



Applied nutritional investigation

Estimation of body fat in adults using a portable A-mode ultrasound



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ABSTRACT

Objective: This study aimed to develop and validate equations to estimate body fat based on anthropometric measurements of subcutaneous fat thickness (SFT) and muscle thickness (MT) measured by A-mode ultrasound (BodyMetrix) in Brazilian adults.

Methods: Individuals ($n = 206$) underwent air-displacement plethysmography for body composition assessment. Arm, thigh, and calf circumferences were also obtained. SFT from triceps, biceps, subscapular, abdominal, thigh, and calf regions and MT from triceps, biceps, thigh, and calf regions were measured by BodyMetrix. Prediction equations were developed by stepwise multiple linear regression using the circumferences, weight, height, SFT, and MT. Lin's concordance correlation coefficient, mean difference, and 95% limits of agreement (95% LOA) were assessed in apparent and internal validity.

Results: The prediction equation for whole-body fat for men included thigh circumference, triceps and thigh SFT, biceps MT, weight, and height. The equation for women included age, calf circumference, abdominal and calf SFT, weight, and height. The prediction equation overestimated men's whole-body fat by 0.5 percentual points, in average, and the lower and upper 95% LOA were -6.8% and 7.7% , respectively. For women, the prediction equation overestimated whole-body fat by 0.1 percentual points, in average. Lower and upper 95% LOA were -6.5% and 6.7% , respectively. Optimism-adjusted results using 500 repetitions with same size samples have shown similar results. Body fat extremes did not influence the whole-body fat estimation.

Conclusions: BodyMetrix A-mode ultrasound, in association with selected conventional anthropometric measurements, proved to be a reliable tool for the estimation of body fat percentage.

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Introduction

Currently, there is a growing importance of body composition evaluation in several fields [1]. Indirect body composition evaluation methods include the air-displacement plethysmography (ADP), dual-energy X-ray absorptiometry (DXA), computerized tomography, magnetic resonance imaging, and hydrostatic weighing (HW) [2]. Even though they are precise and accurate

methods, they are also expensive, not portable, and logistically difficult.

Concerning whole-body fat evaluation, ADP, HW, and DXA are considered gold-standard methods [2–4]. However, their use is limited in the epidemiologic field, some clinical settings, private practice, and gyms because of the problems described above. Thus, it is necessary to develop or improve low-cost methods, which could be used in epidemiological studies. The bioelectrical impedance (BIA) is a largely used low-cost and portable method [2]. Its estimation of body composition compartments (fat and fat-free mass) is based on total body water [2]. Although it has

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importance in the field use, the method requires several assumptions before test, such as fasting, no exercise previously, no alcohol drinking, and absence of condition that affects hydration (phase of menstrual cycle, diseases, etc.) [5]. All these assumptions reduce the confiability of BIA in epidemiological and clinical settings, as well as make its standardized use difficult.

Ultrasound has emerged in the last decades as a suitable tool to use in the assessment of body fat [6]. B-mode ultrasound has been included as an alternative body composition tool because it may overcome some of the previously cited limitations in the measurement of the subcutaneous fat thickness (SFT) [7]. However, A-mode ultrasound remains to be adequately validated as a suitable body composition method.

BodyMetrix BX-2000 (IntelaMetrix, Inc., Livermore, CA, USA), using A-mode ultrasound with a 2.5 MHz transducer, is a portable, practical, and relatively inexpensive tool designed specifically for body composition assessment. Compared with the widely used caliper rule, A-mode ultrasound can reduce limitations concerning the inability to control inter- and intra-subject variation in the skinfold compressibility. It can also decrease the failure to palpate the fat-muscle interface [7], through direct real-time observation. Also, BodyMetrix can measure not only the SFT but also the adjacent muscle thickness (MT), which could help in the prediction of the fat compartment.

This study aimed to develop and validate prediction equations to estimate the whole-body fat percentage based on anthropometric measurements, SFT, and MT measured by BodyMetrix in Brazilian adults.

Materials and methods

A convenience sample was invited to participate in this study, carried out in Pelotas, a southern Brazilian city. Two hundred six subjects were enrolled in this study (104 women). According to the eutrophic and overweight body composition differences, 49 women and 51 men of the chosen sample were overweight (body mass index ≥ 25 kg/m²). The study was approved by the School of Medicine Ethics Committee of the Federal University of Pelotas. All participants signed the informed consent form. In the research clinic, the volunteers underwent anthropometric examination, ADP, and measurements of subcutaneous fat and MT by a portable A-mode ultrasound device.

Body fat percentage was measured by BodPod (Life Measurement, Inc., Concord, CA, USA), an ADP-based equipment. Subjects wore tight-fitting clothes and a swim cap to minimize air from clothing and hair. Temperature and pressure were kept according to the manufacturer's recommendations. The isothermal effects related to skin surface area were reduced by corrective equations as appropriate in adults, and thoracic gas volume was predicted by applying equations including sex, age, and height [8], which was measured to the nearest 1 mm using a wooden stadiometer. Body density was calculated using body mass and volume obtained through BodPod. Body fat percentage was calculated using the Siri equation: %G = [(4.95/body density) – 4.50] \times 100 [9].

Arm, thigh, and calf circumferences were measured on the right side of the participants by a trained and experienced anthropometrist. Arm circumference was measured at the midpoint between the tip of the acromial process and the tip of the olecranon. Thigh circumference was measured at a point situated two-thirds between the edge of the iliac crest and the proximal border of the patella (upper knee). Calf circumference was measured at the largest circumference, with the subjects standing with their legs in a relaxed position approximately 20 cm apart.

SFT and MT were measured at triceps, biceps, subscapular, abdominal, thigh, and calf sites on the right side using the BodyMetrix BX2000 ultrasound. During the measurements, participants were in the standing position with both legs and arms relaxed. Ultrasound gel was applied on the transducer, which was placed perpendicular to the point of skin contact at each of the sites and then moved slowly, sliding vertically down or horizontally, within 5 mm from the measurement site, according to the manufacturer's instructions and the site of measurement.

Tricipital SFT and MT were measured on the posterior surface of the arm and at the midpoint between the tip of the acromial process and the tip of the olecranon. SFT and MT at the biceps site were measured on the anterior surface of the arm, in the greatest point of the apparent circumference of the biceps muscle. Subscapular and abdominal SFT were measured on points 5 cm below the inferior

angle of the scapula and 2 to 3 cm right to the umbilicus, respectively. Thigh SFT and MT were measured on a point situated two-thirds between the edge of the iliac crest and the proximal border of the patella. Calf SFT and MT were measured on the posterior surface of the lower leg at the largest circumference of the calf.

Statistical analyses were performed using Stata 12 software (StataCorp, College Station, TX, USA). Initially, demographic and anthropometric characteristics of the sample were described by sex—statistical significance of difference of measurements, obtained using the Student's *t* test, between men and women were shown. Equations for men and women were developed separately by a stepwise multiple linear regression analysis (backward selection). The final model included only significant variables ($P < 0.05$) associated with body fat percentage. Linear relationship between the fat and MT measured by A-mode ultrasound and total body fat percentage was tested using the command *fracpoly*. Validity analyses were carried out using apparent and internal validity techniques. Concordance between results from each equation in the prediction of body fat percentage and results from BodPod was assessed using Lin's concordance correlation coefficient. Body fat percentages measured by BodPod and estimated by equations were presented, and Bland-Altman plots were employed using the mean difference between methods and the 95% limits of agreement (95% LOA) calculated.

These procedures were applied to the own original sample in the apparent validity analysis. Internal validity of the model was evaluated in 500 repetitions, through same-size samples' bootstrapping [10]. A bootstrap sample is a random sample with replacement. It includes the same number of participants as the original sample, but some are excluded and others included once, twice, and so on. The model was developed in the bootstrap sample, and validated in the original sample. The so-called optimism is obtained by the difference between the models. Optimism-adjusted Lin's concordance correlation coefficient, mean difference, and 95% LOA were then calculated based on the difference between model performance in the bootstrap sample and the original data set.

Results

Table 1 illustrates the sample's characteristics. Men and women were significantly different in most of the measurements. Women presented higher SFT in all sites, whereas MT in all locations was greater in men. The differences justify the creation of an equation for each sex separately.

Tests proved that the relationship between the anthropometric measurements, including fat and MT measured by A-mode ultrasound, and total body fat percentage was linear. Equations for fat prediction are shown in Table 2. The final equation for men included thigh circumference, triceps and thigh SFT, biceps MT, weight, and height. On the other hand, the final equation for women included age, calf circumference, abdominal and calf SFT, weight, and height. Adjusted R^2 was higher for women than for men (0.811 versus 0.730).

Table 1
Summary of the characteristics of adults enrolled in the study

	Mean (SD)		<i>P</i>
	Men	Women	
Age, y	30.0 (8.1)	31.9 (9.9)	0.126
Body mass index, kg/m ²	25.7 (3.7)	24.8 (4.3)	0.120
Arm circumference, cm	33.4 (3.3)	30.3 (4.2)	<0.001
Thigh circumference, cm	54.5 (4.3)	52.7 (5.0)	0.006
Calf circumference, cm	37.6 (2.8)	36.0 (3.0)	<0.001
Subcutaneous fat thickness, mm			
Triceps	6.5 (2.5)	13.3 (3.9)	<0.001
Biceps	3.9 (1.7)	7.7 (4.1)	<0.001
Subscapular	7.2 (2.0)	9.9 (4.2)	<0.001
Abdominal	21.4 (10.7)	28.2 (15.4)	<0.001
Thigh	7.4 (2.3)	13.3 (4.3)	<0.001
Calf	4.7 (1.3)	7.3 (2.3)	<0.001
Muscle thickness, mm			
Triceps	38.9 (10.3)	19.5 (7.6)	<0.001
Biceps	38.5 (7.6)	28.0 (12.8)	<0.001
Thigh	39.2 (8.3)	28.7 (10.0)	<0.001
Calf	63.4 (12.0)	55.7 (7.5)	<0.001

Table 2

Prediction equations of body fat percentage based on demographic and anthropometric measurements and fat and muscle thickness measured by BodyMetrix

Equation	Full model	Adj. R^2	Final equation	Adj. R^2
1 (men)	Age, AC, TC, CC, TSFT, BSFT, SSSFT, ASFT, THSFT, CSFT, TMT, BMT, THMT, CMT, weight, and height	0.714	$-0.71 (\text{TC}) + 0.40 (\text{TSFT}) + 1.01 (\text{THSFT}) - 0.16 (\text{BMT}) - 37.23 (\text{height}) + 0.61 (\text{weight}) + 73.23$	0.730
2 (women)	Age, AC, TC, CC, TSFT, BSFT, SSSFT, ASFT, THSFT, CSFT, TMT, BMT, THMT, CMT, weight, and height	0.808	$0.12 (\text{age}) - 0.76 (\text{CC}) + 0.24 (\text{ASFT}) + 1.10 (\text{CSFT}) - 27.33 (\text{height}) + 0.30 (\text{weight}) + 67.63$	0.811

AC, arm circumference; ASFT, abdominal subcutaneous fat thickness; BMT, biceps muscle thickness; BSFT, biceps subcutaneous fat thickness; CC, calf circumference; CMT, calf muscle thickness; CSFT, calf subcutaneous fat thickness; SSSFT, subscapular subcutaneous fat thickness; TC, thigh circumference; THMT, thigh muscle thickness; THSFT, thigh subcutaneous fat thickness; TMT, triceps muscle thickness; TSFT, triceps subcutaneous fat thickness

Age is expressed in years; circumference, cm; subcutaneous fat thickness, mm; muscle thickness, mm; height, m; and weight, kg

Alternative equations were also considered. With weight and height alone (without the other variables) in the fat prediction equation, the adjusted R^2 found was only 0.522 and 0.664 for men and women, respectively. When the anthropometric measurements together with weight and height were included in the equation, the increase was small (Fig. 1).

Table 3 shows the concordance parameters between prediction equations from anthropometric measurements, including fat and MT measured by A-mode ultrasound, and body fat obtained from ADP using apparent and internal validity. The fat percentage was, on average, similar in both methods.

Concerning the equation created for men, the mean difference of predicted fat was 0.5 percentual points higher in the equation compared with the ADP estimation (95% confidence interval: -0.2 to 1.2). The optimism-adjusted mean difference (resulted from simulation with 500 samples) remained the same. The 95% lower and upper limits of the agreement were -6.8% and 7.7% , with a slight modification in the optimism-adjusted analysis. Lin's concordance correlation coefficient was 0.853 and 0.855 in analyses, accounting for the original sample and optimism-adjusted estimate, respectively.

The mean difference of predicted fat was 0.1 percentual points higher in the prediction equation than in ADP estimation in women (95% confidence interval: -0.6 to 0.7). As observed in men, the optimism-adjusted mean difference remained the same. The 95% lower and upper LOA (-6.5 and 6.7 , respectively, in the apparent validity analysis) had a slight modification in results from internal validity. Lin's concordance correlation coefficient was 0.903 in the sample analysis and 0.904 in the optimism-adjusted estimate (Table 3).

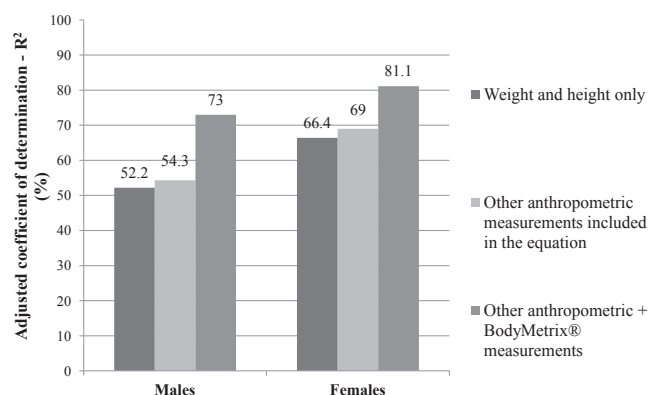


Fig. 1. Adjusted coefficients of determination (R^2) of prediction of fat percentage by weight and height only, in addition to other anthropometric measurements included in the equation and with inclusion of BodyMetrix measurements in Brazilian men and women.

Bland-Altman plots (Fig. 2) show that the differences between each prediction equation and the measured fat percentage by ADP do not significantly change according to the fat percentage. In other words, there is not a systematic bias associated with the quantity of body fat of the subjects for both sexes.

Discussion

This study has developed and assessed the validity of equations for prediction of total fat percentage in healthy Brazilian adults based mainly on A-mode ultrasound measurements. BodyMetrix A-mode portable ultrasound device can be useful in the whole-body fat assessment in adults if used in association with other anthropometric measurements. Men and women have different anthropometric patterns, and this justifies the need of creation of two separate equations. Women showed a higher adjusted coefficient of determination (R^2) and lower mean difference of fat percentage according to the equation. The fat percentage difference was proximately null when comparing the prediction equations and the gold-standard method for both sexes. The high agreement found between fat percentages based on the equations and the internal validity analyses reinforces the consistency of the findings. These equations are probably valid for both eutrophic and overweight subjects, because body fat extremes do not influence the fat percentage estimation of the equations compared with ADP measurement. Stratified analyses by nutritional status were not performed because of sample size, although an equal proportion of eutrophic and overweight individuals was represented in the sample.

Four previous articles concerning the use of BodyMetrix were identified in the literature [11–14]. However, all studies were carried out with relatively small sample sizes and used equations based on skinfold caliper sites for the prediction of body fat, such as the seven-sites Jackson-Pollock equation [15]. In this study, the sample size was larger—with, therefore, better precision of the estimates. Also, we have considered the importance of other simple anthropometric measurements in association with the measurement of the SFT. Furthermore, the methodological differences between ultrasound and skinfold caliper techniques also justify the elaboration of new predictive equations other than the already established skinfold-based equations. Finally, these previous articles used only SFT measurements [11–14], whereas the present study took into consideration that the knowledge of the muscle compartments measurements could be important in the prediction of the fat percentage.

Previous studies have evaluated the use of ultrasonography in the prediction of adipose tissue, with positive results concerning the validity [16–19]. For example, Abe et al. [16] found an adjusted R^2 similar to ours in the comparison between equations based on B-mode ultrasound SFT measurements and body

Table 3

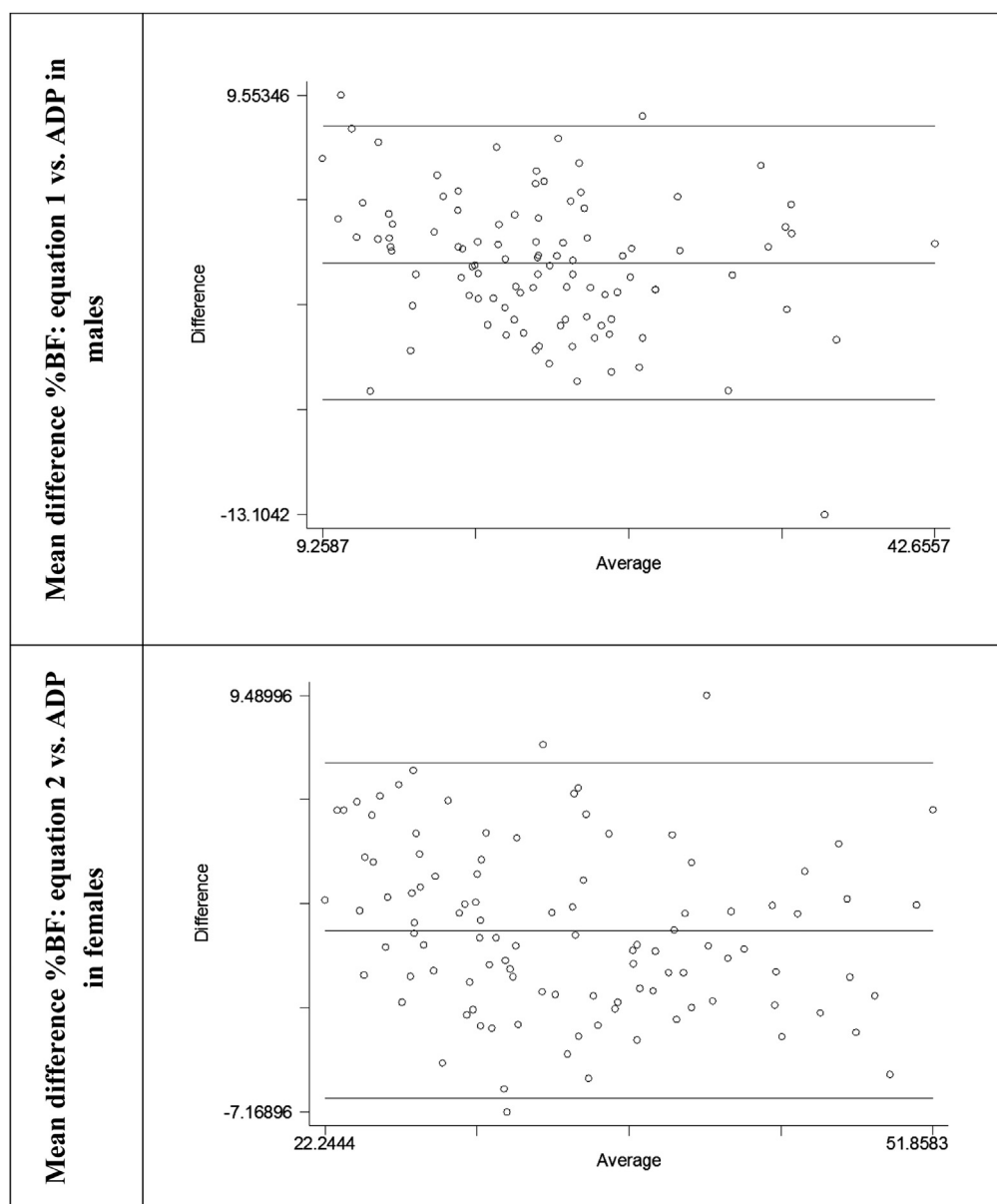
Concordance between body fat percentages measured by ADP and estimated by prediction equations in adults in apparent validity and internal validity analyses using bootstrap technique

	Equation	Mean (SD)	Minimum to maximum	Mean difference (95% CI)	Optimism-adjusted mean difference	95% LOA	Optimism-adjusted 95% LOA	CCC	Optimism-adjusted CCC
Men	%BF by ADP	21.2 (7.3)	5.5–43.2	0.5 (–0.2 to 1.2)	0.5	–6.8 to 7.7	–6.7 to 7.6	0.853	0.855
	%BF by equation 1	21.7 (6.4)	8.7–43.4						
Women	%BF by ADP	34.1 (8.0)	20.4–52.6	0.1 (–0.6 to 0.7)	0.1	–6.5 to 6.7	–6.6 to 6.7	0.903	0.904
	%BF by equation 2	34.2 (7.3)	22.9–54.3						

%BF, body fat percentage; ADP, air-displacement plethysmography; CCC, Lin's concordance correlation coefficient; CI, confidence interval; LOA, limits of agreement

density measured by HW. Also using B-mode ultrasound (with a 12 MHz linear array transducer), another study found evidence that ultrasound may be an accurate and reliable tool to measure total and regional body composition [18].

The use of A-mode portable ultrasound is relatively innovative. The BodyMetrix transducer has a 2.5 MHz frequency, and its technology allows the real-time observation of tissue boundaries: skin-subcutaneous fat, fat-muscle tissue, and muscle-bone



%BF – body fat percentage; ADP – air-displacement plethysmography.

Fig. 2. Bland-Altman plots of the difference between body fat percentage estimated by prediction equations and measured by air-displacement plethysmography in adults.

tissue boundaries [6]. It is used with a common laptop or desktop by USB. Its practicality enables its use in different settings, such as epidemiologic fieldwork, clinical practice, gyms, and sports training centers, with a considerably smaller cost than B-mode ultrasound devices.

The principles involved in the SFT use for prediction of total body fat are the same for both skinfold measurements and ultrasonography techniques. However, the skinfold methodology is more susceptible to technical error, because the technician must have a good ability to detect, pinch, measure, and read the skinfold thickness without direct observation of the fat-muscle limit and inner tissue compression [7,20]. Previous studies have compared the techniques [13,17,19,21,22]. Two studies from the 1980s reported that the measurement of subcutaneous fat with a skinfold caliper or an ultrasound had the same degree of accuracy in the estimation of body fat [19], but that ultrasound was superior to the caliper in measuring subcutaneous fat of obese individuals [17]. In the recent past, a study carried out with primiparous teenagers suggested that skinfold caliper measurements should be interpreted cautiously because of the poor estimation of the amount of subcutaneous adipose tissue gained during gestation in relation to ultrasonography measurements [22]. However, all of these studies were performed with B-mode ultrasound.

The only identified study that evaluated A-mode ultrasound adopted three sites of SFT measurement [13]. Despite good reliability found between ultrasound measurements taken on different visits, it stated that portable ultrasound was not a valid tool for the estimation of total body fat compared with the skinfold estimate [13]. This study, however, was carried out with only 11 subjects and did not use any gold-standard method to allow a better comparison between the instruments.

Our findings suggest that results from the equations are not influenced by body fat extremes in the estimation of body fat. ADP—our reference method—has limitations in the estimation of total body fat in obese individuals, because the hydration status of obese individuals is higher than that in eutrophic subjects, and total body water has a relevant influence on the density estimated by this method [23]. As possible potential limitations, our study did not carry out subgroup analyses according to the subjects' nutritional status. However, our equations do not imply any additional bias in the obese other than the already known bias from the ADP technique. Another point to be considered concerns the impossibility of using an at least three-compartment method of body composition assessment. Nevertheless, ADP is a largely used method, and it was possible to estimate how the accuracy of our method was in comparison to a recognized technique.

Finally, a cross-validation sample was not possible because of logistical aspects, but must be discussed. The precision of estimations was our priority, as well as the similar distribution of men and women and normal and overweight individuals in our sample. On the other hand, the internal validity assessment of predictive statistical models is a common [24–26] and recommended alternative [10] that allows an adequate estimate of performance for subjects who are similar to those in the original sample. Our optimism-adjusted results were nearly identical to those shown in the development sample, and so there was no need for correction.

Conclusions

Our findings support that the A-mode portable ultrasound device can be useful in whole-body fat assessment in adults. It

has shown good performance if used after an adequate training and also in association with anthropometric measurements. It poses an attractive alternative in the epidemiological and clinical settings of body composition because it seems to be more reliable than the skinfold measurement and less expensive than other methods such as DXA or ADP.

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Authors' contributions: R.M.B., M.C.G., S.P.O., M.C.F.A., and T.G.B.-S. conceptualized the study. R.M.B., S.P.O., and T.G.B.-S. supervised the fieldwork. M.O.X. and R.B.B. performed the experiments. R.M.B. conducted the statistical analyses. R.M.B., M.C.G., and M.C.F.A. wrote the article. All authors revised the last version of the article.

Conflict of interest: The authors declare that they have no conflict of interest.

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