Annex 1 Supplementary methodological information

Inclusion criteria

- Age between 18 and 85 years
- Not being pregnant
- No history of smoking
- Being of Spanish-European offspring
- Not having been professional sportsman/woman (amateurs sportsman/woman were allowed if they had not taken part in any official competition in the last two years)
- No acute diseases within 3 month previous to the enrollment
- No chronic condition (except essential hypertension in individuals over 50 years only if they were just on one drug different form beta-blockers or calcium antagonists and did not present an hypertensive response to exercise)
- No past use of any respiratory or cardiac medicines or other drugs with potential muscle toxicity
- No current use of any medications except antacids or sleeping pills (apart from antihypertensive drugs as described above)
- No history of significant thoracic or locomotor trauma
- No thoracic pain
- Absence of respiratory symptoms according to the questionnaire of the European Community for Coal and Steel (ECSC) on respiratory symptoms(1),
- Cardiorespiratory physical examination without abnormalities
- Normal baseline and exercise ECG
- Exhaled carbon monoxide lower than 7 ppm or blood carboxyhemoglobin inferior to 2%
- Baseline arterial blood saturation > 96%
- Normal baseline spirometry
- Normal baseline blood cell count and hemoglobin according to local laboratories.

Measurements:

- **Total body mass** (kg) was established to the nearest 0.1 kg using a calibrated balance and **height** determined to the nearest 0.5 cm using a stadiometer; both measurements were performed following standardized techniques at each of the eight participating laboratories with the subjects in light clothes and without shoes.
- **Age** was defined as the difference between exercise test and birth date divided by 365.25. Spirometry was performed according to Spanish and ERS/ATS guidelines (2, 3).
- **Exhaled carbon monoxide or arterial blood carboxyhemoglobin** was measured before the exercise test with the available equipment at each center.

- **Physical activity** was estimated by the 27-question international physical activity questionnaire (IPAQ-27)(4)
- **Progressive exercise tests** were carried out on electromagnetically braked cycle-ergometers (see below) breath-by-breath using computer based exercise systems (see below). Cycle-ergometer seat adjustments, ECG monitoring and safety measures were standardized in accordance with published guidelines (5). Standard written and oral instructions were provided to the participants regarding communication during the test safety and what was expected from the subject along the test (5, 6). The incremental test consisted of a 2-minute resting period, followed by 3 minutes of unload pedaling after which the power was progressively increased. Finally 3 minutes of the recovery phase were also recorded. Patients wore a low dead-space face mask adapted to the size of their faces. The speed of the ramp was calculated for a test duration of about 10 minutes (5). Participants wore a low dead space face mask.
  The speed of the ramp was calculated according the following formula(4):

  - Unloaded oxygen uptake ($\dot{V}O_2$) ($l \cdot min^{-1}$) = 0.15+(0.006 x weight in kg)
  - estimated $\dot{V}O_2$ ($l \cdot min^{-1}$) =(height in cm –age in years) x0.02 (male) y 0.014 (female)
  - Ramp= ($\dot{V}O_2$ peak - $\dot{V}O_2$ unloaded) x 10

$\dot{V}O_2$ in $l \cdot min^{-1}$ and carbon dioxide production ($\dot{V}CO_2$) in $l \cdot min^{-1}$ were measured at standard temperature and pressure dry (STPD). Data were averaged every 10s and correction for the dead-space was applied. The last 10 s average was considered to be representative of the subject's peak $\dot{V}O_2$. Criteria for a peak test were the observation of a $\dot{V}O_2$ plateau or a respiratory exchange ratio (RER)> 1,1 together with a heart rate > 90% of predicted (220 - age in years) and the subjective observation by the conductors of a good subject performance.

- Quality control (QC) was carried out according to published recommendations(6) in summary after the system was warmed-up for 30 min a three point verification (at flow between 0,5 and 2 $l \cdot min^{-1}$ 4-6 $l \cdot min^{-1}$ and >8 $l \cdot min^{-1}$) of the linearity of the flow-meters and two point calibration check of the gas analyzers with precision gases (i.e error < 1%) and measurements of the time delay were performed. All this procedures were completed within the 30 minutes previous to the test. Monthly biological quality controls were performed every month during the study at each center according to standardized procedures(6) throughout the study. Values for $\dot{V}O_2$ peak /WR at 30 and 70 w had to be between 9 and 11 $ml^{-1} \cdot min^{-1} \cdot$ w A centralized QC reviewed the first BC for each lab before starting the study and all the biological controls thereafter.
- Statistical analysis: Scale variables are presented as mean with standard deviation (SD) within parenthesis and categorical variables as percentages with their standard error.
  - The equations for prediction were developed using a multivariate forward stepwise linear regression. Gender was coded as 1 for men and 0 for women. The model was set as follows: $Y = \beta 0 - \beta 1X1 - \beta 2X2 - \beta 3X3\ldots\ldots$, where Y = directly measured $\dot{V}O_2$ max; β = regression coefficient for each of the

independent variables. Bivariate interaction terms were initially included in the model. The removal procedure was carried out based on the minimum probability of a p-to-remove value of 0.05 for the variables and 0.01 for the interaction terms. For all data the coefficient of determination (R^2) is reported with the standard error of the estimate (SEE, i.e. the square root of the residual sum of squares/N-k-1, where k is the number of predictors). After the determination of regression equations, an investigation of possible violations of the normal model was performed with analysis of the studentized residuals. The presence of possible influential points was analyzed by their leverage and Cook's distance(7) Critical values were derived from appropriate tables accounting for the sample size and number of independent variables. After reviewing the values of influential cases none were excluded. Collinearity among independent variables was investigated by examining, the inflation factor of each variable (FIV) and the condition number (CN). Predetermined criteria to consider excessive collinearity were a FIV>10 and a CN>30(7).

- Agreement between our equation and other prediction equations was tested by Bland–Altman plots and intraclass correlation coefficient. For comparison of predicted $\dot{V}O_2$peak we used the widely used Hansen-Wasserman (8, 9) and the recently published equations from the Fitness Registry and the Importance of Exercise National Database (FRIEND Registry) for cycle-ergometer(10, 11).

o

Table 1 Systems and cycle-ergometers employed in this study

	System	Maker	Cycle-ergometer	Maker
Lab 1	Vyntus CPX	Carefusion (Yorba Linda, CA , USA)	VIA sprint 150 P	Ergoline (Bitz ,Germany)
Lab2	Oxyxon Pro	Carefusion (Yorba Linda, CA , USA)	VIA Sprint 150 P	Ergoline (Bitz ,Germany)
Lab 3	MasterScreen CPX	Carefusion (Yorba Linda, CA , USA)	VIA Sprint 150 P	Ergoline (Bitz ,Germany)
Lab 4	Ergocard Clinical	Medisoft (Sorinnes, Belgium)	Lode	Corival CPET Groninger, The Netherlands
Lab 5	MasterScreen CPX	Carefusion (Yorba Linda, CA , USA)	VIA Sprint 150 P	Ergoline (Bitz ,Germany)
Lab 6	CPX Breeze	Medgraphics (St Paul, MN, USA)	Ergoselect 100P	Ergoline (Bitz ,Germany)
Lab 7	QUARK PFT /Suite 10.0 b	COSMED (Rome, Italy)	Ergoline 900L	Ergoline (Bitz ,Germany)
Lab 8	CPX Breeze	Medgraphics (St Paul, MN, USA)	Ergoselect 100P	Ergoline (Bitz ,Germany)

1. Minette A. Questionnaire of the European Community for Coal and Steel (ECSC) on respiratory symptoms. 1987--updating of the 1962 and 1967 questionnaires for studying chronic bronchitis and emphysema. Eur Respir J. 1989;2(2):165-77.
2. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-38.
3. Garcia-Rio F, Calle M, Burgos F, Casan P, Del Campo F, Galdiz JB, et al. Spirometry. Spanish Society of Pulmonology and Thoracic Surgery (SEPAR). Arch Bronconeumol. 2013;49(9):388-401.
4. Kim Y, Park I, Kang M. Convergent validity of the international physical activity questionnaire (IPAQ): meta-analysis. Public Health Nutr. 2013;16(3):440-52.
5. ATS/ACCP Statement on cardiopulmonary exercise testing. Am J Respir Crit Care Med. 2003;167(2):211-77.
6. Wanger J. Cardiopulmonary Exercise Test. In: Society AT, editor. ATS Pulmonary Function Laboratory Management and Procedure Manual. Third ed. New York: American Thoracic Society; 2016.
7. K.E. KDGKLLM. Applied regression analysis and other multivariable methods Boston, MA, USA: PWS Publishing Co; 1988.
8. Hansen JE, Sue DY, Wasserman K. Predicted values for clinical exercise testing. Am Rev Respir Dis. 1984;129(2 Pt 2):S49-S55.
9. Wasserman K, Hansen JE, Sue DY, Stringer WW, Sietsema KE, Sun XG. Principles of Exercise Testing and Interpretation: Including Pathophysiology and Clinical Applications. Fifth ed. Philadelphia: Lippincott Williams & Wilkins; 2011 2011.
10. Kaminsky LA, Imboden MT, Arena R, Myers J. Reference Standards for Cardiorespiratory Fitness Measured With Cardiopulmonary Exercise Testing Using Cycle Ergometry: Data From the Fitness Registry and the Importance of Exercise National Database (FRIEND) Registry. Mayo Clin Proc. 2017;92(2):228-33.

11. Kokkinos P, Kaminsky LA, Arena R, Zhang J, Myers J. A new generalized cycle ergometry equation for predicting maximal oxygen uptake: The Fitness Registry and the Importance of Exercise National Database (FRIEND). Eur J Prev Cardiol. 2018;25(10):1077-82.