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Allometrically adjusted handgrip strength and chair stand test cut points to identify sarcopenia in older Portuguese adults

Pontos de corte da força de preensão manual e teste de sentar e levantar da cadeira ajustados alometricamente para identificar sarcopenia em idosos portugueses

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Abstract - Absolute muscle strength or adjusted by body mass index (BMI) are useful to identify sarcopenia. However, these values are not accurate for older adults with extreme body sizes because the nonlinear relationship between strength and body size. The purpose was to determine cut-off points for identify sarcopenia in older adults using allometric coefficients to normalise handgrip strength (HGS) and 30-sec chair stand test (30-s CST) by body size. Allometric exponents were proposed with log-linear models for body-size variables (body mass, height and BMI). The remotion of body-size effect on muscle strength with allometric normalisation was tested by partial correlation. Cut-off points for low muscle strength were established by ROC curve and Youden index considering functional limitation (six-minute walk test<400m). Allometric exponents provided for body-size variables range from -0.01 to 2.28 (HGS) and -0.27 to 0.21 (30-s CST). The effect of body size on muscle strength was removed with allometric normalisation (r<0.30). Cut-off points accuracy was always adequate (AUC≥0.78; p<0.001). In conclusion, cut-off points of HGS and 30-s CST allometrically normalised were proposed to identify sarcopenia in Portuguese older adults and allometry maintained adequate the accuracy (AUC>70%). Allometry removed influence of body size on the expression of HGS and 30-s CST and permits evaluate muscle strength regardless of body-size.

Keywords: Disability evaluation; Frailty; Functional status; Geriatric assessment; Mobility limitation; Multidimensional scaling analysis.

Resumo - A força muscular absoluta ou ajustada pelo índice de massa corporal (IMC) é útil para identificar a sarcopenia. No entanto, esses valores não são precisos para idosos com tamanhos corporais extremos devido à relação não linear entre força e tamanho corporal. O objetivo foi determinar os pontos de corte para identificar a sarcopenia em idosos usando coeficientes alométricos para normalizar a força de preensão manual (FPM) e teste de sentar e levantar da cadeira em 30 segundos (30-s CST) por tamanho corporal. Os expoentes alométricos foram propostos com modelos log-lineares para variáveis de tamanho corporal (massa corporal, estatura e IMC). A remoção do efeito do tamanho corporal na força muscular com normalização alométrica foi testada por correlação parcial. Os pontos de corte para baixa força muscular foram estabelecidos pela curva ROC e índice de Youden considerando a limitação funcional (teste de caminhada de seis minutos<400m). Os expoentes alométricos fornecidos para variáveis de tamanho corporal variam de -0,01 a 2,28 (FPM) e -0,27 a 0,21 (30-s CST). O efeito do tamanho corporal na força muscular foi removido com normalização alométrica (r<0,30). A precisão dos pontos de corte sempre foi adequada (AUC≥0,78; p<0,001). Em conclusão, foram propostos pontos de corte para FPM e 30-s CST normalizados alometricamente para identificar sarcopenia em idosos portugueses e a alometria manteve a precisão adequada (AUC>70%). A alometria removeu a influência do tamanho corporal na expressão da FPM e 30-s CST e permite avaliar a força muscular independentemente do tamanho corporal.

Palavras-chave: Avaliação da deficiência; Fragilidade; Estado funcional; Avaliação geriátrica; Limitação da mobilidade; Análise de escalonamento multidimensional.

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INTRODUCTION

Muscle strength tests have been widely applied to identify age-related conditions, such sarcopenia¹, which is associated with older adults' vulnerability, poor health outcomes, increased health-related costs and long-term care admission². Longitudinal analysis has shown that the identification of sarcopenia using handgrip strength (HGS) also predicts mobility limitations, a potential drivers of disability in older adults³. Despite its simplicity and low cost, HGS measurement requires a hand dynamometer, not always available in clinical practice and there are some results bias according to selected protocol⁴. In addition, current HGS reference values used to identify sarcopenia are based on absolute results⁵⁻⁷ or normalised by body mass index (BMI)⁸. Absolute (non-normalised) value is inaccurate in older adults with small body sizes (e.g., lighter or shorter)9-11. Even when these older adults sustained their motor independence they are misclassified as "low strength level" when compared to their heavier and taller peers from the non-normalised reference values¹². To overcome this problem, the utilization of the ratio standard, e.g., the ratio of HGS to body mass or BMI, has been proposed based on the relationship of muscle strength with the body-size indexes⁹⁻¹¹. However, this strategy overestimates/underestimates the real strength of light/short or tall/ heavy older¹².

An alternative to the HGS is the chair stand test (CST) that does not require specific instruments for the clinical practice and is considered a fair substitute to identify sarcopenia¹. The EWGSOP2 recommends the 5-repetition sit-up test to screening sarcopenia¹. However, there are no cut-off points of 30-s CST to identify sarcopenia. One topic that merits consideration is that 30-s CST results is influenced by older adult's height that varies between participants while the seat height do not¹³. In other words, the difficulty imposed by a single seat height for taller participants is superior compared to the difficulty imposed for the smaller ones. Considering this limitation, the traditional approach based in the absolute number of repetitions (reps) on the 30-CST to identify sarcopenia should be normalised according to the older adult's height or adjusted to their seat's height.

Allometric models (Y = aX^b)¹⁴ perceive the nonlinear relationship between muscle strength and body-size variables and can overcome the limitations pointed out for non-normalised HGS and 30-CST^{9-11, 13}. In this sense, the development of cut-off points for muscle strength with allometric scaling might be more appropriate to identify sarcopenia.

Thus, the purpose of this study was to a) establish allometric coefficients to normalize handgrip strength and 30-sec chair stand test according to body-size variables for older adults and b) determine cut-off points for low muscle strength of the allometrically adjusted handgrip strength and 30-sec chair stand test of this population. The hypothesis of this study was that allometric normalisation removes the effect of body size on the expression of muscle strength. The use of these cut-off points for low grip strength should allow identification of the frequency of sarcopenia in older adults regardless of body size and without bias.

METHOD

Ethical statement

Procedures carried out in full compliance with the Helsinki Declaration and the study protocol was approved by the "Instituto Português do Desporto e Juventude (IPDJ)" ethics committee (ID number: 309/DD/2018). All participants read and signed the consent form before the testing procedures.

Study population and design

This cross-sectional research encompasses 729 older adults (514 males and 215 females) from the North of Portugal who took part in a national survey examining a representative sample of the Portuguese population including five mainland geographical areas. Data were collected between March 2017 and November 2018.

Inclusion criteria were age ≥50 years old, independence to perform daily living activities and all physical fitness test measurements. Exclusion criteria were any physical incapacity impairing physical fitness measurements (wheelchair users, amputated) and dementia or any mental condition that impaired older adults' comprehension.

Anthropometry

Height (cm) was measured using a calibrated portable stadiometer (Seca 217, Hamburg) with 0.1 cm resolution. Body weight (kg) was measured to the nearest 0.1 kg (Tanita, Inner Scan BC-522, Tokyo, Japan). Body mass index (BMI) was calculated dividing body mass by height squared in meters (kg/m²).

Handgrip strength

Handgrip strength was determined using a digital handgrip dynamometer (Jamar Plus+, Fabrication Enterprises, Inc. 12-0604-USA). Participants were standing, with elbow flexed at 90° and forearm and wrist in neutral position. The participant was instructed to squeeze the dynamometer as strongly as possible. Two trials were performed with the dominant upper limb with a one-minute interval between attempts. The highest result was considered for analysis.

30-sec chair stand test

Participants were asked to sit and stand continuously within 30 sec in a 43-cm armless chair with their arms crossed at the wrists and held against the chest. The score was the total number of stands executed correctly¹⁵.

Physical activity level

To consider physical activity level in the generation of allometric exponents, physical activity was measured using the Portuguese validated short-version

of the International Physical Activity Questionnaire (IPAQ-SV). The IPAQ-SV comprises the frequency and duration of vigorous and moderate physical activity and walking. Daily duration of each intensity of physical activity was derived multiplying the number of days per week by the time per day in each intensity. To bivariate analysis, the four possible classifications of physical activity level were dichotomized (0=sedentary and irregularly active; 1=active and very active).

Six-minute walk test and classification of functional limitation

The six-minute walk test was used to verify mobility capacity and functional limitation (walk less than 400m in six minutes)¹⁶, with a dichotomous classification for its presence (1) or absence (0). It was executed in a rectangular course (10m x 30m) with markings every five meters. Verbal encouragement was given every 30s. The participants were asked to walk as fast as possible for six minutes.

Statistical analyses

Descriptive statistics (mean, 95% CI and standard deviation) were used as appropriate. Gender differences were examined by independent samples t-test. Log-linear regression models generated allometric exponents. The independent variables (body mass, height and BMI) and dependent variables (HGS and 30-s CST) were converted in their natural logarithm (ln). Thus, each specific allometric exponent (In HGS, In 30-s CST, In body mass, In height and In BMI) was developed. Confounders were age (continuous), gender (females=0, males=1) and PAL (inactive=0; active=1). An interaction with all independent variables was computed for each model (BMI example: ln BMI*age*gender*PAL). The same was done for the ln of body mass and ln height. Enter method was used (all models are shown in the Supplement B). Additional models without interaction variable also where generated and adopted if interaction variable was not statistically significant, allowing application of allometric exponents regardless of gender, age or PAL. The interactions are statistically significant, this strategy would allow the generation of gender-specific allometric exponents. We calculated Variance Inflation Factor (VIF) to warrant no multicollinearity (VIF<5) between independent variables¹⁷. The allometric exponents were used for normalisation of HGS (Equation 1) and 30-s CST (Equation 2) according to each body-size:

Allometrically Adjusted HGS =
$$\left[HGS(kg) \right] / Body size variable^{Allometric exponent}$$
(1)

Allometrically Adjusted 30s CST =
$$\begin{bmatrix} 30s \text{ CST} (repetitions) \end{bmatrix} / \begin{bmatrix} Body \text{ size variable}^{Allometric exponent} \end{bmatrix}$$
 (2)

Ability of allometric normalisation to remove the effect of body size on HGS and 30-s CST was verified by partial correlation analysis (controlling gender, age and physical activity effects) between normalised HGS and 30-s CST with body-size variables¹⁸. Allometric normalisation isolate the effect of body-size variables on HGS and 30-s CST when Pearson correlation of the product moment was negligible (r<0.30)^{10,18}.

The cut-off points for low muscle strength of absolute and allometrically adjusted HGS and 30-s CST were based on dichotomous classification of functional limitation^{6,12}. Receiver operating characteristic (ROC) curve was used to test the sensitivity and specificity in distinguishing functional limitation from absolute and allometrically adjusted HGS and 30-s CST. Cut-off points were selected with Youden Index considering largest area under the curve (AUC)¹⁹.

All procedures were carried out with IBM Statistical Package for the Social Sciences (SPSS) software version 23 (Chicago, USA). Statistical significance level was fixed at p<0.05. This manuscript was written following the requirements of the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) for cross-sectional studies checklist.

RESULTS

The data cleaning process is illustrated by the Supplement A, after applying inclusion and exclusion criteria. From the initial 729 participants, data from 379 participants (aged between 50 and 95 years old) were included for analysis.

Sample characteristics is presented in Table 1. Men compared to women were heavier, taller, stronger (HGS and 30-s CST) and had better mobility (p<0.05). However, there were no differences for age and BMI.

| | | Women (n=367) | | | Men (n=154) | | | |
|--------------------------------------|-------|--------------------|-------|--------|--------------------|-------|--------------|--|
| Variables | Mean | 95% CI LL to UL | SD | Mean | 95% CI LL to UL | SD | (<i>p</i>) | |
| Age, years | 73.5 | 72.9 to 74.2 | 7.1 | 73.8 | 72.9 to 74.7 | 6.8 | 0.654 | |
| Body mass, kg | 66.1 | 65.2 to 67.1 | 10.8 | 77.8* | 76.0 to 79.6 | 13.1 | <0.001 | |
| Height, m | 1.5 | 1.5 to 1.5 | 0.1 | 1.7* | 1.6 to 1.7 | 0.1 | <0.001 | |
| BMI, kg/m2 | 27.9 | 27.5 to 28.3 | 4.3 | 28.3 | 27.6 to 29.1 | 5.3 | 0.294 | |
| HGS ^a , kg | 21.0 | 20.5 to 21.5 | 5.2 | 34.5* | 33.1 to 35.9 | 8.7 | <0.001 | |
| 30-s CST, reps | 15.7 | 15.2 to 16.2 | 5.5 | 17.2* | 16.5 to 17.9 | 5.0 | 0.001 | |
| Six-minute walk test, m | 450.6 | 435.6 to 465.5 | 149.3 | 507.3* | 483.9 to 530.7 | 149.8 | <0.001 | |
| <400 m (functional limitation), % | | 23.2% | | | 15.3% | | | |
| Physical activity level | | | | | | | | |
| Sedentary and irregularly active, % | | 40.8% | | | 23.6% | | | |
| Active and very active, % | | 59.2% | | | 76.4% | | | |

Table 1. Characteristics of the sample.

*p<0.05 (in the comparison between women and men); ^a= n for women: 263 and men: 122. SD=standard deviation; CI: confidence interval; lower: lower band; upper: upper band; HGS: Handgrip strength; CST: Chair stand test; reps=repetitions.

Table 2 shows allometric exponents for ln HGS and ln 30-s CST according to the independent variables (ln body mass, ln height and ln BMI). Any significant interaction variable was produced previously (results with interaction terms are

shown in the Supplement B. The allometric exponents (regression coefficient) varied between -0.01 and 2.28 for HGS, and between -0.27 and 0.21 for 30-s CST (Table 2).

| Dependent variable | Independent | Unit | Regression | 95% CI | |
|-----------------------|--------------|-------|------------------|----------------|--|
| | variable | Unit | Coefficient (SE) | lower to upper | |
| HGS | In body mass | kg | 0.26* (0.44) | 0.07 to 0.45 | |
| | In height | m | 2.28** (0.36) | 1.56 to 2.98 | |
| | In BMI | kg/m2 | -0.01 (0.10) | -0.21 to 0.18 | |
| 30-s CST | In body mass | kg | -0.23 (0.14) | -0.51 to 0.05 | |
| | In height | m | 0.21 (0.59) | 0.28 to 1.71 | |
| | In BMI | kg/m2 | -0.27 (0.14) | -0.55 to 0.02 | |

Table 2. Allometric exponents (regression coefficients) for body-size variables.

*p<0.05; **p<0.001 (statistically significant regression coefficient). All models were adjusted for confounders: age (continuous), gender (0= females, 1= males) and physical activity level (0= inactive; 1= active); SE: standard error; CI: confidence interval; lower: lower band; upper: upper band; HGS: Handgrip strength; CST: Chair stand test.

Correlations between allometrically adjusted HGS, 30-s CST and body-size variables ranged between -0.09 and 0.19, as shown in Table 3. Although some correlations were statistically significant, all were negligible¹⁸, confirming the adequate procedure of allometric normalisation for all body-size variables.

Table 3. Correlations between absolute and adjusted handgrip strength (HGS) and 30-sec chair stand test (30-s CST) by body-size variables exhibited by gender.

| | | | I | HGS | | 30-s CST | | | |
|-------|-----------|----------|-------------------|-------------|-----------|----------|--------------------|-------------|-----------|
| | | Absolute | /Body mass0.26 | /Height2.28 | /BMI-0.01 | Absolute | /Body mass-0.23 | /Height0.21 | /BMI-0.27 |
| Women | Body mass | 0.18** | 0.01 | | | -0.19** | -0.09 | | |
| | Height | 0.40** | | 0.02 | | -0.06 | | -0.08 | |
| | BMI | -0.01 | | | -0.01 | -0.16** | | | -0.05 |
| Men | Body mass | 0.30** | 0.14 | | | 0.02 | 0.15* | | |
| | Height | 0.52** | | 0.19* | | 0.12 | | 0.09 | |
| | BMI | 0.01 | | | 0.02 | -0.08 | | | 0.05 |

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).

Figure 1 exhibit ROC curve analysis of absolute or allometrically adjusted HGS (letter "a" and "b") and 30-s CST (letter "c" and "d") to identify low muscle strength in Portuguese older adults. The cut-off points for low muscle strength (HGS and 30-s CST) according to each gender are presented in Table 4.



Figure 1. ROC curve analysis of absolute or allometrically adjusted handgrip strength (HGS; letter "a" and "b") and 30-sec chair stand test (30-s CST; letter "c" and "d") to identify low muscle strength in Portuguese older adults.

Table 4. Cut-off points for low muscle strength for absolute or allometrically adjusted handgrip strength (HGS) and 30-sec chair stand test (30-s CST).

| | | | Women | | | | | Men | | |
|--------------------------------|--------|------|-------|-------------------|---------------|--------|------|------|-------------------|----------------|
| Variable | AUC | Sens | Spec | 95% CI | Cut points | AUC | Sens | Spec | 95% CI | Cut points |
| HGSª | 0.85** | 81.7 | 72.7 | 0.800 to 0.890 | ≤ 20.0 | 0.84** | 64,0 | 92.8 | 0.762 to 0.899 | ≤ 26.3 |
| HGS/Body mass0.26 | 0.86** | 73.1 | 83,0 | 0.811 to 0.898 | ≤6,32 | 0.84** | 84,0 | 71.1 | 0.763 to 0.900 | ≤ 10.18 |
| HGS/ Height2.28 | 0.82** | 62.4 | 87.7 | 0.766 to 0.863 | ≤6,89 | 0.78** | 52,0 | 90.7 | 0.694 to 0.848 | ≤ 8.48 |
| HGS/BMI- 0.01 | 0.85** | 75.3 | 78.2 | 0.799 to 0.889 | ≤ 20,1 | 0.84** | 64,0 | 92.8 | 0.761 to 0.899 | ≤ 27.5 |
| 30-s CST⁵ | 0.88** | 65.8 | 92.3 | 0.844 to 0.913 | ≤ 12.0 | 0.83** | 83.9 | 65.9 | 0.757 to 0.882 | ≤ 16.0 |
| 30-s CST/ Body mass-0.23 | 0.88** | 79.6 | 79.9 | 0.845 to 0.914 | ≤38.0 | 0.84** | 67.7 | 87,0 | 0.770 to 0.892 | ≤38.0 |
| 30-s CST// Height0.21 | 0.87** | 85.2 | 72.3 | 0.836 to 0.907 | ≤ 13.9 | 0.81** | 83.9 | 65.9 | 0.741 to 0.871 | ≤ 14.6 |
| 30-s CST/ BMI-0.27 | 0.88** | 83.3 | 74.9 | 0.837 to 0.907 | ≤ 36.6 | 0.83** | 67.7 | 85.4 | 0.757 to 0.883 | ≤ 33.8 |

**Statistically significant AUC at the 0.01 level (2-tailed); ^a n for women: 263 and men: 122; ^b n for women: 367 and men: 154. HGS: Handgrip strength; CST: Chair stand test.

All cut-off points of the models proposed for women and men showed acceptable AUC $(>.70)^{20}$. When AUC is 0.87, it means there is an 87% chance that the model will be able to distinguish between positive and negative primary outcome²¹.

DISCUSSION

To the best of our knowledge, this is the first study that proposed allometric coefficients for HGS and 30-s CST normalisation and established the minimal threshold to identify low muscle strength and sarcopenia in older adults. The negligible correlations between allometrically normalised HGS, 30-s CST and body size confirm the noramlisation of body-size effect on expression of HGS and 30-s CST. Thus, the identification of sarcopenia in older adults are more precise, regardless of body-size variability. Older adults with extreme body size (e.g., light and short or tall and heavy) can be evaluated with less chance of bias.

According to the best of our knowledge, studies proposing minimal threshold for low muscle strength allometrically normalised based on 30-s CST are not yet available. We found only one study that proposed minimal threshold for low muscle strength allometrically normalised based on dynamic knee extension¹². However, it is difficult to compare available findings once knee extension performed in leg extension machine is not comparable with the 30-s CST. Both tests involve different loads (external for knee extension and body mass for CST) and movements (hip extension in CST, not present in extensor chair).

Considering HGS, previous studies have already proposed allometric exponents to normalize HGS9-11, and they are comparable with the ones proposed by us. Allometric (curvilinear) or power relationship between two variables is confirmed when allometric exponents vary from 0.00 to 0.9922; and a linear relationship is assumed if the exponent is $\geq 1.00^{22}$. The allometric relationship between HGS and body mass was confirmed in previous studies, with exponents of 0.33 (95% CI = 0.14 to 0.48)¹⁰, 0.40 (95% CI = 0.26 to $(0.78)^9$ and $(0.63)^9$ CI = $(0.31)^{11}$. Similarly, in our study, the allometric relationship between HGS and body mass is accepted because the exponent was 0.26 (Table 2). Conversely, in our study, height had a linear relationship with HGS (Table 2) once the exponent was 2.28, exceeding the unit (\geq 1.00). The same linear relationship between HGS and height was previously found (allometric exponent: 1.84; 95% CI: 1.23 to 2.45)¹⁰. Our proposed allometric exponents (0.26 for body mass and 2.28 for height) were always within the 95% CI indicated by the literature for body mass (95% CI = 0.14 to 0.91)⁹⁻¹¹ and height $(95\% \text{ CI} = 1.23 \text{ to } 2.45)^{10}$.

As previously stated, we did not find previously published allometric exponents to normalize 30-s CST. Nevertheless, absolute normative values for HGS²³ and 30-s CST²⁴ were proposed for older Portuguese adults, allowing comparisons with the absolute values of our study. The 30-s CST performance of our sample (~74 years old) for women (16 rep) and men (17 rep) was close to the 50th percentile reported in the normative study²⁴ for the age of 70-74: 17 (women) and 18 rep (men). This demonstrates the similarity and representativeness of our data when compared to normative studies of older Portuguese adults, allowing for a possible extrapolation of the allometric exponents generated. As a practical example, we hypothesize the performance in the HGS

of an older woman with BMI lower than average (27 kg/m²). In the HGS she performed a total of 19.5 kg, being classified as having low muscle strength according to the absolute criteria proposed in our study (≤ 20.0 kg). However, when normalised by BMI (20.2 kg/kg/m²), is rated above the established cut-off point (≤ 20.1 kg/kg/m²). Mistakes in classifying low muscle strength (false positive) due to not considering body size would include older adults who do not need special care, unnecessarily burdening health systems²¹.

This is the first study that proposed cut-off points of allometrically adjusted HGS and 30-s CST in representative samples of older adults living in developed countries. Allometry remove body-size effects on HGS^{10, 11, 25}, meanwhile HGS and 30-s CST are representative of muscle strength and were associated to height^{5, 13} and body mass²⁶. In the present study we recruited a sample of developed country with a large age-range (50 to 95 years old), characteristic not always covered in studies with older adults. But this study is not without limitations. The cross-sectional design could have underestimated the individual decline of muscle strength with aging. It is desirable that future studies consider longitudinal design to solve this issue. Another limitation topic is the gender imbalance (more participants men than women), inverse of what would be expected with women having a longer life expectancy than men. Only physically independent older adults were recruited, which can underestimate the presence of sarcopenic older adults. However, a considerable quantity of older women (23.2%) and men (15.3%) have functional limitation, one associate indicator of sarcopenia¹. Fixed cut-off points are more usable for clinicians in clinical practice and a standardised value requires more computation, however an easy excel routine can be made to solve this issue¹².

Low muscle strength is part of the algorithm to identify sarcopenia¹ due to association with premature death, complications in institutionalization, poor cognition, fractures and physical independence^{27, 28}. The reduction in muscle strength, body mass, height and BMI expected with aging may improve prediction of adverse outcomes compared to absolute strength. Using the absolute muscle strength recommended by the EWGSOP2¹ derived from English sample⁵ to identify sarcopenia can overestimate the incidence of low strength in Portuguese older adults, as there is a difference of 4 kg in HGS between populations²³. Thus, normalize muscle strength by body size can reduce the differences between countries²³. The specific characteristics of the population directly impact muscle strength values, requiring that the existing cut-off points for sarcopenia be normalized^{12, 29}. Therefore, future studies should a longitudinal design, populacional-specificity, and test the ability to predict poor health outcomes (depressive symptoms, history of falls, hospitalization or mortality factor risks) considering strength adjusted for body size.

CONCLUSION

Sarcopenia cut-off points with HGS and 30-s CST allometrically normalised were proposed for Portuguese older adults and allometry preserve adequate accuracy (AUC>70%). Our hypothesis was confirmed, that allometry normalise the effect of body size on HGS and 30-s CST, making the classification of the strength without influence of body size.

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COMPLIANCE WITH ETHICAL STANDARDS

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Ethical approval

The study was approved by the "Instituto Português do Desporto e Juventude (IPDJ)" institutional review board (ID number: 309/DD/2018) and written informed consent was obtained from all subjects. The protocol was written in accordance with the standards set by the Declaration of Helsinki.

Conflict of interest statement

The authors have no conflict of interests to declare.

Author Contributions

Conception and design of the experiment: PPA, LB. Carrying out the experiments: PPA, LB. Data analysis PPA, DRLM. Contribution with reagents/ research materials/analysis tools: JM. Writing of the article: PPA, LB, JM, DRLM. All authors read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

Supplementary material accompanies this paper. Supplementary Files. Free access in https://osf.io/9gxr7/ This materials are available as part of the online article from http://www.scielo.br/rbcdh