**UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL**

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Carolina Carvalho Martins

# AVALIAÇÃO DO ESCORE DE ADERÊNCIA À DIETA ESTILO MEDITERRÂNEA E SUAS ASSOCIAÇÕES COM COMPOSIÇÃO CORPORAL E DENSIDADE MINERAL ÓSSEA EM UMA AMOSTRA DE MULHERES NA PÓS-MENOPAUSA

Porto Alegre, 2018

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Trabalho de conclusão de curso apresentado à Comissão de Graduação do curso de Nutrição da Universidade Federal do Rio Grande do Sul como requisito parcial e obrigatório para obtenção do título de Bacharel em Nutrição.

Carolina Carvalho Martins

Orientadora: Prof.ª Dr.ª Poli Mara Spritzer

Co-orientadora: Dr.ª Thaís Rasia da Silva

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Trabalho de Conclusão de Curso apresentado à Faculdade de Medicina da Universidade Federal do Rio Grande do Sul para obtenção do Grau de Nutricionista

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**BANCA EXAMINADORA**

Msc.Tayane Fighera – Unidade de Endocrinologia Ginecológica, PPG Ciências Médicas: Endocrinologia, Universidade Federal do Rio Grande do Sul

Prof.ª Dr.ª Michele Drehmer – Departamento de Nutrição, Universidade Federal do Rio Grande do Sul

Prof.ª Dr.ª Poli Mara Spritzer – Unidade de Endocrinologia Ginecológica, PPG Ciências Médicas: Endocrinologia, Universidade Federal do Rio Grande do Sul (Orientadora)

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## RESUMO

A diminuição da produção de estrogênio durante a transição da menopausa tem sido associada à perda de densidade mineral óssea (DMO) e massa magra, aumentando o risco de osteoporose e fraturas. A dieta mediterrânea vem sendo associada a diversos benefícios à saúde, no entanto, poucos dados estão disponíveis sobre dieta mediterrânea e composição corporal na pós-menopausa. Portanto, o objetivo deste estudo foi investigar a associação entre o escore de aderência à dieta mediterrânea, a composição corporal e a DMO em uma amostra de cento e cinco mulheres pós-menopáusicas aparentemente saudáveis do sul do Brasil (idade 55,2 ± 4,9 anos, índice de massa corporal 27,2 ± 4,6 kg/m²). A DMO, % de gordura corporal e índice de massa magra apendicular (ALMI, kg/m²) foram avaliados por absorciometria de raios-x de energia dupla (DXA), taxa metabólica em repouso por calorimetria indireta, atividade física habitual por pedômetro e a ingestão dietética foi medida por um questionário de freqüência alimentar validado. A pontuação do escore da Dieta Mediterrânea (MDS) foi baseada na ingestão dos seguintes componentes da dieta: vegetais e legumes, frutas, cereais, peixes, álcool, azeite, produtos lácteos e carnes. O escore foi gerado a partir da distribuição em tercis de ingestão (tercil 1=1 ponto, tercil 2=2 pontos, tercil 3=3 pontos), exceto para lácteos e carnes, onde o maior tercil foi considerado 1 e o menor 3. O consumo de vinho sendo zero ou > 20 g de álcool/dia foi codificado como 1 e até 20g de álcool/dia codificado como 3. As participantes foram divididas de acordo com a mediana do MDS (<15 ou ≥ 15). Foram observados maiores valores de DMO da coluna (1,076 ± 0,149 vs. 0,997 ± 0,143 g/cm²; P = 0,007) e ALMI (6,6 ± 0,8 vs. 6,3 ± 0,7 kg/m²; P = 0,039) nas participantes com maior MDS. As mulheres com maior MDS tiveram mais anos de estudo [11 (5-12,7) vs. 6 (5-9 anos); P = 0,022]. Análises de regressão mostraram uma associação positiva e independente do MDS sobre a DMO da coluna (média de diferença 0.088 g/cm$^2$, 95% CI, 0.028 - 0.147; P=0.004) e ALMI (média de diferença 0.296 kg/m$^2$, 95% CI, 0.002 - 0.591; P=0.049); esse modelo foi ajustado para média de passos, uso de terapia de reposição hormonal prévia e história de tabagismo. Em conclusão, os resultados do presente estudo sugerem que a

ingestão de um padrão alimentar de dieta estilo mediterrânea pode ser considerado como um fator de estilo de vida associado a melhor massa muscular e DMO e ser utilizado para prevenção primária de osteoporose e fraturas na pós-menopausa.

Palavras-chave: Pós-menopausa.  Dieta mediterrânea. Composição corporal.

## ABSTRACT

Declines in estrogen production during post menopause have been associated with loss in bone mineral density (BMD) and lean mass, increasing the risk for osteoporosis and fractures. Mediterranean diet (MD) has been associated with several health benefits, however, few data are available about the impact of Mediterranean diet on BMD and body composition in postmenopausal women. Therefore, the aim of this study was to investigate the association between MD score, body composition, and BMD in a sample of one hundred five postmenopausal women from Southern Brazil (age 55.2 ± 4, 9 years, body mass index 27.2 ± 4.6 kg/m²). BMD, % body fat, and appendicular lean mass index (ALMI, kg/m²) were assessed by dual-energy x-ray absorptiometry, resting metabolic rate by indirect calorimetry, habitual physical activity by pedometer, and dietary intake assessed by a validated food frequency questionnaire. Scoring of MD was based on the intake of the following dietary components: vegetables and fruits, fruits, cereals, fish, alcohol, olive oil, dairy products and meats. The score was generated from the distribution of tertiles of intake (tertil 1 = 1 point, tertil 2 = 2 points, tertil 3 = 3 points), except for dairy and meats, where the highest tertile was considered 1 and the lowest 3. Wine consumption being zero or> 20 g alcohol / day was coded as 1 and up to 20 g alcohol / day coded as 3. Participants were stratified according to median MDS (<15 or ≥ 15). Higher values of lumbar BMD (1.076 ± 0.149 vs. 0.997 ± 0.143 g/cm², P = 0.007) and ALMI (6.6 ± 0.8 vs. 6.3 ± 0.7 kg/m², P = 0.039) in participants with higher MDS. Women with higher adherence to MD had more years at school [11 (5 – 12.7) vs. 6 (5 – 9) years; P=0.022]. The regression analysis showed a positive and independent association of MDS on the lumbar BMD (mean difference 0.088 g / cm2, 95% CI, 0.028 - 0.147, P = 0.004) and ALMI (mean difference 0.296 kg / m2, 95% CI, 0.002 - 0.591, P = 0.049); this model was adjusted by the mean of steps by day, previous hormone therapy and previous smoking. In conclusion, the present results suggest that the MD pattern may be regarded as a positive lifestyle factor associated with muscle mass and BMD and could be used for prevention of osteoporosis in the post menopause.



Keywords: post-menopause. Mediterranean diet. body composition.

## SUMÁRIO

## 1. REVISÃO DA LITERATURA

A menopausa é definida como a ausência permanente das menstruações, em decorrência da diminuição da função folicular ovariana ou remoção cirúrgica dos ovários. A pós-menopausa abrange os estágios inicial ou recente e tardio. O estágio pós-menopáusico recente abrange os primeiros 3-4 anos depois do último sangramento menstrual, e a menopausa tardia são os anos posteriores (Soules *et al.*, 2001). O período da peri e pós-menopausa traz intensas modificações hormonais, principalmente a diminuição nos níveis de estrogênio (Clarkson, 2007), porém o impacto clínico dessas alterações hormonais é variável entre diferentes mulheres, etnias e populações, e apresenta influências de fatores socioculturais e psicológicos (Obermeyer e Sievert, 2007).

Além dos impactos sobre o sistema reprodutivo, a da peri e pós-menopausa também se associa a mudanças na composição corporal. Essas alterações se caracterizam por aumento de peso e da adiposidade corporal, resultando em um padrão de acumulo de gordura central ou de distribuição andróide (Misso *et al.*, 2005; Donato *et al.*, 2006; Douchi *et al.*, 2007), além de redução da massa muscular (Mazo *et al.*, 2007; Kang *et al.*, 2017; Orsatti *et al.*, 2017).

Mulheres na pós-menopausa em comparação com mulheres na pré-menopausa apresentam duas vezes mais risco de insuficiência coronariana (Grodstein *et al.*, 2000) sendo a doença cardiovascular a principal causa de morte em mulheres na pós-menopausa (Mosca *et al.*, 2006). A presença de obesidade abdominal, dislipidemia, hipertensão arterial, hiperglicemia em jejum ou intolerância à glicose agrava ainda mais o risco de DCV imposto pela menopausa (Creatsas *et al.*, 2005).

A idade por si só também está diretamente associada com a progressiva redução de massa de músculo esquelético e redistribuição da gordura corporal com maior acúmulo na região intra-abdominal (Chaves *et al.*, 2013). A perda da massa, força e função do músculo esquelético, que ocorre com o envelhecimento, contribui para o aparecimento de sarcopenia, fragilidade óssea, osteoporose, fraturas e mortalidade (Kelaiditi *et al.*, 2016), por

conseguinte, causa redução na qualidade de vida e aumento do risco de morbi-mortalidade em idosos (Gobbo *et al.*, 2012).

### 1.1 Avaliações de composição corporal e densidade mineral óssea

A redução da massa óssea parece ser consequência da diminuição de estrogênios (Riggs et al., 1998), aumento na produção de mediadores inflamatórios e alterações da ingestão protéico-calórica, que ocorrem durante o processo de envelhecimento (Silva et al., 2006). A redução da DMO é mais pronunciada após a menopausa (Cummings et al., 1995). Sendo o mesmo ainda considerada o melhor preditor para risco de fraturas por fragilidade (Guthrie et al., 1998; Svejme et al., 2013). Poulles e colaboradores avaliaram ao longo de 27 meses, 230 mulheres com idade entre 48 e 66 anos divididas em três grupos de acordo com o *status* menopausal e demonstrando que a diminuição de DMO na coluna lombar inicia antes da menopausa, com um aumento importante no período peri-menopausa, sendo em média de 2,3% ao ano (Pouilles et al., 1993). Estudos recentes com mulheres pós-menopáusicas sem doença clínica evidente, provenientes do sul do Brasil, demonstrou que menor DMO estava associada com tempo de menopausa, baixa massa muscular e gorda (Silva et al., 2015). Neste estudo, a ingestão de calorias e macronutrientes específicos, bem como a atividade física habitual, não interferiram na DMO. No entanto, são necessários estudos adicionais para determinar se a ingestão adequada de grupos de alimentos específicos eo tipo de atividade física podem exercer alguma influência sobre a DMO.

A massa magra apendicular (ALM), obtida através da absorciometria de raios x de dupla energia (DXA), representa a soma do tecido magro de braços e pernas, pode ser padronizada para altura ao quadrado ou IMC, e é frequentemente utilizada para estimar a massa muscular (Cawthon, 2015). Tem sido sugerido que valores de ALM/IMC menores que 0,512 para mulheres definem baixa massa muscular, por já terem sido associados ao prejuízo de mobilidade e aumento do risco de fragilidade (Cummings *et al.*, 2014; Cawthon, 2015).

## 1.2 Dieta Mediterrânea

A dieta é um potencial fator para a modificação da composição corporal, e para recuperação de massa óssea, principalmente em mulheres com idade mais avançada (Rivas *et al.*, 2013). Um dos primeiros grandes estudos realizados na região do Mediterrâneo (Cresta *et al.*, 1969.) para avaliação do padrão de dieta, acompanhou o consumo semanal durante um ano através de inquéritos individuais e por família; observou-se que os padrões alimentares dessa região eram caracterizados por uma elevada ingestão de cereais, legumes, frutas, peixes e um consumo baixo de carnes, laticínios e doces. Anos depois, a partir de diversos estudos (Keys, 1970; Keys e Keys, 1975; Keys, 1980), houve a constatação da menor mortalidade, nos países do Sul da Europa, associada ao padrão alimentar dessa região.

A Dieta Mediterrânea (MD) tradicional é caracterizada por elevado consumo de frutas e vegetais, oleaginosas, cereais e azeite de oliva, com baixo consumo de gordura saturada, moderadamente alta ingestão de peixes, baixo a moderado consumo de laticínios, baixa ingestão de carnes vermelhas e processados, além de ingestão regular de vinho, de forma moderada (Oldways, 2009). Sendo uma das dietas mais conhecidas e bem estudadas, diversas versões do escore de MD têm sido associados à diminuição do risco de doenças crônicas (Sofi *et al.*, 2010; Echeverria *et al.*, 2017), como as doenças cardiovasculares (Llaneza *et al.*, 2010; Bihuniak *et al.*, 2016), câncer (Sofi *et al.*, 2008), diabetes mellitus tipo 2 (DM2) e obesidade (Giugliano e Esposito, 2008); (Buckland *et al.*, 2008).

Os possíveis mecanismos para esses efeitos seriam a substituição de ácidos graxos saturados por insaturados, que resultaria em uma melhora da sensibilidade à insulina, possibilitando redução do risco de DM2 (Riserus *et al.*, 2009; Salas-Salvadó *et al.*, 2016; Guasch-Ferré *et al.*, 2017); relacionada também à manutenção do peso corporal e redução da gordura corporal central (Gillingham *et al.*, 2011; Alvarez-Perez *et al.*, 2016). Além destes efeitos, as gorduras insaturadas demonstram contribuir para a redução da pressão sanguínea e de triglicerídeos (Poudyal *et al.*, 2011; Salas-Salvadó *et al.*, 2016). Outro mecanismo possível para estes efeitos seria em relação às fibras,

presentes nos cereais integrais e tubérculos, que também podem reduzir o risco de DM2, através da melhora da sensibilidade à insulina e efeitos anti-inflamatórios (Weickert e Pfeiffer, 2008; Guasch-Ferré *et al.*, 2016; Salas-Salvadó *et al.*, 2016). A MD também é rica em polifenóis, que são compostos bioativos, com ação antioxidante, e que estão presentes em frutas e vegetais. Há evidências crescentes de que eles podem ter uma série de benefícios, como a diminuição de pressão arterial, melhora de perfil lipídico e efeitos anti-inflamatórios (Del Rio *et al.*, 2013; Salas-Salvadó *et al.*, 2016). Alimentos e bebidas ricas em polifenóis também podem reduzir a resposta glicêmica pós-prandial e a hiperglicemia de jejum, sugerindo uma diminuição no risco de DM2 (Salas-Salvadó *et al.*, 2016). O consumo de ácidos graxos poliinsaturados, provenientes dos peixes e alguns vegetais vêm sendo associado a menor mortalidade na população que aderiu a uma dieta estilo mediterrânea. (Pérez-López *et al.*, 2009).

A MD também vem sendo recentemente associada ao aumento de massa magra (Rivas *et al.*, 2013; Kelaiditi *et al.*, 2016; Nikolov *et al.*, 2016; Isanejad *et al.*, 2017; Stefan *et al.*, 2017; Tian *et al.*, 2017) e a proteção e preservação da massa óssea (Kontogianni *et al.*, 2009; Garcia-Martinez *et al.*, 2014; Chen *et al.*, 2016; Kelaiditi *et al.*, 2016; Moradi *et al.*, 2017; Rahi *et al.*, 2017) e diminuição da massa de gordura (Llaneza et al., 2010; Cespedes Feliciano et al., 2016; Echeverria et al., 2017).

No estudo de Kontogianni (Kontogianni *et al.*, 2009), a aderência à MD não mostrou efeitos significativos na DMO das 2.020 mulheres gregas estudadas, mas alguns componentes como peixes, azeite de oliva e baixo consumo de carne vermelha, demonstraram estar positivamente associados à DMO da coluna lombar. Um trabalho realizado na China, com 2.371 idosos, demonstrou associação positiva entre a MD e DMO em todos os sítios, além disso a maior aderência à dieta foi associada com uma diminuição de 7% no risco de fraturas de quadril ao longo de 3 anos de acompanhamento (Chen *et al.*, 2016).

Nikolov e colaboradores (Nikolov *et al.*, 2016) realizaram um estudo com 1.509 participantes com idade entre 60 e 80 anos, e observaram uma

associação positiva entre escore de MD e ALM. No estudo de Kelaiditi (Kelaiditi *et al.*, 2016), um resultado semelhante foi obtido em relação ao índice de massa livre de gordura, obtida através de bioimpedância elétrica, e aderência à MD em 2570 mulheres em idade reprodutiva e na pós-menopausa Já no esudo de Tian (Tian *et al.*, 2017), onde foram recrutados 3.289 chineses idosos, avaliados por 3 anos, foi observada uma associação positiva entre escore de MD e ALM, sugerindo que a adesão à MD seja de grande utilidade na manutenção da massa muscular. Além destes efeitos, também foi observada uma relação positiva entre aderência à MD e aumento da força muscular em um estudo com 554 mulheres na pós-menopausa (Isanejad *et al.*, 2017).

Tendo em vista as considerações acima, o objetivo deste trabalho foi de investigar a associação da MD, composição corporal e DMO em uma amostra de mulheres na pós-menopausa sem doenas clínicas.

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*Article*

# Mediterranean dietary pattern is associated with bone mineral density and skeletal muscle mass in postmenopausal women: a cross-sectional study

**Carolina C Martins[1], BSc, Thaís R Silva, PhD [1] and Poli M Spritzer, MD, PhD [1,2*]**

[1] Gynecological Endocrinology Unit, Division of Endocrinology, Hospital de Clínicas de Porto Alegre (HCPA); emaildacarolmartins@gmail.com; thaisrasia@gmail.com; spritzer@ufrgs.br

[2] Laboratory of Molecular Endocrinology, Department of Physiology, Universidade Federal do Rio Grande do Sul (UFRGS); spritzer@ufrgs.br

* Correspondence: spritzer@ufrgs.br; Tel.: +55 51 3359.8027 - Fax: +55 51 3359.8777

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**Abstract:** Declines in estrogen production during post menopause have been associated with loss in bone mineral density (BMD) and in lean mass, increasing the risk for osteoporosis. Mediterranean diet (MD) has been related to lower risk for chronic diseases. However, few data are available regarding MD and body composition in post menopause. Therefore, the aim of this study was to investigate the association between MD, body composition, and BMD in a sample of postmenopausal women with no clinical diseases. BMD, % body fat, and appendicular lean mass index (ALMI, ALM/h2) were assessed by dual-energy x-ray absorptiometry, and dietary intake assessed by a validated food frequency questionnaire. Multivariate regression analysis showed an independent, positive contribution of MD score to lumbar spine BMD (mean difference 0.088 g/cm², 95% CI, 0.028-0.147, P=0.004) and ALMI (mean difference 0.296 kg/m², 95% CI, 0.002-0.591, P=0.049); the model was adjusted for previous hormone therapy, previous smoking behavior, and habitual physical activity, estimated by the mean steps a day. In conclusion, the present results suggest that the intake of a Mediterranean dietary pattern may be regarded as a positive lifestyle factor in the context of non-pharmacological strategies for primary prevention of osteoporosis and fractures in the post-menopause.

**Keywords:** post-menopause; Mediterranean diet; body composition

## 1. Introduction

Bone mineral density (BMD) decreases with increasing age, especially after menopause [1, 2] and is related to the decline in ovarian function. Lower BMD remains the major risk factor for osteoporotic fracture [3] and has been attributed to a number of additional factors: genetic, nutritional, or the prolonged use of certain medication [4]. Declines in endogenous estrogen production during the menopausal transition and post-menopause have been also associated with skeletal muscle mass loss [5-7]. Appendicular lean mass (ALM) derived from whole body dual-energy x-ray absorptiometry (DXA), which represents the sum of lean tissue in the arms and legs, may be scaled to height squared or body mass index (BMI), is often used to estimate muscle mass [7, 8]. The age-related loss of skeletal muscle mass is associated with mobility impairment [9, 10] and are also associated with an increased risk of osteoporosis and fractures [11].

Diet may exert influence on BMD and muscle mass in both pre- and postmenopausal women. In this sense, several studies had previously underline the importance of adequate calcium and vitamin D levels in the prevention of osteoporosis and fractures in the peri- and post-menopause [12, 13] and protein intake has been associated with muscle mass [7, 12, 13]. However, the relevance of the quality of diet, measured as dietary patterns, has recently been recognized [14], because the traditional analysis of the effect of a single nutrient does not allow to examine the complex interactions between nutrients and non-nutrients contained in food [15]. Mediterranean diet (MD) is characterized by high intakes of fruits and vegetables, oilseeds, cereals and olive oil, with low saturated fat consumption, moderately high fish intake, low to moderate consumption of dairy products, low intake of red meat, as well as moderate, but regular, intake of wine [16]. MD has been related to positive health outcomes, several versions of MD scores (MDS) have been associated with lower risk of cardiovascular disease [17, 18], neurodegenerative diseases [19], cancer [20], and with lower all-cause mortality in individuals with CVD [21].

Only few studies reported the relationships between MD and BMD or body composition. While MD, especially high consumption of fish and olive oil and low red meat intake, was positively related to bone mass, MDS was not associated with lumbar spine BMD in a cross-sectional study with a Mediterranean women sample [22]. In turn, a recent study with Chinese adults reported that the increased adherence to the MD was associated with higher BMD in all sites. [23]. Regarding body composition, significant positive association between MDS and bioelectrical impedance analysis of fat-free mass index [24], and positive effect on ALM [25] have been observed. Therefore, the aim of this study was to investigate whether the MD adherence score is associated with body composition, and BMD in a sample of postmenopausal women with no clinical evidence of disease, living in a non-Mediterranean region.

## 2. Materials and Methods

### *2.1. Participants and Design*

This cross-sectional study was performed in the Gynecological Endocrinology Unit at Hospital de Clínicas de Porto Alegre, Brazil, from October 2010 to February 2012. Inclusion criteria were as follows: menopause, defined as last menstrual period at least 1 year before the beginning of the study plus FSH levels > 35 IU/L; and age between 45 and 65 years. Women using hormone therapy in the past 3 months, with diabetes or previous diagnosis of heart disease and current smokers were excluded.

One hundred and nineteen postmenopausal women, fulfill all the inclusion criteria and were consecutively enrolled, and 105 women completed the study protocol. Eleven candidates were excluded (five with diabetes, one with hyperthyroidism, two with untreated hypothyroidism, two with breast cancer, and one who was premenopausal). An additional three participants dropped out because they were unable to commit to the study. The local Ethics Committee approved the study protocol, and written informed consent was obtained from every participant. Details of the participants are described elsewhere [26].

### *2.2. Anthropometric and body composition measurements, blood pressure and resting metabolic rate*

Anthropometric measurements were performed in duplicate. Body weight, height and waist circumference (measured at the midpoint between the lower rib margin and the iliac crest, perpendicular to the long axis of the body) were measured [27].

Blood pressure was measured after a 10-minute rest, in the sitting position, with feet on the floor and the arm supported at heart level. Two measurements were performed at 10-minute intervals, using an automatic blood pressure monitor (Omron HEM742, Rio de Janeiro, Brazil).

Bone mineral density (BMD), % body fat, % trunk fat mass, and appendicular lean mass (ALM) (kg) were assessed by DXA (GE Lunar Prodigy, Radiation Corporation, Madison, WI,

USA). ALM was scaled to height squared and body mass index (BMI) to estimate skeletal muscle mass [8]. BMD was measured in lumbar spine (L1-L4), total femoral and femoral neck, and expressed as g/cm² and t-score [2].

Resting metabolic rate (RMR) was obtained by indirect calorimetry (Fitmate®, Cosmed, Rome, Italy), as previously reported [28].

*2.3. Dietary assessment and Mediterranean diet score*

Dietary intake in the previous month was assessed with a validated food frequency questionnaire [29] consisting of 121 items. Total energy intake, carbohydrates, proteins, lipids (saturated, monounsaturated fatty acids, and polyunsaturated fatty acids), fiber, alcohol consumption and micronutrients were assessing using the Brazilian Table of Food Composition [30]. Vitamin D, E, and A were assessed using the United States Department of Agriculture (USDA) [31] National Nutrient Database for Standard Reference.

MDS represents a summary value of consumed foods and characterizes a measure of adherence to a predefined MD [32]. Higher scores indicate diet quality and a higher intake of beneficial foods. MDS was calculated according to the scale reported by Funtikova et al. [33]. Adherence to MD was based on the intake of the following components: cereals, vegetables, fruits, meat, dairy products, fish, red wine and olive oil. Each food group was stratified by into tertiles. The lowest tertile was coded as 1, medium as 2 and the highest as 3 for cereals, fruits, vegetables, fish, and olive oil. For meat (including red meat, poultry and sausages) and dairy products, the score was inverted, with the highest tertile being coded as 1 and the lowest as 3. For alcohol, up to 20 g/d was coded as 3, and more or less than this daily portion was coded as 1. The possible scores ranged between 8 and 23, the latter reflecting the maximal adherence. Nuts are very expensive food item in Brazil, and it is not frequently consumed by our population, therefore we were not enable to measure nuts intake in this study.

*2.4. Physical activity assessment*

Habitual physical activity was measured by the average number of steps provided by six consecutive days of wearing a digital pedometer (BP 148, Tech Line, São Paulo, Brazil) [26, 34, 35]. The device was configured individually according to weight (kg) and individual step length.

*2.5. Statistical analysis*

Results are presented as mean ± standard deviation (SD), or median and interquartile range, depending on the Gaussian or non-Gaussian distribution of variables (Shapiro-Wilk test). Non-Gaussian variables were log-transformed for statistical analysis and back-transformed into their original units for reporting. To compare the differences between groups, according the median of MDS (<15 or ≥ 15), the 2-tailed Student's *t* test was used. Chi square was calculated for comparisons of dichotomous variables. A multiple linear regression model was also developed to explore the relationship between BMD and skeletal muscle index, defined as ALM scaled to height squared, as dependent variables and MDS as independent variable, adjusted for factors that may influence BMD and ALM, such as age, previous hormone therapy and previous smoking behavior. All analyses were performed using the Statistical Package for the Social Sciences 19.0 (SPSS, Chicago, IL, USA). Data were considered to be significant at $p \leq 0.05$.

**3. Results**

The mean age of participants was 55.2±4.9 y, body mass index 27.2±4.6 kg/m2, and the mean time since menopause was 5.5 ± 1.0 years [26].

Mean energy intake was 1865 ± 622 kcal/day, and mean of MDS was 14.8 ± 3.1. We found no differences in energy and macronutrients intake between groups, except for fiber intake that was higher in the higher MDS group. Regarding the micronutrients, we observed that higher MDS group had higher intake of magnesium, selenium, folate, vitamin A, E and C. Concerning MD components, vegetables, fruits, and fish were more consumed by the higher MDS group and dairy products were less consumed compared to lower MDS group (data not shown).

Table 1 presents the characteristics of postmenopausal women stratified by median MDS. No differences were observed between the groups regarding age, time since menopause, skin color, waist circumference, % body fat, % trunk fat, ALM, blood pressure, mean steps a day, RMR, previous hormone therapy and previous smoking behavior. However, years at school, ALM/height squared and BMD lumbar spine were higher in women with higher MDS. The prevalence of low bone mass was lower in women with higher adherence to MD when compared to less adherents. (Figure 1).

**TABLE 1** Characteristics of postmenopausal women stratified by Mediterranean Diet scores

| | **MD score** | | |
|---|---|---|---|
| | <15 | ≥15 | *P* |
| | (n=51) | (n=54) | |
| Age, y | 54.6 ± 4.9 | 55.7 ± 4.8 | 0.710 |
| **Years at school[a]** | **6 (5 – 9)** | **11 (5 – 12.7)** | **0.022** |
| White, n (%)[b] | 45 (88) | 47 (87) | 0.544 |
| Time since menopause, y[a] | 5 (2.6 – 10) | 6 (3 – 10) | 0.592 |
| BMI, kg/m² [a] | 26.3 (24.2 – 28.1) | 27.1 (23.9 – 31.3) | 0.122 |
| Waist circumference, cm | 85.9 ± 10.2 | 87.4 ± 13.6 | 0.517 |
| %Body fat[a] | 44.4 (38.3 - 47.4) | 43.0 (38.1 – 46.9) | 0.970 |
| Fat mass (kg) | 26.3 ± 8.3 | 28.2 ± 10.5 | 0.301 |
| Appendicular Lean Mass, kg | 16.0 ± 2.0 | 16.7 ± 2.3 | 0.069 |
| **ALM/height squared** | **6.3 ± 0.7** | **6.6 ± 0.8** | **0.039** |
| ALM/weight | 0.242 ± 0.03 | 0.243 ± 0.03 | 0.862 |
| ALM/BMI | 0.607 ± 0.08 | 0.609 ± 0.08 | 0.876 |
| **Lumbar spine BMD, g/cm²** | **0.997 ± 0.143** | **1.076 ± 0.149** | **0.007** |
| Femoral neck BMD, g/cm² | 0.870 ± 0.110 | 0.890 ± 0.119 | 0.298 |
| Total Femoral BMD, g/cm² | 0.925 ± 0.122 | 0.956 ± 0.121 | 0.297 |
| **Low bone mass, n (%)[b]** | **40 (78)** | **31 (60)** | **0.032** |
| Sistolic blood pressure mm Hg[a] | 126 (120 – 140) | 120 (110 – 140) | 0.108 |
| Diastolic blood pressure mm Hg[a] | 80 (80 – 81) | 80 (70 -80) | 0.076 |
| Mean steps a day[a] | 5764 (3601 – 7888) | 4930 (3585 – 8298) | 0.580 |
| RMR (kcal/d) | 1233.0 ± 155.1 | 1291.9 ± 226.1 | 0.135 |
| Previous smoking behavior, n (%)[b] | 17 (34) | 20 (40) | 0.339 |
| Previous hormone therapy, n (%) [b] | 13 (26) | 20 (38) | 0.202 |

Student *t* test; BMD, bone mineral density; BMI, body mass index; BW, body weight; DBP, diastolic blood pressure; RMR, resting
metabolic rate; SBP, systolic blood pressure.
[a]Variables analyzed after log transformation.

[b]x2 test.

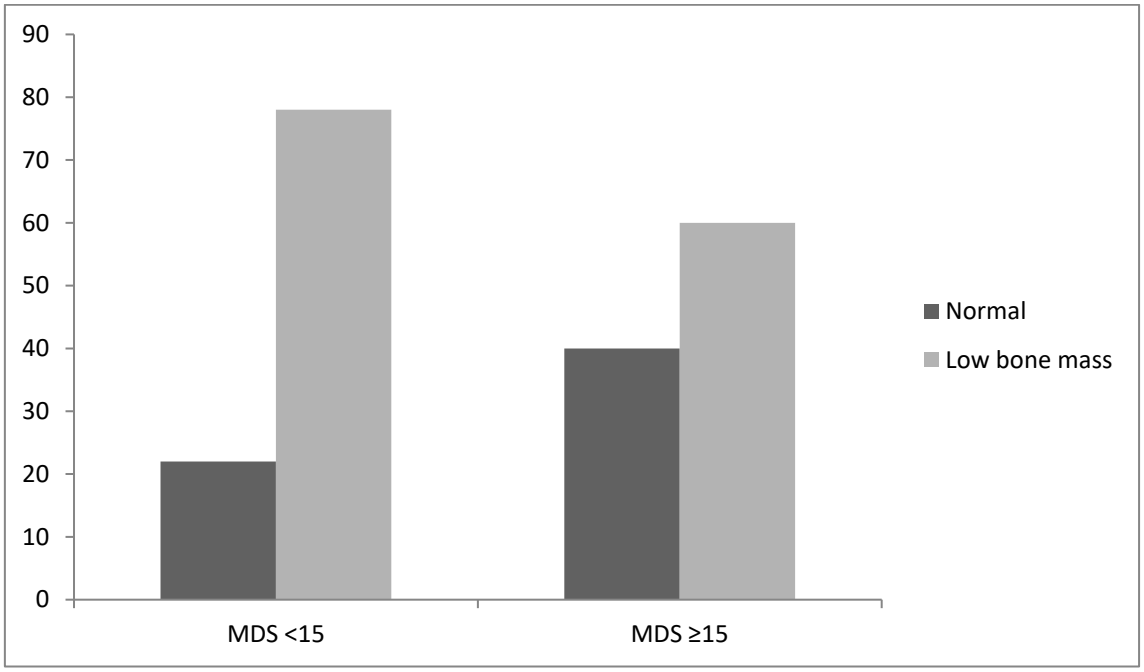


**Figure 1.** Prevalence of low bone mass in women grouped according to MDS.

As shown in Table 2, a multiple linear regression model was set up with BMD lumbar spine (g/cm²) and skeletal muscle index as the dependent variables to test the hypothesis that MDS might be influencing bone and muscle mass. There was an independent, positive contribution of MDS (mean difference 0.088, 95% CI 0.028 – 0.147, *P*=0.004) to lumbar BMD, and an independent, positive contribution of MDS to skeletal muscle index (mean difference 0.296, 95% CI -0.020 – 0.591, *P=0.049*), with both models being adjusted for previous hormone therapy, previous smoking behavior and mean steps a day.

**TABLE 2.** Multiple linear regression analysis with Lumbar spine BMD (g/cm²) and skeletal muscle index as the dependent variable

| | Lumbar spine BMD (g/cm²) | | Skeletal Muscle Index | |
|---|---|---|---|---|
| *Variables* | Mean difference[a] (95% CI) | *P* | Mean difference[a] (95% CI) | *P* |
| MDS | | | | |
| *Unadjusted* | **0.079 (0.022 to 0.137)** | **0.007** | **0.300 (0.015 to 0.585)** | **0.039** |
| *Adjusted*[b] | **0.088 (0.028 to 0.147)** | **0.004**[c] | **0.296 (0.020 to 0.591)** | **0.049**[d] |

MDS: Mediterranean dietary score categories (≥15 compare to <15); SMI = ALM/m²

[a]Unstandardized B.

[b]Model adjusted by previous hormone therapy, previous smoking behavior, and mean steps a day

[c]$R^2$=0.13.

[d]$R^2$=0.12.

**4. Discussion**

In the present study a positive association between lumbar spine BMD and skeletal muscle index with MDS was found in apparently healthy postmenopausal women, most of them without impairment of muscle mass or osteoporosis, even after adjustments for confounding factors. Women with higher adherence to MD presented lower prevalence of low bone mass and had better school education. While the association between BMD, and MDS has been noted, to the best of our knowledgement, this is the first study to describe a positive impact of MD on lean mass in postmenopausal women from non-Mediterranean region and with different diet patterns.

Similar protective association with BMD was observed in a cross-sectional study including 200 healthy pre- and postmenopausal Spanish women, and related to the consumption of vegetables and fruits [36]. Adherence to MD, especially high consumption of fish and olive oil and low red meat intake, was positively associated with bone mass in a cross-sectional study of 196 Greek women [22].

Of the eight food groups included in the MDS, no individual food showed correlation with BMD (data not shown), pointing out the relevance of the quality of a diet, as a whole, measured as MDS, in opposition to the estimation of single food intake for bone health. The potential beneficial associations between the MDS with BMD may result from their nutrients and other nutritional components, such as magnesium [37], vitamin A [38], and C [39, 40]. In fact, in the Fourth Korean National Health and Nutrition Examination Survey, in which dietary information was assessed using a 24-h dietary recall questionnaire, the dietary vitamin C intake was positively associated with BMD in postmenopausal women, and inadequate vitamin C intake could increase the risk of osteoporosis [40]. In addition, the significant amount of antioxidants in the MD can provide resistance against the inhibition of osteoblastic cell differentiation, promoted by oxidative stress [41]. Adherence to the MD was also associated with lower levels of pro-inflammatory cytokines in previous studies [42, 43]. Higher production of pro-inflammatory cytokines was associated with high bone reabsorption rates and increased risk of osteoporosis [44]. In our study, BMD lumbar spine was higher in women with higher MDS and the frequency of low bone mass (osteopenia and osteoporosis) was lower in women with higher adherence to MD.

Saturated fat intake has been reported to be associated with a higher risk of bone loss and osteoporotic fractures [45]. Indeed, the low red meat and low to moderate dairy products content of MD may explain, at least in part, the potential benefit to bone health, as found in the present study. Interestingly, differences in the severity of osteoporosis across the European Union Countries have been reported, with a lower incidence of the disease in the Mediterranean area and this effect has been mainly attributed to specific eating pattern [46, 47]. The potential protective effect of the MD against the risk of hip fractures has been also studied in other regions and the adherence to MD was associated with a lower risk in men and women [48, 49]. Taken together, these data and the results of the present study highlight the potential importance of adherence to the MD on bone health.

Regarding lean mass, we found a positive association of skeletal muscle index (ALM/height squared) with higher MD adherence score in our postmenopausal women aging 55.2 ± 4.9 years old. In older women (65–72 years), a recent study reported that those with lower adherence to MD lost more lean mass (ALM/height squared and total body lean mass) as compared to women with higher adherence over the 3-year follow-up [50]. Also, in a cross-sectional data from the Berlin Aging Study II with old men and women (68.2 ± 3.7 years), higher adherence to a Mediterranean-style diet was associated with a positive effect on ALM/BMI only in women [25]. MDS was also associated with ALM/BMI and ALM/height squared in 3289 Chinese adults aged 40 to 75 years, particularly in male and younger subjects [51]. The impact of the adherence to the MD on preventing muscle mass loss may be due to similar mechanisms attributed to BMD stability, that is, by decreasing the high levels of pro-inflammatory cytokines and reactive oxygen species production that are imputed to the decrease of muscle mass [52]. In

fact, MD has been shown to be associated with lower levels of pro-inflammatory cytokines [42, 43] and higher levels of antioxidants, which can provide resistance against oxidative stress [41].

A strength of the present study is its sample of healthy postmenopausal women, who were mostly non-obese and presented normal lean and bone mass, allowing us to show the relationship between BMD, skeletal muscle mass and MDS, in an earlier stage of life, before the development of sarcopenia, osteoporosis or frailty. Limitations include the cross-sectional design, which precludes conclusions regarding the direction of cause and effect. Another limitation is the incapability to assesses nuts intake, however nuts are very expensive food item in Brazil, and it is not frequently consumed by our population, Our semi quantitative food frequency questionnaire is a robust validated in Brazil, food frequency questionnaire [29], assessing 121 items of food consumption during the preceding month was administered by a trained nutritionist, who interviewed each participant for approximately 50 minutes. This procedure probably enhanced the quality and reliability of our dietary data.

## 5. Conclusions

In conclusion, lumbar spine BMD and skeletal muscle index were positively associated with MDS in the present sample of healthy postmenopausal women. The present results suggest that the intake of a Mediterranean dietary pattern may be regarded as a positive lifestyle factor in the context of non-pharmacological strategies for primary prevention of osteoporosis and fractures in the postmenopausal period. Finally, because this is a palatable dietary pattern, adherence to a MD-style diet can be easily achieved by the general population outside the Mediterranean basin.

## 6. Patents

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