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Original Article

Non-invasive Functional Evaluation of the Reserve in Fatigue and the Diaphragm Structure using Transthoracic Echography in B and M Modes

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A R T I C L E   I N F O

A B S T R A C T

The diaphragm is the principal respiratory muscle. Its special characteristics have made it difficult to design instruments capable of performing a non-invasive evaluation of its structure and function in humans. The present study was designed to evaluate the potential use of ultrasound as a non-invasive method to fulfil these objectives.

Methods: The study consisted of three phases: (1) ultrasound study in autopsy samples (n=10) of a segment of the thoracic-abdominal wall, from the bottom to the parietal peritoneum (i.e., thoracic wall, diaphragm, pleura and peritoneum structures), (2) static ultrasound study of the previous structures and the diaphragm in healthy subjects (n=10) to standardised lung volumes; and (3) dynamic ultrasound study of the contraction-relaxation of the diaphragm in the same subjects, calculating its maximum velocity of relaxation (MVrdi, mm/sec) during a specific inspiratory resistance test.

Results: The ultrasound enabled the pleural and peritoneal limits of the diaphragm to be identified, and quantify its thickness (Tdi), both *ex-vivo* and *in-vivo*, in all cases. The dynamic study of the Tdi showed a linear increase directly associated with the lung volume measurement, as well as a cyclical increase during inspiratory movements at rest. In the resistance test, the MVrdi was maximal with low loads and gradually decreased until reaching a minimum nadir ($\Delta \approx -70\%$ of the initial value) in claudication (fatigue). The MVrdi has a high precision in diagnosing claudication.

Conclusions: Transthoracic ultrasound of the diaphragm is a non-invasive method that gives promising results in the structural and functional evaluation (i.e. fatigue risk) of that muscle. These findings are of pathophysiological interest and could be of use in the clinical care context.

Evaluación funcional no-invasiva de la reserva ante la fatiga y la estructura del diafragma mediante ecografía transtorácica en modos B y M

R E S U M E N

El diafragma es el principal músculo respiratorio. Sus especiales características han dificultado el diseño de instrumentos capaces de evaluar su estructura y función de forma no invasiva en humanos. El presente estudio fue diseñado para evaluar la potencial utilidad de la ecografía como un método no invasivo para cumplir dichos objetivos.

Métodos: El estudio incluyó tres fases: 1) estudio ecográfico en muestras necrópsicas (n = 10) de un segmento de pared toraco-abdominal, incluyendo desde piel hasta peritoneo parietal (e.d., estructuras de pared torácica, diafragma, pleura y peritoneo); 2) estudio ecográfico estático de las estructuras anteriores y el

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diafragma en individuos sanos (n = 10) a volúmenes pulmonares normalizados, y 3) estudio ecográfico dinámico de la contracción-relajación del diafragma en los mismos individuos, con cálculo de la velocidad máxima de relajación (VMARdi, mm/seg) en el curso de una prueba específica de resistencia inspiratoria.
Resultados: La ecografía permitió identificar con claridad los límites pleural y peritoneal del diafragma, y cuantificar su grosor (Gdi), en todos los casos, tanto ex-vivo como in-vivo. El estudio dinámico del Gdi mostró un incremento lineal en relación directa con el volumen pulmonar de medición, además de un incremento cíclico durante los movimientos inspiratorios en reposo. En la prueba de resistencia, la VMARdi fue máxima ante cargas bajas y disminuyó progresivamente hasta alcanzar un nadir mínimo ($\Delta \approx$ – 70% del valor inicial) en la claudicación (fatiga). La medición de VMARdi tiene una alta precisión diagnóstica de claudicación.
Conclusiones: La ecografía transtorácica del diafragma es un método no invasivo que ofrece resultados prometedores en la evaluación estructural y funcional (e.d., riesgo de fatiga) de dicho músculo. Estos hallazgos son de interés fisiopatológico y podrían ser de utilidad en el contexto asistencial clínico.

Introduction

The respiratory muscles are contractile elements that generate the airflow necessary for gas exchange in the lungs. From the embryological and functional point of view, respiratory muscles are skeletal muscles, and as such, possess two fundamental functional characteristics: strength and endurance. The diaphragm is the primary respiratory muscle. As it contracts, the inferior ribs ascend and the abdomen is caudally displaced. As a result, intrathoracic pressure decreases causing air to be inspired. This muscle is anatomically divided into two sections, one costal and one crural, which are embryologically and functionally related.[1,2]

Maximal inspiratory pressure, measured at the mouth (PI max)[3] or oesophagus (PI, esmax)[4] is normally used to measure the power of the inspiratory muscles. However, the force created by the diaphragm is difficult to measure specifically, requiring a more invasive technique. This consists of measuring the maximal transdiaphragmatic pressure (Pdimax), by inserting probes into the oesophagus and stomach, usually through the nostrils.[5] In addition, in order to assess the stamina of the respiratory muscles, respiration tests with inspiratory[6] and/or expiratory[7] loads are needed, using both incremental (maximal) and constant (submaximal) loads until the onset of claudication or muscle fatigue.

Muscle fatigue is considered to be the temporary incapacity of a muscle to make a contractile effort. Fatigue can be reversed with rest,[8] which differentiates it from muscle weakness. Several techniques have been proposed for detecting fatigue, but all imply a certain level of invasiveness. This has, therefore, hampered their clinical application.[9,10] Using an oesophageal probe, Moxham et al[11] were able to demonstrate that the maximum velocity of inspiratory muscle relaxation (maximum relaxation rate or MRR) can serve as an early indicator of fatigue.

Ultrasound is an imaging technique that has proved useful in assessing muscular structure and function for several medical specialties.[12] Its advantages are low cost, reproducibility, safety, and non-invasiveness. With the introduction of M-mode ultrasound, it is now possible to analyse the dynamics of the contraction and relaxation of muscles such as the myocardial muscle.[13] Also, transthoracic ultrasounds have been used occasionally to evaluate diaphragm displacement during respiratory movement.[14] Due to the biological plausibility and technical basis that these concepts offer, we could hypothesize that thoracic transparietal ultrasound could be used to identify the human diaphragm at the thoracic zone of apposition, to quantify its thickness as an indication of the trophic state of the diaphragm, and to quantify the velocity of muscle contraction and relaxation.[15] As such, the general objective of this study was to estimate the capacity of bidimensional and M-mode transthoracic ultrasound to specifically assess the structure and functional state of the human diaphragm by measuring its thickness and maximum relaxation rate, both at rest and during the experimental protocol to induce inspiratory muscle fatigue in healthy individuals (tables 1 and 2).

Table 1
Demographic characteristics, lung function, respiratory muscle function, and *in vivo* ultrasound characteristics of the diaphragm

General characteristics	Units of measurement	
Study population	(No.)	10
Sex	(m:f)	10:0
Age	(years)	34 (9)
BMI	(kg/m²)	25 (3)
FEV_1	(%ref)	90 (7)
FVC	(%ref)	102 (12)
PEmax	(%ref)	96 (8)
PI max	(%ref)	105 (11)
In Vivo, at rest study		
TdiRV	mm	1.38 (0.41)
TdiCKF	mm	1.88 (0.41)
TdiTLC	mm	4.37 (0.97)
In Vivo study during exercise		
Imposed external inspiratory load	(g)	424 (90)
	(cmH_2O)	102 (22)
Tlim	(min)	13 (3)
Inspiratory Diaphragm (i.e., TV)	mm	2.88 (0.78)
MVRdi		
Maximum	mm/sec	8.19 (2.43)
Minimum	mm/sec	1.74 (0.36)
First tercile of exercise	mm/sec	6.62 (2.11)
Second tercile of exercise	mm/sec	5.11 (1.87)
Third tercile of exercise	mm/sec	3.00 (1.06)
During claudication	mm/sec	1.74 (0.36)

BMI: body mass index; FEV_1: forced expiratory volume in 1 second; FRC: functional residual capacity; FVC: forced vital capacity; Tdi: thickness of the diaphragm; PEmax: maximum expiratory pressure, measured at the mouth; PI max, maximum inspiratory pressure, measured at the mouth (Valsalva and Müller manoeuvres, respectively); RV: residual volume; TLC: total lung capacity; Tlim: duration of inspiratory endurance; MVRdi: Maximum velocity of relaxation of the diaphragm; TV: tidal volume.

Table 2
Correlation matrix between inspiratory muscular force (PI max) and the ultrasound variables for the diaphragm

Variables	PI $_{max}$ (%ref)	Tdi$_{RV}$	Tdi$_{FRC}$	Tdi$_{TLC}$	Tdi$_{TV}$
PI max (%ref)	1.000	−0.267	−0.159	0.371	0.105
TdiRV	−0.267	1.000	0.896**	0.675**	0.385**
TdiFRC	−0.159	0.896**	1.000	0.673**	0.300**
TdiTLC	0.371	0.675**	0.673**	1 000	0.399**
TV Tdi	0.105	0.385**	0.300**	0.399**	1 000

FRC: functional residual capacity; Tdi: thickness of the diaphragm; PI max: maximum inspiratory pressure measured at the mouth (Müller manoeuvre); RV: residual volume; TLC: total lung capacity; TV: tidal volume.
** $P<.001$.

Method

Scope of the study. This study was carried out in a tertiary hospital (Hospital del Mar, IMAS, Barcelona, Spain), with the participation of the departments of Pneumology, Cardiology, Radiology, and Pathological Anatomy, and an associated research institute (IMIM, Barcelona).

Ethical aspects and study design. This analytical cross-sectional study was designed in accordance with the legislation and rules for human research (Helsinki Declaration[16]), and was approved by the Clinical Research and Ethics Committee at the hospital. All patients agreed to participate in the study and signed an informed consent. We also obtained consent from the responsible relative for the *ex vivo* model. The study was composed of three phases defined according to the technical characteristics and specific objectives of each one. Stage I: *ex vivo* model. This was carried out on 10 anatomical human parts from necropsies of male cadavers. In order to minimise the effects of rigor mortis, the study was performed within the first four hours after death. A pathologist performed the procedure in all cases in the autopsy room at our institution. The anatomical fragments included the thoracic and abdominal walls (approximately 20x20 cm), including the skin, subcutaneous cellular tissue, ribs, muscles, parietal pleura, and parietal peritoneum. The axial plane limits for cutting the fragments were marked by the anterior and posterior axillary lines. The upper limit was marked off by a horizontal line along the 8th intercostal space, and the lower limit was the ipsilateral iliac crest. The fragments were submerged in a tank with a saline solution at room temperature, and then studied using an immersion ultrasound technique described below. The different tissue components (skin, subcutaneous cellular tissue, ribs, muscles, parietal pleura, parietal peritoneum) were then dissected in order to verify their appearance in the ultrasound and clarify their specific identification. Stage II: static *in vivo* model. We took ultrasound images in ten healthy volunteers that corresponded to the different structures identified in the previous phase, as well as the changes in each with relation to five standardised lung volumes, from residual volume (RV) to total lung capacity (TLC), at intervals equivalent to 20% of the forced vital capacity (FVC). All volunteers were healthy males, with no special training for muscle strength or resistance tests. Stage III: dynamic *in vivo* model. We also used these same volunteers for an ultrasound assessment of diaphragm relaxation during an inspiratory muscular resistance test (from start to claudication). The test was performed using a threshold valve, breathing against a submaximal constant load at 60% PI max[17] as specified below. During this phase, we used M-mode ultrasound imaging to quantify the diaphragm relaxation rate, using a parallel method to the one previously described for intrathoracic pressure measurements.

Procedures and techniques. We performed an anthropometric study on each patient, along with forced spirometry tests and evaluations of inspiratory muscular force and resistance.

1. *Anthropometrics.* We included conventional variables: weight, height, body mass index (BMI), fat free mass index (FFMI), as well as the thoracic perimeter at the level of the lower third of the thorax (normalisation in apnoea and functional residual capacity [FRC]).
2. *Forced Spirometry.* This was performed using a Datospir 92 spirometer (SIBEL, Barcelona), in accordance with SEPAR regulations.[18] The results obtained are expressed as percentages relative to reference values.[19]
3. *Global inspiratory muscular force* (measure of PI max). The patients performed a maximum inspiratory effort from RV against an occluded airway (Biopac Systems, Inc., Santa Barbara, CA). The maximum value from five valid manipulations was expressed as a percentage of the reference value.[20]

4. *Specific test of inspiratory resistance.* Volunteers respired against submaximal inspiratory loads equivalent to 60% of their PI max.[21] Briefly, during the test, the patients respired trough a two-way valve, with a second valve connected to the inspiratory circuit with a threshold opening, similar to the technique described by Nickerson et al.[22] The respiratory pattern was left to the choice of the patient. During the test, we continuously measured the pressure in the mouth using a manometer (Biopac Systems® Inc. Santa Barbara, CA U.S.A.) connected to the circuit. The test was terminated upon claudication, defined as the inability to establish airflow in at least three maximum inspiratory efforts[21] We measured PI max at 1, 5, and 10 min after claudication in order to identify the recovery of inspiratory strength after the inspiratory resistance test.
5. *Transthoracic ultrasound of the diaphragm.* In the three phases of the study, we used a curvilinear ultrasonic transducer, with a frequency of 7.5 MHz (Toshiba®, SSH140A; Japan). The transducer was placed perpendicularly to the plane of the skin (both in the *ex vivo* and *in vivo* studies) at the height of the zone of apposition of the diaphragm. This was defined by the caudal muscular portion at the "lung point" (i.e. the costophrenic angle, at approximately the height of the 10th intercostal space on the non-dominant side) and the midaxillary line on the dominant side. The study was performed under immersion in the *ex vivo* models, whereas the *in vivo* study used a transducing gel interface applied over the skin. The variables evaluated under the bidimensional mode were first: diaphragm thickness (Tdi) in both the *ex vivo* and *in vivo* models; second: we quantified the changes in Tdi in healthy volunteers while they performed inspiration and expiration cycles with a permeable glottis at the previously described different lung volumes. Tdi was defined as the distance between the midpoints of the pleural and peritoneal refractive lines (external and internal limits, respectively) in the transverse ultrasound images obtained of the muscle. Finally, using M-mode ultrasound, we calculated the third variable, defined as the maximum velocity of relaxation of the diaphragm (MVRdi) during inspiratory exercise in the external load test. This variable was quantified in mm/sec in each respiratory cycle using computer software incorporated into the echocardiograph equipment. The rate was defined as the hypotenuse of a right-angled triangle whose height is determined by the change in Tdi (mm) and whose width represents time (fig. 4).

Statistical analysis. The data are expressed as mean (standard deviation). The level of correlation between quantitative variables was evaluated using Pearson's correlation coefficient. The changes in MVRdi were assessed using a repeated measures analysis of variance (ANOVA). The estimation of conditional probabilities at each point in time when claudication occurred and the limit of the product of these probabilities for estimating the rate of claudication at each point were quantified using the Kaplan-Meier model. The precision of the technique for detecting claudication was evaluated using ROC curve analysis. We analysed the reliability of the measurements, assessing both intra- and inter-observer variability, by calculating intraclass correlation coefficients, using an absolute agreement model for two factors (mixed effects). We established $P < .05$ as the value for statistical significance in all cases.

Results

Thickness of the diaphragm (Tdi). The *ex vivo* ultrasound of the different anatomical components allowed us to identify the different layers in all the cases that make up the structures of the thoracic wall at the height of the zone of apposition. Figure 1 shows a representative image of the diaphragm, both in relation to the other structures of the thoracic wall as well as after the dissection of the muscle. The

diaphragm showed a refraction in the ultrasound typical of muscle tissue (fig. 1A), with clearly defined borders. As such, the peritoneal and pleural interfaces allowed for the delineation of the medial and lateral borders of the diaphragm to be specified, which in turn provided a measure of thickness (fig. 1B). In the intact *ex vivo* piece, the mean muscle thickness was 4.1 (0.1) mm, which was not significantly modified after dissection (changes <0.1 mm).

Changes in diaphragm thickness during the respiratory cycle. Healthy volunteers (No.=10, all males, age, 34 [9]) all had normal anthropometric values (weight range: 63-77 kg, BMI: 25 [3] kg/m²). Spirometry values also fell within the normal range (FEV1: 87 [4]% pred; FEV1/FVC, 76 [3]%). The diaphragm was clearly identifiable in the ultrasound image taken in all the individuals, with a mean Tdi at FRC of 1.88 (0.41) mm. Respiratory movements were associated with thickness changes in the diaphragm (fig. 2), with a direct correlation (r=0.865, *P*<.001) between thickness and lung volume when the measurement was taken. As such, the minimum values observed corresponded to RV (1.38 [0.41] mm) and the maximum values corresponded to TLC (4.37 [0.97] mm) (fig. 3).

Measuring the maximum velocity of relaxation of the diaphragm (MVRdi). The M-mode ultrasound allowed for the quantification and monitoring of MVRdi during the muscular resistance test. This had a mean duration of 13 (3) min (range: 8-18 min). The initial MVRdi values (first tercile of the test) were 6.62 (2.11) mm/sec (range: 6.27-7.07 mm/sec), with a progressive and linear reduction during the rest of the test (second tercile, MVRdi = 5.11 [1.87] mm/sec; third tercile, MVRdi = 3.00 [1.06] mm/sec, *P*<.001). We observed a direct linear correlation (r=0.658, *P*<.000) between duration of respiration against external inspiratory load and MVRdi, with a mean decrease of 0.28 mm/sec for each minute of respiration against inspiratory loads. The minimum value reached for MVRdi was 3.00 (1.06) mm/sec, and the minimum value corresponded to respiratory claudication in all cases

(figs. 4 and 5). Inspiratory claudication was associated in all cases with a reversible deterioration of maximal inspiratory pressure (PI max). This was always associated with MVRdi values below 4 mm/sec (*P*<.001; fig. 5) and 70% below the initial MVRdi value. The PI max recovered its initial values at 10 min after claudication. This represents a clinically accepted indication that claudication was partially or totally caused by global inspiratory fatigue (including the diaphragm). The ROC analysis between MVRdi and claudication produced an area below the curve of 0.978 with a 95% confidence interval of 0.947-0.994 and a value of *P*<.0001. Based on the ROC curve coordinates, the cut-off point of 2.1 mm/sec of MVRdi allows for the results of the continuous scale to be summarised with the greatest possible sensitivity (100%, 95% CI, 59.0-100) and specificity (93.8%, 95% CI, 89.4-96.7) to detect claudication. These results all indicate that this diagnostic technique offers a high level of precision, which is corroborated by a curve that is very close to the upper left border.

Analysis of the reliability of measurements. The concordance between results obtained by a single observer as well as the comparison with a second observer demonstrate a high intraclass correlation coefficient (ICC) for both the values of Tdi and MVRdi. Specifically, the Tdi measurements at different lung volumes had an intra-observer ICC of 0.996 (95% CI, 0.991- 0.998), and an inter-observer ICC of 0.992 (95% CI, 0.992-0.998). The inter-observer analysis of the MVRdi measurements produced an ICC of 0.859 (95% CI, 0.658-0.943).

Discussion

The information provided by this study can be summarised in four fundamental findings. Firstly, ultrasound images can clearly identify the borders and thickness of the diaphragm, both in autopsy pieces and in healthy volunteers. The second finding is represented
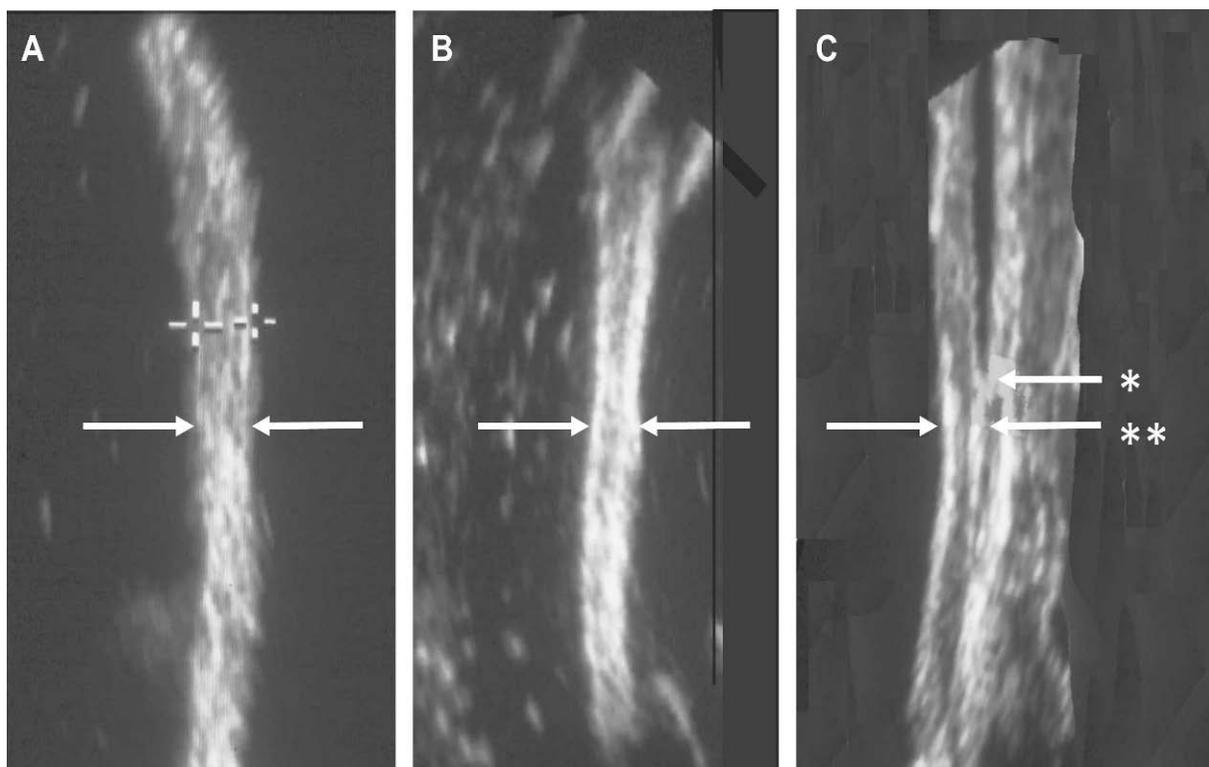

Figure 1. *Ex vivo* phase of the study. Representative images of the diaphragm and neighbouring structures at the height of the thoracic zone of apposition of the muscle obtained using immersed ultrasound. The dissected diaphragm has a refraction typical of muscle tissue (A), whose limits can be easily defined (cross indicators). Two limits of greater density can be seen defined by the peritoneal and pleural serosa (B). In the intact piece (C), the lower point of the lung indicates the lateral costophrenic angle (*). The peritoneal and pleural interface allows us to define the medial and lateral borders of the diaphragm (**).
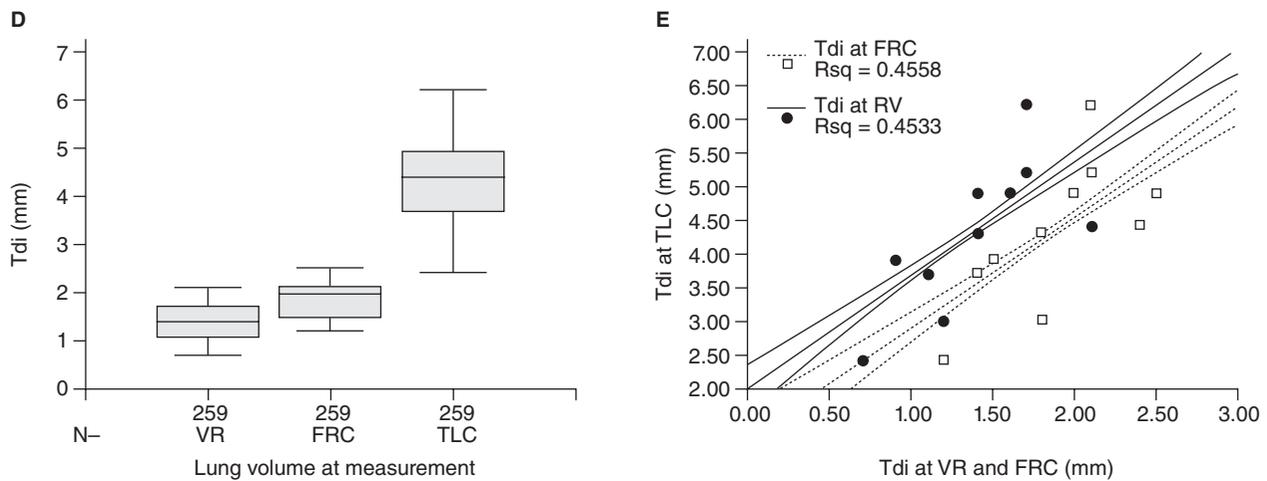
Figure 2. *In vivo* static phase of the study. Representative images of the diaphragm and neighbouring structures at the height of the thoracic zone of apposition obtained using bidimensional ultrasound in healthy volunteers. The diaphragm has a minimum thickness at residual volume RV (A), intermediate thickness at functional residual capacity (FRC) (B), and maximum thickness at total lung capacity (TLC) (C). Figure 2D shows the absolute values obtained with these lung volumes. Figure 2E shows a linear correlation, along with 95% CI between maximum Tdi (at TLC) and the Tdi obtained during apnoea manoeuvres for FRC and RV (*P* <.05 for both). Abbreviations: No.: number of measurements; Tdi: thickness of the diaphragm; RV: residual volume; TLC: total lung capacity; FRC: functional residual capacity.
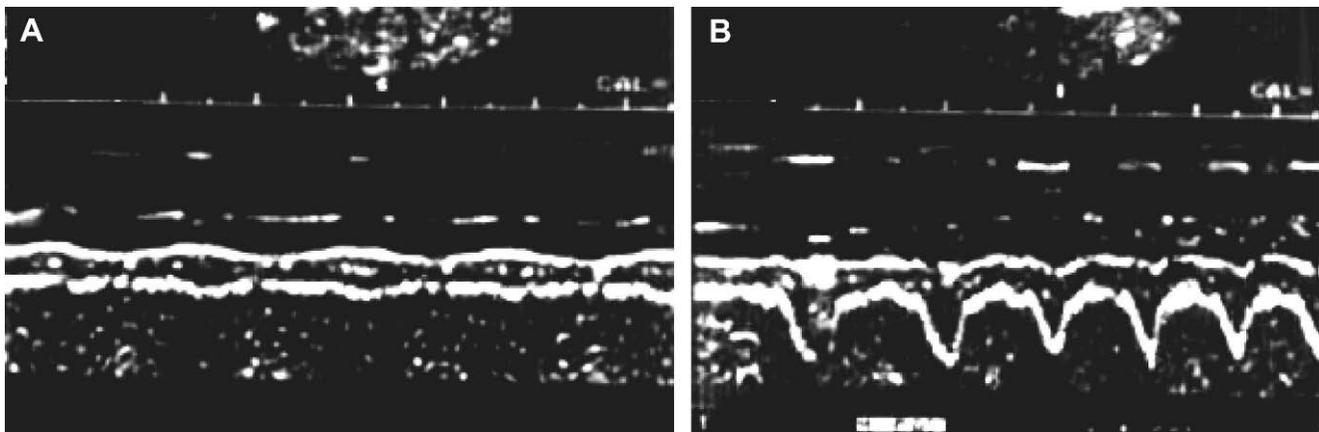
Figure 3. *In vivo* phase of the study and typical dynamic images obtained using M-mode ultrasound (guided by B-mode) of the changes in the thickness of the diaphragm (Tdi) during respiration at rest (A) and during respiration with specific inspiratory loads (B). The change (%) in thickness during inspiratory loads showed no correlation with thickness at rest (C). Abbreviations: Tdi: thickness of the diaphragm; FRC: functional residual capacity.

A Initial MVRdi

B Initial vs final MVRdi

C

D
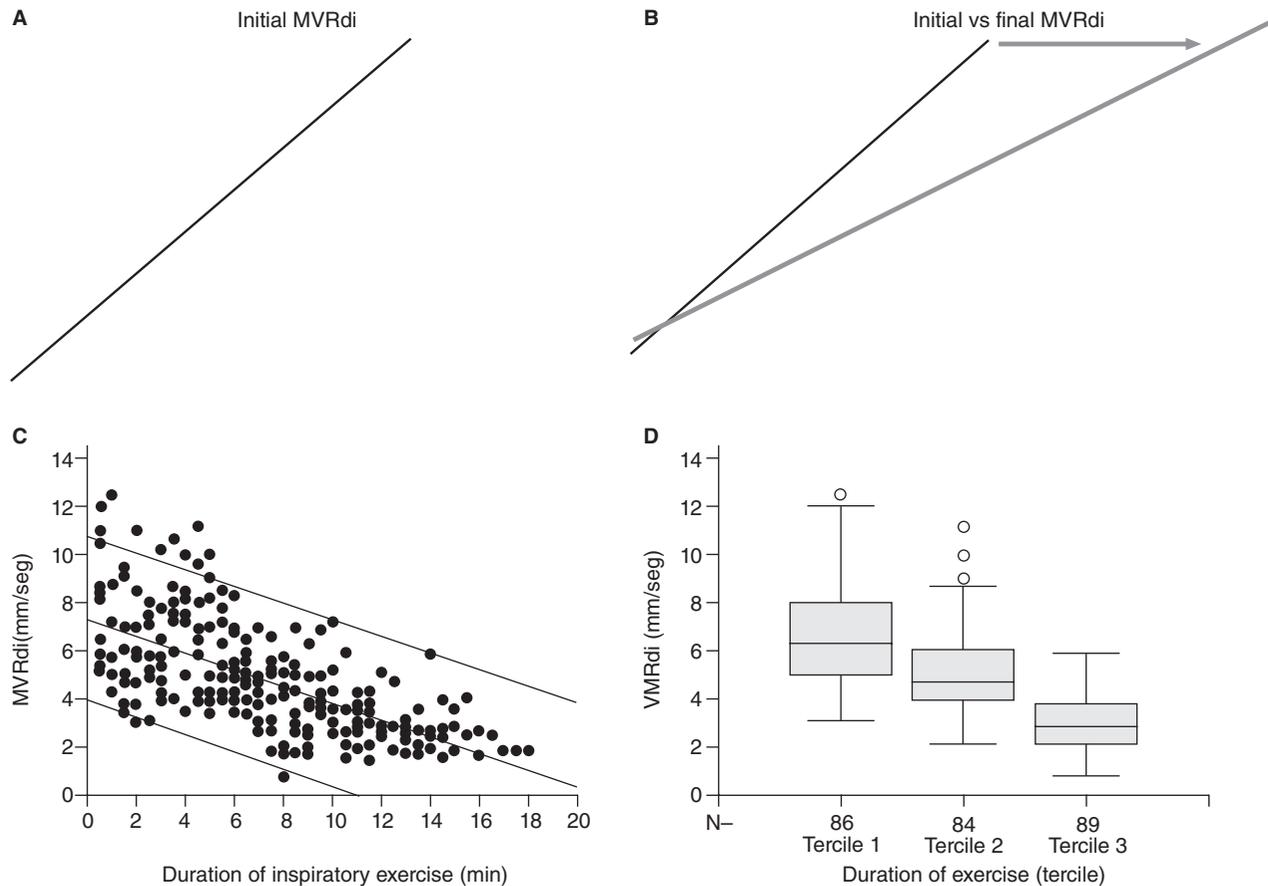
Figure 4. Quantification of the velocity of relaxation of the diaphragm (MVRdi), *in vivo* study. Representative images of the diaphragm obtained using an M-mode transthoracic ultrasound in healthy volunteers while performing resistance tests. Measurements obtained at the start (A) and end (claudication [B]) of the test. There is a linear correlation (r=0.658, *P* <.000) between the duration of inspiratory effort and MVRdi (C). By dividing the test into terciles according to duration (D), we see that the MVRdi has progressively lower values as the subject approaches claudication. Abbreviations: Tdi: thickness of the diaphragm; RV: residual volume; TLC: total lung capacity; MVRdi: maximum velocity of relaxation of the diaphragm.

by the evidence that the thickness of the diaphragm measured with ultrasound varies significantly during the respiratory cycle, tracing muscular contraction. Thirdly, our study confirms that the thickness of the diaphragm has a direct relationship with lung volume. Finally, our study shows that the maximum velocity of relaxation of the diaphragm diminishes progressively with a linear tendency during respiration with external inspiratory loads, reaching minimum values that coincide with respiratory claudication.

Ultrasound imaging is a widely used diagnostic technique in different pathologies and various scenarios in a variety of medical specialities. For example, echocardiography has acquired a major role in the functional evaluation of heart dynamics in cardiology. Our study confirmed part of the results obtained by Ueki et al[23] in that Tdi values change in relation to respiratory movements. However, this information on thickness is limited if the study is trying to measure muscle function and resistance against fatigue. Following the reasoning used in recent years when analysing diastolic function of the left ventricle using the relaxation slope of the posterior wall with M-mode ultrasound, we have approached the evaluation of diaphragmatic function in the same fashion, that is, by measuring the slope of relaxation.[15] Our study has provided two novel concepts. Ours is the first study to evaluate the ultrasound image of the diaphragm through a study of autopsy samples (*ex vivo*, No.=10) under liquid immersion (i.e. phase I). These findings validate the methodology used in the *in vivo* study. Secondly, this is the first study to propose and assess the evaluation of the maximum velocity of relaxation of the diaphragm using M-mode ultrasound as an index

of muscle function and resistance against fatigue, to which we have given the acronym MVRdi.

Autopsy studies: Validation of the ultrasound structure of the diaphragm. The methodology used in the study on fragments obtained from necropsies allowed for the precise identification and characterization of the diaphragm and the different adjacent structures in the zone of apposition in the ultrasound images. This made it possible to study the samples when they were complete as well as after the sequential dissection of the different tissues. Only after this *ex vivo* evaluation did we proceed with the *in vivo* study. Since we did not perform any histological or molecular studies, we cannot comment on the subcellular trophic state of the muscle (e.g. size of fibres, number of fibres, interfibrillar space). However, in the case of the necropsies, the appearance of the diaphragm was that of a normal muscle, without macroscopic signs of atrophy or hypertrophy. One point to take into account is that autopsy samples can have artefacts of muscle contraction with possible changes in ultrasound refraction due to necrosis and/or rigor mortis. Therefore, all samples were studied within the first few hours after the patient had passed away.

In vivo studies: Dynamic changes in the diaphragm observed using ultrasound during ventilatory movements and muscle fatigue. It is known that the diaphragm shortens during contraction. This causes a subsequent increase in thickness (reflecting the superpositioning of actin and myosin myofilaments).[24] Our study has demonstrated that this change in thickness of the diaphragm is definitely produced and can be quantified using ultrasound. These
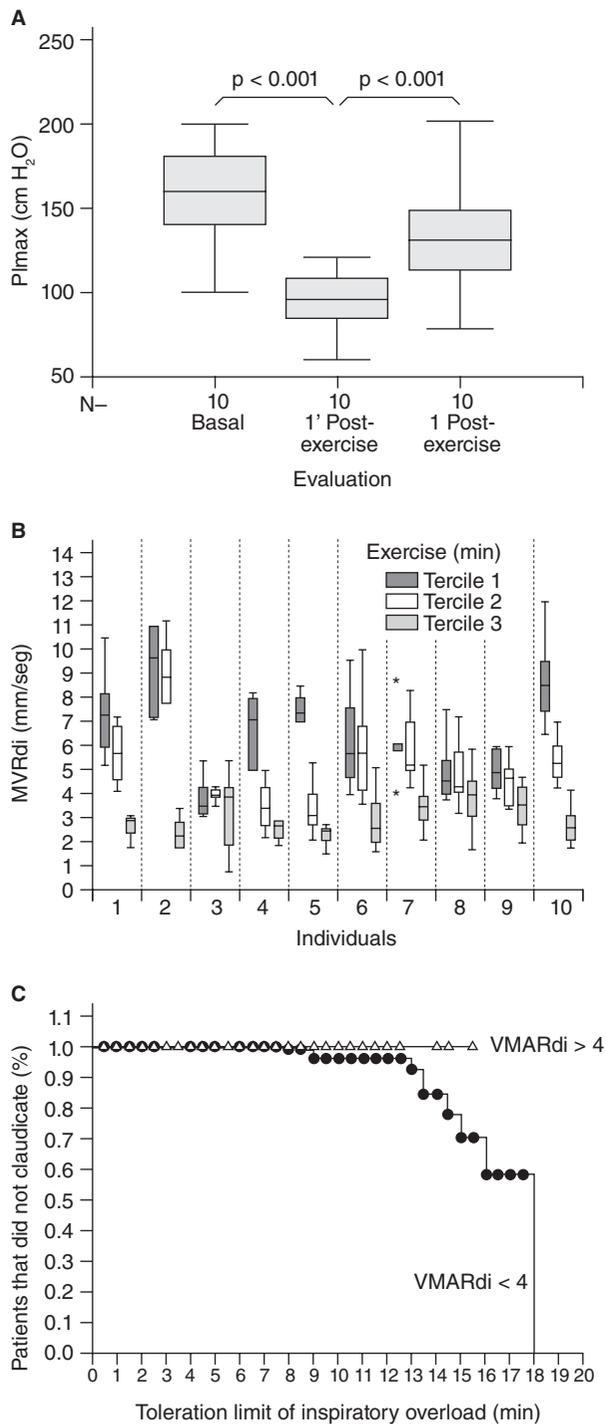
Figure 5. Risk of claudication of the diaphragm and MVRdi. Claudication caused by the muscular resistance test was associated with a transient reduction in inspiratory muscular force (i.e., inspiratory muscular fatigue) (A). The continuous quantification of MVRdi during the resistance test demonstrated that maximum values occurred during the initial stages of the exercise (i.e., the first tercile of time performing the respiratory exercises without additional loads, and which progressively dropped) (B). Claudication was associated with a reduction in MVRdi of up to 70% below the initial values. In no cases was claudication produced at higher than 4 mm/sec (survival function, Kaplan Meier *P*=.217) (C). Abbreviations: Tdi: thickness of the diaphragm; MVRdi: maximum velocity of relaxation of the diaphragm.

results concur with previous studies that have demonstrated that the diaphragm increases in thickness when performing inspiratory movements under normal conditions, and that kinetics of the diaphragm are altered if inspiratory resistance is changed (e.g.

occlusion of the nose).[25] Our results show that the change in Tdi maintains a linear relationship with lung volume at the time of measurement, which seems logical given that the muscle shortens and increases in thickness progressively, allowing for an increase in lung volume. Recently, Boussuguess et al described the M-mode ultrasound as a very reproducible technique for evaluating the cranio-caudal excursion of the diaphragm during at-rest and maximum (sniff) respiration.[26]
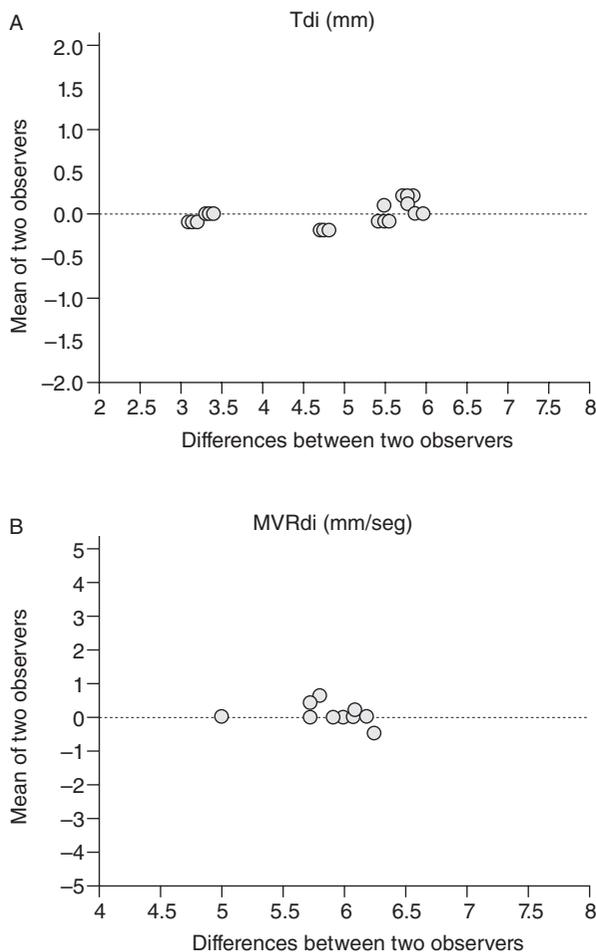
One of the main innovations was that this study allowed for the contraction-relaxation cycle to be evaluated fnctionally, with special emphasis on the analysis of relaxation, during the execution of a standardised experimental protocol of induction of inspiratory muscular fatigue. To our knowledge, this is the first study that establishes a method based on velocity of relaxation as a potentially useful parameter for estimating the functional state of the muscle and the risk of failure against ventilatory loads. In this respect, the study was guided by the knowledge that muscular relaxation is an active phase from both the metabolic and structural points of view, including energy consumption and specific cellular and molecular events. Changes in muscle relaxation represent one of the earliest events as muscles start to fatigue.[9,11] In this sense, fatigue of the respiratory muscles had already been shown to change the velocity of relaxation, expressed as the variable of maximal relaxation rate (MRR).[11] This variable has been described for the curves of oesophageal pressure and nasal pressure, but has seen little use in the normal clinical setting. However, any temporal measurement of the relaxation phase could be potentially useful for this objective. Our research using the M-mode ultrasound technique to gain an image of the muscle appears to confirm this hypothesis. The MVRdi (a variable that could be considered as an ultrasound expression of MRR) progressively diminished in all individuals as the ventilatory resistance test continued. Moreover, this decrease reached a minimum value at the moment of claudication, close to a mean reduction of 70% with respect to its initial value.

In every test for acute muscle overload, it is assumed that various different factors exist such as perceptual and neuropsychological factors. These can also have an effect on the completion of the test (learning the test, sensation of dyspnoea, perception of pressure, etc.).[27] In this first study in the field, we cannot ensure specifically that claudication was produced by diaphragmatic fatigue in all cases. However, both the reduction in initial PI max and its rapid recovery would indicate the presence of respiratory muscle fatigue.[5] In addition, our protocol of respiratory muscle overload was similar to that used in previous studies. These studies confirmed that claudication in these tests tends to be produced by respiratory muscle fatigue.[28,29] Therefore, we can reasonably suggest that the changes in MVRdi reflect changes associated with the development and disappearance of diaphragmatic fatigue.

Potential clinical implications. The findings from our study suggest that transthoracic ultrasound could have a clinically useful role in the non-invasive assessment of respiratory muscles, and of the diaphragm in particular. An added value of our study is that, until now, no technical alternatives have existed to assess the structure and function of the diaphragm in normal clinical practice. Following our study with healthy patients, we believe that it is necessary to assess the reproducibility and applicability of the ultrasound of the diaphragm in patient care, in particular for those with a high risk of diaphragm dysfunction. The possibility that ultrasounds of the diaphragm could provide new information on the relationship between muscle overload and pathologies such as malnutrition, Chronic Obstructive Pulmonary Disease (COPD), chronic heart failure, kyphoscoliosis, and acute situations that produce a high risk of failure in this muscle (such as weaning from mechanical ventilation) is very promising.

Although the proposed variables (Tdi for thickness and MVRdi for functional competence) are non-invasive and relatively easy to measure, there are some considerations that limit their immediate

application in the clinical setting. One of these is related to the absence of information with regard to the inter-day reproducibility of the results in a single patient. There is also a lack of studies that assess the applicability of the technique in other situations of diaphragmatic overload, such as general exercise, acute or chronic situations of hypoxemic or hypercapnic stimuli, and situations of exogenous ventilatory overload (e.g. sepsis). Another potential limitation of diaphragm ultrasound lies in the difficulty of performing the procedure in certain situations, such as some thoracic conditions (kyphoscoliosis, pulmonary emphysema), secuelae from pneumonectomy, subjacent pneumonia, and pleural effusion. We are unaware of the degree to which body position affects the reliability of the ultrasound technique used for assessing the diaphragm. Finally, severe lung hyperinflation can produce caudal displacement of the zone of apposition, making transverse access to the flattened diaphragm very difficult. However, this limitation seems unlikely, since the thickness of the diaphragm in our study was easily measured even at TLC in all individuals. We must also mention that echocardiography is an operator-dependent technique. This characteristic forms part of the limitations of the study. We have included the inter- and intra-observer correlation analyses, which have excellent results in terms of concordance. One must keep in mind that this concordance is analysed by measurements obtained by specialists in this technique. As such, we consider it to be imperative that specific training be given before it enters into general use (fig. 6).

Conclusions

This study shows that transthoracic ultrasound imaging of the diaphragm is a non-invasive method that allows for a generic structural assessment to be performed based on muscle thickness in the different phases of the contraction-relaxation cycle. Moreover, the M-mode technique allows for a promising estimate of the functional state of the diaphragm using maximum velocity of relaxation as a potential estimator of the risk of inspiratory claudication under strain. These findings have obvious physiopathological interest and could be very useful in the context of clinical health care.

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Figure 6. Bland and Altman Graphs of the concordance in inter-observer measurements. The Tdi values for multiple lung volumes resulted in an intra-observer ICC value of 0.996 (95% CI, 0.991-0.998) and the inter-observer value was 0.992 (95% CI, 0.992-0.998) (A). The inter-observer analysis for MVRdi values resulted in an ICC of 0.859 (95% CI, 0.658-0.943) (B).

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