities (LE). These enlargements appear to be caused primarily by the decrease of the inspiratory diaphragm position, although changes of expiratory position in LE conditions also seem to contribute to the DE enlargement. An additional observation is that the diaphragm does not function as one cohesive unit, in which the entire diaphragm responds to ventilatory and postural demands equally. The area of the diaphragm where the most significant, experimentally induced changes in position occurred (i.e., those elicited during UE and LE maneuvers) is the apex (point C), representing the middle part of the diaphragm and crural or posterior portion (point D) of the diaphragm. It appears that individual sections of the diaphragm contribute differently to postural function based on the non-uniform changes seen at the designated points of the diaphragm. Finally, we have demonstrated that changes in DEs and tidal volumes (corrected for body surface area, $V_T$/BSA) are well correlated all three experimental conditions used in this study. Surprisingly, only the LE condition revealed a significant difference between percentage increase of DEs and $V_T$ (see Fig. 6).

Despite the contribution of especially Hodges´s group (7,9-15,29,30) to the advancement of understanding the postural (complex) role of the diaphragm has been invaluable the significant enlargement of the DEs in postural limb activities proved directly (by dynamic MRI) has not been previously reported. Other authors such as McKeough and co-workers (26) have also demonstrated the direct relationship between extremity activity and diaphragm function. McKeough reported that shoulder flexion in healthy adult subjects affects static lung volumes; they proved significant increase in functional residual capacity with increased shoulder flexion. The discrepancy between UE and LE condition described in the present paper probably reflects the condition, in which upper vs. lower extremities were recruited. Isometric flexing of the arms in the UE condition may present a barrier for performing complex and coordinated thoracic movements.

We found that for postural function, individual sections of the diaphragm are involved differently (non-uniformly), i.e. the most prominent changes of diaphragm position induced during UE and LE maneuvers are at the apex (point C) and the posterior (crural) part (point D) of the diaphragm (Fig. 4, Fig. 5). The observed contributions of particular points (B, C and D) to the resultant diaphragm motion (Tables 2 and 4) provide additional, most statistically stringent support for this proposition. The theory of non-uniform recruitment of costal and crural portions of the diaphragm (24) has been previously investigated and re-confirmed (8, 23, 33, 34). The idea that the diaphragm is a functionally dual system where costal and crural portions function mechanically in serial mode, but ventilatory (pneumatically) in parallel mode is confirmed in this study, too.

On the contrary, its is problematic to evidence if a diaphragm position *per se* might be argued as a measure for stabilizing function of diaphragm without diaphragm EMG and/or transdiaphragmatic pressure was measured. We already previously measured active diaphragm contractions (by dynamic MRI) during tidal breathing vs. Valsalva maneuver simultaneously with EMG and spiroghraphic assessments (22); we proved that resultant diaphragm motions are caused by its active contraction.

Another point of discussion is the hydraulic effect created by the diaphragm and abdominal muscles that may assist in spinal stabilization. This concept has been repeatedly studied (6, 20, 22, 27) focusing on the co-activation and sensory-motor control between the diaphragm, abdominal muscles and pelvic floor muscles, which is of great clinical importance. Recently, the central coordination of the diaphragm and abdominal muscles was experimentally verified (28) demonstrating that the activation of the diaphragm and some abdominal muscles is centrally mediated during stabilization of the trunk during respiration and postural activity. Elevated intra-abdominal pressure via contraction of the diaphragm substantially contributes to the stiffness and stability of the spine (10, 14, 27). On the contrary, limb function may be compromised as both respiratory and postural demands are placed on the diaphragm and other muscles involved during limb movement (12).

We found a significant difference between the percent increase of DEs and of $V_T$ where the changes in DEs vs. $V_T$ were reciprocal, a finding that we consider central in this study (see Fig.7). Other studies that examined respiratory function have provided pertinent data concerning lung function without considering diaphragm function related to posture. While

Iwasawa and co-workers measured only respiratory rate (18), Kondo (23) measured ventilation just during deep breathing with a pneumotachometer and an adapted differential pressure transducer. Also Chu´s group (3) measured lung volumes with MRI using a semi-automated computerized method for delineating the lungs and summing cross-sectional areas. We believe spirometric recordings in this study and also in our recent paper (22) obtained satisfactory data of volume-time parameters. Our methodological approach introduces new information for objectifying postural activity of the diaphragm seen in the significant differences between the postural demands of UE and LE conditions. We feel it is highly probable that differences in the order and location of diaphragm recruitment, in each of these conditions, is due to the postural requirements of UE and LE function.

Respiration plays a significant role in postural control, however, the postural demands of the activity performed can influence the function of the diaphragm. Consequently, sitting requires less instantaneous activation of postural mechanisms compared to standing (2) possibly due to exclusion of specific muscle groups needed for upright posture. Therefore, postural loading of the LEs most likely requires greater input from the postural mechanisms than the UE (11, 14). Therefore, postural activation during LE conditions, compared to tidal breathing, elicited not only diaphragmatic contractions, but also positional changes of lowering in both the inspiratory and expiratory position of diaphragm regardless of tidal volume. Although the expiratory position of diaphragm in the LE condition does not reach the expiratory position in TB condition, the diaphragm during the LE maneuver does not relax fully and remains in higher tonic state of activity. We believe, this may confirm that the diaphragm is critically involved in stabilizing the spine during postural activity. This is in agreement with previous report, in which was proven that the diaphragm and transversus abdominis (but not other abdominal muscles) continuously contribute to respiration and postural control. The combined tonic and phasic activity of these muscles represents important feedback for central nervous system to coordinate respiration and control of the spine during limb movements (11).

There are several limitations to this study. *First*, ideally, the entire rib cage including the whole range of DEs should be imaged. Due to the limited size of FOV (34) an isolated analysis of diaphragm was performed focusing on excursion. We agree DEs alone are not sufficient to understand all mechanical actions of the rib cage and related musculature including the diaphragm in terms of the multibody mechanical system theory (16). We have also limited the DE measurements to 3 points, which is similar to other authors and was sufficient for our study (34). We did not replicate the finding of Gierada's (8) proposed "the saddle shape" of the diaphragm during inspiration; nor did we detect "an upper rib cage paradox” demonstrated by coordinated contraction of the upper rib cage and diaphragm muscles (4).

*Second*, while the instructions for carrying out “an isometric flexion of extremities against therapist’s resistance” were identical for each subject, each participant has a different subjective ability to balance external pressure and/or resistance. Regardless of the space limitations with the subjects´ supine posture on the MRI floor, the individualized and properly balanced external pressure performed by the same therapist was sufficient to ensure that each subject received the same amount and direction of force. We followed standardized requirements of current MRI methodology to reduce variation in our future studies on diaphragm motion and function (3).

*Third*, the information about laterality of the diaphragm motion is equivocal. Similar to Suga (33) we could not detect the previously reported finding of asymmetric excursions between hemidiaphragms (8, 34, 36).

*Fourth*, for final assessment of diaphragm motion we cannot exclude the effect of an intra-abdominal mass, especially in cases of central obesity. To ensure that the population of subjects was as uniform as possible, the mean BMI of our study subjects fell within the normal range.

*Fifth*, we cannot ignore the possibility of rib cage distortion during the experimental conditions. Macklem´s group, using a three-compartment chest wall model in 5 normal men during tidal breathing (20), first notified a passive expiratory action of abdominal-apposed rib cage compartment on the diaphragm. Despite testing their subjects in a seated position, the passive stretch of the abdominal muscles most likely exceeded the insertional transdiaphragmatic pressure. This suggests that passive stretching of the abdominal muscles is important in the prevention of rib cage distortion during tidal breathing (20). We did not consider the changes in rib cage geometry that may have resulted from the passive stretch of the abdominal muscles.

Future studies should, *first*, examine the relationships between the diaphragm, abdominal and pelvic floor muscles, regarding their functional and neurosmuscular co-activation, especially with respect to their stabilizing function of the spine (11, 22). *Second*, investigate the relationships between diaphragm contraction, intra-abdominal pressure and limb contraction both in supine, upright as well as sidelying positions. *Third*, investigate whether the rate of flattening and diaphragmatic contour during postural activity is different in patients who have objective clinical findings indicating non-physiological overload of the spine, e.g., due to dysfunctional muscle coordination. *Fourth,* investigate relationships between diaphragm contraction shapes (i.e., quantity and/or quality) during postural activities in subjects with severe vertebrogenic disorders.

## CONCLUSION

A dynamic MRI study with synchronous ventilation measurement during postural upper and lower limb activities provided more detailed information on diaphragmatic motion. Inspiratory diaphragm position (DP) in both isometric postural limb activities is significantly lower compared to that of tidal breathing. Expiratory DP reached significantly lower level only if the lower extremities are recruited. The apex and crural regions of the diaphragm predominantly contribute to the resultant diaphragm position. Comparisons between percent change of diaphragmatic excursions and tidal volumes in both isometric limb activities might contribute to the concept of the postural function of the diaphragm. These findings might have great significance for assessment of diaphragm behavior in clinical situations such as vertebrogenic disorders.

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