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AVALIAÇÃO DA APTIDÃO CARDIORRESPIRATÓRIA PELO *INCREMENTAL SHUTTLE WALKING TEST* EM MULHERES SAUDÁVEIS

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Dissertação apresentada ao Programa de Pós-Graduação em Reabilitação e Desempeno Funcional da Universidade Federal dos Vales do Jequitinhonha e Mucuri, como requisito parcial para obtenção do título de Mestre.

Orientadora: Profª. Drª. Vanessa Amaral Mendonça
Coorientador: Prof. Dr. Hércules Ribeiro Leite

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"Avaliação da aptidão cardiorrespiratória pelo *Incremental Shuttle Walking Test* em mulheres saudáveis."

Dissertação apresentada ao MESTRADO EM REABILITAÇÃO E DESEMPENHO FUNCIONAL, nível de MESTRADO como parte dos requisitos para obtenção do título de MAGISTER SCIENTIAE EM REABILITAÇÃO E DESEMPENHO FUNCIONAL

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DIAMANTINA

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RESUMO

O *Incremental Shuttle Walking Test* (*ISWT*) tem sido sugerido como uma boa opção para avaliar a aptidão cardiorrespiratória na população saudável. Já se sabe que nos homens o *ISWT* é um teste máximo, porém em mulheres ainda existe essa lacuna. Deste modo, este estudo teve como objetivo comparar o *ISWT* com o teste de exercício cardiopulmonar (TECP) e desenvolver uma equação de predição do consumo pico de oxigênio (VO_2 pico) em mulheres saudáveis. **Métodos:** No primeiro estágio, o VO_2 pico, o quociente respiratório pico (R pico), a frequência cardíaca máxima (FC máx) e a porcentagem da FC máx predita (% da FC máx prevista) foram avaliados no TECP e *ISWT*. No segundo estágio, foi elaborada uma equação (n = 54) para predizer o VO_2 pico. No terceiro, a validação desta equação foi realizada por outras 20 participantes. **Resultados:** Não houve diferenças significativas entre o *ISWT* e TECP para os valores de VO_2 pico, FC máx e % da FC máx prevista (P> 0,05), mas a medida de R pico foi significativamente maior no *ISWT* (1,22±0,13 no *ISWT* vs. 1,18±0,1 no TECP; P = 0,022). Além disso, houve uma correlação positiva moderada entre os testes para as variáveis VO_2 pico (r = 0,51; P = 0,0007), FC máx (r = 0,65; P <0,0001) e R pico (r = 0,55; P = 0,0002) e a análise de *Bland-Altman* demonstrou concordância VO_2 pico (bias = -0,14). A distância percorrida no *ISWT* e a idade explicaram 36,3% (R quadrado ajustado = 0,363) da variância do VO_2 pico. A equação foi: VO_2 pico (previsto) = 19,793 + (0,02 x distância percorrida) - (0,236 x idade). Não houve diferença estatisticamente significativa entre o VO_2 pico medido diretamente com o estimado pela equação elaborada e a análise de *Bland-Altman* mostrou concordância entre as medidas, com um bias de 1,5 ml / kg / min. **Conclusão:** O *ISWT* é um teste máximo, mostrando resultados semelhantes aos do TECP e a equação prevista é válida e aplicável para avaliação do VO_2 pico em mulheres jovens saudáveis.

Palavras-chave: *Incremental Shuttle Walking Test*, VO_2 pico, mulheres.

ABSTRACT

The Incremental Shuttle Walking Test (ISWT), have been suggested as a good option for evaluating cardiorespiratory fitness in the healthy population. It is already known that in men the ISWT is a maximum test, but in women there is still this gap. Thus, this study aimed to compare the ISWT with the cardiopulmonary exercise testing (CEPT) and to develop an equation for peak oxygen uptake (VO_2 peak) prediction in healthy women. **Methods:** In the first stage, the VO_2 peak, respiratory exchange ratio (R peak), heart rate max (HR max) and percentage of predicted HR max (% predicted HR max) were evaluated in CEPT and ISWT. In the second stage, an equation was elaborated (n = 54) to predict the VO_2 peak. In the third, the validation of this equation was performed by another 20 participants. **Results:** There were no significant differences between the ISWT and CEPT for VO_2 peak, HR max and % predicted HR max values ($P>0.05$), except for R peak measure that was 1.22(0.13) in ISWT vs. 1.18(0.1) in CEPT ($P=0.022$). Therefore, both tests showed a moderate positive correlation for VO_2 peak ($r=0.51$; $P=0.0007$), HR max ($r=0.65$; $P<0.0001$) and R peak ($r=0.55$; $P=0.0002$) and the Bland-Altman analysis demonstrated agreement for VO_2 peak (bias = -0.14). The distance walked on ISWT and age explained 36.3% (Adjusted R Square = 0.363) of the variance in VO_2 peak. The equation was VO2 peak (predicted) = 19.793 + (0.02 x distance walked) - (0.236 x age).There was no statistically significant difference between VO_2 peak measured directly with that estimated by the elaborated equation and the Bland-Altman analysis showed an agreement with a bias of 1.5 ml/kg/min.

Conclusion: ISWT is a maximal showing similar results as the CEPT, and the predicted equation is valid and applicable for evaluation of VO_2 peak in young healthy women.

Keywords: Incremental Shuttle Walking Test, VO_2 peak, women.

SUMÁRIO

1 INTRODUÇÃO

A aptidão cardiorrespiratória (ACR) pode ser definida como a capacidade de executar um exercício de intensidade moderada a alta, de natureza dinâmica, com participação de grandes grupos musculares por períodos de tempo prolongados. É um importante componente da aptidão física relacionada à saúde, que reflete as capacidades funcionais dos sistemas respiratório, cardiovascular e musculoesquelético (ACSM, 2014).

O padrão ouro para avaliação da ACR é a obtenção do consumo máximo de oxigênio (VO_2 máx) (ATS, 2003) que reflete a máxima capacidade em absorver, transportar e consumir o oxigênio (WASSERMAN, 2012). O VO_2 máx é representado por um platô no gráfico de VO_2 versus trabalho; entretanto, em testes clínicos, um platô pode não ser encontrado antes que outros sintomas (como frequência cardíaca ou volume sistólico) levem à interrupção do exercício (ATS, 2003). Por isso, de maneira prática, o VO_2 máx é considerado sinônimo do VO_2 pico, ou seja, é equivalente ao maior valor de VO_2 obtido no pico do esforço (ATS, 2003; HERDY & CAIXETA, 2016).

Os principais determinantes do VO_2 pico são os fatores genéticos, a quantidade de massa muscular, a idade, o sexo e o peso corporal, além de poder ser afetado pelo treinamento (ATS, 2003). O mesmo pode ser avaliado através da medida direta dos gases exalados ou por equações de predição pré-estabelecidas.

A mensuração direta do VO_2 pico envolve laboratórios especializados com equipamentos de alto custo e profissionais especializados (teste de esforço cardiopulmonar-TECP), o que nem sempre está disponível na prática clínica (ACSM, 2003). Diante disso, testes de campo são cada vez mais utilizados para avaliação da ACR, como o *Incremental Shuttle Walking Test* (*ISWT*).

O *ISWT* foi desenvolvido por Singh *et al*. em 1992 para avaliar a ACR de portadores de doença pulmonar obstrutiva crônica. Trata-se de um teste simples no qual o indivíduo deve caminhar/correr em terreno plano uma distância conhecida de 10 metros, ao redor de uma marcação de dois cones. Originalmente o teste é composto por 12 estágios de 1 minuto, sendo que o ritmo da passada é ditado por sinais sonoros que vão se tornando mais próximos a cada estágio, levando o voluntário a caminhar em uma velocidade cada vez maior para alcançar o próximo cone antes ou junto do próximo sinal, impondo um esforço progressivo (SINGH *et al*., 1992).

Ressalta-se que a avaliação da ACR tem sido frequentemente realizada tanto na prática clínica quanto em investigações científicas, com o intuito de fornecer parâmetros para

a prescrição e elaboração de programas de exercícios (PALANGE *et al.*, 2007; ATS, 2006). Nesse contexto, o *ISWT* pode ser uma ferramenta simples e barata para a avaliação da ACR em diferentes populações.

A característica incremental do *ISWT* explica as fortes correlações encontradas entre esse teste e o TECP em pacientes com doenças cardiopulmonares (SINGH *et* al., 1994; MORALES *et al*., 2000; ONORATI *et al*., 2003;) e indivíduos saudáveis (DOURADO *et al*., 2011; NEVES *et al.*, 2015). Além disso, alguns estudos demonstraram que o *ISWT* é um teste máximo para crianças e idosos (DOURADO *et al*., 2013; LANZA *et al*., 2015; PINHO *et al*., 2015; VARDHAN *et al*. 2017).

Recentemente, nosso grupo de pesquisa demonstrou que em homens jovens saudáveis o *ISWT* foi capaz de promover respostas cardiorrespiratórias máximas. Ainda, foi desenvolvida uma equação de predição do VO_2 pico que demonstrou viabilidade para sua avaliação, com base em variáveis obtidas durante o *ISWT* (NEVES *et al.*, 2015). Porém, uma limitação desse estudo foi não incluir mulheres na amostra.

Considerando que os valores de VO_2 para mulheres representam cerca de 70% da média dos valores para homens (NUNES *et al*., 2005), a investigação do comportamento do VO_2 durante o *ISWT* na população feminina é válida e necessária. Os poucos estudos publicados avaliaram apenas mulheres obesas (PEIXOTO-SOUZA *et al.*, 2015; JÜRGENSEN *et al.*, 2016), permanecendo uma lacuna na literatura sobre o *ISWT* em mulheres saudáveis.

Diante disso, o objetivo deste estudo foi avaliar a ACR de mulheres jovens saudáveis durante o TECP e o *ISWT* através da medida direta dos gases exalados, a fim de classificar a intensidade do *ISWT* e elaborar uma equação de predição para o VO_2 pico para essa população.

2 REFERÊNCIAS

American College of Sports Medicine. **Diretrizes do ACSM para os testes de esforço e sua prescrição.** Rio de Janeiro: Guanabara Koogan, 2003.

American College of Sports and Medicine (ACSM). **Diretrizes do ACSM para os testes de esforço e sua prescrição.** Balady, Guanabara Koogan, 2014.

American Thoracic Society/ American College of Chest Physicians. ATS/ACCP statement on cardiopulmonary exercise testing. **Am J Respir Care Med**. 167 (2): 211-77, 2003

American Thoracic Society Documents (ATS). **American Thoracic Society/European Respiratory Society Statement on Pulmonary Rehabilitation**. 2006. Disponível em: https://www.atsjournals.org/doi/pdf/10.1164/rccm.200508-1211ST. Acesso em: 09 jan 2018.

Dourado, V.Z.; Vidotto, M.C.; Guerra, R.L.F. Reference equations for the performance of healthy adults on field walking tests. **J Bras Pneumol**. 37: 607–614, 2011.

Dourado, V.Z. and Guerra, R.L.F.. Reliability and validity of heart rate variability threshold assessment during an incremental shuttle-walk test in middle-aged and older adults. **Braz J Med and Biol Res**. 46: 194-199, 2013.

Herdy, A.H. and Caixeta, A. Classificação Nacional da Aptidão Cardiorrespiratória pelo Consumo Máximo de Oxigênio. **Arq Bras Cardiol**. 106(5):389-395, 2016.

JÜRGENSEN, S. P. *et al.* Does the incremental shuttle walk test require maximal effort in young obese women?. **Brazilian Journal of Medical and Biological Research.** São Carlos, v.49, n. 8, p. 1-7, 2016.

Lanza, F.C.; Zagatto, Edo P.; Silva, J.C.; Selman, J.P.; Imperatori, T.B.; Zanatta, D;J. *et al*. Reference Equation for the Incremental Shuttle Walk Test in Children and Adolescents. **J Pediatr**. Nov; 167(5):1057-61, 2015.

Morales, F.J., Montemayor, T.; Martinez, A. Shuttle versus six-minute walk test in the prediction of outcome in chronic heart failure. **Int J Cardiol**. 76: 101–105, 2000.

Neves, C.D.C.; Lacerda, A.C.; Lage, V.K.; Lima, L.P.; Fonseca, S.F.; de Avelar, N.C., *et al*. Cardiorespiratory responses and prediction of peak oxygen uptake during the shuttle walking test in healthy sedentary adult men. **PLOS ONE**. 6;10(2):e0117563, 2015.

Nunes, R.A.M.; Pontes, G.F.R.; Dantas, P.M.S.; Fernandes Filho, J. Referencial table of cardiopulmonary fitness. **Fitness & Performance Journal**. 4(1), 27 – 33, 2005.

Onorati, P.; Antonucci, R.; Valli, G.; Berton, E.; De Marco, F., *et al*. Non-invasive evaluation of gas exchange during a shuttle walking test vs. a 6-min walking test to assess exercise tolerance in COPD patients. **Eur J Appl Physiol**. 89: 331–336, 2003.

Palange, P. *et al*. Recommendations on the use of exercise testing in clinical practice. **European Respiratory Journal**. Roma, v. 29, n. 1, p. 185-209, 2007.

Peixoto-Souza, F. S. *et al*. Reproducibility of the incremental shuttle walk test for women with morbid obesity. **Physiother Theory Pract**. São Paulo, v. 31, n. 6, p. 428-432, 2015.

Pinho, T.M.P. **Predictive Equation for Incremental Shuttle Walk Test in Adolescents**. Universidade de Aveiro. 2015 Available from: http://hdl.handle.net/10773/14580.

Singh, S. J. *et al*. Development of a shuttle walking test of disability in patients with chronic airways obstruction. **Thorax**. Leicester, v. 47, n. 12, p. 1019–1024, 1992.

Singh, S.J.; Morgan, M.D.; Hardman, A.E.; Rowe, C.; Bardsley, P.A. Comparison of oxygen uptake during a conventional treadmill test and the shuttle walking test in chronic airflow limitation. **Eur Respir J**. 7:2016–2020, 1994.

Vardhan, V.; Palekar, T.; Dhuke, P.; Baxi, G. Normative Valeus of Incremental Shuttle Walk Test in Children AND Adolescents: Observational study. **Int J Pharma Bio Sci**. 8(4): (B) 478-483, 2017.

Wasserman K. **Principles of exercise testing and interpretation: including pathophysiology and clinical applications**. 5th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2012.

3 ARTIGO CIENTÍFICO 1

Cardiorespiratory fitness assessment and prediction of peak oxygen consumption by Incremental Shuttle Walking Test in healthy women

Short Title: Incremental Shuttle Walking Test in healthy women

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Abstract

Introduction: The Incremental Shuttle Walking Test (ISWT), have been suggested as a good option for evaluating cardiorespiratory fitness in the healthy population. It is already known that in men the ISWT is a maximum test, but in women there is still this gap. Thus, this study aimed to compare the ISWT with the cardiopulmonary exercise testing (CEPT) and to develop an equation for peak oxygen uptake (VO_2 peak) prediction in healthy women.

Methods: In the first stage, the VO_2 peak, respiratory exchange ratio (R peak), heart rate max (HR max) and percentage of predicted HR max (% predicted HR max) were evaluated in CEPT and ISWT. In the second stage, an equation was elaborated (n = 54) to predict the VO_2 peak. In the third, the validation of this equation was performed by another 20 participants.

Results: There were no significant differences between the ISWT and CEPT for VO_2 peak, HR max and % predicted HR max values ($P>0.05$), except for R peak measure that was 1.22(0.13) in ISWT vs. 1.18(0.1) in CEPT ($P=0.022$). Therefore, both tests showed a moderate positive correlation for VO_2 peak ($r=0.51$; $P=0.0007$), HR max ($r=0.65$; $P<0.0001$) and R peak ($r=0.55$; $P=0.0002$) and the Bland-Altman analysis demonstrated agreement for VO_2 peak (bias = -0.14). The distance walked on ISWT and age explained 36.3% (R^2 Adjusted= 0.363) of the variance in VO_2 peak. The equation was VO2 peak (predicted) = 19.793 + (0.02 x distance walked) - (0.236 x age).There was no statistically significant difference between VO_2 peak measured directly with that estimated by the elaborated equation and the Bland-Altman analysis showed an agreement with a bias of 1.5 ml/kg/min.

Conclusion: ISWT is a maximal test showing similar results as the CEPT, and the predicted equation is valid and applicable for evaluation of VO_2 peak in young healthy women.

Keywords: Incremental Shuttle Walking Test, VO_2 peak, women.

Introduction

Cardiorespiratory fitness (CRF) is defined as the ability to sustain dynamic exercise by large muscle groups over time at moderate to high intensity levels [1]. Furthermore, CRF have been used to measure exercise capacity and provide information about physical limitation, morbidity prognosis, and responsiveness to treatment [2]. The current gold standard for the evaluation of CRF is the direct measurement of maximal oxygen uptake (VO_2max) which represents the maximal achievable level of oxidative metabolism involving large muscle groups [3]. However, in clinical testing situations, the exercise usually is limited by symptoms before the individual achieve the VO_2max. Consequently, VO_2 peak is often used as an estimate for VO_2max and they are used interchangeably [3].

The laboratory assessment of CRF through maximal tests on treadmills or cycle ergometers (cardiopulmonary exercise testing-CEPT) has a high cost [4] and require specialized professionals and equipments that is not always available [5]. Thus, field tests were developed and have been increasingly used in clinical practice, such as the Six-minute walk test and the Incremental Shuttle Walking Test (ISWT). ISWT was created by Singh et al. [6] to assess the CRF of patients with chronic pulmonary obstructive disease (COPD) and later used in other conditions or healthy subjects [7, 8, 9, 10, 11].

Several studies had already shown strong correlations between the performance on CEPT and ISWT [5, 12, 13, 14]. Some studies have showed that the ISWT is a maximal test in the pediatric and elderly population [15, 16, 17, 18], however the intensity of ISWT was often indirectly assessed by predictive equations [15, 16, 17]. Hence, our study group compared cardiorespiratory responses between ISWT and CEPT in healthy young adult men [14] and adolescent boys (data not published), where the results showed moderate to high significant correlation and agreement, concluding that the ISWT is a maximal test in these

subjects. In addition, a VO_2 peak prediction equation based on ISWT variables was developed and it demonstrated feasibility and validity [14]. However, this study did not include women in the assessments, remaining a gap in the literature about the ISWT in healthy women.

In this paper, we evaluate the CRF in healthy young women by comparing and correlating VO_2 peak, respiratory quotient peak (R peak), maximum heart rate (HR max) and percentage of predicted maximum heart rate (% predicted HR max), between ISWT with CEPT through direct analysis of the exhaled gases, aiming to classify the ISWT intensity and to elaborate a predictive equation to estimate the VO_2 peak in young adult women population.

Materials and methods

Design

This was a cross-sectional study divided into three stages: (1) To compare the CEPT and the ISWT, evaluate the correlation and agreement between the variables VO_2 peak, R peak, HR max and % predicted HR max, as well as determine the ISWT intensity in the female population; (2) To elaborate an equation to predict the VO_2 peak; and (3) validate the equation. The sample size was calculated using the statistical program G.Power 3.1 and was based on the relationship between the number of variables to be included in the multiple regression analysis and the minimum number of observations required, considering an effect size of 0.68 and power of 0.99, it was necessary 54 volunteers in order to develop a linear model containing up to 4 variables [14]. To validate the equation, another 20 volunteers were required [14].

Subjects

Women between 18 and 45 years of age were recruited by convenience from Diamantina city, Minas Gerais state, Brazil. The inclusion criteria were: self-report of no acute or chronic diseases; eutrophic according to the body mass index (BMI between 18.5 and 24.9 kg/m²); no smoker; sedentary (not performing physical activity for 30 minutes or more at least three times a week) [19]. The participants were excluded from the study if did not reach the maximal test values on the treadmill (% predicted HR max higher than 90%) and those who failed to understand the tests. This study was approved by the Ethics and Research Committee of Universidade Federal dos Vales do Jequitinhonha e Mucuri, Brazil (protocol 1.184.419/2015) and conducted in accordance with the Resolution Nº 466/12 of the National Health Council and the Declaration of Helsink. The participants were informed about the procedures and potential risks associated with the study and all gave written informed consent.

Assessment of cardiorespiratory fitness

To evaluate the cardiorespiratory fitness, all participants were instructed to avoid physical activity and intake caffeine and alcohol in the 24 h prior to the test, to get at least 8 hours of sleep the night before, to eat a light meal and to ingest 500 ml of water two hours before the tests [19]. During all tests performed, the exhaled gases were collected and assessed by a portable telemetric gas analysis system (K4b2, Cosmed, Rome, Italy). Among other variables, VO_2, R and HR breath-by-breath were monitored. The data were tabulated and was defined as VO_2 peak and R peak the highest value of these measures at peak effort [20]. Predicted HR max was calculated by the equation HR max = 220 – age [21].

The first phase of the study was performed on three consecutive days. On the first day, the anthropometric variables weight, height and BMI, were measured and a familiarization was performed. On subsequent days, the CEPT or the ISWT was performed by randomization.

The ISWT was performed in a 10-m course identified by two cones placed 0.5 m from each end point, with an initial speed of 0.5 m/s, increasing 0.17 m/s every minute. The protocol used was composed of 15 stages of 1 min, to prevent the ceiling effect [10, 22] and the walking speed was dictated by a sound [6]. The test was interrupted if the volunteer did not reach the cone once, at the request of the volunteer or for some other reported symptom (dyspnea, dizziness, vertigo, and angina). The CEPT protocol was based on the progression of the ISWT, with the same initial speed and the same speed increase every minute, without changing the incline of the treadmill. The criteria for interrupting the CEPT was systolic blood pressure (SBP) greater than 210 mm Hg; diastolic blood pressure greater than 120 mm Hg; sustained decrease in SBP; angina; dyspnea; cyanosis; nausea; dizziness; or by volunteer's request [19].

In the second and third stage, the participants performed two ISWT with an interval of 30 minutes between then [23] and the results of the test with the longest walking distance were used for the statistical analysis. To validate the equation, a different group of women was selected according to the same inclusion criteria of the study. The VO_2 peak obtained by the gas analyzer was compared with the VO_2 peak predicted by the elaborated equation.

Statistical analysis

Statistical analysis was performed with the Statistical Package for Social Sciences programs version 22.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0 (Inc., USA). Data were presented as mean (standard deviation). In the first stage the normality of the data was calculated by Shapiro-Wilk test. As the data presented normal distribution, the comparison between the means of the physiological variables evaluated (VO_2 peak, R peak, HR max and % predicted HR max) were performed using Paired T-test. The correlation analysis of the variables collected was performed by Pearson`s correlation. The agreement of

the variables collected was performed by the Bland-Altman analysis. In the second stage, the Kolmogorov-Smirnov test was used, and the analysis of multiple linear regression was performed with the variables age, weight, height and distance walked defined a priori to elaborate the VO_2 peak prediction equation. For the validation of the equation, the Shapiro-Wilk test was performed and then the paired T-test to compare the mean values of the VO_2 peak values obtained by the equation with those obtained by the analyzer of gases. In addition, the comparison

between the women of first and third stages were realized using the Independent test t or Mann-Whitney test, according of normality of data. The level of statistical significance adopted was $P < 0.05$.

Results

First stage: Comparison between CEPT and ISWT

The general characteristics of the participants of first and second stage and their performance on ISWT are showed in table 1.

Table 1. General characteristics of participants study.

Variable	*N=54*
Age (years)	26.41± 5.6 (24.89-27.92)
Weight (kg)	56.56 ± 9.1 (54.08-59.05)
Height (m)	1.63 ± 0.1 (1.608-1.641)
BMI (kg/m²)	21.86 ± 1.8 (21.38-22.33)

Distance walked (m)	821.10 ± 118.9 (788.7-853.6)
Walking speed (m/s)	2.06 ± 0.2 (2.013-2.104)

The data is presented as mean ± SD (95% CI). BMI = body mass index.

Forty volunteers performed both ISWT and CEPT and their cardiorespiratory responses are presented in table 2. There was no statistically significant difference for any of the variables, except for the R peak, which was higher in the ISWT. According to the percentage of predicted HR max (above 90%) and R peak (> 1.1), the ISWT could be considered a test of maximum intensity [14, 24, 25]. Blood pressure and heart rate were monitored during all tests and there were no intercurrences.

Table 2. Comparison between the cardiorespiratory variables obtained in the ISWT and in the CEPT.

Outcome	Groups		Comparison between interventions
	ISWT (n = 40)	CEPT (n = 40)	P-value
VO_2 peak (mL/kg/min)	30.20(4.78)	30.35(4.81)	0.842
R peak	1.22(0.13)	1.18(0.1)	0.022*
HR max (bpm)	187.6(9.26)	186.7(9.63)	0.460
Predicted HR max (%)	97.25(4.51)	96.77(4.69)	0.463

The data is presented as mean (SD). *P<0.05. ISWT = Incremental Shuttle Walking Test; CEPT= cardiopulmonary exercise test; VO_2 = oxygen uptake; R = respiratory exchange ratio; HR = heart rate; Paired-t test.

Significant correlations were found for the variables VO_2 peak, HR max and R peak (Fig 1). The Bland-Altman analysis also demonstrated agreement between the VO_2 peak in the ISWT and in the CEPT (Fig 2).

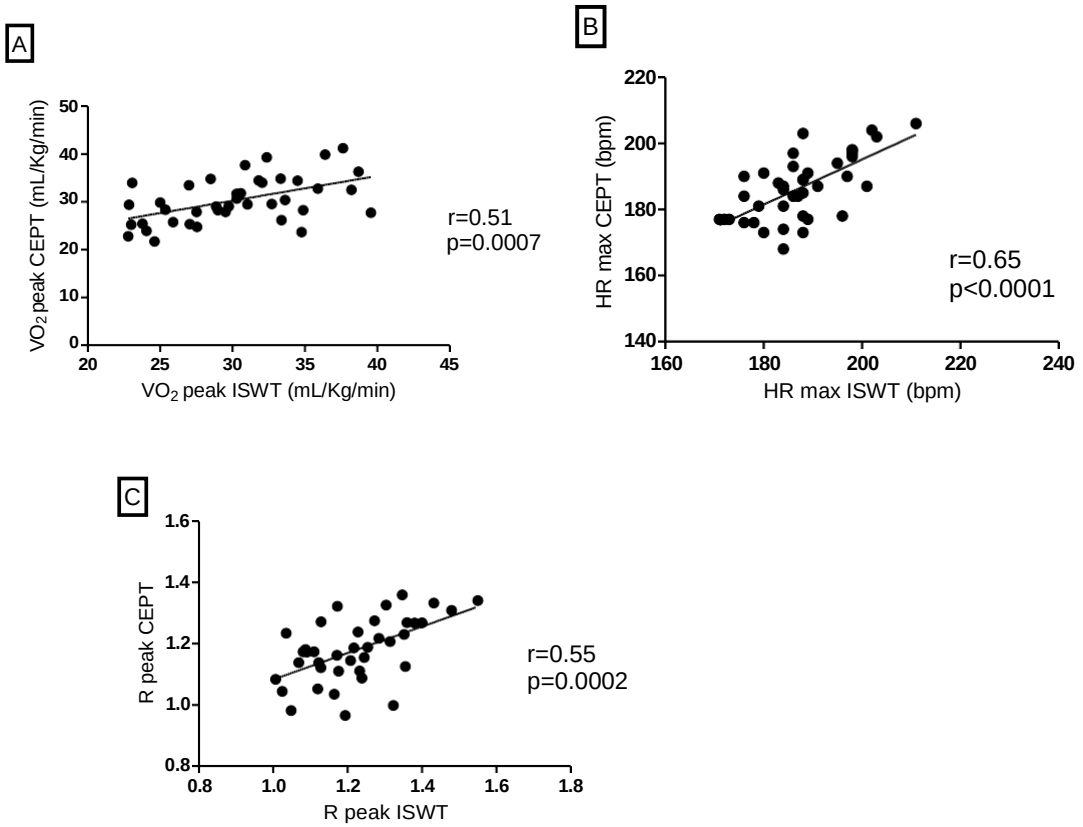
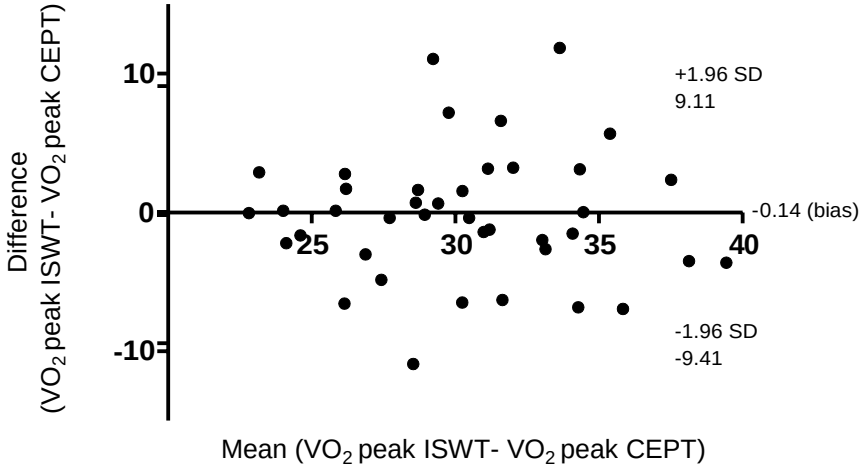


Fig 1. Correlation between (A) VO_2 peak, (B) HR max and (C) R peak in the ISWT and the CEPT. ISWT = Incremental Shuttle Walking Test; CEPT= cardiopulmonary exercise test; VO_2 = oxygen uptake; HR max = maximum heart rate; R = respiratory exchange ratio.



153

154 **Fig 2. Bland-Altman agreement of VO_2 peak in the ISWT and the CEPT.** ISWT =
155 Incremental Shuttle Walking Test; CEPT= cardiopulmonary exercise test; VO_2 = oxygen
156 uptake.

157 Second stage: Reference equation for VO_2 peak

158 The univariate analysis was performed with the variables age, weight, height and
159 distance walked (N=54). A model of stepwise linear multiple regressions showed distance
160 walked on ISWT and age explained 36.3% (Adjusted R Square = 0.363) of the variance in
161 VO_2 peak. The reference equation for the VO_2 peak in the ISWT was:

162 VO_2 peak (predicted) = 19.793 + (0.02 x distance walked) - (0.236 x age)

163 Third stage: Validation of the reference equation

164 The characteristics of the volunteers who participated in the equation validation are
165 present in table 3.

166 Table 3. General characteristics of the study participants.

Variable	*N=20*
Age (years)	25.85 ± 5.6 (23,24-28,46)

Weight (kg)	55.84 ± 5.7 (53.16-58.51)
Height (m)	1.62 ± 0.04 (1.594-1.638)
BMI (kg/m²)	21.34 ± 1.5 (20.61-22.07)
Distance walked (m)	865 ± 100.2 (818.1-911.9)
Walking speed (m/s)	2.11 ± 0.14 (2.049-2.181)

The data is presented as mean ± SD (95% CI). BMI = body mass index.

There was no statistically significant difference between the participants of equation elaboration and validation for age, weight, height, BMI, Distance walked and Walking speed (P>0.05; data not show).

When the reference equation was applied in this group, there was no statistically significant difference of the VO_2 peak obtained by the use of the gold standard method in comparison to that obtained by the equation [32.50 (5.6) mL/kg/min and 30.99 (2.6) mL/ kg/ min, respectively; p = 0.178)]. It was possible to verify the agreement between these measures by the Bland-Altman method, in which a bias of 1.5 mL/kg/min was observed, representing a difference of 3.6% between the ways of measuring VO_2 peak (Fig 3).

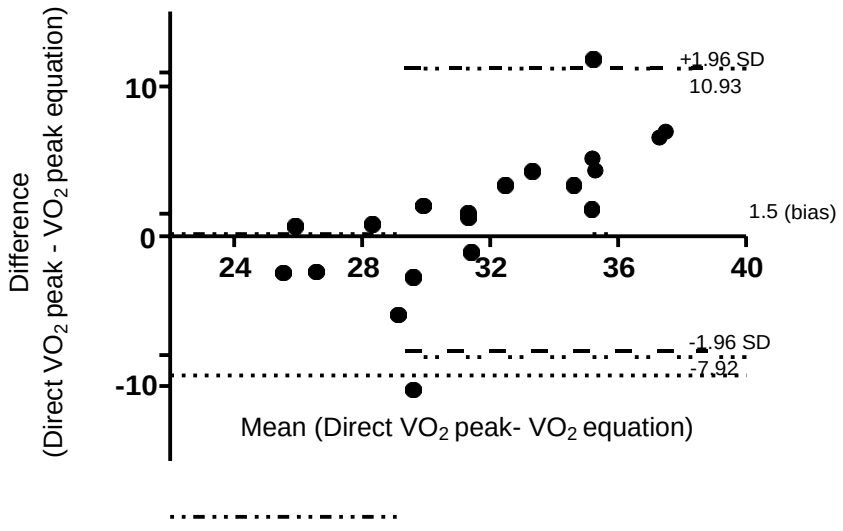


Fig 3. Bland-Altman agreement of VO_2 peak in the validation of the reference equation. ISWT = Incremental Shuttle Walking Test; CEPT= cardiopulmonary exercise test; VO_2 = oxygen uptake.

Discussion

In the present study it was observed that the direct VO_2 peak measurement was concordant between the CEPT and the ISWT and that ISWT was a maximum test in the young healthy women population. Considering that the direct analysis of VO_2 is not feasible for clinical practice, an equation was elaborated and validated to estimate this measure. The distance walked on ISWT and age were the variables that composed the equation. Furthermore, there was agreement between VO_2 peak measured directly and that estimated by the elaborated equation, indicating its validity and applicability in this population.

In the last years the ISWT has been applying in the healthy population [10, 11, 18, 26]. However, as far as we know, this is the first study that a comparison between CEPT and ISWT was performed in healthy young women. Initial investigations were carried out in patients with COPD, cystic fibrosis and chronic heart failure, showing strong and significant correlations for VO_2 peak of CEPT and ISWT [7, 9, 12].

In a study recently published by our research group, male healthy adults showed HR max, VO_2 peak and R peak values with strong and significant correlations and agreement between the ISWT and the CEPT, with ISWT being a maximal test for this population [14]. Considering that the maximum VO_2 values for women are about 70% of the average values for men [27] and that is not known whether ISTW is a maximum test for healthy young women, we initially investigated the intensity of ISTW.

Since the values of HR max above 90% of predicted and R peak > 1.1 [14, 24, 25], we establish that this is a maximum test for this population, and similar VO_2 peak results were found between CEPT and ISWT. Further tests carried out with patients with cardiopulmonary diseases concurred with our findings [6, 12, 28, 29]. However, data on the validity of the ISWT to evaluate VO_2 peak in healthy individuals are scarce in the literature [18]. Gonçalves et. al [30], studying subjects of both sexs, different age (≥ 18 years old), who presented comorbidities such as arterial hypertension, peripheral vascular disease, arthritis and cardiopathies, also concluded that ISWT above 12 levels requires maximum effort in these individuals.

As the direct analysis of the exhaled gases has a high cost, the use of prediction equations becomes more applicable due to the feasibility and low cost. Considering our results that ISTW is a maximum test to healthy women, its usefulness is reinforced as a simple way of measuring CRF. In this context, an equation was then elaborated for the prediction of VO_2 peak in ISTW.

In our study, age and distance walked accounted for more than 30% of VO_2 peak variance. In the literature it is reported that beyond gender, other factors that influence VO_2 peak as genetic factors, age, weight, and training [31]. Findings similar to our study were found in obese women, where there was a significant correlation between the VO_2 peak in the cardiopulmonary exercise test with the ISWT VO_2 peak and the ISWT distance [5]. In this same study, the variables age and distance walked by the ISWT explained the predictive model for the VO_2 peak.

Only two other studies have published a reference equation for VO_2 peak using ISWT, highlighting the variables distance and body mass in the prediction [11, 32]. In the study of Dourado et. al [11] the distance in the ISWT was selected, the maximum walking velocity, and distance in the ISWT × body mass as the only determinants of the peak VO_2. This is

consistent with the variables selected in our study. However, they did not compare to another cardiopulmonary exercise test, nor did they validate the equation.

As age is a determining factor for VO_2 peak, it is important to highlight that several studies have used the ISWT in the older population [10, 11, 22, 23, 26] or in children and adolescents [15-17], and some evaluated stratifying age groups [2, 30]. Due to the influence of cardiorespiratory fitness on functional independence, there is great interest in describing age-related changes in maximum oxygen consumption. Evidences support a 10% per decade decline in VO_2 max in men and women regardless of activity level [33]. For all the facts reported, it makes sense for age to be a predictor of VO_2 peak in the elaborated equation.

Our study presents differentials when proposing a prediction equation for VO_2 peak, the main variable for evaluation of cardiorespiratory fitness [19, 34], since most of the studies with ISWT focus on the prediction of walking distance [2, 10, 15, 17, 22, 23]. In addition, those who did the VO_2 peak prediction equation for women did not validate it [5, 11]. The equation developed in this study was validated in other volunteers and the VO_2 peak values obtained by the equation and the values of VO_2 peak obtained by the gas analyzer were similar, indicating that the application of the equation is feasible to estimate the VO_2 peak of the chosen population.

The limitation of the study was the level of physical activity having been self-reported, but this strategy is adopted in scientific studies [35, 36].

Conclusion

The Incremental Shuttle Walking Test was concordant with the CEPT, requiring maximum effort in young health women. The elaborated equation is valid and applicable, being a simple and inexpensive tool to evaluate the cardiorespiratory fitness in the study population.

Acknowledgments

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References

1. Després JP. Physical Activity, Sedentary Behaviours, and Cardiovascular Health: When Will Cardiorespiratory Fitness Become a Vital Sign? Can J Cardiol 2016 Apr; 32(4):505-13.

2. Agarwal B, Shah M, Andhare N, Mullerpatan R. Incremental Shuttle Walk Test: Reference values and predictive equation for healthy Indian adults. Lung India 2016 Jan-Feb;33(1):36-41.

3. American Thoracic Society/ American College of Chest Physicians. ATS/ACCP statement on cardiopulmonary exercise testing. Am J Respir Care Med. 2003; 167 (2): 211-77.

4. American College of Sports Medicine. Diretrizes do ACSM para os testes de esforço e sua prescrição. Rio de Janeiro: Guanabara Koogan, 2003.

5. Jürgensen SP, Trimer R, Di Thommazo-Luporini L, Dourado VZ, Bonjorno-Junior JC, Oliveira CR, et al. Does the incremental shuttle walk test require maximal effort in

young obese women? Braz J Med Biol Res [online]. 2016; vol.49, n.8, e5229. Epub 11-Jul-2016.

6. Singh SJ, Morgan MD, Scott S, Walters D, Hardman AE. Development of a shuttle walking test of disability in patients with chronic airways obstruction. Thorax 1992; 47: 1019-1024.

7. Bradley J, Howard J, Wallace E, Elborn S. Validity of a modified shuttle test in adult cystic fibrosis. Thorax 1999; 54 (5): 437 – 439.

8. Morales FJ, Martínez A, Méndez M, Agarrado A, Ortega F, Fernández-Guerra J et al . A shuttle walk test for assessment of functional capacity in chronic heart failure. Am Heart J 1999;138 (2): 291 – 298.

9. Lewis ME, Newall C, Townsend JN, Hill SL, Bonster RS. Incremental Shuttle walk test in the assessment of patients for heart transplantation. Heart 2001; 86:183-7.

10. Dourado VZ, Vidotto MC, Guerra RLF. Reference equations for the performance of healthy adults on field walking tests. J Bras Pneumol 2011; 37: 607–614.

11. Dourado VZ, Guerra RL, Tanni SE, Antunes LC, Godoy I. Reference values for the incremental shuttle walk test in healthy subjects: from the walk distance to physiological responses. J Bras Pneumol 2013; 39: 190–197.

12. Singh SJ, Morgan MD, Hardman AE, Rowe C, Bardsley PA. Comparison of oxygen uptake during a conventional treadmill test and the shuttle walking test in chronic airflow limitation. Eur Respir J 1994; 7: 2016-2020.

13. Jürgensen SP et al. Shuttle walking test in obese women: test-retest reliability and concurrent validity with peak oxygen uptake. Clin Physiol Funct Imaging 2015. 35:120–126.

14. Neves, CDC, Lacerda AC, Lage VK, Lima LP, Fonseca SF, de Avelar NC, et al. Cardiorespiratory responses and prediction of peak oxygen uptake during the shuttle walking test in healthy sedentary adult men. PLoS One 2015 Feb 6;10(2):e0117563.

15. Lanza FC, Zagatto Edo P, Silva JC, Selman JP, Imperatori TB, Zanatta DJ, et al. Reference Equation for the Incremental Shuttle Walk Test in Children and Adolescents. J Pediatr. 2015 Nov; 167(5):1057-61.

16. Pinho TMP. Predictive Equation for Incremental Shuttle Walk Test in Adolescents Universidade de Aveiro. 2015 Available from: http://hdl.handle.net/10773/14580.

17. Vardhan V, Palekar T, Dhuke P, Baxi G. Normative Valeus of Incremental Shuttle Walk Test in Children AND Adolescents: Observational study. Int J Pharma Bio Sci 2017 October; 8(4): (B) 478-483.

18. Dourado VZ, and R.L.F. Guerra. Reliability and validity of heart rate variability threshold assessment during an incremental shuttle-walk test in middle-aged and older adults. Braz J Med and Biol Res 2013; 46: 194-199.

19. ACSM, American College os Sports Medicine. Diretrizes do ACSM para os testes de esforço e sua prescrição. Balady, Gary J, 6ªed., Guanabara Koogan; 2014.

20. Herdy AH, Caixeta A. Classificação Nacional da Aptidão Cardiorrespiratória pelo Consumo Máximo de Oxigênio. Arq Bras Cardiol 2016; 106(5):389-395.

311 21. Karvonen MJ, Kental E, Mustala. The effects of on heart rate a longitudinal study.
312 Ann Med Exper Fenn 1957 35: 307–315.

313 22. Jürgensen SP, Antunes LC, Tanni SE, Banov MC, Lucheta PA, Bucceroni AF, Godoy
314 I, Dourado VZ. The incremental shuttle walk test in older Brazilian adults. Respiration
315 2011;81(3):223-8.

316 23. Probst VS, Hernandes NA, Teixeira DC, Felcar JM, Mesquita RB, Gonçalves CG et
317 al. Reference values for the incremental shuttle walking test. Respiratory medicine
318 2012; 106(2): 243-248.

319 24. Robergs RA, Dwyer D, Astorino T. Recommendations for improved data processing
320 from expired gas analysis indirect calorimetry. Sports Med 2010; 40: 95–111.

321 25. Sawyer BJ, Blessinger JR, Irving BA, Weltman A, Patrie JT, Gaesser GA. Walking
322 and running economy: inverse association with peak oxygen uptake. Med Sci Sports
323 Exerc 2010; 42: 2122–2127.

324 26. Harrison SL, Greening NJ, Houchen-Wolloff L, Bankart J, Morgan MD, Steiner MC,
325 et al. Age-specific normal values for the incremental shuttle walk test in a healthy
326 British population. J Cardiopulm Rehabil Prev 2013; 33: 309–313.

327 27. Nunes RAM, Pontes GFR, Dantas PMS, Fernandes Filho, J. Referencial table of
328 cardiopulmonary fitness. Fitness & Performance Journal 2005; 4(1), 27 – 33.

329 28. Onorati P, Antonucci R, Valli G, Berton E, De Marco F, Serra P, et al. Non-invasive
330 evaluation of gas exchange during a shuttle walking test vs. a 6-min walking test to
331 assess exercise tolerance in COPD patients. Eur J Appl Physiol 2003; 89: 331-336.

29. Palange P, Forte S, Onorati P, Manfredi F, Serra P, Carlone S. Ventilatory and metabolic adaptations to walking and cycling in patients with COPD. J Appl Physiol 2000; 88:1715-1720.

30. Gonçalves CG, Mesquita R, Hayashi D, Merli MF, Vidotto LS, Fernandes KBP, et al. Does the Incremental Shuttle Walking Test require maximal effort in healthy subjects of different ages? Physiotherapy 2015 Jun;101(2):141-6.

31. American Thoracic Society/ American College of Chest Physicians. ATS/ACCP statement on cardiopulmonary exercise testing. Am J Respir Care Med. 2003; 167 (2): 211-77.

32. Jurio-Iriarte B, Gorostegi-Anduaga I, Aispuru GR, Pérez-Asenjo J, Brubaker PH, Maldonado-Martín S. Association between Modified Shuttle Walk Test and Cardiorespiratory Fitness in Overweight/Obese Adults with Primary Hypertension: EXERDIET-HTA Study. J Am Soc Hypertens 2017 Apr; 11(4):186-195.

33. Hawkings S,Wiswell R. Rate and Mechanism of Maximal Oxygen Consumption Decline with Aging Implications for Exercise Training. Sports Med 2003; 33(12):877-88.

34. American Thoracic Society/European Respiratory Society. American Thoracic Society/European Respiratory Society statement on pulmonary rehabilitation. Am J Resp Crit Care Med 2006; 173: 1390–1413.

35. Lima VP, Velloso M, Almeida FD, Carmona B, Ribeiro-Samora GA, Janaudis-Ferreira T. Test–retest reliability of the unsupported upper-limb exercise test (UULEX) and 6-min peg board ring test (6PBRT) in healthy adult individuals. Physiotherapy Theory and Practice 2018 Jan; 19:1-7.

36. Robles P, Araujo T, Brooks D, Zabjek K, Janaudis-Ferreira T, Marzolini S, Goldstein R, Mathur S. Cardiorespiratory Responses to Short Bouts of Resistance Training Exercises in Individuals With Chronic Obstructive Pulmonary Disease: A COMPARISON OF EXERCISE INTENSITIES. J Cardiopulm Rehabil Prev 2017 Sep; 37(5):356-362.

ANEXO A- COMPROVANTE DE APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA

UNIVERSIDADE FEDERAL DOS VALES DO JEQUITINHONHA E MUCURI (FAFEID-UF)

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO DA APTIDÃO CARDIORRESPIRATÓRIA DURANTE O SHUTTLE WALKING TEST EM MULHERES SAUDÁVEIS

Pesquisador: VANESSA A MENDONÇA

Área Temática:

Versão: 2

CAAE: 45623315.9.0000.5108

Instituição Proponente: Universidade Federal dos Vales do Jequitinhonha e Mucuri

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.184.419

Data da Relatoria: 24/08/2015

Apresentação do Projeto:

A avaliação da aptidão cardiorrespiratória (ACR) tem sido comumente realizada com o objetivo de fornecer informações acerca da tolerância reduzida ao exercício em diversas condições patológicas, bem como fornecer parâmetros para a prescrição e elaboração de um programa de exercícios. Dentre os diversos testes de campo para avaliação da ACR de pacientes cardiopulmonares, destaca-se o Shuttle Walking Test (SWT), um teste estrutura incremental e progressiva, com a passada ditada externamente. A aplicação do SWT com intuito de avaliar a ACR em indivíduos

saudáveis tem sido implementada nos últimos anos, no entanto, a aplicação deste em mulheres saudáveis ainda é pouco conhecida e a intensidade do exercício ainda não está bem estabelecida. Sendo assim, este projeto tem como objetivo avaliar a ACR durante o Shuttle Walking Test em mulheres saudáveis. Métodos: Serão convidadas a participar desse estudo 53 mulheres adultas, saudáveis, sedentárias, com idade entre 18-45 anos. Cada voluntária passará pelas três etapas do estudo, sendo elas: (1) avaliação da composição corporal e familiarização; (2) avaliação da ACR por meio de teste incremental em esteira e (3) avaliação da ACR por meio do SWT. A ordem de realização das etapas 2 e 3 será aleatória e balanceada. Durante ambos os testes o consumo máximo de oxigênio (VO2 máx) será continuamente monitorado pelo analisador de gases. Além

disso, antes, durante e após os testes serão avaliadas e monitoradas a percepção subjetiva do esforço, a pressão arterial e a frequência cardíaca máxima. Durante o SWT serão registradas as etapas e voltas alcançadas no teste para cálculo da distância percorrida. Resultados esperados: Espera-se que o SWT produza respostas cardiorrespiratórias máximas e que a equação de referência será viável para a predição do VO2 pico de mulheres saudáveis.

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar a aptidão cardiorrespiratória durante o Shuttle Walking Test em mulheres saudáveis.

Objetivo Secundário:

Comparar o consumo pico de oxigênio (VO2máx), o quociente respiratório pico (Rpico) e a frequência cardíaca máxima mensurados durante o Shuttle Walking Test com teste incremental em esteira em mulheres saudáveis. - Verificar se há correlação e concordância entre variáveis cardiorrespiratórias (VO2pico, R pico e FC máx) mensuradas durante o Shuttle Walking Test e teste incremental em esteira em mulheres saudáveis.- Propor equação matemática que permita estimar o consumo pico de oxigênio a partir do Shuttle Walking Test em mulheres saudáveis.

Avaliação dos Riscos e Benefícios:

Adequados. Os pesquisadores afirmam que como as voluntárias realizarão dois testes progressivos, após a realização dos mesmos, elas podem estar sujeitas a riscos relacionados à prática de atividade física como tonturas, dispnéia, cansaço intenso e fadiga muscular. Entretanto, tais sintomas são minimizados com o repouso após a

realização do teste. A pressão arterial e a freqüência cardíaca serão monitoradas continuamente antes, durante e após o teste. Para recuperação as mesmas serão orientados a permanecer sentadas, mantendo-se a freqüência respiratória em padrão fisiológico. Caso haja dor muscular após o teste, essa poderá ter duração de até 03 dias. Para isso, para reduzir o sintoma, as voluntárias serão orientadas a aplicar bolsa de gelo no local da dor, durante 30 minutos. Deve-se ressaltar que haverá controle da realização dos procedimentos, os pesquisadores serão treinados previamente, os equipamentos são modernos e haverá também controle dos dados vitais da voluntária, diminuindo assim a probabilidade de intercorrências para os mesmos durante o teste. Também será permitida a interrupção do procedimento se necessário. Todo o material utilizado será devidamente higienizado e desinfetado. Os testes serão aplicados por um pesquisador devidamente treinado, uma vez que segundo as Diretrizes do American College of Sports Medicine (ACSM, 2003), a supervisão médica em testes máximos e submáximos para pacientes de baixo

risco são desnecessários. De forma a minimizar os riscos, como estabelecido pelos critérios de inclusão do estudo, somente serão incluídas as voluntárias que se enquadrarem na categoria de baixo risco, como demonstrado adiante: -Pacientes com baixo risco: sujeitos 45 anos de idade, que são assintomáticos e não satisfazem mais de um limiar dos fatores de risco (ACSM, 2003). Incluem-se como fatores de risco: -História familiar: Infarto agudo do miocárdio, revascularização coronariana ou morte súbita antes dos 55 anos de idade no pai ou em outro parente masculino de primeiro grau, ou antes de 65 anos de idade na mãe ou em outro parente feminino de primeiro grau; -Fumo de cigarros: fumante atual de cigarros ou aqueles que deixaram de fazê-lo no transcorrer dos 6 meses precedentes; Hipertensão: PAS > 140 mmHg ou PAD > 90 mmHg, confimadas por 2 mensurações ou por utilização medicamentosa; -Obesidade: (IMC > 30, ou circunferência cintura > 102 cm em homens e 88 cm em mulheres); - Sedentarismo. Para garantir a segurança do voluntário, os testes serão realizados em um local onde uma rápida e apropriada resposta emergencial possa ser realizada. No ambiente das avaliações estará disponível uma fonte de oxigênio (via cateter nasal). Caso necessário, imediatamente após alguma intercorrência será acionado por telefone o serviço de emergência do SAMU. Contudo, deve-se ressaltar que as avaliações propostas já foram realizadas em estudos anteriores do nosso grupo de pesquisa, os quais foram publicados na literatura, relatando ausência de intercorrências (Neves et al., 2015).

Como benefícios, os pesquisadores apontam o conhecimento da atual aptidão física da voluntária, a utilização dos dados das avaliações realizadas (composição corporal e aptidão cardiorrespiratória) para auxiliar na elaboração de um programa de exercícios, bem como a motivação para o início da atividade física. Para a comunidade científica, espera-se que os achados do presente projeto possam contribuir para um melhor entendimento sobre a real intensidade do SWT em mulheres saudáveis, promovendo informações que auxiliem futuros estudos.

Comentários e Considerações sobre a Pesquisa:

A avaliação da aptidão cardiorrespiratória consistirá da realização de dois testes cardiorrespiratórios, um teste incremental na esteira e um teste de campo de caminhada (Shuttle Walking Test - SWT). Durante ambos os testes o consumo (captação, transporte e utilização de oxigênio) máximo de oxigênio (VO2 máx) será continuamente monitorado através da espirometria de circuito aberto, pelo sistema de telemetria do analisador de gases K4b2 (COSMED). Para isso, as voluntárias irão respirar utilizando uma máscara facial e portarão de um colete que contém a

unidade do equipamento. Shuttle Walking Test: O SWT consiste em caminhar em terreno plano percorrendo de maneira repetida uma distância conhecida de 10 metros, ao redor de uma marcação de dois cones, separados a uma distância de 9 metros. A velocidade da caminhada é ditada por um sinal sonoro, onde a sonorização acústica única indica o tempo em que o paciente deve percorrer a distância predeterminada, alcançar o cone e mudar de
direção retornando ao outro cone, enquanto que a sinalização acústica tripla indica a necessidade de aumentar a velocidade para percorrer a distância entre os cones. Durante o teste, os sinais sonoros vão se tornando mais próximos a cada minuto, levando a voluntária a caminhar em uma velocidade cada vez maior. A velocidade inicial é de 0,5 m/s e aumenta em 0,17 m/s a cada minuto, com a duração máxima de 15 minutos. Não são permitidas pausas durante o teste, de forma que a voluntária permaneça marchando no lugar até ouvir o próximo sinal sonoro e reiniciar a
caminhada. A prova chegava ao fim caso o indivíduo não seja capaz de alcançar por uma vez o cone ou se o mesmo desejar interromper a prova por sintomas (fadiga, cansaço, vertigem, tontura) podendo ser desencadeados ao aumentar a velocidade da caminhada. Trata- se, portanto, de uma prova incremental com estágios de até 15 níveis de velocidade. Serão registradas as etapas e voltas alcançadas no teste para cálculo da distância percorrida, além da pressão arterial (PA) no início e final do teste e a freqüência cardíaca (FC) a cada mudança de nível de velocidade. Teste Incremental na Esteira: O protocolo utilizado no teste incremental na esteira será baseado na progressão do SWT, sendo composto por estágios de 1 minuto, com incremento da velocidade a cada minuto, sem aumento da inclinação da esteira. A velocidade inicial será de 0,5 m/s com acréscimos de 0,17 m/s a cada estágio.Inicialmente a voluntária será mantida em repouso, sentada por 10 minutos. Serão medidas FC, PA e percepção
subjetiva do esforço (PSE). A percepção subjetiva do esforço será avaliada pela escala de Borg que classifica a intensidade do exercício em uma escala de 6 a 20, variando de muito, muito leve a muito, muito difícil. Todas as medidas serão realizadas no repouso, ao final de cada estágio e na recuperação. Após o repouso inicial, a voluntária será orientada sobre o teste e posteriormente posicionado na esteira para realização do mesmo. Durante todo o teste a voluntária será questionada quanto à sintomatologia e possibilidade de dar continuidade ao procedimento. Aumento da PA sistólica (PAS) acima de 210 mmHg, da PA diastólica (PAD) acima de 120 mmHg, queda sustentada da PAS, angina, dispnéia, cianose, palidez, náusea, tontura, vista turva, desejo da voluntária,fadiga, claudicação, câimbra, PSE através do Borg acima de 18 e FC acima de 90% da FC máx prevista para a idade (220 – idade) serão os critérios para interrupção do teste (ACSM,

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Continuação do Parecer: 1.184.419

2003). Assim que concluído o teste, a voluntária realizará a recuperação ativa (05 minutos de caminhada em baixa velocidade) e passiva (05 minutos de repouso sentada).

Considerações sobre os Termos de apresentação obrigatória:

Foi apresentado o Projeto de Pesquisa, Folha de Rosto, Cronograma, TCLE e carta de concordância dos setores com assinatura dos responsáveis. O TCLE está adequado (informações necessárias para os sujeitos da pesquisa, linguagem acessível e contato do CEP/UFVJM atualizado, conforme a Resolução 466/12).

Recomendações:

- Segundo a Carta Circular nº. 003/2011/CONEP/CNS, de 21/03/11, há obrigatoriedade de rubrica em todas as páginas do TCLE pelo sujeito de pesquisa ou seu responsável e pelo pesquisador, que deverá também apor sua assinatura na última página do referido termo.
- Relatório final deve ser apresentado ao CEP ao término do estudo em 30/09/2016. Considera-se como antiética a pesquisa descontinuada sem justificativa aceita pelo CEP que a aprovou.

Conclusões ou Pendências e Lista de Inadequações:

O projeto atende aos preceitos éticos para pesquisas envolvendo seres humanos preconizados na Resolução 466/12 CNS.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

DIAMANTINA, 13 de Agosto de 2015

Assinado por:
Disney Oliver Sivieri Junior
(Coordenador)

ANEXO B- NORMAS PARA SUBMISSÃO REVISTA PLOS ONE

Style and Format

File format	Manuscript files can be in the following formats: DOC, DOCX, or RTF. Microsoft Word documents should not be locked or protected. LaTeX manuscripts must be submitted as PDFs. Read the LaTeX guidelines.
Length	Manuscripts can be any length. There are no restrictions on word count, number of figures, or amount of supporting information. We encourage you to present and discuss your findings concisely.
Font	Use a standard font size and any standard font, except for the font named "Symbol". To add symbols to the manuscript, use the Insert → Symbol function in your word processor or paste in the appropriate Unicode character.
Headings	Limit manuscript sections and sub-sections to 3 heading levels. Make sure heading levels are clearly indicated in the manuscript text.
Layout and spacing	Manuscript text should be double-spaced. Do not format text in multiple columns.
Page and line numbers	Include page numbers and line numbers in the manuscript file. Use continuous line numbers (do not restart the numbering on each page).
Footnotes	Footnotes are not permitted. If your manuscript contains footnotes, move the information into the main text or the reference list, depending on the content.
Language	Manuscripts must be submitted in English. You may submit translations of the manuscript or abstract as supporting information. Read the supporting information guidelines.
Abbreviations	Define abbreviations upon first appearance in the text. Do not use non-standard abbreviations unless they appear at least three times in the text. Keep abbreviations to a minimum.
Reference style	PLOS uses "Vancouver" style, as outlined in the ICMJE sample references. See reference formatting examples and additional instructions below.
Equations	We recommend using MathType for display and inline equations, as it will provide the most reliable outcome. If this is not possible, Equation Editor or Microsoft's Insert→Equation function is acceptable. Avoid using MathType, Equation Editor, or the Insert→Equation function to insert single variables (e.g., "$a^2 + b^2 = c^2$"), Greek or other symbols (e.g., β, Δ, or ′ [prime]), or mathematical operators (e.g., x, ≥, or ±) in running text. Wherever possible, insert single symbols as normal text with the correct Unicode (hex) values. Do not use MathType, Equation Editor, or the Insert→Equation function for only a portion of an equation. Rather, ensure that the entire equation is included. Equations should not contain a mix of different equation tools. Avoid "hybrid" inline or display equations, in which part is text and part is MathType, or part is MathType and part is Equation Editor.

Manuscript Organization

Manuscripts should be organized as follows. Instructions for each element appear below the list.

Beginning section	*The following elements are required, in order:* • Title page: List title, authors, and affiliations as first page of manuscript • Abstract • Introduction
Middle section	*The following elements can be renamed as needed and presented in any order:* • Materials and Methods • Results • Discussion • Conclusions (optional)
Ending section	*The following elements are required, in order:* • Acknowledgments • References • Supporting information captions (if applicable)
Other elements	• Figure captions are inserted immediately after the first paragraph in which the figure is cited. Figure files are uploaded separately. • Tables are inserted immediately after the first paragraph in which they are cited. • Supporting information files are uploaded separately.

Viewing Figures and Supporting Information in the compiled submission PDF
The compiled submission PDF includes low-resolution preview images of the figures after the reference list. The function of these previews is to allow you to download the entire submission as quickly as possible. Click the link at the top of each preview page to download a high-resolution version of each figure. Links to download Supporting Information files are also available after the reference list.

Parts of a Submission

Title

Include a full title and a short title for the manuscript.

Title	Length	Guidelines	Examples
Full title	250 characters	Specific, descriptive, concise, and comprehensible to readers	Impact of cigarette smoke exposure on innate

		outside the field	immunity: A *Caenorhabditis elegans* model Solar drinking water disinfection (SODIS) to reduce childhood diarrhoea in rural Bolivia: A cluster-randomized, controlled trial
Short title	100 characters	State the topic of the study	Cigarette smoke exposure and innate immunity SODIS and childhood diarrhoea

Titles should be written in sentence case (only the first word of the text, proper nouns, and genus names are capitalized). Avoid specialist abbreviations if possible. For clinical trials, systematic reviews, or meta-analyses, the subtitle should include the study design.

Author list

Author names and affiliations

Enter author names on the title page of the manuscript and in the online submission system.

On the title page, write author names in the following order:

- First name (or initials, if used)
- Middle name (or initials, if used)
- Last name (surname, family name)

Each author on the list must have an affiliation. The affiliation includes department, university, or organizational affiliation and its location, including city, state/province (if applicable), and country. Authors have the option to include a current address in addition to the address of their affiliation at the time of the study. The current address should be listed in the byline and clearly labeled “current address.” At a minimum, the address must include the author’s current institution, city, and country.

If an author has multiple affiliations, enter all affiliations on the title page only. In the submission system, enter only the preferred or primary affiliation. Author affiliations will be listed in the typeset PDF article in the same order that authors are listed in the submission.

Corresponding author

The submitting author is automatically designated as the corresponding author in the submission system. The corresponding author is the primary contact for the journal office and the only author able to view or change the manuscript while it is under editorial consideration.

The corresponding author role may be transferred to another coauthor. However, note that transferring the corresponding author role also transfers access to the manuscript. (To designate a new corresponding author while the manuscript is still under consideration, watch the video tutorial below.)

Only one corresponding author can be designated in the submission system, but this does not restrict the number of corresponding authors that may be listed on the article in the event of publication. Whoever is designated as a corresponding author on the title page of the manuscript file will be listed as such upon publication. Include an email address for each corresponding author listed on the title page of the manuscript.

Consortia and group authorship

If a manuscript is submitted on behalf of a consortium or group, include the consortium or group name in the author list, and provide the full list of consortium or group members in the Acknowledgments section. The consortium or group name should be listed in the manuscript file only, and not included in the online submission form. Please be aware that as of October 2016, the National Library of Medicine's (NLM) policy has changed and PubMed will only index individuals and the names of consortia or group authors listed in the author byline itself. Individual consortium or group author members need to be listed in the author byline in order to be indexed, and if included in the byline, must qualify for authorship according to our criteria.

Author contributions

Provide at minimum one contribution for each author in the submission system. Use the CRediT taxonomy to describe each contribution. Read the policy and the full list of roles.

Contributions will be published with the final article, and they should accurately reflect contributions to the work. The submitting author is responsible for completing this information at submission, and we expect that all authors will have reviewed, discussed, and agreed to their individual contributions ahead of this time.

PLOS ONE will contact all authors by email at submission to ensure that they are aware of the submission.

Cover letter

Upload a cover letter as a separate file in the online system. The length limit is 1 page.

The cover letter should include the following information:

- Summarize the study's contribution to the scientific literature
- Relate the study to previously published work
- Specify the type of article (for example, research article, systematic review, meta-analysis, clinical trial)
- Describe any prior interactions with PLOS regarding the submitted manuscript
- Suggest appropriate Academic Editors to handle your manuscript (see the full list of Academic Editors)
- List any opposed reviewers

Title page

The title, authors, and affiliations should all be included on a title page as the first page of the manuscript file.

Abstract

The Abstract comes after the title page in the manuscript file. The abstract text is also entered in a separate field in the submission system.

The Abstract should:

- Describe the main objective(s) of the study

- Explain how the study was done, including any model organisms used, without methodological detail
- Summarize the most important results and their significance
- Not exceed 300 words

Abstracts should not include:

- Citations
- Abbreviations, if possible

Introduction

The introduction should:

- Provide background that puts the manuscript into context and allows readers outside the field to understand the purpose and significance of the study
- Define the problem addressed and why it is important
- Include a brief review of the key literature
- Note any relevant controversies or disagreements in the field
- Conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved

Materials and Methods

The Materials and Methods section should provide enough detail to allow suitably skilled investigators to fully replicate your study. Specific information and/or protocols for new methods should be included in detail. If materials, methods, and protocols are well established, authors may cite articles where those protocols are described in detail, but the submission should include sufficient information to be understood independent of these references.

Protocol documents for clinical trials, observational studies, and other non-laboratory investigations may be uploaded as supporting information. Read the supporting information guidelines for formatting instructions. We recommend depositing laboratory protocols at protocols.io. Read detailed instructions for depositing and sharing your laboratory protocols.

Human or animal subjects and/or tissue or field sampling

Methods sections describing research using human or animal subjects and/or tissue or field sampling must include required ethics statements. See the reporting guidelines for human research, clinical trials, animal research, and observational and field studies for more information.

Data

PLOS journals require authors to make all data underlying the findings described in their manuscript fully available without restriction, with rare exception.

Large data sets, including raw data, may be deposited in an appropriate public repository. See our list of recommended repositories.

For smaller data sets and certain data types, authors may provide their data within supporting information files accompanying the manuscript. Authors should take care to maximize the

accessibility and reusability of the data by selecting a file format from which data can be efficiently extracted (for example, spreadsheets or flat files should be provided rather than PDFs when providing tabulated data).

For more information on how best to provide data, read our policy on data availability. PLOS does not accept references to "data not shown."

Cell lines

Methods sections describing research using cell lines must state the origin of the cell lines used. See the reporting guidelines for cell line research for more information.

Laboratory Protocols

To enhance the reproducibility of your results, we recommend and encourage you to deposit laboratory protocols in protocols.io, where protocols can be assigned their own persistent digital object identifiers (DOIs).

To include a link to a protocol in your article:

1. Describe your step-by-step protocol on protocols.io
2. Select Get DOI to issue your protocol a persistent digital object identifier (DOI)
3. Include the DOI link in the Methods section of your manuscript using the following format provided by protocols.io: http://dx.doi.org/10.17504/protocols.io.[PROTOCOL DOI]

At this stage, your protocol is only visible to those with the link. This allows editors and reviewers to consult your protocol when evaluating the manuscript. You can make your protocols public at any time by selecting Publish on the protocols.io site. Any referenced protocol(s) will automatically be made public when your article is published.

New taxon names

Methods sections of manuscripts adding new taxon names to the literature must follow the reporting guidelines below for a new zoological taxon, botanical taxon, or fungal taxon.

Results, Discussion, Conclusions

These sections may all be separate, or may be combined to create a mixed Results/Discussion section (commonly labeled "Results and Discussion") or a mixed Discussion/Conclusions section (commonly labeled "Discussion"). These sections may be further divided into subsections, each with a concise subheading, as appropriate. These sections have no word limit, but the language should be clear and concise.

Together, these sections should describe the results of the experiments, the interpretation of these results, and the conclusions that can be drawn.

Authors should explain how the results relate to the hypothesis presented as the basis of the study and provide a succinct explanation of the implications of the findings, particularly in relation to previous related studies and potential future directions for research.

PLOS ONE editorial decisions do not rely on perceived significance or impact, so authors should avoid overstating their conclusions. See the *PLOS ONE* Criteria for Publication for more information.

Acknowledgments

Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution.

Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named.

References

Any and all available works can be cited in the reference list. Acceptable sources include:

- Published or accepted manuscripts
- Manuscripts on preprint servers, providing the manuscript has a citable DOI or arXiv URL. Read the Preprint Policy.

Source	Format
Published articles	Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (*Ailuropoda melanoleuca*). Genet Mol Res. 2011;10: 1576-1588. Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. Mol Immunol. 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005. *Note: A DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers. When providing a DOI, adhere to the format in the example above with both the label and full DOI included at the end of the reference (doi: 10.1016/j.molimm.2014.11.005). Do not provide a shortened DOI or the URL.*
Accepted, unpublished articles	Same as published articles, but substitute "Forthcoming" for page numbers or DOI.
Online articles	Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. Global Health. 2005;1: 14. Available from: http://www.globalizationandhealth.com/content/1/1/14
Books	Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.
Book chapters	Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. AIDS and the historian. Bethesda: National Institutes of Health; 1991. pp. 21-28.
Deposited articles (preprints, e-prints, or arXiv)	Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity; 1991. Preprint. Available from: arXiv:1403.3301v1. Cited 17 March 2014.
Published media (print or online newspapers and magazine articles)	Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 29 Jan 2014. Available from: http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html Cited 17 March 2014.
New media (blogs, web sites, or other	Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available from:

Do not cite the following sources in the reference list:

written works)	http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/.
Masters' theses or doctoral dissertations	Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available from: http://cumincad.scix.net/cgi-bin/works/Show?2e09
Databases and repositories (Figshare, arXiv)	Roberts SB. QPX Genome Browser Feature Tracks; 2013 [cited 2013 Oct 5]. Database: figshare [Internet]. Available from: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214
Multimedia (videos, movies, or TV shows)	Hitchcock A, producer and director. Rear Window [Film]; 1954. Los Angeles: MGM.

- Unavailable and unpublished work, including manuscripts that have been submitted but not yet accepted (e.g., "unpublished work," "data not shown"). Instead, include those data as supplementary material or deposit the data in a publicly available database.
- Personal communications (these should be supported by a letter from the relevant authors but not included in the reference list)

References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., "We used the techniques developed by our colleagues [19] to analyze the data"). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

Do not include citations in abstracts or author summaries.

Make sure the parts of the manuscript are in the correct order *before* ordering the citations.

Formatting references

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the "Vancouver" style. Example formats are listed below. Additional examples are in the ICMJE sample references.

Journal name abbreviations should be those found in the National Center for Biotechnology Information (NCBI) databases.

Supporting Information

Authors can submit essential supporting files and multimedia files along with their manuscripts. All supporting information will be subject to peer review. All file types can be submitted, but files must be smaller than 10 MB in size.

Authors may use almost any description as the item name for a supporting information file as long as it contains an "S" and number. For example, "S1 Appendix" and "S2 Appendix," "S1 Table" and "S2 Table," and so forth.

Supporting information files are published exactly as provided, and are not copyedited.

Supporting information captions

List supporting information captions at the end of the manuscript file. Do not submit captions in a separate file.

The file number and name are required in a caption, and we highly recommend including a one-line title as well. You may also include a legend in your caption, but it is not required.

In-text citations

We recommend that you cite supporting information in the manuscript text, but this is not a requirement. If you cite supporting information in the text, citations do not need to be in numerical order.

Figures and Tables

Figures

Do not include figures in the main manuscript file. Each figure must be prepared and submitted as an individual file.

Cite figures in ascending numeric order upon first appearance in the manuscript file.

Figure captions

Figure captions must be inserted in the text of the manuscript, immediately following the paragraph in which the figure is first cited (read order). Do not include captions as part of the figure files themselves or submit them in a separate document.

At a minimum, include the following in your figure captions:

- A figure label with Arabic numerals, and "Figure" abbreviated to "Fig" (e.g. Fig 1, Fig 2, Fig 3, etc). Match the label of your figure with the name of the file uploaded at submission (e.g. a figure citation of "Fig 1" must refer to a figure file named "Fig1.tif").
- A concise, descriptive title

The caption may also include a legend as needed.

Tables

Cite tables in ascending numeric order upon first appearance in the manuscript file.

Place each table in your manuscript file directly after the paragraph in which it is first cited (read order). Do not submit your tables in separate files.

Tables require a label (e.g., "Table 1") and brief descriptive title to be placed above the table. Place legends, footnotes, and other text below the table.

Data reporting

All data and related metadata underlying the findings reported in a submitted manuscript should be deposited in an appropriate public repository, unless already provided as part of the submitted article.

Repositories may be either subject-specific (where these exist) and accept specific types of structured data, or generalist repositories that accept multiple data types. We recommend that authors select repositories appropriate to their field. Repositories may be subject-specific (e.g., GenBank for sequences and PDB for structures), general, or institutional, as long as DOIs or accession numbers are

provided and the data are at least as open as CC BY. Authors are encouraged to select repositories that meet accepted criteria as trustworthy digital repositories, such as criteria of the Centre for Research Libraries or Data Seal of Approval. Large, international databases are more likely to persist than small, local ones.

To support data sharing and author compliance of the PLOS data policy, we have integrated our submission process with a select set of data repositories. The list is neither representative nor exhaustive of the suitable repositories available to authors. Current repository integration partners include Dryad and FlowRepository. Please contact data@plos.org to make recommendations for further partnerships.

Instructions for PLOS submissions with data deposited in an integration partner repository:

- Deposit data in the integrated repository of choice.
- Once deposition is final and complete, the repository will provide you with a dataset DOI (provisional) and private URL for reviewers to gain access to the data.
- Enter the given data DOI into the full Data Availability Statement, which is requested in the Additional Information section of the PLOS submission form. Then provide the URL passcode in the Attach Files section.

If you have any questions, please email us.

Accession numbers

All appropriate data sets, images, and information should be deposited in an appropriate public repository. See our list of recommended repositories.

Accession numbers (and version numbers, if appropriate) should be provided in the Data Availability Statement. Accession numbers or a citation to the DOI should also be provided when the data set is mentioned within the manuscript.

In some cases authors may not be able to obtain accession numbers of DOIs until the manuscript is accepted; in these cases, the authors must provide these numbers at acceptance. In all other cases, these numbers must be provided at submission.

Identifiers

As much as possible, please provide accession numbers or identifiers for all entities such as genes, proteins, mutants, diseases, etc., for which there is an entry in a public database, for example:

- Ensembl
- Entrez Gene
- FlyBase
- InterPro
- Mouse Genome Database (MGD)
- Online Mendelian Inheritance in Man (OMIM)
- PubChem

Identifiers should be provided in parentheses after the entity on first use.

Striking image

You can choose to upload a “Striking Image” that we may use to represent your article online in places like the journal homepage or in search results.

The striking image must be derived from a figure or supporting information file from the submission, i.e., a cropped portion of an image or the entire image. Striking images should ideally be high resolution, eye-catching, single panel images, and should ideally avoid containing added details such as text, scale bars, and arrows.

If no striking image is uploaded, we will designate a figure from the submission as the striking image.

Additional Information Requested at Submission

Funding Statement

This information should not be in your manuscript file; you will provide it via our submission system.

This information will be published with the final manuscript, if accepted, so please make sure that this is accurate and as detailed as possible. You should not include this information in your manuscript file, but it is important to gather it prior to submission, because your financial disclosure statement cannot be changed after initial submission.

Your statement should include relevant grant numbers and the URL of any funder's web site. Please also state whether any individuals employed or contracted by the funders (other than the named authors) played any role in: study design, data collection and analysis, decision to publish, or preparation of the manuscript. If so, please name the individual and describe their role.

Competing Interests

This information should not be in your manuscript file; you will provide it via our submission system.

All potential competing interests must be declared in full. If the submission is related to any patents, patent applications, or products in development or for market, these details, including patent numbers and titles, must be disclosed in full.

Manuscripts disputing published work

For manuscripts disputing previously published work, it is *PLOS ONE* policy to invite a signed review by the disputed author during the peer review process. This procedure is aimed at ensuring a thorough, transparent, and productive review process.

If the disputed author chooses to submit a review, it must be returned in a timely fashion and contain a full declaration of all competing interests. The Academic Editor will consider any such reviews in light of the competing interest.

Authors submitting manuscripts disputing previous work should explain the relationship between the manuscripts in their cover letter, and will be required to confirm that they accept the conditions of this review policy before the manuscript is considered further.

Related manuscripts

Upon submission, authors must confirm that the manuscript, or any related manuscript, is not currently under consideration or accepted elsewhere. If related work has been submitted to *PLOS ONE* or

elsewhere, authors must include a copy with the submitted article. Reviewers will be asked to comment on the overlap between related submissions.

We strongly discourage the unnecessary division of related work into separate manuscripts, and we will not consider manuscripts that are divided into "parts." Each submission to *PLOS ONE* must be written as an independent unit and should not rely on any work that has not already been accepted for publication. If related manuscripts are submitted to *PLOS ONE*, the authors may be advised to combine them into a single manuscript at the editor's discretion.

PLOS does support authors who wish to share their work early and receive feedback before formal peer review. Deposition of manuscripts with preprint servers does not impact consideration of the manuscript at any PLOS journal.

Authors choosing bioRxiv may now concurrently submit directly to select PLOS journals through bioRxiv's direct transfer to journal service.

Guidelines for Specific Study Types

Human subjects research.

Manuscripts should conform to the following reporting guidelines:

- Studies of diagnostic accuracy: STARD
- Observational studies: STROBE
- Microarray experiments: MIAME
- Other types of health-related research: Consult the EQUATOR web site for appropriate reporting guidelines

Methods sections of papers on research using human subjects or samples must include ethics statements that specify:

- The name of the approving institutional review board or equivalent committee(s). If approval was not obtained, the authors must provide a detailed statement explaining why it was not needed
- Whether informed consent was written or oral. If informed consent was oral, it must be stated in the manuscript:
    - Why written consent could not be obtained
    - That the Institutional Review Board (IRB) approved use of oral consent
    - How oral consent was documented

For studies involving humans categorized by race/ethnicity, age, disease/disabilities, religion, sex/gender, sexual orientation, or other socially constructed groupings, authors should:

- Explicitly describe their methods of categorizing human populations
- Define categories in as much detail as the study protocol allows
- Justify their choices of definitions and categories, including for example whether any rules of human categorization were required by their funding agency
- Explain whether (and if so, how) they controlled for confounding variables such as socioeconomic status, nutrition, environmental exposures, or similar factors in their analysis

In addition, outmoded terms and potentially stigmatizing labels should be changed to more current, acceptable terminology. Examples: “Caucasian” should be changed to “white” or “of [Western] European descent” (as appropriate); “cancer victims” should be changed to “patients with cancer.”

For papers that include identifying, or potentially identifying, information, authors must download the Consent Form for Publication in a PLOS Journal, which the individual, parent, or guardian must sign once they have read the paper and been informed about the terms of PLOS open-access license. The signed consent form should not be submitted with the manuscript, but authors should securely file it in the individual's case notes and the methods section of the manuscript should explicitly state that consent authorization for publication is on file, using wording like:

The individual in this manuscript has given written informed consent (as outlined in PLOS consent form) to publish these case details.

For more information about *PLOS ONE* policies regarding human subjects research, see the Publication Criteria and Editorial Policies.

Clinical trials

Clinical trials are subject to all policies regarding human research. *PLOS ONE* follows the World Health Organization's (WHO) definition of a clinical trial:

A clinical trial is any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes [...] Interventions include but are not restricted to drugs, cells and other biological products, surgical procedures, radiologic procedures, devices, behavioural treatments, process-of-care changes, preventive care, etc.

All clinical trials must be registered in one of the publicly-accessible registries approved by the WHO or ICMJE (International Committee of Medical Journal Editors). Authors must provide the trial registration number. Prior disclosure of results on a clinical trial registry site will not affect consideration for publication. We reserve the right to inform authors' institutions or ethics committees, and to reject the manuscript, if we become aware of unregistered trials.

PLOS ONE supports prospective trial registration (i.e. before participant recruitment has begun) as recommended by the ICMJE's clinical trial registration policy. Where trials were not publicly registered before participant recruitment began, authors must:

- Register all related clinical trials and confirm they have done so in the Methods section
- Explain in the Methods the reason for failing to register before participant recruitment

Clinical trials must be reported according to the relevant reporting guidelines, i.e. CONSORT for randomized controlled trials, TREND for non-randomized trials, and other specialized guidelines as appropriate. The intervention should be described according to the requirements of the TIDieR checklist and guide. Submissions must also include the study protocol as supporting information, which will be published with the manuscript if accepted.

Authors of manuscripts describing the results of clinical trials must adhere to the CONSORT reporting guidelines appropriate to their trial design, available on the CONSORT Statement web site. Before the paper can enter peer review, authors must:

- Provide the registry name and number in the methods section of the manuscript

- Provide a copy of the trial protocol as approved by the ethics committee and a completed CONSORT checklist as supporting information (which will be published alongside the paper, if accepted). This should be named S1 CONSORT Checklist.
- Include the CONSORT flow diagram as the manuscript's "Fig 1"

Any deviation from the trial protocol must be explained in the paper. Authors must explicitly discuss informed consent in their paper, and we reserve the right to ask for a copy of the patient consent form.

The methods section must include the name of the registry, the registry number, and the URL of your trial in the registry database for each location in which the trial is registered.

Animal research

Manuscripts reporting animal research must state in the Methods section:

- The full name of the relevant ethics committee that approved the work, and the associated permit number(s).
- Where ethical approval is not required, the manuscript should include a clear statement of this and the reason why. Provide any relevant regulations under which the study is exempt from the requirement for approval.
- Relevant details of steps taken to ameliorate animal suffering.

Authors should always state the organism(s) studied in the Abstract. Where the study may be confused as pertaining to clinical research, authors should also state the animal model in the title.

To maximize reproducibility and potential for re-use of data, we encourage authors to follow the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for all submissions describing laboratory-based animal research and to upload a completed ARRIVE Guidelines Checklist to be published as supporting information.

Non-human primates

Manuscripts describing research involving non-human primates must report details of husbandry and animal welfare in accordance with the recommendations of the Weatherall report, *The use of non-human primates in research* (PDF), including:

- Information about housing, feeding, and environmental enrichment.
- Steps taken to minimize suffering, including use of anesthesia and method of sacrifice, if appropriate.

Random source animals

Manuscripts describing studies that use random source (e.g. Class B dealer-sourced in the USA), shelter, or stray animals will be subject to additional scrutiny and may be rejected if sufficient ethical and scientific justification for the study design is lacking.

Unacceptable euthanasia methods and anesthetic agents

Manuscripts reporting use of a euthanasia method(s) classified as unacceptable by the American Veterinary Medical Association or use of an anesthesia method(s) that is widely prohibited (e.g., chloral hydrate, ether, chloroform) must include at the time of initial submission, scientific

justification for use in the specific study design, as well as confirmation of approval for specific use from their animal research ethics committee. These manuscripts may be subject to additional ethics considerations prior to publication.

Humane endpoints

Manuscripts reporting studies in which death of a regulated animal (vertebrate, cephalopod) is a likely outcome or a planned experimental endpoint, must comprehensively report details of study design, rationale for the approach, and methodology, including consideration of humane endpoints. This applies to research that involves, for instance, assessment of survival, toxicity, longevity, terminal disease, or high rates of incidental mortality.

Full details of humane endpoints use must be reported for a study to be reproducible and for the results to be accurately interpreted.

For studies in which death of an animal is an outcome or a planned experimental endpoint, authors should include the following information in the Methods section of the manuscript:

- The specific criteria (i.e. humane endpoints) used to determine when animals should be euthanized.
- The duration of the experiment.
- The numbers of animals used, euthanized, and found dead (if any); the cause of death for all animals.
- How frequently animal health and behavior were monitored.
- All animal welfare considerations taken, including efforts to minimize suffering and distress, use of analgesics or anaesthetics, or special housing conditions.

If humane endpoints were not used, the manuscript should report:

- A scientific justification for the study design, including the reasons why humane endpoints could not be used, and discussion of alternatives that were considered.
- Whether the institutional animal ethics committee specifically reviewed and approved the anticipated mortality in the study design.

Observational and field studies

Methods sections for submissions reporting on any type of field study must include ethics statements that specify:

- Permits and approvals obtained for the work, including the full name of the authority that approved the study; if none were required, authors should explain why
- Whether the land accessed is privately owned or protected
- Whether any protected species were sampled
- Full details of animal husbandry, experimentation, and care/welfare, where relevant

Paleontology and archaeology research

Manuscripts reporting paleontology and archaeology research must include descriptions of methods and specimens in sufficient detail to allow the work to be reproduced. Data sets supporting statistical and phylogenetic analyses should be provided, preferably in a format that allows easy re-use. Read the policy.

Specimen numbers and complete repository information, including museum name and geographic location, are required for publication. Locality information should be provided in the manuscript as legally allowable, or a statement should be included giving details of the availability of such information to qualified researchers.

If permits were required for any aspect of the work, details should be given of all permits that were obtained, including the full name of the issuing authority. This should be accompanied by the following statement:

All necessary permits were obtained for the described study, which complied with all relevant regulations.

If no permits were required, please include the following statement:

No permits were required for the described study, which complied with all relevant regulations.

Systematic reviews and meta-analyses

A systematic review paper, as defined by The Cochrane Collaboration, is a review of a clearly formulated question that uses explicit, systematic methods to identify, select, and critically appraise relevant research, and to collect and analyze data from the studies that are included in the review. These reviews differ substantially from narrative-based reviews or synthesis articles. Statistical methods (meta-analysis) may or may not be used to analyze and summarize the results of the included studies.

Reports of systematic reviews and meta-analyses must include a completed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist and flow diagram to accompany the main text. Blank templates are available here:

- Checklist: PDF or Word document
- Flow diagram: PDF or Word document

Authors must also state in their "Methods" section whether a protocol exists for their systematic review, and if so, provide a copy of the protocol as supporting information and provide the registry number in the abstract.

If your article is a systematic review or a meta-analysis you should:

- State this in your cover letter
- Select "Research Article" as your article type when submitting
- Include the PRISMA flow diagram as Fig 1 (required where applicable)
- Include the PRISMA checklist as supporting information

Meta-analysis of genetic association studies

Manuscripts reporting a meta-analysis of genetic association studies must report results of value to the field and should be reported according to the guidelines presented in *Systematic Reviews of Genetic Association Studies* by Sagoo *et al.*

On submission, authors will be asked to justify the rationale for the meta-analysis and how it contributes to the base of scientific knowledge in the light of previously published results. Authors will also be asked to complete a checklist (DOCX) outlining information about the justification for the

study and the methodology employed. Meta-analyses that replicate published studies will be rejected if the authors do not provide adequate justification.

Personal data from third-party sources

For all studies using personal data from internet-based and other third-party sources (e.g., social media, blogs, other internet sources, mobile phone companies), data must be collected and used according to company/website Terms and Conditions, with appropriate permissions. All data sources must be acknowledged clearly in the Materials and Methods section.

In the Ethics Statement, authors should declare any potential risks to individuals or individual privacy, or affirm that in their assessment, the study posed no such risks. In addition, the following Ethics and Data Protection requirements must be met.

For interventional studies, which impact participants' experiences or data, the study design must have been prospectively approved by an Ethics Committee, and informed consent is required. The Ethics Committee may waive the requirement for approval and/or consent.

For observational studies in which personal experiences and accounts are not manipulated, consultation with an Ethics or Data Protection Committee is recommended. Additional requirements apply in the following circumstances:

- If information used could threaten personal privacy or damage the reputation of individuals whose data are used, an Ethics Committee should be consulted and informed consent obtained or specifically addressed.
- If authors accessed any personal identifying information, an Ethics or Data Protection Committee should oversee data anonymization. If data were anonymized and/or aggregated before access and analysis, informed consent is generally not required.

Cell lines

Authors reporting research using cell lines should state when and where they obtained the cells, giving the date and the name of the researcher, cell line repository, or commercial source (company) who provided the cells, as appropriate.

Authors must also include the following information for each cell line:

For *de novo* (new) cell lines, including those given to the researchers as a gift, authors must follow our policies for human subjects research or animal research, as appropriate. The ethics statement must include:

- Details of institutional review board or ethics committee approval; AND
- For human cells, confirmation of written informed consent from the donor, guardian, or next of kin

For established cell lines, the Methods section should include:

- A reference to the published article that first described the cell line; AND/OR
- The cell line repository or company the cell line was obtained from, the catalogue number, and whether the cell line was obtained directly from the repository/company or from another laboratory

Authors should check established cell lines using the ICLAC Database of Cross-contaminated or Misidentified Cell Lines to confirm they are not misidentified or contaminated. Cell line authentication is recommended – e.g., by karyotyping, isozyme analysis, or short tandem repeats (STR) analysis – and may be required during peer review or after publication.

Blots and gels

Manuscripts reporting results from blots (including Western blots) and electrophoretic gels should follow these guidelines:

- In accordance with our policy on image manipulation, the image should not be adjusted in any way that could affect the scientific information displayed, e.g. by modifying the background or contrast.
- All blots and gels that support results reported in the manuscript should be provided.
- Original uncropped and unadjusted blots and gels, including molecular size markers, should be provided in either the figures or the supplementary files.
- Lanes should not be overcropped around the bands; the image should show most or all of the blot or gel. Any non-specific bands should be shown and an explanation of their nature should be given.
- The image should include all relevant controls, and controls should be run on the same blot or gel as the samples.
- A figure panel should not include composite images of bands originating from different blots or gels. If the figure shows non-adjacent bands from the same blot or gel, this should be clearly denoted by vertical black lines and the figure legend should provide details of how the figure was made.

Antibodies

Manuscripts reporting experiments using antibodies should include the following information:

- The name of each antibody, a description of whether it is monoclonal or polyclonal, and the host species.
- The commercial supplier or source laboratory.
- The catalogue or clone number and, if known, the batch number.
- The antigen(s) used to raise the antibody.
- For established antibodies, a stable public identifier from the Antibody Registry.

The manuscript should also report the following experimental details:

- The final antibody concentration or dilution.
- A reference to the validation study if the antibody was previously validated. If not, provide details of how the authors validated the antibody for the applications and species used.

Small and macromolecule crystal data

Manuscripts reporting new and unpublished three-dimensional structures must include sufficient supporting data and detailed descriptions of the methodologies used to allow the reproduction and validation of the structures. All novel structures must have been deposited in a community endorsed database prior to submission (please see our list of recommended repositories).

Small molecule single crystal data

Authors reporting X-Ray crystallographic structures of small organic, metal-organic, and inorganic molecules must deposit their data with the Cambridge Crystallographic Data Centre (CCDC), the Inorganic Crystal Structure Database (ICSD), or similar community databases providing a recognized validation functionality. Authors are also required to include the relevant structure reference numbers within the main text (e.g. the CCDC ID number), as well as the crystallographic information files (.cif format) as Supplementary Information, along with the checkCIF validation reports that can be obtained via the International Union of Crystallography (IUCr).

Macromolecular structures

Authors reporting novel macromolecular structures must have deposited their data prior to submission with the Worldwide Protein Data Bank (wwPDB), the Biological Magnetic Resonance Data Bank (BMRB), the Electron Microscopy Data Bank (EMDB), or other community databases providing a recognized validation functionality. Authors must include the structure reference numbers within the main text and submit as Supplementary Information the official validation reports from these databases.

Methods, software, databases, and tools

PLOS ONE will consider submissions that present new methods, software, or databases as the primary focus of the manuscript if they meet the following criteria:

Software submissions

Manuscripts whose primary purpose is the description of new software must provide full details of the algorithms designed. Describe any dependencies on commercial products or operating system. Include details of the supplied test data and explain how to install and run the software. A brief description of enhancements made in the major releases of the software may also be given. Authors should provide a direct link to the deposited software from within the paper.

Database submissions

For descriptions of databases, provide details about how the data were curated, as well as plans for long-term database maintenance, growth, and stability. Authors should provide a direct link to the database hosting site from within the paper.

New taxon names

Zoological names

When publishing papers that describe a new zoological taxon name, PLOS aims to comply with the requirements of the International Commission on Zoological Nomenclature (ICZN). Effective 1 January 2012, the ICZN considers an online-only publication to be legitimate if it meets the criteria of archiving and is registered in ZooBank, the ICZN's official registry.

For proper registration of a new zoological taxon, we require two specific statements to be included in your manuscript.

In the Results section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Anochetus boltoni Fisher sp. nov. urn:lsid:zoobank.org:act:B6C072CF-1CA6-40C7-8396-534E91EF7FBB

You will need to contact Zoobank to obtain a GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper.

Please also insert the following text into the Methods section, in a sub-section to be called "Nomenclatural Acts":

All PLOS articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Botanical names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature, and apply only to seed plants, ferns, and lycophytes.

Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the Results section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Solanum aspersum S.Knapp, sp. nov. [urn:lsid:ipni.org:names:77103633-1] Type: Colombia. Putumayo: vertiente oriental de la Cordillera, entre Sachamates y San Francisco de Sibundoy, 1600-1750 m, 30 Dec 1940, J. Cuatrecasas 11471 (holotype, COL; isotypes, F [F-1335119], US [US-1799731]).

Journal staff will contact IPNI to obtain the GUID (LSID) after your manuscript is accepted for publication, and this information will then be added to the manuscript during the production phase

In the Methods section, include a sub-section called "Nomenclature" using the following wording:

All PLOS articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Fungal names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature.

Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the Results section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Hymenogaster huthii. Stielow et al. 2010, sp. nov. [urn:lsid:indexfungorum.org:names:518624]

You will need to contact either Mycobank or Index Fungorum to obtain the GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper. Effective January 2013, all papers describing new fungal species must reference the identifier issued by a recognized repository in the protologue in order to be considered effectively published.

In the Methods section, include a sub-section called “Nomenclature” using the following wording (this example is for taxon names submitted to MycoBank; please substitute appropriately if you have submitted to Index Fungorum):

All PLOS articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Qualitative research

Qualitative research studies use non-quantitative methods to address a defined research question that may not be accessible by quantitative methods, such as people's interpretations, experiences, and perspectives. The analysis methods are explicit, systematic, and reproducible, but the results do not involve numerical values or use statistics. Examples of qualitative data sources include, but are not limited to, interviews, text documents, audio/video recordings, and free-form answers to questionnaires and surveys.

Qualitative research studies should be reported in accordance to the Consolidated criteria for reporting qualitative research (COREQ) checklist. Further reporting guidelines can be found in the Equator Network's Guidelines for reporting qualitative research.