

Time constant of inspiratory muscle relaxation in cystic fibrosis

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BACKGROUND: Patients with cystic fibrosis (CF) are at increased risk of inspiratory muscle fatigue and respiratory failure. The time constant (τ) of the inspiratory muscle relaxation is a simple bedside test of muscle fatigue. We have compared patients with CF and healthy controls regarding τ and hypothesized that it is negatively associated with severity of lower airway obstruction.

METHODS: For this cross-sectional study, τ after maximal inspiration and spirometric indices (forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC)) were measured.

RESULTS: Fifty-three CF patients (median age 14 y (interquartile range: 11–19.5)) and 53 age- and sex-matched healthy control subjects (14 y (11–19.5)) were recruited. Application of a general linear model revealed that health status (CF vs. non-CF) had a significant effect on τ ($P < 0.001$), but age group and the interaction of age group with health status did not have significant effects on τ ($P = 0.10$ and $P = 0.71$, respectively). Participants with CF had significantly higher τ (253 (188–406)) than control subjects (117 (81–185)) ($P < 0.001$) and τ was negatively related to FEV₁ ($r = -0.205$; $P = 0.031$) and FVC ($r = -0.294$; $P = 0.002$).

CONCLUSION: Patients with CF have higher τ than healthy controls but the correlation of τ with expiratory flow function is modest.

Respiratory failure constitutes the commonest cause of death in patients with cystic fibrosis (CF). Although respiratory failure in patients with CF primarily results from lower airway and lung parenchyma failure, respiratory muscle dysfunction significantly contributes to the development and the severity of the clinical picture. Respiratory muscle fatigue has been attributed to lower airway obstruction (1), lung hyperinflation (2), malnutrition (3), and chronic respiratory infection (4,5). Early recognition of respiratory muscle impairment may allow timely application of strategies aimed at preserving the functional integrity of the respiratory muscles, such as inspiratory muscle training (6) and noninvasive ventilation (7). Respiratory muscle dysfunction can be evaluated using a variety of measures, such as maximal respiratory pressures (8), the tension-time index of respiratory muscles (9), diaphragmatic electromyography (10), and inspiratory muscle endurance during exercise (11). However, the majority of these measures

require the use of complex laboratory equipment and methodology and frequently involve invasive placement of catheters or electrodes, or repeated maximal forced manoeuvres, which could be challenging for young or unwell patients with CF.

Evaluation of the time constant of muscle relaxation (τ , tau) may potentially be an alternative method for the assessment of respiratory muscle fatigue in patients with CF, which can be easily measured by portable manometry. The basic principle on which the method is based is that when skeletal muscles contract against increased external loads, their relaxation slows as a result of ATP depletion (12). ATP depletion affects the function of the calcium pumps in the sarcoplasmic reticulum (13) and results in slower calcium uptake by muscle fibers (14). In terms of physical properties, fatigued muscle fibers take longer to relax after contraction and this reduction in the relaxation rate can be quantified by an increase in τ . Rising τ values signal worsening inspiratory muscle function and potentially future respiratory failure.

τ has not been previously studied in patients with CF. In the current investigation, we hypothesized that: (i) patients with CF have higher τ than healthy controls; and (ii) τ is negatively correlated with expiratory flow function (i.e., spirometric) indices and somatic muscular indices. A secondary objective of this study was to assess whether τ can be utilized as a predictor of future hospitalization(s).

RESULTS

Patients and Age Groups

During the study period, 53 subjects were recruited in the study. The control group consisted of 53 healthy individuals matched to patients for age and gender. Ages ranged from 7 to 34 y. Anthropometric, nutrition, pulmonary function and respiratory muscle function data in the CF and control groups, and relevant statistical comparisons are summarized in **Table 1**. The two study groups did not differ in terms of maximal inspiratory pressure (P_{imax}).

Effects of CF Status and Age on τ

Values for τ according to age and health status are summarized in **Table 2**. Patients with CF had higher τ than healthy controls ($P < 0.001$), but the 7–11-y-old group did not differ from the 12–34-y-old group in terms of τ ($P > 0.05$). Application of the general linear model revealed that health

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Table 1. Anthropometric, nutrition, pulmonary function, and respiratory muscle function data in the CF and the control groups

	Healthy subjects n = 53	Cystic fibrosis n = 53	P value
Male gender (%)	31 (58.5)	29 (54.7)	NS
Age, years	14 (11–19.5)	14 (11–19.5)	NS
Height, cm	161 (147–172)	156 (140–165)	0.045
Weight, kg	53 (43–64)	48 (35–59)	NS
BMI, z-score	0.53 (-0.41 to 0.93)	0.14 (-0.63 to 0.72)	NS
MAMC, cm	25.0 (23.0–27.0)	22.0 (19.0–24.5)	<0.001
TST, mm	14 (10–20)	12 (9–15)	0.036
UAMA, mm ²	3225 (2664–4047)	2449 (1855–3257)	<0.001
FVC, z-score	N/A	-0.90 (0.33 to -3.03)	N/A
FVC, % predicted	N/A	96 (72–111)	N/A
FEV ₁ z-score	N/A	-1.74 (-0.38 to -3.97)	N/A
FEV ₁ % predicted	N/A	88 (61–101)	N/A
FEF _{25–75%} z-score	N/A	-2.36 (-0.02 to -4.05)	N/A
FEF _{25–75%} % predicted	N/A	52 (29–85)	N/A
RR	18 (14–22)	18 (15–23)	NS
TV, L	0.605 (0.440–0.795)	0.460 (0.370–0.700)	0.025
P _{imax} , cmH ₂ O	86 (61–108)	74 (61–100)	NS
T	117 (81–185)	253 (188–406)	<0.001

Continuous data are presented as median (interquartile range). FEF_{25–75%}, forced expiratory flow between 25 and 75% of vital capacity; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; MAMC, midarm-muscle-circumference; RR, respiratory rate; TST, triceps-skinfold-thickness; TV, tidal volume; UAMA, upper arm muscle area.

Table 2. Values of τ in younger children aged 7–11 y and older children and young adults aged 12–34 y in the CF and the control groups

	Healthy subjects		P value	CF		P value
	Age 7–11 y; n = 17	Age 12–34 y; n = 36		Age 7–11 y; n = 17	Age 12–34 y; n = 36	
τ	92 (71–137)	130 (94–211)	0.053	229 (144–388)	284 (204–407)	0.223

Data are presented as median (interquartile range). CF, cystic fibrosis.

status (CF vs. non-CF) had a significant effect on τ ($P < 0.001$; **Figure 1**), but age group and the interaction of age group with health status did not have significant effects ($P = 0.10$ and $P = 0.71$, respectively).

Effects of Anthropometric Characteristics, Nutritional Status, Somatic Muscle Mass, and Spirometric Indices on τ

When the whole cohort was analyzed, there was no significant relation between τ and BMI z-score, upper arm muscle area (UAMA), respiratory rate (RR), or tidal volume (TV). When only the CF group was considered, there was a significant negative relation between τ and forced expiratory volume in 1 sec (FEV₁) z-score ($r = -0.205$, $P = 0.031$) and between τ and forced vital capacity (FVC) z-score ($r = -0.294$, $P = 0.002$)

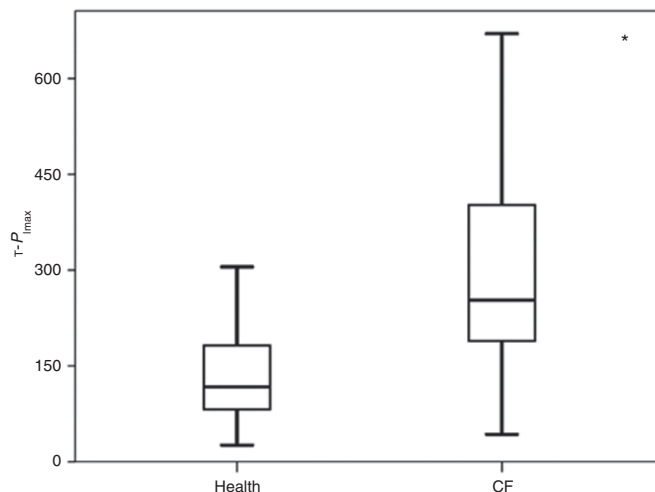


Figure 1. Time constant of relaxation of the inspiratory muscles after a forced maximal inspiration (τ) in health and in cystic fibrosis. Horizontal lines represent the 5th, 25th, 50th, 75th, and 95th percentile of τ values. * $P < 0.001$.

but not between τ and BMI z-score, UAMA, RR, TV, or forced expiratory flow between 25 and 75% of vital capacity (FEF_{25–75%}).

High FEV₁, τ , and Risk for Future Hospitalization

During the 12 mo following respiratory measurements, 27 (50.9%) subjects were hospitalized at least once due to exacerbation of respiratory symptoms. Receiver operating characteristic curve analysis to assess the performance of τ in predicting hospital admission produced an area under the curve of 0.579. The 90th centile of τ in the control participants was equal to 289 and this value was used to define τ as high or low in participants with CF. Twenty-one of 53 (39.6%) subjects with CF had high τ and there was no significant association between presence of high τ and future hospitalization (odds ratio (OR) = 1.56 (95% confidence interval (CI): 0.80–3.04); $P = 0.186$). Twenty-six (49.1%) of subjects with CF had low FEV₁. Although low FEV₁ was associated with future hospitalization (OR = 2.48 (95% CI: 1.31–4.70); $P = 0.002$), there was no effect of the interaction between τ and FEV₁ on the risk of hospitalization (OR = 0.99 (95% CI: 0.99–1.01); $P > 0.05$).

DISCUSSION

We have demonstrated that patients with CF have higher τ of the inspiratory muscle relaxation after forced inspiration than age- and sex-matched control subjects without lung disease. Furthermore, we have found that in patients with CF the more severe the airway obstruction the higher the τ values or in other words the slower the relaxation of inspiratory muscles following a maximum inspiratory effort against an occluded airway. However, this correlation was only modest, suggesting that factors other than the severity of lower airway obstruction may be important determinants of τ . Assessment of inspiratory muscle relaxation in patients with CF by measurement of the time constant of relaxation has not been reported in the literature previously.

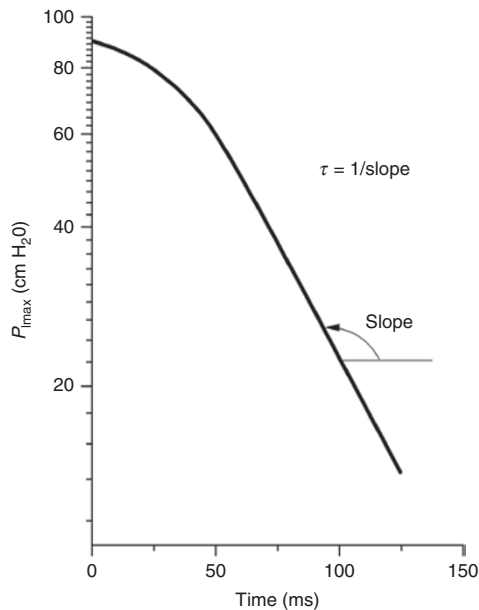


Figure 2. Ln-transformed P_{imax} vs. time: τ is calculated from the lower 60% portion of the curve when the decay is monoexponential ($\tau = 1/\text{slope}$).

When inspiratory muscle function was evaluated in subjects without CF based on measurement of τ , increased values were demonstrated following induced inspiratory muscle fatigue (15–18). In agreement with τ measurements in healthy participants of the present cohort, τ values in the range of 50–100 have been found in other reports, both pre- and postinduction of fatigue (15,17,18). Of note, our subjects with CF had much higher τ values than healthy control participants, potentially suggesting that these patients are at increased risk of inspiratory muscle fatigue and respiratory failure compared to the general population.

Determinants of respiratory muscle compromise in CF have been reported in detail (1–3,9,19,20). Airway obstruction impacts on inspiratory muscle function by increasing the load against which the respiratory muscles are forced to operate (1) and hyperinflation places the respiratory muscles in mechanical disadvantage (2). Moreover, malnutrition affects the diaphragmatic pressure-generating capacity (20), given that decreased somatic muscle mass indices have been consistently associated with decreased maximal respiratory pressures in CF patients (3,9). Furthermore, chronic respiratory infection (5) and nutritional deficiencies (19) might also be implicated in the pathophysiology of respiratory muscle dysfunction in CF. Our study identified that FEV_1 and FVC were associated only modestly with τ in CF. The relatively modest association of τ with FEV_1 and FVC implies that high values of τ and possible respiratory failure are determined by additional factors other than spirometry. Thus, τ might be conveying information that is not readily available through other methods, and has the potential to fit an unmet clinical need in managing patients with CF.

Inspiratory muscle impairment in patients with CF has been studied in the literature using maximal respiratory pressures (8), diaphragmatic electromyography (10), the tension-time

index of the respiratory muscles (2,3,9), and endurance testing by breathing against increased external loads (11,21,22). Although maximal respiratory pressures describe the pressure-generating capacity of the respiratory muscles at a given point of time, they would not be appropriate for the assessment or prediction of respiratory muscle function over time (14). Surface diaphragmatic electromyography can be affected appreciably by electrical “noise” from neighboring or underlying muscle groups or by levels of subcutaneous fat. Moreover, the noninvasive tension-time index of the respiratory muscles has been explored in CF patients in a number of studies and high values of TT_{mus} have been reported, also indicating an increased risk of inspiratory muscle fatigue and respiratory failure (2,3,9). Unfortunately, measurement of the tension-time index requires the utilization of pneumotachograph and differential-pressure transducers, i.e., a technical setup which is only found in specialized research laboratories. Direct measurements of respiratory muscle endurance via breathing against imposed external resistances would be a laborious task for patients with CF who are already using an appreciable fraction of their reserves to cope with everyday activity. Finally, portable equipment can be used to assess respiratory muscle function by measurement of the maximum relaxation rate (23), an index whose utility might be questionable due to the limited number of integer values that it attains in health and in CF. For these reasons, we suggest that measurement of τ , for the assessment of inspiratory muscle function in CF patients has considerable advantages.

In terms of clinical applicability, measurement of τ can be easily performed at the bedside and in the community and bears minimal running cost. Serial determination of τ , especially in patients with severe airway obstruction, could possibly allow timely implementation of therapeutic interventions for unloading the respiratory muscles and increasing inspiratory muscle efficiency. Inspiratory muscle training (6), aerobic exercise (24), and noninvasive ventilation (7) are good examples of such interventions. Respiratory failure in patients with CF is the combined result of lower airway, lung parenchymal and respiratory muscle dysfunction, making abnormal τ values a plausible predictor of respiratory failure in these patients (25). However, the specific values of τ which would bear clinical implications or predict fatigue of the inspiratory muscles, respiratory failure and need for mechanical ventilation in CF patients have not been studied in the literature so far.

Although increased τ values would likely have a bearing on severity of illness and probably aggressiveness of therapy at the time of admission, in our cohort of patients with CF, high τ was not a significant predictor of hospital admission due to exacerbation of respiratory symptoms. We propose that risk of hospital admission might be determined not only by τ values but also by the interrelation of τ , nutritional status and expiratory flow function as reflected by spirometric indices. In addition, other processes including lung tissue damage and remodeling, asynchronous and ineffective breathing patterns, and alveolar recruitment may also contribute to respiratory failure in CF. Thus, further research should clarify the relative contribution

of these factors in predicting hospital admission. For example, further research in this field might aim in determining a fatigue threshold for τ via regression analysis of τ against validated indices such as the tension-time index of the respiratory muscles, for which a fatigue threshold has already been established (26), or via measuring τ in patients with impending or established respiratory failure prior to initiation of mechanical ventilation. Additionally, future studies could further clarify the effect of lung hyperinflation and chronic airway infection on τ .

In conclusion, this study has demonstrated slower relaxation of inspiratory muscles in patients with CF relative to healthy control subjects but a modest association of inspiratory muscle dysfunction with the severity of lower airway obstruction. Regular determination of τ is feasible and could potentially yield important information on the state of respiratory muscle function longitudinally.

METHODS

Subjects

CF patients attending regularly the clinic at the Cystic Fibrosis Center of the Aghia Sophia Children's Hospital (Athens, Greece) were eligible for recruitment from January until October 2010. The control group consisted of healthy children and young adults without respiratory problems matched for age and gender that were enrolled at the general pediatrics outpatient clinics. CF diagnosis was based on positive sweat chloride test (sweat chloride concentration > 60 mEq/l) and expanded mutation analysis. Exclusion criteria for participation in the study were: (i) acute exacerbation within 30 d prior to the assessment; (ii) use of systemic corticosteroids; and (iii) inability to provide reproducible flow-volume curves in spirometry. Height and weight were measured and BMI z-score was calculated (27). UAMA was calculated from the triceps-skinfold-thickness and the midarm-muscle-circumference (28).

The study protocol was approved by the Aghia Sophia Children's Hospital Research Ethics Committee. Informed written consent was obtained from all adult patients and all parents of children who were <18 y of age. Consent was obtained from older children and adolescents.

Maneuvers Performed by CF Patients and Control Participants

Following measurements of BMI and UAMA, all participants underwent respiratory muscle testing. Testing was completed in the morning hours, with the subject in the sitting position and while wearing a nose clip. During the evaluation, both CF patients and control subjects were in stable clinical condition. RR and TV were measured with a pneumotachograph (Mercury F100L, GM Instruments, Kilwinning, Scotland) connected to a differential pressure transducer (DP45, range \pm 3.5 cm H₂O, Validyne, Northridge, CA). P_{imax} was measured from a side port on the pneumotachograph, using a differential pressure transducer (DP45, range \pm 225 cm H₂O, Validyne). The signals from the differential pressure transducers were amplified, using a carrier amplifier (Validyne CD 280, Validyne). The amplified signals were recorded and displayed in real time on a computer running Labview software (National Instruments, Austin, TX) with analog-to-digital sampling at 100 Hz (16-bit NI PCI-6036E, National Instruments).

τ was assessed using a handheld respiratory manometer (MicroRPM, CareFusion, San Diego, CA). Each subject performed at least three reproducible maximal inspiratory manoeuvres, followed by normal quiet expiration, during which inspiratory muscle relaxation was assessed. P_{imax} was measured while performing a maximal inspiratory effort from residual volume against an occluded airway. The occlusions were performed with a unidirectional valve (Intersurgical, Berkshire, UK) connected to the mouthpiece (total dead-space 8 ml). Care was taken to eliminate any leak around the mouthpiece; a small circuit leak allowed for avoidance of glottic closure (14). Only

maximal pressure manoeuvres with plateau pressure of at least 1 s were accepted for subsequent analysis (14). In addition to respiratory muscle testing, patients with CF underwent spirometry according to the European Respiratory Society guidelines (MasterScreen Pulmonary Function Analyzer; Jager AG, Wurzburg, Germany) (29).

Calculation of τ

τ was assessed after maximal inspiration against an occluded airway performed as described above, using the PUMA PC analysis software (Carefusion). τ is a unitless variable and is equal to the reciprocal of the slope of the ln-transformed pressure decline as a function of time at the lower 60% part of the curve (Figure 2). The slope is defined as the limiting ratio of the logarithmic pressure difference between two different times over the time interval separating these two instances. It is equal to the tangent of the angle that the tangent to the graph makes with the horizontal time axis. At the lower part of the curve, the pressure decay is monoexponential and the trace follows a straight line (14). The PUMA software automatically traced and selected the slope, and τ was expressed in absolute values. Only those pressure traces with smooth pressure decay were studied. For each subject, the mean τ value of at least three maximal reproducible manoeuvres was recorded.

Spirometry

FEV₁, FEF₂₅₋₇₅, and FVC were measured in patients with CF. Spirometric indices were expressed as z-scores (30). Spirometry was completed without administration of a bronchodilator.

Primary and Secondary Outcome Measures and Explanatory Variables of the Study

τ was the primary outcome measure of the study. Health status (CF vs. non-CF), age, BMI z-score, UAMA, RR, TV, FEV₁, FEF₂₅₋₇₅, and FVC were explanatory variables that were assessed for their potential effect on the primary outcome measure. Patients with CF were followed for 1 y after completion of measurements to record hospitalizations due to respiratory symptom exacerbation according to the clinical impression of the CF attending physician. Subsequently, τ was evaluated as predictor of at least 1 hospitalization for respiratory symptom exacerbation during the 12 mo following respiratory muscle testing.

Sample Size Calculation

The sample size calculation was based on the assumption that a difference in τ between CF patients and controls equal to 26 was clinically significant (15). The SD obtained from pilot data collection was equal to 30. The required sample size to detect an increase in τ of 26 with 95% power at the 1% level of statistical significance was 48 subjects in each study group.

Data Analysis

CF subjects and controls were compared regarding anthropometrics, somatic muscle indices, P_{imax} , τ , and breathing cycle components. Data were tested for normality with the Kolmogorov-Smirnov test and by visual inspection of their distribution curves. Differences between the two study groups or between younger (7–11-y old) and older participants (12–34-y old) were tested for significance using the Mann-Whitney rank sum test or χ^2 test, as appropriate. Since younger children have significantly different respiratory muscle function than older children and young adults (9), a general linear model was applied to evaluate the possible effect of: (i) health status (CF diagnosis vs. non-CF); (ii) age group (7–11 y of age vs. 12–34 y of age); and (iii) the interaction of health status with age, on τ . Kendall-tau rank correlation coefficient was used to examine the relationship of τ with BMI z-score, UAMA, RR, and TV in both patients with CF and control participants. For CF patients only, the correlations of τ with FVC z-score, FEV₁ z-score, and FEF₂₅₋₇₅ z-score were also assessed.

The performance of τ in predicting hospital admission due to respiratory exacerbation was assessed by receiver operating characteristic curve analysis. FEV₁ z-score \geq 1.64 was considered normal and <1.64 was defined as low (30). In addition, the 90th centile of τ in the control participants was used as cut-off value to define τ in CF patients as high or low. OR and the corresponding 95% CI for at least 1 hospitalization due to respiratory symptoms exacerbation occurring over the year following respiratory measurements in CF subjects with

high vs. low τ was calculated (univariate logistic regression analysis). Multivariable logistic regression analysis was carried out to explore the effects of FEV₁ z-score and the interaction between FEV₁ z-score and τ on the risk of hospitalization. P values less than 0.05 were considered significant. Statistical analysis was performed using SPSS 17.0 (SPSS, Chicago IL).

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REFERENCES

- Hart N, Polkey MI, Clément A, et al. Changes in pulmonary mechanics with increasing disease severity in children and young adults with cystic fibrosis. *Am J Respir Crit Care Med* 2002;166:61–6.
- Hayot M, Guillaumont S, Ramonatxo M, Voisin M, Préfaut C. Determinants of the tension-time index of inspiratory muscles in children with cystic fibrosis. *Pediatr Pulmonol* 1997;23:336–43.
- Hahn A, Ankermann T, Claass A, Mann M, Lindemann H, Neubauer BA. Non-invasive tension time index in relation to severity of disease in children with cystic fibrosis. *Pediatr Pulmonol* 2008;43:973–81.
- Divangahi M, Matecki S, Dudley RW, et al. Preferential diaphragmatic weakness during sustained Pseudomonas aeruginosa lung infection. *Am J Respir Crit Care Med* 2004;169:679–86.
- Dassios TG, Katelari A, Doudounakis S, Dimitriou G. Chronic Pseudomonas aeruginosa infection and respiratory muscle impairment in cystic fibrosis. *Respir Care* 2014;59:363–70.
- Enright S, Chatham K, Ionescu AA, Unnithan VB, Shale DJ. Inspiratory muscle training improves lung function and exercise capacity in adults with cystic fibrosis. *Chest* 2004;126:405–11.
- Fauroux B. Why, when and how to propose noninvasive ventilation in cystic fibrosis? *Minerva Anestesiol* 2011;77:1108–14.
- Heinzmann-Filho JP, Marostica PJ, Donadio MV. Ventilatory muscle strength in cystic fibrosis patients: a literature review. *Monaldi Arch Chest Dis* 2012;77:134–8.
- Dassios T, Katelari A, Doudounakis S, Mantagos S, Dimitriou G. Respiratory muscle function in patients with cystic fibrosis. *Pediatr Pulmonol* 2013;48:865–73.
- Reilly CC, Jolley CJ, Elston C, Moxham J, Rafferty GF. Measurement of parasternal intercostal electromyogram during an infective exacerbation in patients with cystic fibrosis. *Eur Respir J* 2012;40:977–81.
- Leroy S, Perez T, Neviere R, Aguilaniu B, Wallaert B. Determinants of dyspnea and alveolar hypoventilation during exercise in cystic fibrosis: impact of inspiratory muscle endurance. *J Cyst Fibros* 2011;10:159–65.
- Edwards RH, Hill DK, Jones DA. Metabolic changes associated with the slowing of relaxation in fatigued mouse muscle. *J Physiol* 1975;251:287–301.
- Westerblad H, Lännergren J. Slowing of relaxation during fatigue in single mouse muscle fibres. *J Physiol* 1991;434:323–36.
- ATS/ERS. Statement on respiratory muscle testing. *Am J Respir Crit Care Med* 2002;166:518–624.
- Esau SA, Bye PT, Pardy RL. Changes in rate of relaxation of sniffs with diaphragmatic fatigue in humans. *J Appl Physiol Respir Environ Exerc Physiol* 1983;55:731–5.
- Aubier M, Murciano D, Lecocguic Y, Viires N, Pariente R. Bilateral phrenic stimulation: a simple technique to assess diaphragmatic fatigue in humans. *J Appl Physiol* (1985) 1985;58:58–64.
- Mador MJ, Kufel TJ. Effect of inspiratory muscle fatigue on inspiratory muscle relaxation rates in healthy subjects. *Chest* 1992;102:1767–73.
- Wilcox PG, Eisen A, Wiggs BJ, Pardy RL. Diaphragmatic relaxation rate after voluntary contractions and uni- and bilateral phrenic stimulation. *J Appl Physiol* (1985) 1988;65:675–82.
- Gontijo-Amaral C, Guimarães EV, Camargos P. Oral magnesium supplementation in children with cystic fibrosis improves clinical and functional variables: a double-blind, randomized, placebo-controlled crossover trial. *Am J Clin Nutr* 2012;96:50–6.
- Hart N, Tounian P, Clément A, et al. Nutritional status is an important predictor of diaphragm strength in young patients with cystic fibrosis. *Am J Clin Nutr* 2004;80:1201–6.
- Keens TG, Krastins IR, Wannamaker EM, Levison H, Crozier DN, Bryan AC. Ventilatory muscle endurance training in normal subjects and patients with cystic fibrosis. *Am Rev Respir Dis* 1977;116:853–60.
- Orenstein DM, Franklin BA, Doershuk CF, et al. Exercise conditioning and cardiopulmonary fitness in cystic fibrosis. The effects of a three-month supervised running program. *Chest* 1981;80:392–8.
- Dassios TG, Doudounakis S, Dimitriou G. Maximum rate of pressure development and maximal relaxation rate of respiratory muscles in patients with cystic fibrosis. *Respir Care* 2013;58:474–81.
- Dassios T, Katelari A, Doudounakis S, Dimitriou G. Aerobic exercise and respiratory muscle strength in patients with cystic fibrosis. *Respir Med* 2013;107:684–90.
- Dassios T. Determinants of respiratory pump function in patients with cystic fibrosis. *Paediatr Respir Rev* 2015;16:75–9.
- Ramonatxo M, Boulard P, Préfaut C. Validation of a noninvasive tension-time index of inspiratory muscles. *J Appl Physiol* (1985) 1995;78:646–53.
- Kuczumski RJ, Ogden CL, Guo SS et al. 2000 CDC Growth Charts for the United States: methods and development. *Vital Health Stat* 2002;11:1–190.
- Frisancho AR. New norms of upper limb fat and muscle areas for assessment of nutritional status. *Am J Clin Nutr* 1981;34:2540–5.
- Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993;16:5–40.
- Quanjer PH, Stanojevic S, Cole TJ, et al.; ERS Global Lung Function Initiative. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012;40:1324–43.