Respiratory Physiology & Neurobiology 260 (2019) 58–62

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

# Reproducibility of diaphragmatic thickness measured by M-mode ultrasonography in healthy volunteers

Simone Scarlata*, Damiana Mancini, Alice Laudisio, Antonelli Incalzi Raffaele

*Geriatrics, Unit of Respiratory Pathophysiology and Thoracic Endoscopy, Campus Bio Medico University and Teaching Hospital, Rome, Italy*

ARTICLE INFO

*Keywords:*
Diaphragm ultrasound
Diaphragmatic thickness
Reproducibility

ABSTRACT

We aimed at testing M-mode ultrasound diaphragmatic thickness reproducibility and its clinical correlates in healthy volunteers.

Sixty-six consecutive healthy volunteers were considered eligible, and enrolled in the study. During a single visit, all participants received three M-mode and B-mode ultrasound evaluations of Diaphragmatic Thickness (DT). We then proceeded to calculate the thickness difference and assess the association of ultrasonographic measurements with demographic and anthropometric data. Variables associated through univariate analyses were entered in multivariable models, and Intraclass Correlation Coefficient (ICC) was performed in order to determine intra- and inter-observer reproducibility.

Intra- and inter-observer agreements showed to be excellent through Cronbach's Alpha, ranging from 0.81 - 0.91 and 0.86 - 0.92, respectively. Mean diaphragmatic thickness measurements were: 2.6 ( ± 0.5) mm at inhalation and 1.8 ( ± 0.4) mm at exhalation. The results we obtained significantly varied according to gender, showing diaphragmatic motion, inspiratory/expiratory thickness and fractional thickening to be significantly lower in women. Moreover, a significantly reduced expiratory diaphragmatic thickness emerged in the subgroup of subjects having a sedentary work ($p = 0.045$). The crude association between expiratory thickness and active work produced a B coefficient of 0.19 (95% CI: 0.04-0.38; $p = 0.045$), which was confirmed after adjustments considering age and sex ($B = 0.20$; 95% CI: 0.01- 0.39; $P = 0.037$).

Diaphragmatic thickness measurements using M-mode are reproducible. Intra and inter-observer agreement is high enough to support the precision of this measurement and provide a further analytic tool for a wider application in clinical practice.

## 1. Introduction

Ultrasonographic diaphragm evaluation has recently gained popularity in clinical practice, especially in critical care environments and in respiratory rehabilitation units, where ultrasound has been used to evaluate Diaphragmatic Thickness (DT) in the zone of apposition. In this area, the diaphragm is seen as a structure made of 3 distinct layers including a non-echogenic central layer bordered by 2 echogenic layers, the peritoneum and the diaphragmatic pleurae (Ayoub et al., 1997).

DT at rest is generally assumed as an index of respiratory muscle mass, which decreases, for example, in case of muscle waste or sarcopenia following chronic or degenerative pathologic conditions or simply in association with senescence (De Bruin et al., 1997; Buchman et al., 2009). Furthermore, at the end of a deep inhalation (total lung capacity, TLC), and together with the difference (thickening difference, TD) between resting thickness and maximal apnoea inspiratory thickness, DT is often used as a surrogate of diaphragm strength and endurance (ATS/ERS statement on respiratory muscle testing, 2002). In clinical practice, DT has shown to decrease rapidly within a few days of mechanical ventilation in about 40% of the patients (Levine et al., 2008; Doorduin et al., 2013). DT measured during resting tidal breathing for a trial of spontaneous breathing has recently shown to predict the success of extubation (DiNino et al., 2014). Another study found that DT predicted the success of a spontaneous breathing trial (Ferrari et al., 2014) These findings suggest that ultrasound can provide very useful information for the management of critically ill patients (Goligher et al., 2015; Lerolle and Diehl, 2011).

However, it still remains unclear whether the routine use of US can significantly impact clinical outcomes of acute respiratory failure (Sferrazza Papa et al., 2016).

* Corresponding author at: Geriatrics- Unit of Respiratory Pathophysiology and Thoracic Endoscopy, Campus Bio Medico University and Teaching Hospital, Via Alvaro del Portillo 200, 00128, Rome, Italy.

*E-mail address:* s.scarlata@unicampus.it (S. Scarlata).

https://doi.org/10.1016/j.resp.2018.12.004
Received 24 November 2018; Accepted 13 December 2018
Available online 13 December 2018
1569-9048/ 

Despite this intriguing evidence, we still lack data with regard to US reproducibility in determining DT. While the reproducibility of the diaphragmatic motion has been recently validated (Scarlata et al., 2018), its measure has been basically tested in ICU unit subjects already suffering from diaphragmatic dysfunctions (Goligher et al., 2015). We are aware of two validation studies reporting normal values for both DT and TD(Vishwanath et al., 2016; Carrillo-Esper et al., 2016). Unfortunately, both of these studies performed a B-mode diaphragm measurement instead of using the recommended, and more precise, M-mode approach (Lerolle and Diehl, 2011).

On these bases, we therefore aimed at testing M-mode assessed ultrasound DT and TD reproducibility with their clinical correlates on a sample of healthy volunteers.

## 2. Materials and methods

Sixty-six consecutive healthy volunteers were considered eligible, and enrolled in the study. The local Ethic Committee approved the study protocol (Prot. 30/17. PAR ComEt CBM) and all subjects signed a written informed consent. We then obtained a clinical trial registration number (ClinicalTrials.gov Identifier: NCT02801058).

Eligibility criteria included: asymptomatic, healthy adults; absence of chronic pain or acute symptomatology during the last 72 h before the visit; no diagnosed pathology.

Exclusion criteria were as follows: pregnancy; breastfeeding; diagnosis of pathological conditions; chronic drug treatment; medical history of abdominal surgery.

We then proceeded to collect a comprehensive clinical history from the subjects and performed physical examinations in order to identify any active or relevant clinical conditions. When available, we also reviewed blood analysis and medical records.

### 2.1. Ultrasound assessment of diaphragm

During a single visit, all participants received three DT ultrasound evaluations (Exagyne - Echo Control Medical- ECM, Angoulême - France). Subjects were examined in the recumbent position. A linear probe was placed on the line between the eighth and ninth intercostal spaces, midway between the antero- and mid-axillary lines, according to suggested recommendations (Sferrazza Papa et al., 2016) The right side appositional area can be visualized 0.5–2 cm below the phrenicocostal sinus (Fig. 1). The diaphragm appears as a three-layered structure immediately below the chest wall (Boon et al., 2013) and consists of a non-echogenic muscular layer bound by peritoneum and pleura echogenic membranes (Ueki et al., 1995). We referred to the diaphragm as the most superficial structure obliterated by the lung edge during inhalation. Through M-mode, we measured diaphragmatic thickness from the middle of the pleural line to the middle of the peritoneal line, at FRC and TLC. TD was defined as the difference in the observed thickness between inhalation and exhalation. The change in diaphragmatic thickness was expressed as the diaphragmatic ratio (DR) between in and expiratory thicknesses (Ueki et al., 1995).

To determine intra-observer variability, each volunteer was examined three times by the same operator, systematically taking three consecutive M-mode and B-mode measurements of DT, TD and DR on the right side. A second operator then took a further DT measurement on the same day.

### 2.2. Statistical analysis

We used SPSS software (version 23.0; SPSS Inc, Chicago, IL) to carry out statistical analyses.

All data are expressed as means ± standard deviations (SD) or 95% confidence intervals (CI) or median and inter-quartile range for continuous variables, or as percentages for categorical variables. Data distribution was analyzed using a Kolmogorov-Smirnov test. Differences between men and women were assessed through either Student's *t*-test or Mann-Whitney U test, as appropriate. To determine the correlation between variables, we adopted the Spearman analysis. Differences were considered significant at $p < 0.05$.

We used linear regression analysis to outline the association of ultrasonographic measurements with demographic and anthropometric data. Variables associated through univariate analyses were entered in multivariable models, while abnormally distributed variables were analyzed after log-transformation. Intra-class correlation coefficient (ICC) was obtained for the assessment of intra-rater reproducibility (Bland and Altman, 1986).

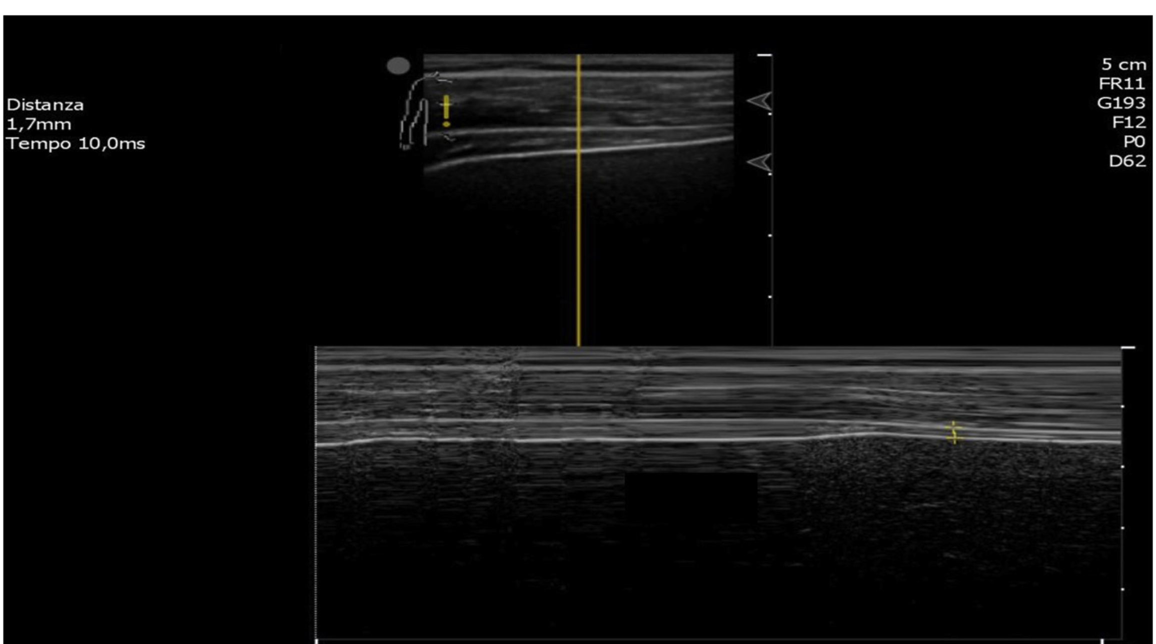


**Fig. 1.** B-mode (upper panel) and M-mode (lower panel) ultrasound view of the diaphragm.

**Table 1**
Characteristics of the study population.

| | N = 66 (%), mean (SD) or median (IQR) |
|---|---|
| Age (years) | 40 (15) |
| Sex (female) | 36 (54) |
| Weight (Kg) | 71 (12) |
| • Men | 72 (14) |
| • Women | 70 (11) |
| Height (cm) | 170 (10) |
| • Men | 171 (10) |
| • Women | 170 (9) |
| Body Mass Index (Kg/h$^2$) | 24.2 (3.5) |
| Cigarette smoke exposure | 16 (24) |
| Sedentary work | 36 (54) |
| Physically active | 34 (51) |
| Diaphragmatic excursion (mm) | 59.6 (14.4) |
| Expiratory thickness (ET) (mm) | 2.6 (0.5) |
| Inspiratory thickness (IT) (mm) | 1.8 (0.4) |
| Diaphragmatic Ratio (IT/ET) | 1.51 (0.24) |
| Thickness difference (mm) | 0.8 (0.3) |

## 3. Results

Correct, right side diaphragm ultrasound visualization and measurement were obtained for all participants. Table 1 summarizes anthropometric and general aspects of the study population. Age, weight, height and BMI were normally distributed. About half of the study population reported to perform physical activity on regular bases, while about 50% reported having a sedentary work. 16 out of 66 reported a current or former cigarette smoke exposure.

The intra-observer agreement on the three measurements was excellent during both exhalation and inhalation, resulting in a Cronbach's Alpha of 0.850 and 0.810, and an intra-class coefficient of 0.860 and 0.800, respectively. These results, together with all the correlation matrices, are listed in Table 2.

Inter-rater agreement summarized in Table 3 shows a very high correlation between the measurements taken by two different operators, having a Cronbach's Alpha of 0.92 and 0.86, and an intra class coefficient of 0.90 and 0.84, for expiratory and inspiratory thicknesses respectively.

Mean diaphragmatic motion was at 59 ( ± 14.4) mm. A statistically significant correlation was observed among diaphragmatic motion amplitude, weight and height (R = 0.36 and 0.35, respectively), while an inverse correlation with age (R = -0.27) was observed. Overall, mean diaphragmatic thickness measurements were: 2.6 ( ± 0.5) mm at inhalation and 1.8 ( ± 0.4) mm at exhalation. Consequently, the mean TD was 0.8 ( ± 0.3) mm and the DR was 1.51 ( ± 0.25) (Table 2). These results significantly varied according to gender, showing diaphragmatic motion, inspiratory/expiratory thickness, and fractional thickening to be significantly lower in women, while no difference was found in the DR (Table 4). No significant correlations were found between thickness indexes and age, weight, height and BMI. A statistically significant reduced expiratory diaphragmatic thickness emerged in the subgroup of subjects having a sedentary work (p = 0.045) with a close-to-significance trend observed, in the same group, also for inspiratory thickness (p = 0.066).

In linear regression models, the crude association between expiratory thickness and active work produced a B coefficient of 0.19 (95% CI: 0.04-0.38; p = 0.045), which was confirmed after adjustments considering age and sex (B = 0.20; 95% CI: 0.01- 0.39; P = 0.037). Similarly, diaphragmatic motion resulted to be associated with height and weight at crude (B = 0.37; 95% CI = 0.09-0.64; P = 0.009) and age and sex adjusted models (B = 0.36; 95% = 0.10-0.62; P = 0.008), however this association was not maintained in the fully adjusted model (B = 0.29; 95% = -0.06-0.63; P = 0.101).

## 4. Discussion

This study provides evidence that measurement of right diaphragmatic thickness and breathing-related variations using M-mode are reproducible and precise. Our conclusion is supported by the high correlation coefficients and the low mean differences observed in our intra- and inter-observer's variability analysis, as well as through the robust Crohnbach's alpha and inter-class coefficients found (Tables 2 and 3), thus confirming the potential of using ultrasound to assess diaphragmatic function and strength in clinical practice.

Normal DT M-mode values were established on the basis of these measurements and resulted comparable with those reported in the existing literature (Vishwanath et al., 2016).

We have analyzed a representative sample of healthy population having a normal distribution in terms of main anthropometric parameters. This allowed us to investigate whether these indexes could be associated with diaphragmatic thickness and represent prediction models.

It is not the first time that a precision study is carried out with exactly this purpose. Vishwanath et al., (2016) have recently evaluated diaphragm thickness through B-mode ultrasound, and measured Diaphragmatic Fraction (DF) in healthy volunteers, describing age and sex-related thickness variability. However, since reproducibility was not assessed, their data are far from being conclusive. Furthermore, data were obtained through a B-mode measurement only, which represents an evident limitation of the procedure and does not allow for a loop recording of inspiratory and expiratory diaphragmatic thickness at the same breath cycle for adequate comparisons.

As expected, gender influences diaphragmatic motion (the excursion being larger in men than in women). Interestingly however, it is unlikely that this variability might be due to sex related differences in anthropometric indexes, since in our study population height and weight were not significantly different in the two groups. Several other mechanisms should then be taken into account, such as: differences in muscle mass/force ratio, perfusion, contractile properties, and metabolism (Hunter, 2014).

Remarkably, no statistically significant association was documented between DT, at FRC or TLC, DR, DF and major population and anthropometric indexes, such as age, weight, height and BMI. This result could indicate that diaphragmatic strength, endurance and related

**Table 2**
Intra-rater agreement between repeated diaphragmatic measurements.

| | Mean (SD) | Cronbach's Alpha | ICC | 95% CI | p-value |
|---|---|---|---|---|---|
| Diaphragmatic excursion (1st measurement) | 60.1 (16.1) | 0.91 | 0.91 | 0.87 - 0.94 | < 0.0001 |
| Diaphragmatic excursion (2nd measurement) | 59.0 (15.2) | | | | |
| Diaphragmatic excursion (3rd measurement) | 59.0 (15.6) | | | | |
| Expiratory thickness (1st measurement) | 2.6 (0.6) | 0.85 | 0.86 | 0.77- 0.90 | < 0.0001 |
| Expiratory thickness (2nd measurement) | 2.5 (0.6) | | | | |
| Expiratory thickness (3rd measurement) | 2.5 (0.6) | | | | |
| Inspiratory thickness (1st measurement) | 1.8 (0.5) | 0.81 | 0.80 | 0.70 – 0.87 | < 0.0001 |
| Inspiratory thickness (2nd measurement) | 1.7 (0.4) | | | | |
| Inspiratory thickness (3rd measurement) | 1.8 (0.5) | | | | |

**Table 3**
Inter-rater agreement between diaphragmatic measurements taken by two different operators.

| | Mean (SD) | Cronbach's Alpha | ICC | 95% CI |
|---|---|---|---|---|
| Mean diaphragmatic excursion (1st operator) | 59.4 (14.1) | 0.90 | 0.88 | 0.85 - 0.94 |
| Mean diaphragmatic excursion (2nd operator) | 57.8 (15.4) | | | |
| Mean expiratory thickness (1st operator) | 2.5 (0.5) | 0.92 | 0.90 | 0.83- 0.97 |
| Mean expiratory thickness (2nd operator) | 2.6 (0.9) | | | |
| Mean inspiratory thickness (1st operator) | 1.8 (0.3) | 0.86 | 0.84 | 0.73 – 0.89 |
| Mean inspiratory thickness (2nd operator) | 1.7 (0.7) | | | |

**Table 4**
Linear regression analysis assessing the association of ultrasonographic measurements with demographic and anthropometric data.

| | Diaphragmatic Motion | Expiratory Thickness | Inspiratory Thickness | Diaphragmatic Ratio | Thickness Difference |
|---|---|---|---|---|---|
| Age | −0.27* | −0.15 | −0.17 | −0.06 | −0.16 |
| Weight | 0.36* | 0.08 | −0.03 | 0.07 | −0.09 |
| Height | 0.35* | −0.03 | −0.03 | 0.08 | −0.01 |
| BMI | 0.11 | 0.23 | 0.14 | −0.04 | −0.04 |
| Sex | | | | | |
| Male | 65.0 (13.0) | 1.87 (0.38) | 2.78 (0.48) | 1.57 (0.04) | 0.91 (0.28) |
| Female | 55.0 (14.1) | 1.67 (0.39) | 2.39 (0.54) | 1.47 (0.04) | 0.72 (0.26) |
| p-value | 0.004 | 0.043 | 0.004 | 0.071 | 0.006 |
| Cigarette smoke exposure | | | | | |
| No | 59.3 (14.8) | 1.77 (0.37) | 2.55 (0.53) | 1.49 (0.23) | 0.78 (0.26) |
| Yes | 60.5 (13.4) | 1.73 (0.47) | 2.61 (0.61) | 1.60 (0.27) | 0.88 (0.34) |
| p-value | 0.760 | 0.717 | 0.734 | 0.123 | 0.243 |
| Sedentary work | | | | | |
| Yes | 57.8 (14.4) | 1.67 (0.36) | 2.46 (0.53) | 1.51 (0.25) | 0.78 (0.28) |
| No | 61.7 (14.3) | 1.87 (0.41) | 2.70 (0.54) | 1.52 (0.23) | 0.84 (0.29) |
| p-value | 0.272 | 0.045 | 0.066 | 0.974 | 0.458 |
| Physically active | | | | | |
| No | 58.5 (13.4) | 1.75 (0.38) | 2.59 (0.55) | 1.56 | 0.83 (0.28) |
| Yes | 60.6 (15.4) | 1.77 (0.41) | 2.55 (0.54) | 1.47 | 0.78 (0.28) |
| p-value | 0.561 | 0.882 | 0.788 | 0.125 | 0.468 |

muscle volume are independent from anthropometric measures, and are rather linked to physical fitness and regular exertion.

In our study, DT main correlates resulted to be sex (for both inspiratory and expiratory thickness) and having a sedentary work, that resulted associated with thinner inspiratory diaphragm only. Such correlation was confirmed both on a crude linear regression model (B = 0.19; 95% CI:0.04-0.38; P = 0.045) and after age and sex adjustments (B = 0.20; 95% = 0.01-0.39; P = 0.037).

The finding of thicker diaphragm at inhalation in more sedentary subjects deserves further comments. Inspiratory thickness has often been used as a proxy for diaphragm trophism, and reduction range with regard to FRC thickness inversely correlates with muscle contractility strength. Indeed, evidence that less fit subjects may display thinner diaphragm at inhalation was already documented by Souza et al., (2014), who found ultrasonographic DT values in physically trained volunteers to be greater than values found in healthy physically inactive controls. This difference may be related to infiltration of muscle fat associated with sedentarism and senescence (Rossi et al., 2011). Similarly, another study (DePalo et al., 2004) reported that strengthening of respiratory muscles through global physical training produces a diaphragm thickness increase of 26% after 16 weeks. Such changes may instead be due to muscle hypertrophy following regular physical activity (Kamen and Knight, 2004).

The demonstration of diaphragmatic thickness determination through ultrasound strengthens the potential of US as a reliable tool for rapid, cost- and time-saving, radiation-sparing evaluations of the respiratory mechanics and performance in a variety of clinical settings.

We are aware that the present study has some limitations. Firstly, the sample of subjects was small. Secondly, the participants were not a random sample taken from the adult population. However, these subjects represented most age groups (ranging from 18 to 78 years of age) and were gender-balanced. Thirdly, we could not test the correlation between DT and indexes of pulmonary functions, such as Forced Expiratory Volume in the first second (FEV1), Forced Vital Capacity (FVC) nor the maximum inspiratory and expiratory pressure. Indeed, a linear correlation between lung volumes and diaphragmatic thickness was already proved (Boussuges et al., 2009), and it has recently been shown that M-mode ultrasound of forced expiratory profile has similarities with the spirometric flow-volume curve (Zanforlin et al., 2014). Still, inter-rater variability has not yet been determined and further studies should be carried out in order to explore this possibility. Finally, we were not able to test many variables potentially affecting the predictivity of diaphragmatic motion models.

## 5. Conclusions

We proved that diaphragmatic excursion measurements using M-mode are reproducible. Intra-observer agreement was high enough to support the precision of this measurement and provide a further analytic tool for a wider application in clinical practice. Future research assessing the inter-rater agreement of this measure should however be performed. The main predictor of diaphragmatic thickness after adjustment resulted to be physical activity, and this should be taken into account in order to design specifically tailored studies and develop reference equations for normality. Nonetheless, in order to obtain highly reliable reference equations, other determinants are yet to be identified.

## References

American Thoracic Society/European Respiratory Society, 2002. ATS/ERS Statement on respiratory muscle testing. Am. J. Respir. Crit. Care Med. 166, 518–624.
Ayoub, J., Cohendy, R., Dauzat, M., Targhetta, R., De la Coussaye, J.E., Bourgeois, J.M., Ramonatxo, M., Prefaut, C., Pourcelot, L., 1997. Non-invasive quantification of diaphragm kinetics using m-mode sonography. Can. J. Anaesth. 44, 739–744.
Bland, J.M., Altman, D.G., 1986. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1, 307–310.

Boon, A.J., Harper, C.J., Ghahfarokhi, L.S., Strommen, J.A., Watson, J.C., Sorenson, E.J., 2013. Two-dimensional ultrasound imaging of the diaphragm: quantitative values in normal subjects. Muscle Nerve. 47, 884–889.

Boussuges, A., Gole, Y., Blanc, P., 2009. Diaphragmatic motion studied by m-mode ultrasonography: methods, reproducibility, and normal values. Chest 135, 391–400.

Buchman, A.S., Boyle, P.A., Leurgans, S.E., Evans, D.A., Bennett, D.A., 2009. Pulmonary function, muscle strength, and incident mobility disability in elders. Proc. Am. Thorac. Soc. 6, 581–587.

Carrillo-Esper, R., Pérez-Calatayud, ÁA., Arch-Tirado, E., Díaz-Carrillo, M.A., Garrido-Aguirre, E., Tapia-Velazco, R., Peña-Pérez, C.A., Espinoza-de Los Monteros, I., Meza-Márquez, J.M., Flores-Rivera, O.I., Zepeda-Mendoza, A.D., de la Torre-León, T., 2016. Standardization of sonographic diaphragm thickness evaluations in healthy volunteers. Respir Care. 61, 920–924.

De Bruin, P.F., Ueki, J., Bush, A., Khan, Y., Watson, A., Pride, N.B., 1997. Diaphragm thickness and inspiratory strength in patients with Duchenne muscular dystrophy. Thorax 52, 472–475.

DePalo, V.A., Parker, A.L., Al-Bilbeisi, F., McCool, F.D., 2004. Respiratory muscle strength training with non-respiratory maneuvers. J. Appl. Physiol. (1985) 96, 731–734.

DiNino, E., Gartman, E.J., Sethi, J.M., McCool, F.D., 2014. Diaphragm ultrasound as a predictor of successful extubation from mechanical ventilation. Thorax 69, 423–427.

Doorduin, J., van Hees, H.W., van der Hoeven, J.G., Heunks, L.M., 2013. Monitoring of the respiratory muscles in the critically ill. Am. J. Respir. Crit. Care Med. 187, 20–27.

Ferrari, G., De Filippi, G., Elia, F., Panero, F., Volpicelli, G., Aprà, F., 2014. Diaphragm ultrasound as a new index of discontinuation from mechanical ventilation. Crit. Ultrasound J. 6 (8).

Goligher, E.C., Laghi, F., Detsky, M.E., Farias, P., Murray, A., Brace, D., Brochard, L.J., Bolz, S.S., Rubenfeld, G.D., Kavanagh, B.P., Ferguson, N.D., 2015. Measuring diaphragm thickness with ultrasound in mechanically ventilated patients: feasibility, reproducibility and validity. Intensive Care Med. 41, 734.

Hunter, S.K., 2014. Sex differences in human fatigability: mechanisms and insight to physiological responses. Acta Physiol. (Oxf). 210, 768–789.

Kamen, G., Knight, C.A., 2004. Training-related adaptations in motor unit discharge rate in young and older adults. J. Gerontol. A Biol. Sci. Med. Sci. 59, 1334–1338.

Lerolle, N., Diehl, J.L., 2011. Ultrasonographic evaluation of diaphragmatic function. Crit. Care Med. 39, 2760–2761.

Levine, S., Nguyen, T., Taylor, N., Friscia, M.E., Budak, M.T., Rothenberg, P., Zhu, J., Sachdeva, R., Sonnad, S., Kaiser, L.R., Rubinstein, N.A., Powers, S.K., Shrager, J.B., 2008. Rapid disuse atrophy of diaphragm fibers in mechanically ventilated humans. N. Engl. J. Med. 358, 1327–1335.

Rossi, A.P., Watson, N.L., Newman, A.B., Harris, T.B., Kritchevsky, S.B., Bauer, D.C., Satterfield, S., Goodpaster, B.H., Zamboni, M., 2011. Effects of body composition and adipose tissue distribution on respiratory function in elderly men and women: the health, aging, and body composition study. J. Gerontol. A Biol. Sci. Med. Sci. 66, 801–808.

Scarlata, S., Mancini, D., Laudisio, A., Benigni, A.M., Antonelli Incalzi, R., 2018. Reproducibility and clinical correlates of supine diaphragmatic motion measured by M-mode ultrasonography in healthy volunteers. Respiration 16, 1–8.

Sferrazza Papa, G.F., Pellegrino, G.M., Di Marco, F., Imeri, G., Brochard, L., Goligher, E., Centanni, S., 2016. A review of the ultrasound assessment of diaphragmatic function in clinical practice. Respiration 91, 403–411.

Souza, H., Rocha, T., Pessoa, M., Rattes, C., Brandão, D., Fregonezi, G., Campos, S., Aliverti, A., Dornelas, A., 2014. Effects of inspiratory muscle training in elderly women on respiratory muscle strength, diaphragm thickness and mobility. J. Gerontol. A Biol. Sci. Med. Sci. 69, 1545–1553.

Ueki, J., De Bruin, P.F., Pride, N.B., 1995. In vivo assessment of diaphragm contraction by ultrasound in normal subjects. Thorax 50, 1157–1161.

Vishwanath, T.Thimmaiah, Geetha, M.J., Jain, Keval P., 2016. Evaluation of thickness of normal diaphragm by B mode ultrasound. Int. J. Contemp. Med. Res. 3, 2658–2660.

Zanforlin, A., Smargiassi, A., Inchingolo, R., di Marco Berardino, A., Valente, S., Ramazzina, E., 2014. Ultrasound analysis of diaphragm kinetics and the diagnosis of airway obstruction: the role of the M-mode index of obstruction. Ultrasound Med. Biol. 40, 1065–1071.