ONLINE SUPPLEMENTARY MATERIAL

METHODS

Table S1. Characteristics of participating centres

Participating centre	Region in Spain	City	Evaluator in charge of maximal respiratory pressures	Recruited volunteers (females/males); n (%)
Faculty of Physiotherapy, University of A Coruña	Galicia	A Coruña	Ana Lista Paz Margarita Barral Fernández	(36/42); 77 (12.6)
FCS Blanquerna, Ramon Llul University	Catalonia	Barcelona	Jordi Vilaró Casamitjana Margarita Barral Fernández	(24/25); 49 (8)
Paraplegics National Hospital of Toledo	Castilla-La Mancha	Toledo	Pilar Bravo Cortés	(26/23); 49 (8)
University Hospital of A Coruña	Galicia	A Coruña	Esther Giménez Moolhuyzen Margarita Barral Fernández	(26/21); 47 (7.7)
Faculty of Health Sciences, University of Malaga	Andalusia	Málaga	Ana Lista Paz	(24/23); 47 (7.7)
Faculty of Health Science, University of Deusto	Basque Country	Donostia-San Sebastián	Ane Arbillaga Etxarri Margarita Barral Fernández	(23/23); 46 (7.5)
Doce de Octubre University Hospital	Community of Madrid	Madrid	Esther García Delgado	(24/21); 45 (7.4)
ONCE University School of Physiotherapy	Community of Madrid	Madrid	Cristina Serrano Veguillas	(19/22); 41 (6.7)
Canarias University Hospital	Canary Islands	Santa Cruz de Tenerife	Carolina González Montañez Margarita Barral Fernández	(22/19); 41 (6.7)
General University Hospital Santa Lucía	Region of Murcia	Cartagena	Margarita Barral Fernández	(20/19); 39 (6.4)
San Jorge University	Aragon	Zaragoza	Marina Francín Gallego Margarita Barral Fernández	(20/18); 38 (6.2)
University of Córdoba	Andalusia	Córdoba	Margarita Barral Fernández	(18/18); 35 (5.7)
Son Espases University Hospital	Balearic Islands	Palma de Mallorca	José Luis Varela Felices	(19/14); 33 (5.4)
Hospital Clínic	Catalonia	Barcelona	Elena Gimeno Santos Rodrigo Torres Castro Margarita Barral Fernández	(14/9); 23 (3.8)

Maximal respiratory pressure assessment protocol

Maximal respiratory pressures were assessed using a MicroRPM® portable digital manometer (Vyaire Medical GmbH, Hoechberg, Germany) with an operating interval range of ±300 cmH_2O and a precision of ±3%. This device has shown good reliability for measuring maximal inspiratory and expiratory pressure (PImax/PEmax) measurements[1] and has been previously used in the determination of other reference equations[2-4]. According to manufacturer's instructions, calibration is factory-set and should remain stable indefinitely, so daily calibration was not needed. In four centres where the manometer was already in place and employed in regular clinical practice for more than one year, a factory calibration was performed to ensure the accuracy of the measurements. PImax/PEmax assessments were performed according to American Thoracic Society and European Respiratory Society (ATS/ERS) recommendations[5,6] and following the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR) protocol[7]. All measurements were taken with the patient in a sitting position using a flanged mouthpiece and nose-clips. PImax/PEmax were performed in a random order, starting from residual volume and total lung capacity, respectively. No visual feedback was provided to the volunteers during the manoeuvres. All participants were vigorously encouraged to make maximal efforts during the tests. Pressures were sustained for 3-5 seconds, and one-second plateau pressure was taken as PImax/PEmax. During PEmax, subjects were asked to hold their cheeks rigid with their hands to avoid leaks and to minimize buccinator muscle contribution. A maximum of 10 repetitions was performed for PImax/PEmax, with a minimum of six acceptable manoeuvres (i.e., without air leaks and with the graph showing a trend to a plateau), three with a variability of <5% (repeatability criteria). The participants had to rest for one minute between each repetition of PImax/PEmax and at least five minutes before changing the pressure measurement. The highest value of the three reproducible manoeuvres was selected. Even when PImax was a negative relative to ambient /atmospheric pressure, we reported it as a positive value for better data interpretation, as in former studies.

Training of assessors and quality control

The thirteen evaluators in the different sites were trained in the protocol procedures by the principal investigator (ALP) during an in-person meeting. Video tutorials of each measurement performed by ALP were also sent to the team *(see Supplementary online video material: https://youtu.be/BMzHJTRYXjE)*. At the end of the training, each evaluator had to perform a pilot study with at least five healthy subjects. A video of the last pilot subject was sent to ALP. Only after the entire process was seen to be correctly performed was the evaluator allowed to start with the measurements. The subjects included in the pilot study are not part of the final sample. To increase the quality control of the study, ALP performed a quality check, observing at least two subject evaluations *in situ*. All the graphs recorded for each subject were visualized by ALP to avoid any bias in selecting the PImax/PEmax manoeuvre. At that point, if the recording had not fulfilled the criteria of the aforementioned protocol, the data were eliminated during the data-cleaning process.

Statistical analysis

Continuous variables are presented as means (standard deviation [SD]), while categorical values are shown as absolute values and percentages. When necessary, the normality assumption of continuous variables was verified numerically using the Shapiro-Wilk test, and graphically using Kernel density estimates[8].

An ANOVA test between nested models was performed to choose the most suitable equation. Moreover, an additional comparison model was constructed using cross-validation techniques[9]. These are a set of methods for measuring the performance of a predictive model, by randomly splitting the data into a training data set and a test data set. Firstly, models are fitted to the training data set, and the predicted values are then checked with the validation set. The preferred model is that which produces the best prediction performance.

We also aimed to define cut-off limits for respiratory muscle weakness by using T-scores of ≥2.5 SD below average peak maximal respiratory pressures achieved at a young age. For that purpose, we used a recent method used by Dodds et al.[10] to define sarcopenia by establishing cut-off limits of T-scores ≥2.5 SD below the peak mean value in handgrip strength achieved at a young age (i.e. 27 kg for males and 16 kg for females). Firstly, sex-specific cross-sectional centiles for PImax/PEmax were produced[10]. Cut-off values for both females and males are derived from a T-score. We computed the mean (SD) to identify the interval of ages where maximum average values were observed. Values 2.5 SD or below these mean average values were taken to represent respiratory muscle weakness. We considered widths of the whole age range (18-80 years) in classes with intervals varying from five to 15 years. The interval with the highest value for the pressure was selected, and complete sets of modal intervals were finally compared to obtain the most relevant age class to compute the maximum pressure and its SD. This procedure was applied for maximal respiratory pressures in females and males, respectively.

Table S2. Linear regression models as maximal respiratory pressure reference equations

	PImax Female				PImax Male				PEmax Female				PEmax Male			
Predictors	*Estimates*	*SEE*	*CI (95%)*	*p-value*	*Estimates*	*SEE*	*CI (95%)*	*p-value*	*Estimates*	*SEE*	*CI (95%)*	*p-value*	*Estimates*	*SEE*	*CI (95%)*	*p-value*
Intercept	61.48	14.57	32.80 – 90.16	**<0.001**	98.60	17.62	63.93 – 133.27	**<0.001**	74.75	19.32	36.74 – 112.75	**<0.001**	58.11	30.15	-1.23 – 117.45	0.055
Age	0.66	0.45	-0.24 – 1.55	0.150	1.18	0.52	0.15 – 2.21	**0.025**	1.67	0.60	0.49 – 2.86	**0.006**	3.71	0.90	1.95 – 5.48	**<0.001**
BMI	1.55	0.46	0.63 – 2.46	**0.001**	0.76	0.58	-0.39 – 1.91	0.193	1.75	0.62	0.54 – 2.96	**0.005**	2.64	1.00	0.66 – 4.61	**0.009**
Age^2	-0.01	0.00	-0.02 – -0.00	**0.010**	-0.02	0.01	-0.03 – -0.01	**0.001**	-0.02	0.01	-0.03 – -0.01	**0.001**	-0.04	0.01	-0.06 – -0.02	**<0.001**
Observations	314				294				313				293			
RSE	22.59				29.92				25.52				43.73			
R^2 / R^2 adjusted	0.128 / 0.119				0.157 / 0.148				0.074 / 0.065				0.091 / 0.081			

Values in columns indicate the estimated terms for the equation in each subgroup, the standard error of the estimate (SEE) of each coefficient, the 95% Confidence Intervals (CI) and p-values for testing the significance of each estimate.
Abbreviations: BMI: body mass index; PEmax: maximal expiratory pressure; PImax: maximal inspiratory pressure; R^2: coefficient of determination, RSE: Residual standard error; SEE: standard error of the estimate.
The reference equations are:
PImax (females)= 61.48 + 0.66 * age + 1.55 * BMI - 0.01 * age^2
PImax (males)= 98.60 + 1.18 * age + 0.76 * BMI – 0.02 * age^2
PEmax (females)= 74.75 + 1.67 * age + 1.75 * BMI – 0.02 * age^2
PEmax (males)= 58.11 + 3.71 * age + 2.64 * BMI – 0.04 * age^2

References

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