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Development & cross-validation of anthropometric predictive models to estimate the appendicular skeletal muscle mass in middle-aged women in Sri Lanka

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Background & objectives: Attempts have been made to estimate appendicular skeletal muscle mass (ASMM) using anthropometric indices and most of these are country specific. This study was designed to develop and cross-validate simple predictive models to estimate the ASMM based on anthropometry in a group of healthy middle-aged women in Sri Lanka.

Methods: The study was conducted on a randomly selected group of community-dwelling women aged 30-60 years. ASMM (kg) quantified with dual-energy X-ray absorptiometry (DXA) ($ASMM_{DXA}$) was used as the reference standard. Anthropometric measurements such as body weight (kg), height (m), limb circumferences (cm) and skinfold thickness (mm) which showed significant correlations with $ASMM_{DXA}$, were used to develop the models. The models were developed using a group of 165 women (aged 30-60 yr) and were cross-validated using a separate sample of women (n=167) (mean age: 48.9±8.56 yr), selected randomly.

Results: Nine anthropometry-based models were developed using weight, height, skinfold thicknesses, circumferences, body mass index, menopausal status (MS) and age as independent variables. Four models which were based on height, weight, triceps skinfold thickness (TSFT), age and MS met all the validation criteria with high correlations (ranged 0.89-0.92) and high predictive values explaining high variance (80-84%) with low standard error of estimate (1.10-1.24 kg).

Interpretation & conclusions: The four models (ASMM 1-ASMM 4) developed based on height, weight, TSFT, age and MS showed a high accuracy in estimating the ASMM in middle-aged women.

Skeletal muscle mass (SMM) is a metabolically active body compartment in the human body, and plays a central role in a number of physiological processes[1]. Approximately 75 per cent of SMM is located in the appendicular region called as appendicular SMM (ASMM)[2], and it is closely related to ambulation, mobility and performance of daily activities. The SMM in women decreases at a rate of one per cent per year after the third decade of age, with an accelerated decline after the fifth decade[3]. In women, changes in

SMM are partly age related and partly related to ovarian failure following menopause[4]. As a result, they are prone to develop sarcopenia which leads to deleterious consequences such as impaired physical performance, physical disability and poor quality of life[5].

Many technologies are used to measure SMM, of those magnetic resonance imaging (MRI) is considered the gold standard due to its high accuracy and lack of radiation exposure[6]. Dual-energy X-ray absorptiometry (DXA) also has a high measurement precision and relatively low radiation hazard[7] than other imaging techniques such as computer tomography (CT). Despite the high accuracy, MRI, CT and DXA are not widely used to estimate SMM due to limitations inherent to these technologies.

Anthropometry offers practical alternative that is simple, valid and inexpensive for measuring SMM[8], especially in resource-poor settings. Several formulae based on anthropometric indices have been developed to estimate ASMM. These formulae, however, have been validated mostly in the European populations, and their applicability to other populations or ethnic groups is uncertain. Country-specific prediction models are more likely to suit their own populations than models developed elsewhere[9].

Hence, country-specific prediction models have been developed in China[10], Japan[7], India[11,12], USA[13], Brazil[14] and Australia[15] using different anthropometric indices such as height, weight, body mass index (BMI), limb circumferences, waist circumference, limb lengths and skinfold thickness as unadjusted indices[12,14,15], adjusted indices for body fat mass, height or arm length[10,13] and in combination with handgrip strength[7]. Only one study could be traced related to SMM in Sri Lanka[16]. This is mainly due to the lack of appropriate technology to measure SMM and non-use of SMM in clinical applications. Therefore, in this study, an attempt was made to develop country-specific formulae, using indices of anthropometry, to estimate ASMM in middle-aged women in Sri Lanka.

Material & Methods

A community-based cross-sectional study was conducted in two stages in the community study area of the Faculty of Medicine, University of Ruhuna in Galle, Sri Lanka, over a period of one and a half years from June 2015 to January 2017. Ethical clearance for the study was obtained from the Ethical Review Committee, Faculty of Medicine, University of Ruhuna, Sri Lanka, and written informed consent was obtained from each participant before the commencement of the study.

Study participants: A group of healthy community-dwelling women aged 30-60 year were selected randomly from the study area. Of the 18 public health midwifery areas, two areas (Godakanda East and Kapuhempala) were selected randomly to recruit women for the development of new models (Stage 1; group A, n=165) and two other areas (Hapugala and Kahaduwawaththa) were selected randomly to recruit women for the validation of the models (Stage 2; group B, n=167). Women who were on pharmacologic doses of medications such as corticosteroids, thyroxin, hormonal contraceptives, hormone replacement therapy, vitamin D and calcium; those who were following dedicated dietary or exercise programmes; pregnant or lactating women were excluded from the study. Women with non-communicable diseases; polycystic ovarian syndrome; chronic inflammatory conditions; malabsorption and chronic diseases involving cardiovascular, nervous, respiratory or gastrointestinal systems were also excluded. Menopausal status (MS) (pre- and post-menopausal) was considered on self-stated menstrual history based on the classification of Stages of Reproductive Aging Workshop[17].

The sample size for the study was calculated based on the BMI [mean and standard deviation (SD)] between pre- and post-menopausal women in a previous study[18]. The formula used for this calculation was as follows: $N=(Z_{\alpha/2}+Z_\beta)^2\times(\sigma1^2+\sigma2^2)/(\mu1-\mu2)^2$, where $Z_{\alpha/2}$=95 per cent confidence interval=1.96, Z_β=80 per cent power=0.84 σ1 and σ2=standard deviations and μ1−μ2=effects size/difference between two means[19]. The minimum recommended number of pre- and post-menopausal women in each category was 155. However, 184 pre-menopausal and 166 post-menopausal women participated in the study. Due to incomplete data, 15 pre-menopausal and three post-menopausal women were excluded from the analysis.

Measured variables: Body weight (kg), height (m), circumferences (cm) and skinfold thickness (mm) at specific sites were measured adhering to the standard protocols[13,20]. Body weight was measured to the nearest 0.1 kg using a digital weighing scale (NAGATA, Tainan, Taiwan) when wearing light clothes. The standing height was measured without footwear and recorded to the nearest 0.1 cm with a stadiometer

(NAGATA, Tainan, Taiwan). BMI was calculated as weight in kilograms divided by height in metres squared (kg/m^2). Limb circumferences at mid-upper arm, mid-thigh and medial calf on the right side were measured in triplicate. Skinfold thicknesses were measured in triplicate over the triceps, calf and thigh, using a skinfold calliper (Skinfold Caliper - Holtain Ltd., UK) to the nearest 1 mm in the right side. The precision errors (CV%) of anthropometric indices included in this study were determined by measuring 30 women twice in the same setting on the same day. The precision errors of measured anthropometric indices ranged from 1.30 to 5.66 per cent.

The limb circumferences (C_{limb}) were corrected for subcutaneous adipose tissue thickness as described previously[21,22]. The corrected muscle (including bone) circumferences (C_m) were calculated as $C_m = C_{limb} - \pi S$ (S: skinfold thickness). C_m was considered as corrected girth (CAG, corrected arm girth; CTG, corrected thigh girth and CCG, corrected calf girth). Further, as appendicular circumferences are uni-dimensional and muscle mass is three dimensional, C_m was squared and multiplied by height to convert to a three-dimensional measures[21,22].

DXA was used as the reference standard to quantify the ASMM. $ASMM_{DXA}$ (kg) was measured with a DXA Scanner (Hologic Discovery W, Hologic Inc., Discovery, Bedford, MA, USA) adhering to the manufacturer's recommendations. The accuracy and precision of measurements were ensured by scanning the full body phantom each scanning day and inspecting the calibration chart from beginning to the end of the study. SMM was analyzed by inbuilt software provided by the manufacturers, and $ASMM_{DXA}$ was determined by the sum of SMM of lower and upper limbs. The physical activity (PA) level was evaluated with the short version of the International Physical Activity Questionnaire (IPAQ)[23] which was forward-backward translated into Sinhala language and pre-tested. Participants were asked to report the time duration spent for walking, moderate intensity activity and vigorous intensity activity during the week prior to the interview. The PA data were converted to minutes per week and expressed as a metabolic equivalent (MET-min/wk) according to the IPAQ guidelines for data processing[23].

Statistical analysis: Data were analyzed using IBM SPSS v20.0 (SPSS Inc., Chicago, IL, USA). Normality of the data distribution was determined with Kolmogorov-Smirnov test with relevant plots. Descriptive statistics, including means (SD), median (interquartile range) or frequency (%), were used to describe the data. The group comparison of continuous data was performed with independent samples *t* test (after log transformation in PA data), and the group comparison of categorical data was performed with Chi-square test to evaluate the suitability for cross-validation.

Development of new models: Data from the 165 middle-aged women in group A were used for the development of new models. Pearson's correlation coefficients (*r*) were estimated between $ASMM_{DXA}$ and anthropometric variables to identify significant associations. Only the variables which showed strong significant associations with ASMM were considered for the development of models. The collinearity between the variables was verified by the variance inflation factor (VIF) and tolerance (T) values. Thus, VIF values <10 were considered as acceptable and tolerance values above 0.1[24]. Based on the above results, regression analyses were performed using $ASMM_{DXA}$ as the dependent variable and weight, triceps skinfold thickness (TSFT) (the strongest correlation among skinfold thicknesses studied), mid-upper arm circumference (MUAC) (the strongest correlation among arm circumferences studied), BMI, in different steps while age and MS also as independent variables in step-wise method. The next step was performed including all the measured variables as the 7th step. After that, another two steps which included corrected girths and three-dimensional figures in combination with age, weight, height and MS were performed as 8th and 9th steps. In the development of models, the variables that were entered and excluded in each step are shown in Table I. Based on the selected variables by the regression models, mathematical equations were developed.

Cross-validation of newly developed models: For the cross-validation of the newly developed models, 167 study participants assigned to group B were studied. Scatter plots were drawn and correlations between the ASMMs predicted by the newly developed models and $ASMM_{DXA}$ were determined. The mean differences between ASMM measured and estimated with developed models were compared by the paired samples *t* test. If the ASMM measured and estimated with developed models were not significantly different, additionally, determination coefficient (R^2) and standard error of estimate (SEE) were determined with

Table I. Entered variables for regression model in nine steps and excluded variables from the model in the development of new models

Step	Variables included in the regression model	Variables excluded from the model
1	Age, TSFT, MUAC, weight	MUAC
2	Age, TSFT, MUAC, weight, height	MUAC, age
3	Age, TSFT, MUAC, weight, MS	MUAC
4	Age, TSFT, MUAC, weight, height, MS	MUAC, TFST, age
5	Age, TSFT, MUAC, BMI	TSFT
6	Age, TSFT, MUAC, BMI, MS	TFST, age
7	Age, TSFT, ThSFT, CaSFT, MUAC, ThC, CaC, weight, height, MS	TSFT, CaSFT, MUAC, ThC, CaC, MS
8	Age, weight, height, MS, CAG, CTG, CCG	Age, MS, CAG
9	Age, Weight, MS, CAG^2 height, CTG^2 height, CCG^2 height	MS, CAG2 height

TSFT, triceps skinfold thickness; ThSFT, thigh skinfold thickness; CaSFT, calf skinfold thickness; MUAC, mid-upper arm circumference; ThC, thigh circumference; CaC, calf circumference; BMI, body mass index, CAG, corrected arm girth, CTG, corrected thigh girth, CCG, corrected calf girth, CAG^2 height/CTG^2 height/CCG^2 height, three-dimensional limb figures; MS, menopausal status

linear regression model. To consider the models to be valid, the validation criteria described by Lohman[25] and followed subsequently by Pereira *et al*[14] were used, *i.e.*, the models tested should not be significantly different from reference standard, SEE should be <3.5 and R^2 should be >0.7. The models which satisfied the above criteria were further tested for repeatability with Bland and Altman plots[26] and by assessing the single-measure intraclass correlation coefficient (ICC) with 95 per cent confidence interval.

To assess the adequacy of the sample, the power of the study was estimated by Post Hoc Compute Achieved Power Analysis using the G*Power software version 3.1.9.2 (Heinrich Heine Universität Düsseldorf, Germany)[27].

Results

Anthropometric indices and basic characteristics of groups A and B are shown in Table II. There was no significant difference between the characteristics, age, MS, anthropometric indices, PA level and $ASMM_{DXA}$ of the two groups, indicating that two groups were similar to perform cross-validation. In group A, all anthropometric indices studied showed positive correlations with $ASMM_{DXA}$ (Table III). Strong correlations were observed between $ASMM_{DXA}$ and weight ($r=0.87$), MUAC ($r=0.73$), BMI ($r=0.67$), TSFT ($r=0.56$) and height ($r=0.55$). Even though the corrected limb girths and three-dimensional figures were calculated expecting better representation of limb muscle mass, the correlations between $ASMM_{DXA}$ and corrected girths or three-dimensional figures were low compared to the correlation between $ASMM_{DXA}$ and crude girth indices (Table III).

The nine models developed to estimate the ASMM are given in Table IV. No significant collinearity was observed between independent variables (VIF <10 and tolerance values above 0.1) (data not shown). The results of the cross-validation (comparison between measured and estimated ASMM) are shown in Table V. The models which had non-significant mean difference between measured and estimated ASMMs were further tested for correlation and repeatability as shown in Table VI.

Of the nine models, only four models, including ASMM 1, ASMM 2, ASMM 3 and ASMM 4, met all the validation criteria; measured and estimated ASMMs were not significantly different (Table V) and had high correlations (r range from 0.89 to 0.92) and high coefficients of determination (80-84% variation of $ASMM_{DXA}$) with low SEEs (1.10-1.24 kg) (Table VI).

An acceptable measurement agreement was observed for models ASMM 1-ASMM 4 when data were examined by the Bland and Altman plots (Figure). Further, ICC for validated models indicated strong measurement concordance (Table VI).

The models derived in regression steps based on BMI in combination with TSFT and MUAC (ASMM 5 and ASMM 6), model based on all the measured limb circumferences and skinfold thicknesses (ASMM 7) and models based on corrected girths and three-dimensional figures (ASMM 8 and ASMM 9), however, did not meet the validation criteria (Table V). The *post hoc* power calculation test was conducted to calculate the sample power of valid anthropometry models by adopting an error probability of 5 per cent for the sample size used. The sample

Table II. Characteristics of the study participants		
Characteristic	Group A (n=165)	Group B (n=167)
	Mean (SD) or median (IQR) or n (%)	Mean (SD) or median (IQR) or n (%)
Age (yr)	49.11 (8.21)	48.9 (8.56)
Height (m)	1.50 (0.06)	1.51 (0.05)
Weight (kg)	57.52 (10.62)	57.32 (11.04)
BMI (kg/m²)	25.36 (4.32)	25.51 (2.84)
TSFT (mm)	19.47 (6.09)	19.31 (6.27)
ThSFT (mm)	27.71 (9.90)	26.92 (9.93)
CaSFT (mm)	18.63 (8.41)	17.80 (8.54)
MUAC (cm)	31.15 (3.85)	30.72 (3.77)
ThC (cm)	49.07 (7.41)	49.33 (7.51)
CaC (cm)	34.40 (5.68)	33.40 (4.24)
ASMM$_{DXA}$ (kg)	15.39 (2.75)	15.51 (2.84)
Walking PA score (MET-min/wk)	346.50 (0.00-1386.00)	346.50 (0.00-981.75)
Moderate PA score (MET-min/wk)	5040.00 (5040.00-5040.00)	5040.00 (5040.00-5040.00)
Vigorous PA score (MET-min/wk)	1680.00 (560.00-3360.00)	1680.00 (560.00-3360.00)
Total PA score (MET-min/wk)	7546.00 (6293.00-9093.00)	7067.00 (5880.00-9093.00)
Pre-menopausal women (%)	84 (50.9)	85 (50.9)
Post-menopausal women (%)	81 (49.1)	82 (49.1)

The group comparison of continuous data was performed with independent samples *t* test (after log transformation in physical activity data). The group comparison of categorical data was performed with Chi-square test. No significant difference observed between the two groups. SD, standard deviation; IQR, interquartile range; TSFT, triceps skinfold thickness; ThSFT, thigh skinfold thickness; CaSFT, calf skinfold thickness; MUAC, mid-upper arm circumference; ThC, thigh circumference; CaC, calf circumference; BMI, body mass index; ASMM$_{DXA}$, appendicular skeletal muscle mass measured with DXA; PA, physical activity

power (1-β error probability) was 1.00 for all four valid models (ASMM 1-ASMM 4).

Discussion

Of the nine anthropometric models developed to estimate ASMM among middle-aged women, only the four models which were based on height, weight, TSFT age and MS met all the validation criteria. Our results were consistent with previous studies from the USA[13], China[10], India[12] and Japan[7] where similar anthropometric measures were used to predict the ASMM. In these analyses, the complex equations which include the skinfold thickness and circumferences have shown improved prediction qualities[10,12,13]. We, however, did not observe such improvement when skinfold thickness and limb circumferences were included in the models. Our results were more compatible with Chinese[10] and Indian[12] studies. The subtle differences seen between the studies could be due to many factors. This could partly be due to the differences in the reference standards used. Some have used MRI as the reference standard[13] while others have

used DXA[10,12]. Variations in the composition of study samples such as ethnicity and age range of individuals[13] could also have contributed to such disparities.

Even though studies have shown that BMI predicts the muscle mass[28] and used it for ASMM prediction models[14,15], in our analysis, the models based on BMI failed to meet the validation criteria. Our observation might be due to the combining effect of menopause and age within the models. BMI, however, is a surrogate of body fat content and reflects global adiposity and its association with ASMM would not be strong. Weak association between BMI and ASMM compared to other anthropometric indices has also been reported in previous studies[13] in support of the findings of the current study. Further, BMI has been identified as an index that assesses total muscularity, rather than the relative distribution of muscle mass within the total body[29], and therefore, BMI is not a proper indicator of ASMM.

Previous studies have used limb girths corrected for local fat content and modified further to reflect

Table III. Correlations between anthropometric characteristics and appendicular skeletal muscle mass measured with DXA in women in group A (n=165)

Characteristics	Correlation with ASMM$_{DXA}$ (Pearson correlation**)
Height (m)	0.55
Weight (kg)	0.87
BMI (kg/m^2)	0.67
TSFT (mm)	0.56
ThSFT (mm)	0.38
CaSFT (mm)	0.43
MUAC (cm)	0.73
ThC (cm)	0.53
CaC (cm)	0.52
CAG	0.60
CTG	0.47
CCG	0.25
CAG^2 height	0.68
CTG^2 height	0.59
CCG^2 height	0.25

**All the variables were significantly correlated at <0.01 level. BMI, body mass index; TSFT, triceps skinfold thickness; ThSFT, thigh skinfold thickness; CaSFT, calf skinfold thickness; MUAC, mid-upper arm circumference; ThC, thigh circumference; CaC, calf circumference; ASMM$_{DXA}$, appendicular skeletal muscle mass measured with DXA; CAG, corrected arm girth; CTG, corrected thigh girth; CCG, corrected calf girth; CAG^2 height/CTG^2 height/CCG^2 height, three-dimensional limb figures

the three-dimensional nature of the muscle mass[10,13]. In our data, the uncorrected indices correlated better with ASMM$_{DXA}$ than the corrected limb girths or three-dimensional figures and models developed with corrected indices or three-dimensional figures did not meet validation criteria. This emphasizes the possibility of using models with simple anthropometric indices which can be easily measured, rather than using corrected indices as also highlighted by Wen *et al*[10].

Furthermore, skinfold callipers are generally not used in clinical settings and skinfold thickness and circumferences are associated with high measurement errors and factors such as loss of muscle mass, increased fat mass and loss of skin elasticity can potentially influence the readings[30]. Therefore, the developed models that include weight and height with age or MS would be more appropriate for the practice than the models that include the skinfold thickness. In our study samples were selected randomly from a semi-urban area of the Galle district which has socio-economic characteristics and disease prevalence similar to national figures[31]. Hence, the anthropometric models developed can be generalized to middle-aged women in other parts of the country as well.

There were several limitations in our study. This study included only middle-aged women and further studies should be done on other age groups and males. Furthermore, these models need to validated further

Step	Model	Models equation	Correlation coefficient (r)*	Determination coefficient (R^2)	SEE (kg)
1	ASMM 1	5.366+0.255 (weight)-0.064 (age)-0.078 (TSFT)	0.90	0.80	1.21
2	ASMM 2	−7.405+0.204 (weight)+8.802 (height)-0.045 (age)	0.91	0.82	1.14
3	ASMM 3	4.917+0.254 (weight)-0.572 (MS)-0.075 (TSFT)-0.037 (age)	0.90	0.81	1.20
4	ASMM 4	−8.394+0.205 (weight)+8.728 (height)-0.772 (MS)	0.91	0.82	1.14
5	ASMM 5	3.966+0.337 (MUAC)-0.075 (age)+0.182 (BMI)	0.76	0.58	1.78
6	ASMM 6	2.022+0.342 (MUAC)-1.271 (MS)+0.181 (BMI)	0.77	0.58	1.77
7	ASMM 7	−2.205+0.244 (weight)+0.055 (height)-0.062 (ThSFT)-0.061 (age)	0.95	0.89	1.04
8	ASMM 8	−9.585+0.171 (weight)+0.095 (height), 0.001 (CTG)+0.001 (CCG)	0.92	0.85	1.08
9	ASMM 9	4.183-0.036 (age)+0.176 (weight)+0.001 (CTG2 height)+0.001 (CCG2 height)	0.90	0.82	1.16

Table IV. Models developed to estimate appendicular skeletal muscle mass of middle-aged women in group A (n=165)

*Correlations were significant at <0.001 level. Weight in kg, height in m, circumference in cm, skinfold thickness in mm. MS, menopausal status: 2, post-menopausal; 1, pre-menopausal; TSFT, triceps skinfold thickness; MUAC, mid-upper arm circumference; BMI, body mass index; CTG, corrected thigh girth; CCG, corrected calf girth; ThSFT, thigh skinfold thickness in mm; SEE, standard error of estimate

Table V. Cross-validation of the developed models; comparison of the measured and estimated appendicular skeletal muscle mass in group B (n=167)

Model	Mean±SD kg	Mean difference±SD	Range of mean difference	Significance (P)
ASMM 1	15.35±2.60	0.16±1.24	−0.02-0.35	0.08
ASMM 2	15.41±2.59	0.10±1.15	−0.07-0.28	0.24
ASMM 3	15.36±2.60	0.14±1.20	−0.03-0.33	0.11
ASMM 4	15.41±2.58	0.09±1.10	−0.06-0.26	0.24
ASMM 5	14.46±2.26	1.20±1.15	1.02-1.38	<0.001
ASMM 6	15.20±2.06	0.31±1.74	0.04-0.58	0.02
ASMM 7	15.15±2.08	0.35±1.73	0.09-0.62	0.008
ASMM 8	17.22±2.39	0.08±1.13	−1.73-−1.37	<0.001
ASMM 9	18.76±3.91	0.70±2.02	−1.69-−1.48	<0.001

Mean comparison was performed with paired samples *t* test. SD, standard deviation; ASMM, appendicular skeletal muscle mass
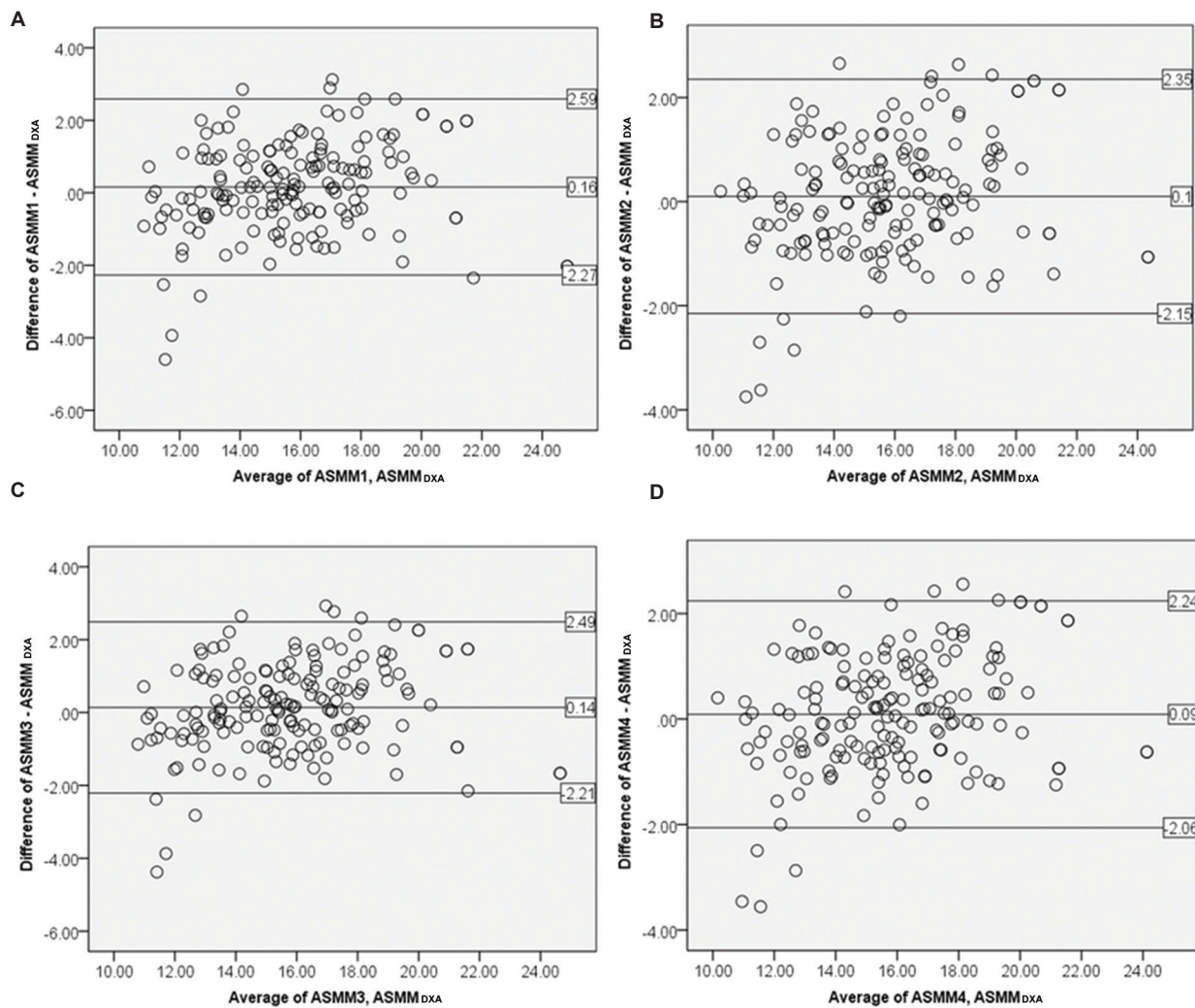

Figure. Agreement between newly developed models and criterion method (ASMM$_{DXA}$) (n=167). (**A**) ASMM 1, (**B**) ASMM 2, (**C**) ASMM 3, (**D**) ASMM 4. ASMM$_{DXA}$, appendicular skeletal muscle mass measured with DXA.

Table VI. Cross-validation of the developed models; regression analysis and repeatability analysis with group B (n=167)

Model	Regression analysis			ICC	95% confidence interval of ICC
	Correlation coefficient (r)*	Determination coefficient (R^2)	SEE (kg)		
ASMM 1	0.89	0.80	1.24	0.84	0.86-0.92
ASMM 2	0.91	0.83	1.16	0.90	0.87-0.93
ASMM 3	0.90	0.82	1.20	0.90	0.87-0.92
ASMM 4	0.92	0.84	1.10	0.91	0.89-0.93

*Correlations were significant at <0.001 level. SEE, standard error of estimate, ICC, intraclass correlation; ASMM, appendicular skeletal muscle mass

especially in individuals with co-morbidities to enhance the validity of the models. As we used the DXA manufacturer's routines to estimate the ASMM without resorting the DXA machine for this specific validation, the between-manufacturer variation could potentially limit the application of the mathematical models we proposed to DXA devices by other manufacturers.

In this study, simple predictive models were developed and validated which used different anthropometric measures to quantify the ASMM of middle-aged Sri Lankan women. These models showed a high accuracy and were suitable for research and were clinical settings. The selection of the model can be based on the availability of anthropometric indices in the particular setting.

***Conflicts of Interest*:** None.

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