

# Fat Mass as an Independent Variable to Assess the Possibility of Predicting the Stability in Postmenopausal Women with and Without Osteoporosis

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## SUMMARY

**Purpose.** We investigated the effect of fat mass value (based on skinfold thickness) and body mass index (BMI) on the center of pressure sway to assess the possibility of predicting the stability of postmenopausal women with and without osteoporosis during the standing position.

**Methods.** A total of 78 postmenopausal women participated voluntarily in the study. Postmenopausal (aged 55–75 years) women were divided into, osteoporotic, (lumbar T-score  $\leq -2.5$ ,  $n = 51$ ), and nonosteoporotic (lumbar T-score  $> -1$ ,  $n = 27$ ) groups. Total body fat mass was assessed based on skinfold measurement. The isometric strength of the lower limb muscle groups was recorded using a digital hand-held dynamometer. Postural sway was determined by measuring the center of pressure (CoP) sway in the anterior-posterior (AP) and mediolateral (ML) direction during a comfortable double stance position, with eyes open.

**Results.** The fat mass value and BMI were significantly lower in the osteoporotic group than in the nonosteoporotic group. The isometric strength of all lower extremity muscle groups was considerably lower in osteoporotic subjects than in the nonosteoporotic group, except for dorsi flexors and knee extensors. In the mediolateral direction, the CoP sway displacement and velocity were significantly higher in the osteoporotic group than in the nonosteoporotic group. Fat mass was a significant independent variable for predicting CoP velocity in the ML direction ( $VIF = 2.51$ ,  $p = 0.050$ ).

**Conclusions.** The low-fat mass value is associated with mediolateral postural instability in postmenopausal women. Losing BMI-fat mass and muscle strength may affect postural instability in postmenopausal women. The cautiously strength-balance exercises may be effective for osteoporotic women to improve stability.

## KEY WORDS

*Osteoporosis; postmenopausal women; fat mass; center of pressure; instability.*

## INTRODUCTION

Estrogen deficiency related to menopause status could induce a net negative bone balance (postmenopausal osteoporosis) (1). Furthermore, if left untreated, half of the postmenopausal women may experience osteoporotic fractures in their remaining lifetime (2).

According to a systematic review in 2018, the overall prevalence of osteoporosis is estimated at 32% (confidence interval 95%) in postmenopausal Iranian women aged 50 to 55 years old (3). Also, it was reported that the economic burden of osteoporosis-related fracture is significant, costing approximately \$ 17.9 billion and £ 4 billion per annum

in the USA and UK, respectively (4), which indicates the profound impact, in terms of mortality and morbidity on individuals, healthcare systems and communities as a whole. Hence, it highlights the importance of monitoring and screening the risk of falling and fracture in this population. The fracture risk assessment algorithm is currently used to estimate fracture risk through Clinical Risk Factors (CRFs) and Bone Mineral Density (BMD). However, it has been reported that the addition of fall risks, as an independent risk factor, to other CRFs and BMD considerably increases the predictive worth of such algorithms (5). Moreover, postural instability has been proposed as the strongest predictor for falls and fractures (6, 7).

The (Center of Pressure) CoP-related parameters extracted from static posturography have been shown as strong predictors of falling risk among healthy community-dwelling elderly (8–10). The CoP parameters may provide valuable information for diagnosing people at risk of falling due to instability. Aging is associated with increased CoP sway in the anterior-posterior/medial-lateral direction. Laughton *et al.* reported that older people who experienced falling had more CoP displacement in the anteroposterior direction (8). Silva *et al.* showed a higher anteroposterior CoP sway in osteoporotic women than in healthy women (9). Sinaki *et al.* revealed that osteoporotic-kyphotic individuals have higher CoP sway velocity and displacement in the medial-lateral direction than healthy age-matched individuals (10). Instability in osteoporotic women, associated with increased CoP sway, can be due to decreased muscle strength, increased fear of falling, weight loss, and muscle/bone mass ratio change (11). Some previous studies demonstrated a relationship between the body's anthropometry and balance control (12, 13). The effect of obesity and BMI (Body Mass Index) on balance control has been regarded (14–16). According to a recent review finding, an increase in BMI and fat mass is proposed to predict postural instability, fall risk, and fracture (17). Neri *et al.* showed a positive association between waist circumference and risk of falls in older women; the obese women exhibited reduced postural balance control and increased fear of falling (18). However, there are conflicting results on the relationship between BMI-fat mass and balance control parameters. Some reports reveal no significant relationship between BMI-fat mass and balance function. Others linked obesity to a fall risk factor related to impaired postural control in older adults (19–22). A low BMI-fat mass has been proposed to be a relevant hip fracture risk factor (23). Furthermore, it was reported that the relationship between BMI-fat mass and risk of fractures is site-specific with both detrimental and protective effects on fracture risk, with the protective effects explained by the endocrinal and load-related mechanical impact of adipose tissue on BMD (24). There

is a paradoxical effect in which the increase in BMI-fat mass leads to a detrimental effect on postural control and a protective effect on BMD. In addition to the impact of fat mass on BMI, it has a gender-dependent effect on balance control (20). Due to the high risk of falling in osteoporotic women and the changes that occur in weight and fat mass in these people, we designed this study to investigate the effect of the fat mass (based on the skinfold thickness) and BMI on the fall efficacy scale (FES) and static stability parameters in postmenopausal women with and without osteoporosis. Measuring the CoP displacement and velocity requires expensive and precise laboratory equipment such as an accurate force plate. Determining the relationship between fat mass (obtained through cheap and accessible tools such as skinfold thickness by caliper) and CoP sway may make it possible to regularly monitor and predict the equilibrium status of women with osteoporosis who are at high risk of falls. In this way, physiotherapists can be more effective in planning balance-strengthening exercises and reducing the risk of falling. So, we hypothesized that fat mass (based on the skinfold thickness) as an independent variable might predict the center of pressure sway in postmenopausal women with and without osteoporosis during the standing position.

## METHODS

### Study design and participants

This cross-sectional study was conducted (May 20, 2019 - Sep 22, 2019) at the Motion Analysis Laboratory of the Physical Therapy Department in Tarbiat Modares University, Tehran, Iran. The Medical Ethics Committee of Hamedan University of Medical Sciences approved the study (IR. UMSHA. 1398.069). The sample size was estimated based on the mean  $\pm$  SD of CoP displacement in the anterior-posterior direction in a double standing position in Torkaman *et al.* (2015) with a 95% confidence interval and 80% power (25). Individuals were classified into osteoporosis ( $n = 51$ , lumbar T-score  $\leq -2.5$ ) and non-osteoporosis ( $n = 27$ , Lumbar T-score  $> -1$ ) groups based on dual-energy X-ray absorption assay, which was performed 3-6 months before the study. A trained physiotherapist performed all assessments from 9.00 a.m. to 1.00 p.m., two sessions apart, within 24 hours.

Among postmenopausal women who became acquainted with the study by distributing promotional cards and counseling at the bone density centers, 84 women were willing to participate in the study voluntarily. The inclusion criteria included females 55–75 years of age, menopausal status for at least one year before the study, with no record of regular physical exercise for at least one year. In addition, the subjects who had secondary osteoporosis, a history of

osteoporotic fracture, osteopenia ( $-2.5 > T\text{-score} \leq -1$ ), the presence of neurogenic or myopathic disorders, diabetes, thyroid disease, rheumatoid diseases, any malignant neoplasia, and the use of drugs known to affect muscle strength were excluded from the study. Thus, a total of 78 volunteers were finally enrolled in the study. The methods and aims of the assessments were entirely explained to the subjects, and they signed consent forms before the evaluations.

### Measurements

All subjects were assessed in two sessions. During the first session, anthropometric parameters, including age (year), height (m), and body mass index (BMI,  $\text{weight}/\text{height}^2$ ,  $\text{kg}/\text{m}^2$ ), were recorded. The skin-fold thickness was measured using a caliper to calculate fat and fat-free mass. The isometric strength was measured for the lower extremity muscular groups. The FES was administered through an interview using the Persian version of the FES questionnaire (26, 27), and finally, the self-reported duration of menopause was determined. After 24 hours, the static postural stability was assessed in the second session.

### Fat mass measure

We measured the skinfold *thickness* in the triceps, supra iliac, and thigh regions using a caliper (Nederland b.v-Pomdernal-Huidplooidikte). Briefly, the skin fold was picked between the thumb and forefinger and pulled away slightly from the underlying tissue in each area. When the caliper jaws were applied to the skin fold, the thumb and forefinger were removed, then reading was taken after 2 or 3 s. All measurements were performed three times on the right side of the body in a standing position. The internal consistency *was excellent* for the triceps, supra iliac, and thigh regions (Cronbach's *alpha* 0.978, 0.979, and 0.939, respectively). First, the average value of fat mass was determined as a percentage of body weight by the nomogram proposed by Pollock *et al.* (26); then, the fat-free mass was calculated by the Siris equation (27).

### Muscular strength measurement

The isometric strength of the hip abduction, adduction, extension and flexion, knee extension and flexion, and dorsi and plantar flexion of the ankle were measured bilaterally. The measurement was done by a digital hand-held dynamometer (Hand-held Dynamometer; Lafayette Instrument Co., Lafayette, IN, USA). An expert physiotherapist assessed each measure three times bilaterally, with at least one min of rest between repeated tests of the same muscle group; the mean value was used for analysis. The duration of each isometric test was 5 s, and the strength value was recorded in kilograms (kg).

### Static postural stability measurement

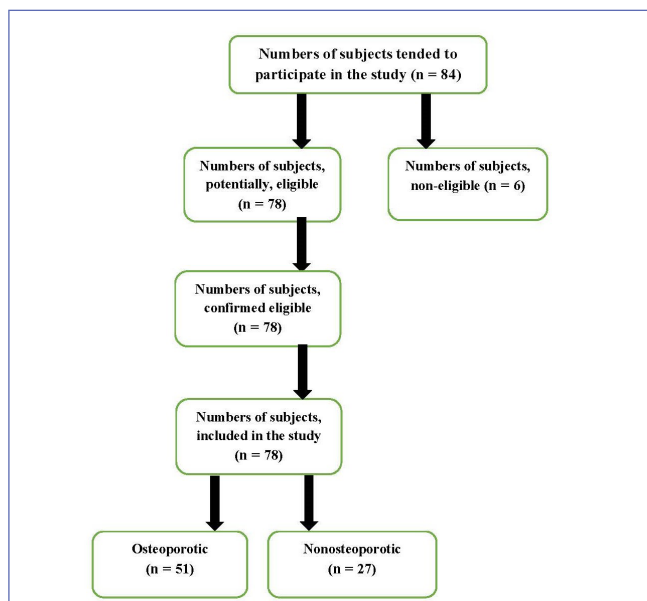
The static postural stability assessment was performed on a force plate (9286B; Kistler Co., Winterthur, Switzerland). Data acquisition was performed by the sampling frequency of 100 Hz. All participants were assessed in a comfortable double stance position; Data were taken with eyes open, and registration time was 20 s. Data were processed by MATLAB software. The CoP displacement (Cm) and mean velocity of the CoP sway (cm/s) were determined in the anterior-posterior (AP) and mediolateral (ML) directions.

### Data analysis

SPSS version of 16 (IBM, Armonk, NY, USA) was used for statistical analysis. The Shapiro-Wilk test showed the normal distribution of data ( $p > 0.05$ ), so the independent-sample t-test was used to compare the variables between groups. The linear regression model was also performed in relation to the fat mass, fat-free mass, T-score value, FES score, and BMI with the CoP displacement and CoP sway velocity. Pearson's coefficient correlation assessed the correlation of the variables. Statistical significance was set at  $p \leq 0.05$ .

## RESULTS

The flow diagram of participants is illustrated in **figure 1**. According to the include and exclude criteria, seventy-eight volunteers enrolled in the study and were assigned to the osteoporotic and nonosteoporotic (normal BMD) groups.



**Figure 1.** Diagram of the number of subjects at each stage of the study.

Anthropometric and basic variables are shown in **table I**. There were no significant differences in age, the duration of menopause, and fat-free mass ( $p > 0.05$ ) between the two groups. The BMI and fat mass values were significantly higher in the nonosteoporotic (normal BMD) group than in the osteoporotic group ( $p = 0.032$  and  $p = 0.040$ , respectively). The FES score in the osteoporotic group was higher than that of the nonosteoporotic group ( $p = 0.012$ ).

According to **table II**, the isometric strength of all evaluated muscle groups was significantly lower in the osteoporotic group compared to the nonosteoporotic group ( $p \leq 0.05$ ), except for dorsi flexors and knee extensor muscle groups, which values indicated no significant difference between two groups ( $p > 0.05$ ).

In the osteoporotic group (**table III**), the ML CoP velocity and displacement were significantly higher than in the nonosteoporotic group ( $p = 0.048$  and  $p = 0.014$ , respectively). The CoP sway velocity and displacement in the AP

direction showed no significant difference between groups ( $p > 0.05$ ).

**Table IV** shows a significant negative correlation between BMI, fat mass, fat-free mass, and lumbar T-score with CoP mean velocity in the ML direction ( $p = 0.016$ ). Conversely, the FES correlated positively and significantly with CoP velocity in the ML direction ( $p \leq 0.05$ ). In addition, FES correlated negatively with fat mass value ( $p = 0.038$ ). Conversely, BMI, fat-free mass, and lumbar T-score showed a significant positive correlation with fat mass value ( $p \leq 0.05$ ).

The correlated parameters with CoP velocity in the ML direction (**table IV**) were entered as the independent predictors in the linear regression analysis model to compare their value to predict the CoP velocity in the ML direction. Fat mass was observed as a significant independent variable for predicting CoP velocity in the ML direction ( $p \leq 0.05$ ) (**table V**).

The collinearity test indicates no significant collinearity for the selected independent variables ( $VIFs \leq 5$ ).

**Table I.** Anthropometric and basic variables between the groups, Mean  $\pm$  SD.

Variables	Osteoporotic (n = 51)	Nonosteoporotic (n = 27)	P-value
Age (years)	58.45 $\pm$ 5.03	56.22 $\pm$ 5.97	0.085
BMI (kg/m <sup>2</sup> )	27.24 $\pm$ 3.34	28.91 $\pm$ 2.70	0.032*
Menopause duration (years)	11.5 $\pm$ 6.88	8.61 $\pm$ 6.78	0.238
Fat mass (kg)	17.02 $\pm$ 4.74	19.36 $\pm$ 4.21	0.040*
Fat-free mass (kg)	49.05 $\pm$ 5.02	51.04 $\pm$ 4.39	0.094
Lumbar T-Score	-2.85 $\pm$ 0.70	0.05 $\pm$ 0.99	< 0.001*
FES score	17.78 $\pm$ 9.42	12.45 $\pm$ 3.43	0.012*

\*Significant difference between two groups ( $p \leq 0.05$ ). BMI: Body Mass Index; FES: Fall efficacy scale.

**Table II.** Isometric Strength of lower extremity muscle groups, Mean  $\pm$  SD.

Muscular groups (Isometric strength) (Kg)	Osteoporotic (n = 51)	Nonosteoporotic (n = 27)	P-value
Dorsi flexors	11.98 $\pm$ 4.86	14.06 $\pm$ 6.37	0.116
Plantar flexors	11.08 $\pm$ 4.63	13.61 $\pm$ 4.18	0.026*
Hip abductors	15.23 $\pm$ 6.95	19.81 $\pm$ 12.01	0.040*
Hip adductors	10.85 $\pm$ 3.51	13.81 $\pm$ 5.76	0.007*
Hip flexors	11.30 $\pm$ 5.86	14.44 $\pm$ 7.87	0.050*
Hip extensors	11.58 $\pm$ 6.35	16.33 $\pm$ 9.10	0.010*
Knee flexors	9.27 $\pm$ 4.33	13.34 $\pm$ 9.83	0.016*
Knee extensors	18.02 $\pm$ 5.49	19.91 $\pm$ 9.33	0.277

\*Significant difference between two groups ( $p \leq 0.05$ ).

**Table III.** Static stability parameters, Mean ± SD.

Variables	Osteoporotic (n = 51)	Nonosteoporotic (n = 27)	P-value
CoP displacement in AP direction (cm)	0.162 ± 0.0047	0.022 ± 0.304	0.183
CoP displacement in ML direction (cm)	0.119 ± 0.0360	0.102 ± 0.030	0.048*
CoP velocity in AP direction (cm/s)	0.0082 ± 0.0019	0.0075 ± 0.0017	0.144
CoP velocity in ML direction (cm/s)	0.590 ± 0.1808	0.486 ± 0.1503	0.014*

\*Significant difference between groups (p ≤ 0.05); CoP: Center of Pressure; AP: Anterior-Posterior; ML: Mediolateral.

**Table IV.** Correlation between anthropometric parameters, FES, and CoP velocity in ML direction.

Variables	CoP velocity in ML direction (cm/s)		Fat mass (kg)	
	PCC	P-value	PCC	P-value
Age (years)	0.198	0.085	0.066	0.583
BMI (kg/m <sup>2</sup> )	- 0.453	0.000*	0.761	0.000*
Fat mass (kg)	- 0.309	0.008*	1	----
Fat-free mass (kg)	- 0.245	0.034*	0.432	0.000*
Lumbar T-score	- 0.239	0.037*	0.312	0.008*
FES	0.284	0.016*	-0.254	0.038*

\*Significant Correlation (p ≤ 0.05). PCC: Pearson Correlation Coefficient; BMI: Body Mass Index; FES: Fall Efficacy Scale.

**Table V.** Regression analysis with Entering method for prediction of CoP sways velocity in ML direction.

Variables	Standardized Beta Coefficient	t	P-value	Collinearity Statistics	
				Tolerance	VIF
BMI (kg/m <sup>2</sup> )	- 0.113	-0.613	0.542	0.337	2.97
Fat mass (kg)	- 0.338	-1.99	0.050*	0.397	2.51
Fat-free mass (kg)	- 0.023	-0.176	0.861	0.661	1.51
Lumbar T-score	- 0.145	-1.26	0.212	0.860	1.16
FES	0.203	1.68	0.098	0.781	1.28

\*Significant Correlation (p ≤ 0.05). VIF: Variance Inflation Factor; BMI: Body Mass Index; FES: Fall Efficacy Scale; t value: t Statistic is a measure of the precision of regression Beta Coefficient.

## DISCUSSION

The present study was designed to investigate the effect of fat mass value (based on skinfold thickness) and BMI on the CoP sway to assess the possibility of predicting the stability of postmenopausal women with and without osteoporosis during the standing position. Although there are more accurate methods for calculating fat mass, such as DEXA, using simple and accessible techniques such as skinfold thick-

ness allows for periodic follow-up of high-risk osteoporotic women. Suppose the relationship between fat mass and the CoP parameters is also determined. In that case, it can effectively predict the balance status, the risk of falling, and planning their therapeutic exercise.

The results revealed that osteoporotic women have lower BMI and fat mass values than nonosteoporotic women. Martinez-Ramirez *et al.* (2017) indicated that higher BMI

and total fat mass are associated with decreased risk of osteoporosis (28). In contrast, it is shown that excess fat mass may not protect against reductions in bone mass (23, 29). The adipose-derived hormones (estrogen, leptin, adiponectin) might affect the relationship between fat and bone mass through bone metabolism involvement. In other words, the adipocyte cells become the alternative estrogen source in estrogen-deficient subjects; thus, the protective role of fat tissue on bone mass might be highlighted in deficient hormonal subjects (29). Hence, postmenopausal women (with estrogen deficiency) with lower fat mass might be at risk of osteoporosis and fractures due to the lack of adequate alternative estrogen sources. The contrasting role of fat mass hormones on the bone and skeletal muscle mass complicates this relationship. In addition to the direct osteogenic effects on osteoblasts, leptin also has osteolytic effects by crossing the blood-brain barrier in the hypothalamus and suppressing serotonin production, which leads to a sympathetic nervous system (SNS) signaling via norepinephrine. This signaling pathway activates osteoclast differentiation, which leads to increased bone resorption (30).

Therefore, the lack of considering a specific optimal BMI-fat mass range for the subjects with hormone deficiency and ignoring the visceral fat mass value might cause inconsistency observed in the fat mass caliper data and bone mass relationship. In addition, high fat mass-BMI may positively affect bone metabolism by increasing bone loading during weight-bearing and daily functional activities. Applying mechanical loading to bones according to mechanostat theory can cause the bone formation and prevent bone resorption (31).

The static stability assessment showed a higher ML velocity sway and ML displacement in the osteoporotic group. This finding supports a recent study, which indicates the relationship between postural instability (increased CoP diversity) and osteoporosis in postmenopausal women (32).

BMI, fat mass, fat-free mass, lumbar T-score, and FES score were significantly associated with ML postural stability parameters. Linear regression analysis showed that fat mass is the only significant predictor of CoP sway velocity in the ML direction. At least, in the BMI range of our study, an increase in fat mass may significantly decrease CoP sway velocity in the ML direction. Our finding is inconsistent with Meng *et al.* (2020). They indicated that the rise in BMI and adiposity in older adults is associated with declines in postural control in the frontal plane (increase in CoP sway parameters) (33). Of course, they measured central adiposity in obese participants with BMI  $\geq 30$  kg/m<sup>2</sup> in both genders, which is different from our study. Rezaei pour *et al.* (2018) showed that the CoP sway velocity is lower in obese elderly females than the normal-weight females in the mediolateral direction (34). The role of obesity in increasing frontal stability is attributed to the

anatomical changes in obesity, such as valgus knees, which keep feet apart, resulting in the frontal base of support expansion and increasing frontal postural stability (12). However, Blaszczyk *et al.* (2009) revealed that the increased fat mass-BMI reduces the risk of falls and fractures in the elderly, unlike young adults (35). Increased body inertia due to the excessive fat mass-BMI and high stance width related to the fat mass accumulation in the lower limbs increases frontal postural stability (35). The gender-dependent distribution of adipose tissue (gynoid type in females *versus* android type in males (36)), the hormonal status and age-dependent effects of fat mass on balance control (as mentioned by Blaszczyk *et al.* (35)), different adiposity measuring, and other associated parameters in the subjects (such as anxiety behaviors and fear of falling) maybe the reasons for the inconsistency observed in the studies about the fat mass and frontal CoP sway parameters relationship.

Interestingly, our results indicated that postmenopausal women with lower fat mass values experienced greater fear of falling (negative, significant correlation). There was a more incredible CoP sway velocity in the frontal plane due to the positive and significant correlation with FES. Subjects with higher self-judged fear values have more CoP sway variability than those with lower fear values (37). It was proposed that the effect of the fear of falling on postural control and fall risk is mediated by the changes in the allocation of attention or impaired attentional processing and related alteration in motor control (38). It was also indicated that the effects of anxiety condition or fear of falling on the balance control related to a complex interaction between neurophysiological changes (increase in muscle spindle sensitivity, the vestibular gain of balance, head and eye reflexes) and alteration in attentional processes (39). The fat mass has been well investigated as an alternative source of estrogen and other fat mass hormones. Some of these hormones, such as leptin, are anxiolytic and antidepressants (40). So, decreasing FES probably, mediated by the more plasma level of anxiolytic leptin in subjects with higher fat mass value. Therefore, the higher fat mass value due to the broader mediolateral base of support and also decreasing of FES may affect the frontal postural stability in postmenopausal women, mechanically and cognitively, which needs to be investigated in future studies.

In addition to the evaluated anthropometric parameters, which correlated to the postural instability, a contaminant decrease in muscle strength has been proposed as an important risk factor for balance impairment in osteoporotic subjects (41). Our results also showed a reduction in the isometric strength of lower limb muscle groups in osteoporotic women. The relation between muscular strength of lower extremity and postural instability in osteoporotic subjects related to the muscular alterations in adaptation to

the bone structure's deterioration, which leads to a change in center of gravity and, consequently, inefficient balance control falling, and fractures (42). For this reason, lower extremity muscle strengthening should be considered in rehabilitation programs to improve postural stability, mobility, and falling risk in older women (43, 44).

Postural instability is a significant risk factor for falls and fractures and may be associated with lower muscle strength, osteoporosis, and low-fat mass in postmenopausal women. Therefore, the BMI-fat mass values in women should maintain an optimal range in the decades following menopause. Furthermore, muscle strengthening may effectively prevent the development of osteosarcopenia in these women. On the other hand, weight loss without muscle strengthening and balance training may increase the risk of falling due to the undeniable role of muscle strength, especially in the lower limb, on the proper posture and balance.

It should be noted that the BMI-fat mass value in the present study was not at the level of obesity, and the positive effects of BMI-fat mass on BMD and postural stability are related to overweight status.

The small sample size for this cross-sectional study, the lack of consideration for visceral fat (waist circumference, as a simple proxy for visceral fat measurement), and the lack of measuring the plasma level of fat mass hormones were important limitations to the present study. DEXA body composition is a precise measurement of segmental body fat distribution in the arms, legs, waist (android), hips (gynoid), and trunk (torso) that we suggest investigating the relation with the stability parameters. Furthermore, assessing the status of sarcopenia compared to measuring muscle strength can provide more accurate information, so it's recommended for future studies. Although the age difference between the two groups was not significant, the wide range of participants' age (55-75 years) and relatively asymmetric age distribution was one of the study's inevitable limitations because women with osteoporosis are generally in the upper range. According to the criteria, we enrolled all eligible volunteer women with osteoporosis and normal BMD. It suggests that future studies limit the age range so that the distribution of individuals is more uniform in terms of age. Another limitation of the study is the exclusion of postmenopausal women with osteopenia. It suggests that the effect of fat mass (based on skinfold thickness) and BMI on CoP sway parameters to be investi-

gated to predict stability in static and dynamic positions in postmenopausal women with normal BMD osteopenia and osteoporosis.

## CONCLUSIONS

The results showed low-fat mass value is associated with mediolateral postural instability in postmenopausal women. Periodic assessment of muscle strength and fat /fat-free mass based on the skinfold thickness may provide important information about postural instability in osteoporotic women. Regarding the loss of BMI-fat mass and decreased muscle strength, it is recommended that strength-balance exercises be considered cautiously for osteoporotic women to improve stability.

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## DATA AVAILABILITY

The data are available under reasonable request to the corresponding author.

## CONTRIBUTIONS

GT, MM, SM: conceptualization, design. MD, SM, MM, ZB: acquisition of data. GT, SM, MD, MM, ZB, HN: data analysis and interpretation. GT, SM, MM, MD, ZB, HN: drafting of the manuscript. GT, SM, MM: critical revision. All authors read and approved the final version of the manuscript.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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