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Pedro Pugliesi Abdalla¹,²,⁴, Ana Claudia Rossini Venturini¹,², André Pereira dos Santos¹,², Marcio Tasinafo²,³, José Augusto Gonçalves Marini²,³, Thiago Cândido Alves², Alcivandro de Sousa Oliveira², Jorge Mota²,⁴, Gareth Stratton⁵, and Dalmo Roberto Lopes Machado¹,²,³,⁴

¹Escola de Enfermagem. Universidade de São Paulo. Ribeirão Preto, São Paulo. Brazil. ²Anthropometry, Training and Sport Study and Research Group (GEPEATE). Universidade de São Paulo. Ribeirão Preto, São Paulo. Brazil. ³Escola de Educação Física e Esporte. Universidade de São Paulo. Ribeirão Preto, São Paulo. Brazil. ⁴Centro de Investigação em Actividade Física, Saúde e Lazer (CIAFEL). Universidade do Porto. Porto, Portugal. ⁵School of Sport Science. Swansea University. Swansea, Wales. United Kingdom

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Correspondence: Pedro Pugliesi Abdalla. Escola de Enfermagem. Universidade de São Paulo em Ribeirão Preto. Av. Bandeirantes, 3900, Campus Universitário, Monte Alegre. 14040-902 Ribeirão Preto, São Paulo. Brazil
e-mail: pedroabdalla11@gmail.com

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**ABSTRACT**

**Introduction:** functional limitation is a result of sarcopenia and is associated with loss of skeletal muscle mass (SMM). Cost-effective methods are important for the identification of sarcopenia.

**Objective:** to propose cutoff points for normalized calf circumference (CC) in order to identify low SMM in older women based on their functional limitation.

**Methods:** in this descriptive, cross-sectional study the CC values of a young female sample (n = 78) were used to establish cutoff points (-2 SD) for low SMM in older women (n = 67). Functional limitation was identified by the six-minute walk test (≤ 400 m). CC was normalized by body mass, height, and BMI. The diagnostic accuracy of CC was calculated with a ROC curve, using functional limitation as standard.

**Results:** cutoff points and area under the curve (AUC) were: CC (≤ 28.5; 0.62); CC·body mass\(^{-1}\) (≤ 0.40; 0.63); CC·height\(^{-2}\) (≤ 8.52; 0.55) and CC·BMI\(^{-1}\) (≤ 1.10; 0.73). Only CC·BMI\(^{-1}\) achieved a desirable accuracy (AUC > 0.7) to distinguish functional limitation.

**Conclusion:** the accuracy attained supports the use of CC·BMI\(^{-1}\) to identify low SMM in older women. In the clinical context it is possible to predict the risk of sarcopenia when sophisticated methods for determining SMM are not available.

**Keywords:** Aged. Anthropometry. Frail elderly. Mobility disability. Muscle mass.

**RESUMEN**

**Introducción:** la limitación funcional es consecuencia de la sarcopenia y se asocia con la pérdida de masa muscular esquelética...
(MME). Los métodos rentables son importantes para la identificación de la sarcopenia. **Objetivo:** proponer puntos de corte para la circunferencia de la pantorrilla (CP), normalizada para identificar un MME bajo en mujeres mayores en función de su limitación funcional. **Métodos:** en este estudio descriptivo de carácter transversal se utilizaron los valores de CP de una muestra de mujeres jóvenes (n = 78) para establecer los puntos de corte (-2 DS) de la MME baja en las mujeres mayores (n = 67). La limitación funcional se identificó mediante la prueba de la marcha de seis minutos (≤ 400 m). La CP se normalizó por la masa corporal, la altura y el IMC. La precisión diagnóstica de la CP se calculó con la curva ROC, utilizando como estándar la limitación funcional. **Resultados:** los puntos de corte y el área bajo la curva (AUC) fueron: CP (≤ 28,5; 0,62); CP·masa corporal$^{-1}$ (≤ 0,40; 0,63); CP·altura$^{-2}$ (≤ 8,52; 0,55) y CP·IMC$^{-1}$ (≤ 1,10; 0,73). Solo el CP·IMC$^{-1}$ logró la precisión deseable (AUC > 0,7) para distinguir la limitación funcional. **Conclusión:** la precisión alcanzada respalda el uso de CP·IMC$^{-1}$ para identificar la MME baja en las mujeres mayores. En el contexto clínico es posible predecir el riesgo de sarcopenia cuando no se dispone de métodos sofisticados para determinar la MME. **Palabras clave:** Envejecido. Antropometría. Ancianos frágiles. Discapacidad de movilidad. Masa muscular.

**INTRODUCTION**
Functional limitation caused by reduced mobility is an age-related phenomenon that negatively affects the physical independence of older adults (1). One of the main consequences of this limitation is sarcopenia, a disease characterized by low muscle strength and reduced amount of skeletal muscle mass (SMM) (2). Sarcopenia affects 10 percent of older adults worldwide (3) and 60 percent of older women in Brazil (4). When untreated, sarcopenia exposes older adults to functional impairment, mobility disorders, increased risk of falls, loss of functional independence, physical frailty, increases in
hospitalization, decreased quality of life, and increased risk of premature death (5). Therefore, the early identification of the disease considerably reduces the economic impact on health systems and on the personal and social burdens of care for older adults (6).

For the diagnostic confirmation of sarcopenia two conditions are required: reduced muscle strength and decreased SMM (2). SMM parameters can be obtained from dual-energy X-ray absorptiometry (DXA), bioelectrical impedance analysis, magnetic resonance imaging, computed tomography, muscle biopsy, or magnetic resonance spectroscopy (2). However, these require skilled health professionals, and are high-cost and difficult to access in the context of regular clinical practice, particularly in low- and middle-income countries (7).

Although the measurement of SMM by DXA is one of the most widely used procedures (4), this test lacks a good relationship with reduced mobility as associated with sarcopenia (8), and thus has limited diagnostic value (8). On the other hand, calf circumference (CC) is a cost-effective and expedient alternative for estimating SMM, a clinically relevant outcome in community-dwelling older adults (2,9). Although CC has a good association with mobility in older adults (10), this relationship is characterized by an inverted U-shaped form (9). Normalizing CC by body size, as recommended, will correct this non-linear relationship (2). Normalization of SMM is already performed on selected DXA variables, such as appendicular skeletal muscle mass (ASMM), usually corrected relative to body size (ASMM·height$^{-2}$, ASMM·body mass$^{-1}$ or ASMM·BMI$^{-1}$) (11). Unfortunately, these variables have weak associations with clinically relevant outcomes in older adults (8,12). Moreover, the identification of a low SMM index (ASMM·height$^{-2}$) includes the absolute value of CC (Table III) (13-22) for generating cutoff points, which will penalize older adults with a smaller body size.

Given the limitations evident in quantifying SMM, the objective of this study was to propose cutoff points for CC normalized by body size to identify low SMM in older women with regard to functional limitation. Our hypothesis is that normalization of CC provides a pragmatic
indicator of functional limitation that, in turn, indicates risk of sarcopenia. Therefore, monitoring functional limitation could alert to the degenerative consequences resulting from the ageing process in women at a public healthcare level.

MATERIALS AND METHODS

Participants and settings (age, gender, country socioeconomic status, and so on)

For this descriptive and cross-sectional study a sample was comprised of two age groups from the same population who attended our laboratory between October 2016 and June 2017: the first group consisted of 79 young women aged between 18 and 30 years (23.9 ± 3.4 years), and the second group of 69 older women aged between 60 and 85 years (69.8 ± 6.0 years). A sample size calculation was previously performed (n = \[\frac{Z_y \text{ SD}}{\varepsilon}\]^2) from the maximum desired error (\(\varepsilon \leq 1\) %), trust level (\(Z_y = 0.95\)) and population variability (SD). The main variable (CC) for the age group of women with greater variability obtained from a compatible population was adopted as reference (SD = 4.29 cm) (21). The minimum sample size was calculated (n = 142).

The voluntary recruitment of participants took place through personal invitations, and electronic and printed dissemination in the community. Young women met the following criteria: not taking antidepressants or stimulants that affect the central nervous system, self-declared to be in good health, not having amputated body parts, not performing more than 10 hours/week of physical training. Older women should be able to walk independently, not have uncontrolled chronic illnesses, acute infections, tumors, back pain, hip, and knee prosthesis, unintentional weight loss of more than three kg in the last three months. Criteria for discontinuing the study were reports of serious balance problems, and sequelae of stroke and cognitive impairment. All participants gave their full and informed consent to
take part, and the study was designed and conducted in accordance
with the Declaration of Helsinki and approved by the Institution’s
Ethics Committees (CAAE: 57511516.5.0000.5659 and
54345016.6.0000.5659).

**Instruments and procedures**
All measurements were taken individually at the University Hospital of
Ribeirao Preto School of Medicine, University of Sao Paulo, Brazil (HC-FMRP-USP), in the morning from 9 am to 11 am). Data collection took
place in a single session, by the same examiners trained in each
measure. The older women cognitive deficit was verified by a
questionnaire. Anthropometric measurements of height to the nearest
0.01 m and body weight (kg) were performed (26) for all women, and
BMI was calculated (kg·m⁻²). Other measures performed are described
below.

**Cognitive assessment**
To ensure the aptitude and cognitive capacity of older women, the
Mini Mental State Examination (MMSE) was used in the reduced
version of 19 points (27). Participants who scored 12 or less were
considered to have a cognitive deficit and were excluded from the
analysis.

**Anthropometric assessments**
From the measurements of body mass in kg and height in cm body
mass index (BMI; kg·m⁻²) was determined. CC was measured
according to a standardized procedure (26) with recording of the
median from 3 measurements. For analyses CC was considered as
absolute (cm) and normalized: CC·body mass⁻¹ in cm·kg⁻¹, CC·height⁻²
in cm·m⁻² and CC·BMI⁻¹ in cm·kg⁻¹·m⁻².

**Six-minute walk test**
To assess physical function the six-minute walk test was performed on a flat, non-slip surface, 30 meters in length, with calibrated markings every three meters. Older women were asked to walk as fast as possible for six minutes and were allowed to rest during the test without stopping the clock. Total distance covered was recorded to the nearest 3 meters. Functional limitation was identified when walking distance was ≤ 400 m (1).

**Statistical analysis**

Descriptive statistics included central tendency and confidence interval (95 % CI). Cutoff points were established for absolute or normalized CC considering -2 SD compared to the mean CC of young and healthy women, as recommended by the EWGSOP for SMM parameters (2). The likelihood of different CC expressions to explain the occurrence of functional limitation was confirmed by logistic regression. Additionally, significance in statistical models was verified using the chi-square ($\chi^2$) test and their coefficients of logistic regression. Finally, the ability of CC to discriminate functional limitation was illustrated using an area under the ROC curve (AUC) value > 0.70 (28). For this analysis we adopted a dichotomous classification for the presence (1) or absence (0) of a functional limitation. All analyses were performed on the SPSS 25.0 and MedCalc 15.2 programs, with a previously established level of significance ($\alpha = 5 \%$).

**RESULTS**

Figure 1 shows the flowchart for recruiting study participants. One hundred and forty-eight women were initially eligible for the studies. After applying exclusion criteria, the analyses were carried out in 145 women (78 young).

Descriptive statistics for MMSE score, chronological age, anthropometric variables, and walking distance are shown in table I. Older women were older, and had greater body mass, BMI, and
absolute CC. Younger women were taller (10 cm) and weighed less (6 kg) than their older counterparts. The higher BMI found in older (≅ 27 kg·m⁻²) compared to younger women (≅ 21 kg·m⁻²) illustrates a greater weight gain with age. Likewise, the highest normalized CC values observed in young women showed an inverse relationship with absolute CC according to age. From a functional perspective nearly two thirds of the older women (39 %; n = 26) did not achieve a good functional score on the walk test.

Normalized CC cutoff points showed an inverse relationship with age when compared to absolute CC (Table I). CC values normalized by body mass (0.40 cm·kg⁻¹), height squared (8.52 cm·m⁻²) or BMI (1.10 cm·kg⁻¹·m⁻²) also had a different relationship for each age group. All mean CC values were within the 95 % confidence threshold, which suggests high reliability even when replicated for other samples of the same population.

The accuracy of detecting a functional limitation using different CC expressions is shown in table II. The p-values of the regressions originating from CC as normalized by body mass (p = 0.027) and BMI (p = 0.001) demonstrate the likelihood of significantly accounting for the occurrence of functional limitation. In fact, only CC·BMI⁻¹ presented an acceptable accuracy (AUC > 0.70), as shown in figure 2. There is statistical significance in the chi-quadratic distribution (Wald = 8.72; p = 0.001). From the beta exponent (b = 0.002) of the CC·BMI⁻¹ variable (Table II) it was possible to calculate the likelihood of decreasing functional limitation (i.e., 0.002 - 1 = -0.998). That is, for every increased tenth of unit (0.1 cm·kg⁻¹·m⁻²) of CC·BMI⁻¹ there is a reduction in the chances of functional limitation by ≅ 10 %. As an example from our findings (Table II), mean CC·BMI⁻¹ in young women (1.6 cm·kg⁻¹·m⁻²) compared to older women (1.3 cm·kg⁻¹·m⁻²) has a difference of 0.3 cm·kg⁻¹·m⁻². This represents a reduction in the chances of functional limitation for young women by around 30 %.

Although the adjustment of CC by body mass (Table II) was significant (χ² = 4.89; p = 0.027), the extremely low odds ratio value (< 0.001)
did not sufficiently explain the occurrence of functional limitation. This finding was confirmed when the analysis of the ROC curve for CC·body mass\(^{-1}\) showed insufficient sensitivity (27 %), specificity (98 %), and AUC (0.63) values. On the other hand, the CC·BMI\(^{-1}\) cutoff point presented an acceptable AUC (0.73), even though its sensitivity (85 %) was greater than its specificity (54 %).

After applying the cutoff point of the CC·BMI\(^{-1}\) (≤ 1.10 cm·kg\(^{-1}\)·m\(^{-2}\)) in our sample, about 12 % of older women were classified with low SMM (n = 7/60). Figure 3 further illustrates the relationship between CC and mobility in older women, as expressed by the distance covered in the six-minute walk test. The inverted U-shaped relationship expressed by absolute CC (a) versus the resulting linearity when normalized by BMI (b) may be seen.

Our model (CC·BMI\(^{-1}\)), in addition to correcting the non-linear relationship between CC and mobility, showed a positive and significant correlation between adjusted CC and mobility (r = 0.48; p < 0.001).

**DISCUSSION**

Our aim was to develop cutoff points for CC normalized by body size to identify low SMM in older women with respect to functional limitation. The cutoff points of CC when normalized by body size were more effective than absolute CC in identifying low SMM in older women. CC·body mass\(^{-1}\) and CC·BMI\(^{-1}\) explained (p < 0.05) the likelihood of occurrence of functional limitation (walking ≤ 400 m in six minutes). But only CC normalized by BMI (1.10 cm·kg\(^{-1}\)·m\(^{-2}\)) achieved an acceptable sensitivity (85 %) and accuracy (AUC > 0.70) (28) to identify the low SMM associated with functional limitation. Furthermore, this model (CC·BMI\(^{-1}\)) was able to linearize the inverted U-shaped relationship usually observed in absolute CC expressions. Absolute CC is accurate to estimate and identify a low level of SMM in older women (15); however, when normalized by body size, particularly BMI (2), it avoids false negatives for functional limitation.
and sarcopenia. An absolute CC value below 27 cm alerts to care needs for older women to perform their daily activities (29). However, when absolute CC is high (i.e., > 38 cm), it also predicts the risk of impaired mobility (10), suggesting the need to create double cutoff points for the absolute CC (one for high values and another for low values), due to a non-linear relationship between absolute CC and mobility (10). This was confirmed in this study when all of our older women with elevated CC (> 38 cm) had functional limitations (walking distance ≤ 400 m), as seen in figure 3a.

A multiple linear regression model proposed from NHANES data (30), including CC in combination with sex, race, and age, was able to predict up to 90% of ASMM as measured by DXA. This approach could be adequate to identify SMM deficit in older women were it not for its weak relationship with functional limitation (8). Absolute CC values could result in prediction errors (Fig. 3a) over time, since higher CCs are generally related to overweight and obesity (BMI > 25 kg·m⁻²) characterizing a negative impact on functional performance (10).

One of the strengths of this study involves a normalization of CC that had not yet been proposed as predictor of SMM, although it has long been recommended (2). Another positive finding was that alternatively to most studies that used ASMM·height⁻² derived from DXA as an SMM indicator, our model was able to discriminate functional limitation using normalized CC in an linear expression approach. The procedure to generate a cutoff point at -2 SD of the mean value for young women is a recommended method to approach SMM parameters (2). This study also has its limitations: for example, the sample was not random or stratified. Another limitation is that muscle function and quality were not analyzed in the present study. These limitations, as well as others such as perceptual-motor factors, affect the relationship between CC and performance, and thus can be aims for future studies. Nevertheless, the application of the model to other populations has an unconfirmed predictive value and requires further development before being adopted into clinical practice.
One implication of our findings for clinical practice supports the use of normalized CC by BMI as a simple and inexpensive indicator for monitoring SMM losses associated with functional capacity in older women. Another implication involves choosing variables that predict incident adverse health-related outcomes in older adults to identify potential sarcopenia by CC, allowing intervention or prophylactic decisions to be made when monitoring the health of older women. Our CC·BMI\(^{-1}\) model classified 12\% of older women with reduced SMM, and 39\% of older women as having functional limitation (walking ≤ 400 m). The cutoff point at -2 SD of mean CC·BMI\(^{-1}\) for young women (≤ 1.10 cm·kg\(^{-1}\)·m\(^{-2}\)) was lower than the cutoff point based on functional limitation (≤ 1.33 cm·kg\(^{-1}\)·m\(^{-2}\)) as estimated by the ROC curve (data not shown), which would classify older women with mobility problems. This corrects the distortion of overestimating functional limitation (39\%) present in the current classification of SMM deficit (12\%), closer to the worldwide prevalence of sarcopenia (≅ 10\%) (3) but still below the prevalence of Brazilian older women (≅ 20\%) (4). But we emphasize that we did not consider muscle strength here, the first criterion for the diagnosis of sarcopenia, but only the second criterion (low SMM) (2). Maybe this should be recommended for further researches.

In conclusion, our hypothesis that normalized CC helps to identify functional limitation as a prognostic tool to estimate the risk of sarcopenia has been confirmed. Our findings support the use of CC normalized by BMI to identify low SMM as an expression of functional limitation in older women, without the inverted U-shaped relationship bias. Monitoring the functional limitations resulting from ageing in women may be feasible using this simple and inexpensive approach, particularly given the high prevalence of sarcopenia in older women in low- to middle-income countries. Its use can be a viable alternative in clinical practice when sophisticated methods for identifying the low SMM associated with functional limitation are not available.
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Table I. A descriptive analysis of young and older women, body measurements, and calf-circumference (CC) cutoff points, absolute and normalized, to identify low skeletal muscle mass (SMM) and functional performance in older women.

| Variables          | Unit       | young women (n = 78) | CC cutoff point | older women (n = 67) | 95% CI     |
|--------------------|------------|-----------------------|-----------------|-----------------------|------------|
|                    |            | mean ± SD             | lowe upp S D    | mean ± SD             | lowe upp S D |
| MMSE score         | (0-19)     | 17.4 ± 17.0           | 17.8 ± 17.0     | 17.0 ± 17.8           | 17.2 ± 6.0 |
| Age (years)        |            | 23.9 ± 3.2            | 24.7 ± 4.8      | 69.8 ± 68.3           | 71.2 ± 6.0 |
| Body mass (kg)     |            | 60.0 ± 8.5            | 61.9 ± 8.8      | 66.6 ± 63.8           | 69.4 ± 11.6 |
| Height (m)         |            | 1.7 ± 0.1             | 1.7 ± 0.2       | 1.6 ± 1.5             | 1.6 ± 0.1 |
| BMI (kg·m⁻²)       |            | 21.8 ± 2.2            | 22.4 ± 2.8      | 27.3 ± 26.2           | 28.4 ± 4.4 |
| CC (cm)            |            | 34.0 ± 2.7            | 34.6 ± 7.0      | 34.8 ± 34.1           | 35.5 ± 2.9 |
| CC·body mass⁻¹ (cm·kg⁻¹) |       | 0.6 ± 0.1             | 0.6 ± 0.1      | 0.40 ± 0.5            | 0.6 ± 0.1 |
| CC·height⁻² (cm·m⁻²) |         | 12.2 ± 1.1            | 12.6 ± 8.8     | 11.3 ± 12.0           | 11.7 ± 1.4 |
| CC·BMI⁻¹ (cm·kg⁻¹·m⁻²) |       | 1.6 ± 1.5             | 1.6 ± 1.0      | 1.3 ± 1.3             | 1.33 ± 0.2 |
| Walking distance*  | (m)        | 413 ± 391             | 436 ± 92       | 6 ± 39                |
| Functional limitation† | (%)    | 39%                   | 10%            | 1%                    |

CI: confidence interval; SD: standard deviation; MMSE: Mini Mental State Examination; BMI: body mass index; CC: calf circumference. *In the six-minute walk test; †Walking distance ≤ 400 m.
Table II. Likelihood of the occurrence of a functional limitation* explained by logistic regression from absolute and normalized calf circumference (CC)

| Dependent variable | Independent variable | $\chi^2$ | $p$-value | Wald | OR |
|--------------------|----------------------|----------|------------|------|----|
| Dichotomous walking distance (1: ≤ 400 m and 0: > 400 m) | CC | 2.79 | 0.095 | 2.56 | 1.16 |
| | CC·body mass$^1$ | 4.89 | 0.027 | 4.33 | 6 | < |
| | CC·height$^2$ | 0.28 | 0.595 | 0.28 | 0.001 | 1.10 |
| | CC·BMI$^1$ | 10.77 | 0.001 | 8.72 | 2 | 0.00 |

*Walking distance ≤ 400 m in the six-minute walk test; BMI: body mass index; $\chi^2$: chi-square; OR: odds ratio.
Table III. Studies that proposed cutoff points to identify low skeletal muscle mass (SMM) by calf circumference (CC), identified from the Receiver Operating Characteristic (ROC) curve analysis.

| Authors                | Origin of older adults | N     | Dependent variable | Cut points \((≤)\) of absolute CC (cm) | AUC  | Sen. (%) | Spec. (%) |
|------------------------|------------------------|-------|--------------------|----------------------------------------|------|----------|-----------|
| Pagotto et al. (2018)  | Brazilians             | 132   | ASMM · height\(^2\) | 34 for men, 33 for women               | 0.75 | 71       | 77        |
| Kim et al. (2018)      | South Koreans         | 657   | ASMM · height\(^2\) | 35 for men, 33 for women               | 0.81 | 92       | 59        |
| Sampaio et al. (2017)  | Brazilians             | 316   | Frailty (23)       | 32 for both sexes                      | 0.67 | 54       | 73        |
| Kusaka et al. (2017)   | Japanese women         | 116   | Sarcopenia (24)    | 32.8 for women                         | 0.79 | 73       | 80        |
| Bahat et al. (2016)    | Turks                  | 406   | SMM · height\(^2\) | 33 for both sexes                      | 0.84 | 100      | 74        |
| Kawakami et al. (2015) | Japanese women         | 526   | Sarcopenia (24)    | 34 for men, 33 for women               | 0.94 | 89       | 88        |
| Barbosa-Silva et al. (2015) | Brazilians         | 189   | ASMM · height\(^2\) | 34 for men, 33 for women               | 0.76 | 61       | 76        |
| Rolland et al. (2003)  | French women           | 145   | ASMM · height\(^2\) | 31 for women                          | -    | 44       | 91        |
| Bonnefoy et al. (2002) | French                 | 911   | ASMM · height\(^2\) | 30.5 for both sexes                    | 0.81 | 73       | 73        |
ASMM: appendicular skeletal muscle mass; SMM: skeletal muscle mass; Sens.: sensitivity; Spec.: specificity. *Calculated from the fat-free mass obtained by bioelectrical impedance analysis. †Serum albumin < 30 g·L⁻¹ or BMI < 19 kg·m⁻².

Fig. 1. Flow chart of women recruited by age groups.
Fig. 2. Accuracy of absolute and normalized calf circumference (CC) to detect functional limitation (≤ 400 m in six-minute walk test) in older women, represented by the ROC curve and area under the curve (AUC).
Fig. 3. Comparison of the inverted U-shaped relationship (a) between absolute CC and mobility (six-minute walk test) with the linear (b) one of the indicator proposed in this study (CC·BMI\(^{-1}\)) in older women (n = 67).