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Motor Reaction Time, Sarcopenia and Functional Skills in Elderly Women: A Cross-Sectional Study

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Abstract

IMPORTANCE: Aging generates changes over the years. Because of this, the musculoskeletal system is directly degraded and suffer deficits in its performance in elderly patients with Sarcopenia, as this condition is characterized by a decrease in muscle mass and function.
OBJECTIVE: Correlate the motor reaction time and functional skills of non-sarcopenic, pre-sarcopenic and sarcopenic elderly women, and analyze influence on the risk of falls.
DESIGN: Cross-sectional observational analytical study, following the methodological strategies of STROBE (Strengthening the Reporting of Observational Studies in Epidemiology), carried out under the approval of the Research Ethics Committee of the Unievangélica University, no. 3.694.235/2019.
SETTING: Participants were evaluated regarding: cognitive status, level of physical activity, fear of falling, body composition, motor reaction time, static and dynamic balance, gait kinetics, strength and endurance of the lower limbs and finally handgrip strength.
PARTICIPANTS: A total of 59 volunteer elderly women were assessed following the diagnostic criteria for sarcopenia proposed by the European Working Group on Sarcopenia in Older People (EWGSOP).
RESULTS: The results show that there was a greater difference in motor reaction time between the non-sarcopenic and sarcopenic elderly women due to the executing organ being damaged by the presence of sarcopenia, causing motor response to slowdown. Functional deficit, fear of falling and greater risk of falls were observed in the sarcopenic group, under the harmful influence of increased motor reaction time.
CONCLUSION: Sarcopenic elderly women present increased motor reaction time, that is, slowed motor responses due to decreased muscle mass, strength and impaired musculature, which generate functional deficits that contribute to an increased risk of falls.

Key words: Aging, motor reaction time, sarcopenia, functional skills/functionality, postural balance, risk of falls.

Introduction

The ability to carry out tasks through interaction with the environment determines an individual's functional capacity. The decrease in functional capacity in old age results from the emergence of morphological and biochemical changes in the musculoskeletal system (1). Aging leads to a decline in this capacity, resulting from demographic, social, psychological, environmental, lifestyle and biological factors, which may include loss of muscle mass, strength and cardiorespiratory endurance (2). This is reflected in the inability to perform tasks that require speed, reasoning, mobility, coordination and balance (3).

According to the European Working Group on Sarcopenia in Older People (EWGSOP), sarcopenia is characterized as a condition that generates a progressive and widespread loss of skeletal muscle mass and strength, which can lead to physical disability, poor quality of life and even death (4, 5). Currently, it is one of the causes for decrease in functional capacity in the elderly, making them weaker, disturbing their activities of daily living, increasing their dependence and, consequently, the risk of falls, prolonging hospital stays (6). Studies show that after the age of 50, there is an annual loss of 1% of muscle mass, 2% of gait speed, and 1.9 to 5.0% of handgrip strength (7).

The decline of neuromuscular integration systems due to advancing age promotes a reduction in the agility of reaction to sensory stimuli and a decrease in the speed of central processes, due to the restriction of the speed at which sensorimotor processes are performed, resulting in a decline in elderly people's performance. The response time comprises the time it takes the body to initiate recovery from imbalance. A person's response time when subjected to imbalance is critical in preventing falls (8). Aging can be characterized by the reduction of connections between neural circuits, whose probability increases over time (9).

Ideal, agile, precise and balanced motor control depends on bringing together the planning organ, the conduction route and the executing organ. It is notorious that the aging process causes neuromuscular alterations; however, there is a lack of studies and research linking sarcopenia to these changes. Thus, the aim of the present study was to correlate motor the reaction time and functional skills of non-sarcopenic, pre-sarcopenic and sarcopenic elderly women, and the influence of this on risk of falls.

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Materials and methods

Design and Ethical Aspects

This is a cross-sectional study of observational analytical character, whose evaluations were carried out in the city of Ceres, state of Goiás, Brazil. The study followed the methodological strategies of Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) (10), and it was carried out with the approval of the Research Ethics Committee of the Unievangélica University, no. 3.694.235/2019. All volunteers consented to the research before the start of data collection by signing the informed consent form (ICF).

Sample

Initially, a pilot study carried out with 15 volunteers (non-sarcopenic = 5; pre-sarcopenic = 5; sarcopenic = 5) guided the sample size. The sample was calculated using the software G *Power 3.1.9.2 (Franz Faul, Universitat Kiel, Germany) (11), considering the intergroup variance of Simple Motor Reaction Time. Thus, the sample required to detect a significant and clinically important difference between groups was N = 45 (n = 15 per group), obtaining effect size (r) = 1.32, $p < 0.05$, power 0.95.

In the end, after invitation, screening and complete assessments, the study sample consisted of 59 elderly women aged over 60 years. For the volunteers to be included, the following criteria were considered: (i) elderly women able to stand and walk independently; (ii) literate or not; (iii) who has agreed to participate in the study and signed the informed consent form; (iv) body mass index (BMI) <30 kg/m2; (v) preserved cognition (Mini Mental State Examination> 18 points) (12).

The sample of this study does not comprise (i) volunteers with neurological disorders and/or sequelae; (ii) vestibular disease; (iii) uncorrected visual impairment; (iv) orthopedic alterations such as amputations, fractures, history of ankle sprain in the last six months; (v) who had drank alcoholic beverages in the 24 hours preceding the assessments; (vi) who reported osteoarthrosis in the spine and/or endoprosthesis in the lower limbs; (vii) with medical diagnosis of rheumatoid arthritis.

Instruments for Diagnosis and Subclassification of Sarcopenia

According to the European Working Group on Sarcopenia in Older People (EWGSOP), three variables are used to diagnose sarcopenia: muscle strength, muscle mass and physical performance. This study used the following tests: Timed Up and Go (TUG), applied to assess the physical performance, mobility and functional balance of the volunteers, through the time spent to perform the task of getting up from a chair, walking 3 meters, turn around, return to the chair and sit down again; Bioimpedance platform, used to calculate body composition through the different levels of electrical waves of biological tissues that are exposed to current frequencies emitted by the platform. This current runs throughout the body and provides estimates of the amount of fat present in the body in proportion to the elderly woman's body weight; Handgrip Strength Dynamometry, for which a Jamar® dynamometer was used to assess the muscle strength of the volunteers, with three measurements being taken and then considered the highest value between them in the dominant and non-dominant hand, with an interval 1 minute between each measurement. The measurement of handgrip strength is as sensitive as the strength of the knee flexors and extensors by isokinetics to relate the gait variables that are altered with age.

The diagnosis of sarcopenia is based on three criteria, with criterion 1 being low amount of muscle mass, criterion 2 low muscle strength, and criterion 3 low functional performance. The classification is defined in different stages: pre-sarcopenia (criterion 1), sarcopenia (criterion 1+2 or 3) and severe sarcopenia (criterion 1+2+3) (13).

After performing the tests, the volunteers were subdivided into non-sarcopenic, those with minimal change in the variables; pre-sarcopenic, those with low muscle mass; and sarcopenic, those with low muscle mass and low muscle strength or physical performance.

Demographic Assessment Instruments, Health Aspects and Functional Skills

Mini Mental State Examination (MMSE)

The MMSE questionnaire was administered to assess cognitive function. The volunteers were asked to answer questions that covered orientation, memory, attention and specific skills, such as naming and understanding, totaling a maximum score of 30 points, with scores below 18 points indicating cognitive impairment (14).

International Physical Activity Questionnaire (IPAQ)

The IPAQ questionnaire was used to determine the level of physical activity. The volunteers were asked to answer the questions considering the time spent doing physical activities in a normal or usual week. The categorical score classifies them as: very active; active; irregularly active; or sedentary. This calculation multiplies the value of energy expenditure in MET of that activity (walking equals 3.3 MET, moderate 4.0 MET, and vigorous 8.0 MET) by the frequency in days per week and the time in minutes declared for each activity (15).

Falls Efficacy Scale International (FES-I-BR)

The FES-I-BR scale was used to assess the level of concern with falling while performing daily living activities. The FES-I consists of 16 items and has four possible answers, with scores from 1 to 4. The total score can, thus, range from 16 to 64, in which 16 corresponds to the absence of concern and 64 to extreme concern with falling while performing the activities mentioned in the questionnaire (16).

Stage Balance Test

The 4-Stage Balance Test was administered in order to assess the static balance of the volunteers, with the following methodology: the volunteer was asked to stand in 4 different positions, without support and with eyes open, and to remain in each position for 10 seconds without moving their feet or needing support. The test assesses how many positions were held for 10 seconds (17).

30-Second Sit-to-Stand Test

The 30-Second Sit-to-Stand Test was used to assess leg strength and endurance. The methodology was as follows: the volunteer was asked to perform the task of sitting on a chair and rising to a full standing position and sitting down again, repeating this action for 30 seconds. The number of times she was able to stand and sit was assessed, and the lower the number of repetitions, the greater the risk of falling for that elderly woman (18).

Motor Reaction Time (MRT)

The motor reaction time test was used to measure the simple motor reaction time and fatigue, using the TRT_S2012 Software with the keyboard, considering the space key as the command point. The volunteer had the goal of responding as quickly as possible to the stimuli generated by the software. There was a quick and short stimulus, with which the simple motor reaction time was measured, and a second stimulus held for a certain time at random, measuring the motor reaction time until fatigue (19).

Gait Analyzer

The Gait Analyzer app was used in order to quantify the degree of gait balance. The test is based on a smartphone app that measures the accelerations produced during gait movements, with the following methodology: with the cell phone attached to the waist in a belt with a compartment, the volunteer was asked to walk at a comfortable speed for at least 20 steps. At the end of the test, the result appears in the app itself, showing all gait variables.

10-Meter Walk Test

The 10-Meter Walk Test was used to assess gait kinematics and spatiotemporal parameters. The test consists of a 20-meter walk, with an initial 5 meters for acceleration and final 5 meters for deceleration, in which the volunteer was instructed to walk at her fastest walking speed, wearing typical shoes and without running for the 20 meters. An auxiliary device could be used if necessary (20).

Statistical Analysis

Statistical analysis was performed using SPSS Statistics version 23.0 (IBM, Chicago, USA). The behavior of the measures was analyzed using the Shapiro-Wilk test, determining the subsequent tests. The sample was characterized by descriptive statistics and reported as mean, standard deviation and confidence interval. One-way analysis of variance (ANOVA) with Tukey's post-hoc was used to analyze the differences between volunteers classified as non-sarcopenic, pre-sarcopenic and sarcopenic. To assess the relationship of discriminative variables, health characteristics and functional skills with motor reaction time, Pearson's product correlation (r) was calculated. A correlation of $r \leq 0.3$ was considered "weak," 0.31 to 0.69 as "substantial," and ≥ 0.7 as "strong" (21). The adopted significance was $p \leq 0.05$.

Results

To compose and carry out this study, initially, 100 eligible elderly women were invited. However, some were not included in the study for meeting the exclusion criteria, due to having neurological diseases, osteoarthritis, rheumatoid arthritis, autoimmune diseases, high BMI, and others for not completing the assessment or not accepting to participate in the study. For these reasons, the study was carried out only with volunteers who met all the inclusion criteria and consented to participate in the study, totaling 59 elderly women, which complies with the sample calculation required in the pilot study.

The results shown in Table 1 demonstrate there is homogeneity between the groups regarding age and body mass index (BMI). For the variables body weight, body fat and muscle mass, however, the difference was significant, especially between the non-sarcopenic and sarcopenic groups, because this condition causes an increase in body fat and a decrease in muscle mass. These findings demonstrate that the decrease in muscle mass is not only a consequence of advancing age, as the present study found that although there was no difference between the ages of the volunteers, there was a difference in muscle mass between the non-sarcopenic, pre-sarcopenic and sarcopenic groups.

Table 3 presents the correlations that show that the longer the motor reaction time, the lower the functional performance of these elderly women. Functional deficit is seen in the reduction of step length and gait speed. On the other hand, body balance shows an improvement with increase in motor reaction time, justified by the women slowing down to perform the movement as a strategy to adjust balance.

In the variables muscle, fat, fear of falling, handgrip strength and the sit-to-stand test, there was no correlation among the women in the sarcopenic group, as all volunteers were equally affected by sarcopenia and were equal in relation to muscle restriction, increased fat and decreased strength, so there was no intragroup difference for these variables (Table 3).

Table 1. Comparison of descriptive data, body composition, cognitive level and fear of falling between non-sarcopenic (n = 15), pre-sarcopenic (n = 19) and sarcopenic (n = 18) groups

		Average (Standard deviation)	CI 95%	p value	Pairwise comparison		
					A/B	A/C	B/C
Age (years)	Non-sarcopenic	72.10 (±7.57)	68.46 – 75.75				
	Pre-sarcopenic	71.74(±10.73)	66.57 – 76.91	0.733	-	-	-
	Sarcopenic	70.19 (±5.71)	67.59 – 72.79				
	Total	71.31 (±8.09)	69.20 – 73.41				
Weight (kg)	Non-sarcopenic	58.84 (±6.12)	55.89 – 61.79				
	Pre-sarcopenic	63.85 (±9.67)	59.18 – 68.51	0.033	0.024	0.008	0.026
	Sarcopenic	67.74 (±13.71)	61.50 – 73.98				
	Total	63.62 (±10.91)	60.78 – 66.46				
BMI (kg/m²)	Non-sarcopenic	24.90 (±2.28)	23.80 – 26.00				
	Pre-sarcopenic	26.24 (±4.01)	24.31 – 28.17	0.277	-	-	-
	Sarcopenic	26.98 (±5.27)	24.58 – 29.38				
	Total	26.07 (±4.11)	25.00 – 27.14				
Fat (% / kg)	Non-sarcopenic	34.37 (±3.50)	32.68 – 36.06				
	Pre-sarcopenic	38.90 (±5.45)	35.51 – 42.30	0.022	0.038	0.009	0.089
	Sarcopenic	39.21 (±5.85)	36.39 – 42.03				
	Total	37.54 (±6.20)	35.93 – 39.16				
Muscle (% / kg)	Non-sarcopenic	27.74 (±1.41)	27.06 – 28.42				
	Pre-sarcopenic	25.10 (±3.08)	23.69 – 26.50	0.009	0.022	0.001	0.004
	Sarcopenic	23.89 (±2.28)	22.79 – 24.99				
	Total	25.88 (±2.68)	25.18 – 26.58				
Visceral Fat (%)	Non-sarcopenic	8.42 (±2.12)	7.40 – 9.44				
	Pre-sarcopenic	9.37 (±2.14)	8.34 – 10.40	0.219	-	-	-
	Sarcopenic	9.81 (±3.11)	8.39 – 11.22				
	Total	9.22 (±2.55)	8.56 – 9.88				
MMSE (score)	Non-sarcopenic	22.89 (±3.16)	21.37 – 24.42				
	Pre-sarcopenic	25.68 (±6.37)	22.61 – 28.75	0.064	-	-	-
	Sarcopenic	26.14 (±1.88)	25.29 – 27.00				
	Total	24.95 (±4.36)	23.81 – 26.08				
FES-I (score)	Non-sarcopenic	30.05 (±7.56)	26.41 – 33.70				
	Pre-sarcopenic	31.24 (±9.46)	26.37 – 36.10	0.381	-	-	-
	Sarcopenic	34.10 (±10.73)	29.21 – 38.98				
	Total	31.89 (±9.39)	29.40 – 34.39				

Note: A- non-sarcopenic; B- pre-sarcopenic; C- sarcopenic; BMI-Body Mass Index; MMSE- Mini Mental State Examination; FES-I-Falls Efficacy Scale International. Comparative analysis between sarcopenia subclassifications performed by One-way Anova test, with Tukey's Post-hoc for comparison between pairs, adopting a significance value of $p \leq 0.05$.

Discussion

Motor reaction time was found to present greater difference between the non-sarcopenic and sarcopenic groups due to the executing organ (the musculature) being impaired by the presence of sarcopenia, causing a slowdown in the motor response in the latter group. In relation to handgrip strength, the difference was significant between all groups due to the decrease in muscle strength being a characteristic of the elderly, but the sarcopenic group had the lowest muscle strength.

In a healthy aging process, pathological and adaptive processes are active in the musculoskeletal system and muscle regeneration pathways are present and active in this natural process; however, with the presence of sarcopenia there is a dysregulation in this regeneration process (22). Although the brain is cortically free of abnormalities, the executing organ, the muscle, is damaged. Due to the inability to regenerate after injuries, it soon becomes slower to perform certain tasks. In fact, elderly women affected by sarcopenia have a deficient and more injured executive organ than elderly women who are not

Table 2. Comparison of motor reaction time and functional abilities between non-sarcopenic (n = 15), pre-sarcopenic (n = 19) and sarcopenic (n = 18) groups

		Average (Standard deviation)	CI 95%	p value	Pairwise comparison		
MRT - Simple (ms)	Non-sarcopenic	563.67 (±143.55)	428.79 – 698.54				
	Pre-sarcopenic	869.00 (±143.29)	598.83 – 974.00	0.003	0.002	<0.001	0.042
	Sarcopenic	1086.21 (±189.62)	898.42 – 1274.00				
	Total	860.29 (±161.70)	731.75 – 988.83				
MRT – Initial fatigue (ms)	Non-sarcopenic	878.60 (±173.48)	671.77 – 985.43				
	Pre-sarcopenic	1152.00 (±174.55)	816.55 – 1317.45	0.029	0.038	0.012	0.068
	Sarcopenic	1351.42 (±166.24)	1174.90 – 1527.94				
	Total	1146.00 (±123.52)	1000.25 – 1291.75				
MRT – Final fatigue (ms)	Non-sarcopenic	928.33 (±125.10)	692.92 – 1003.75				
	Pre-sarcopenic	872.06 (±102.65)	572.37 – 1171.74	0.435	-	-	-
	Sarcopenic	1085.26 (±190.43)	848.88 – 121.61				
	Total	966.19 (±114.19)	823.04 – 1109.34				
Muscle strength (kg/f)	Non-sarcopenic	24.16 (±1.76)	22.35 – 25.97				
	Pre-sarcopenic	22.74 (±1.33)	21.61 – 23.86	0.000	<0.001	<0.001	<0.001
	Sarcopenic	18.71 (±1.45)	17.60 – 19.83				
	Total	21.76 (±2.70)	20.80 – 22.73				
30-second sit to stand (repetitions)	Non-sarcopenic	9.47 (±2.09)	8.47 – 10.48				
	Pre-sarcopenic	8.74 (±1.98)	7.30 – 10.17	0.807	-	-	-
	Sarcopenic	9.10 (±1.66)	6.98 – 11.22				
	Total	9.10 (±1.42)	8.21 – 9.99				
TUG (seconds)	Non-sarcopenic	9.26 (±1.97)	8.31 – 10.21				
	Pre-sarcopenic	10.79 (±1.26)	9.22 – 12.36	0.257	-	-	-
	Sarcopenic	9.81 (±1.14)	8.38 – 11.24				
	Total	9.95 (±1.88)	9.20 – 10.70				
4-Stage Balanced test (score)	Non-sarcopenic	2.84 (±0.90)	2.41 – 3.28				
	Pre-sarcopenic	3.26 (±0.99)	2.79 – 3.74	0.364	-	-	-
	Sarcopenic	3.14 (±0.91)	2.73 – 3.56				
	Total	3.08 (±0.93)	2.84 – 3.33				
Gait speed (m/s)	Non-sarcopenic	1.67 (±0.48)	1.45 – 1.89				
	Pre-sarcopenic	163 (±0.50)	1.39 – 1.87	0.054	-	-	-
	Sarcopenic	1.32 (±0.48)	1.09 – 1.55				
	Total	1.54 (±0.50)	1.41 – 1.67				
Step length (m)	Pre-sarcopenic	0.40 (±0.11)	0.12 – 0.68				
	Pre-sarcopenic	0.16 (±0.17)	0.12 – 0.34	0.251	-	-	-
	Sarcopenic	0.21 (±0.12)	0.17 – 0.41				
	Total	0.25 (±0.13)	0.13 – 0.37				
Cadence (steps/min)	No-sarcopenic	116.87 (±8.52)	112.15 – 121.58				
	No-sarcopenic	109.74 (±5.38)	102.32 – 117.15	0.205	-	-	-
	Sarcopenic	108.00 (±7.94)	99.83 – 116.17				
	Total	111.02 (±5.18)	106.91 – 115.12				

Note: A- No-sarcopenic; B- No-sarcopenic; C- Sarcopenic; SRT-Simple Reaction Time; FRT-Fatigue Reaction Time; MRT – Motor Reaction Time; HGS- Handgrip Strength; TUG-Timed Up and Go. Comparative analysis between sarcopenia subclassifications performed by One-way Anova test, with Tukey's Post-hoc for comparison between pairs, adopting a significance value of $p \leq 0.05$.

Table 3. Analysis of the correlation of motor reaction time with body composition, fear of falling and intragroup functional abilities [non-sarcopenic (n = 15), pre-sarcopenic (n = 19) and sarcopenic (n = 18)]

	Non-sarcopenic			Pre-sarcopenic			Sarcopenic		
	TER	TRD-I	TRD-F	TRE	TRD-I	TRD-F	TRE	TRD-I	TRD-F
Muscle (% / kg)	-0.235	-0.199	-0.684	-0.350	-0.891	-0.251	-	-	-
Fat (% / kg)	-	-	-	-0.606	0.644	0.360	-	-	-
FES-I (score)	0.743	0.319	0.643	0.621	0.900	0.034	-	-	-
Muscle strength (kg/f)	-0.777	-0.611	-0.719	-0.325	-0.541	0.275	-	-	-
30-Second Sit to Stand (repetitions)	-0.565	-0.337	-0.756	-0.851	-0.820	0.165	-	-	-
TUG (seconds)	0.991	0.904	-0.112	0.722	0.763	0.361	-0.408	-0.032	0.765
4-Stage Balanced test (score)	-0.728	-0.968	0.352	0.220	-0.143	-0.901	0.532	0.231	-0.482
Gait speed (m/s)	0.118	-0.526	-0.975	0.355	-0.142	-0.49	-0.496	-0.050	-0.584
Step length (m)	-0.988	-0.977	0.352	-0.620	-0.054	-0.548	-0.675	0.509	0.238
Cadence (steps/min)	0.939	0.993	-0.529	-0.512	-0.474	-0.008	-	-	-

Note: A- non-sarcopenic; B- pre-sarcopenic; C- sarcopenic; FES-I - Falls Efficacy Scale International; HGS-Handgrip strength; TUG-Timed Up and Go; - no significant correlation ($p \leq 0.05$). Pearson's correlation test, considering p values < 0.05 as significant and weak correlation at r < 0.3, moderate of 0.3 to 0.6, and strong > 0.6.

affected by the condition.

The presence of sarcopenia reduces the ability of motoneurons to innervate regenerating muscle fibers. There are also evident changes in the expression of several cytokines known to play important roles in establishing and maintaining neuromuscular connectivity during development and adulthood (23).

Arnold et al. (24) measured the muscle activation time of sarcopenic elderly people, the time spent between receiving the stimulus and performing the movement and found results similar to those presented in the present study. They found that the muscles of elderly people with sarcopenia are always inflamed due to injuries that cannot be effectively regenerated, causing the motor reaction time to be longer and, consequently, making the elderly person slower.

Other studies corroborate our results, showing that with the presence of sarcopenia, in addition to the executing organ being damaged and its regeneration capacity diminished, the motoneurons responsible for joining information from the brain to the muscle are also impaired (22, 25). Due to these two aspects, sarcopenic elderly people are slowed down, with which there is an increase in motor reaction time, as the muscle does not regenerate after an injury and the impaired motoneurons cannot generate impulses as fast as in non-sarcopenic elderly people.

The findings presented in this study and in the literature prove that with the increase in motor reaction time and the increase in time to perform functional activities such as the TUG test, added to the fear of falling represented by the high score on the FES-I scale, elderly women have an increased risk of falls (17, 26, 27). The justification may be associated with the muscle base, which when impaired will slow the movement and not allow a quick adjustment response to any disturbance.

For reading and interpreting the data, it should be considered that the study was carried out only with women and the methodological design adopted does not allow establishing a cause-and-effect relationship. However, as far as the authors are aware, there are no other studies that sought to relate neuromuscular disorders generated by sarcopenia to changes in the motor reaction time of elderly women.

Conclusion

The findings of the present study highlight that, in the presence of sarcopenia, there is a slowdown in motor responses due to a decrease in muscle mass, muscle strength, impaired and injured muscles. In addition, with the increase in motor reaction time, there are functional deficits, corroborating the increased risk of falls in elderly women with sarcopenia.

Conflicts of Interest: The authors declare that they have no conflicts of interest to this work.

Ethical standards: The research complied with the current laws of Brazil. The study was approved by the Research Ethics Committee of the Unievangélica University of Goiás, and adhered to the principles of the Declaration of Helsinki.

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