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ASSOCIAÇÕES ENTRE DIETA, COMPOSIÇÃO CORPORAL E DENSIDADE MINERAL ÓSSEA EM MULHERES NA PÓS-MENOPAUSA

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Esta tese de doutorado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia: Faculdade de Medicina, Universidade Federal do Rio Grande do Sul. Será constituída de uma introdução, no formato de revisão sistemática e dois artigos originais. O artigo do capítulo 2 foi submetido para a revista *Menopause.* O artigo do capítulo 3 está publicado na revista *BMC Endocrine Disorders*.

SUMÁRIO

LISTA DE TABELAS E FIGURAS

CAPÍTULO 1

CAPÍTULO 2

CAPÍTULO 3

LISTA DE ABREVIATURAS

AFM: abdominal fat mass

ALM: appendicular lean mass

BMD: bone mineral density

BMI: body mass index

BW: body weight

CD: Control diet

DHEAS: dehydroepiandrosterone sulfate

DXA: dual-energy X-ray absorptiometry

FSH: follicle stimulating hormone

HDL: high-density lipoprotein

HOMA: homeostasis model assessment

HP: high protein

LAP: lipid accumulation product

LDL: low-density lipoprotein

PA: physical activity

RDA: recommended dietary allowances

RMR: resting metabolic rate

SHBG: sex-hormone binding globulin

SMI: skeletal muscle index

us-CRP: ultrasensitive C reactive protein

WC: waist circumference

RESUMO

A menopausa é definida como a ausência permanente das menstruações, em decorrência da diminuição da função folicular ovariana ou remoção cirúrgica dos ovários. O declínio da produção endógena de estrogênio durante a transição menopáusica tem sido associado à perda de densidade mineral óssea (DMO) e de massa muscular esquelética. Além disso, não só o status menopáusico, mas também o envelhecimento promove outras modificações na composição corporal, como o aumento e redistribuição da massa de gordura, resultando em um padrão de acumulação de gordura central ou de distribuição androide. Essas alterações de composição corporal levam a efeitos deletérios na saúde dessas mulheres, como aumento do risco de doenças cardiovasculares, fraturas e mortalidade.

Estudos de intervenção na população em geral tem demonstrado que dietas hiperproteicas em comparação com dietas de baixo teor de proteínas levam a maior perda de circunferência de cintura e gordura abdominal durante o seu seguimento. Ainda não foram publicadas revisões sobre o efeito de dietas hiperproteicas sobre gordura abdominal ou adiposidade central em mulheres de meia-idade. Portanto, o objetivo da introdução, sendo o capítulo 1 desta tese, foi conduzir uma revisão sistemática de ensaios clínicos randomizados para identificar os efeitos de dietas hiperproteicas comparadas a dietas controles sobre gordura abdominal em mulheres de meia-idade. Devido ao número limitado de evidências identificadas, não foi possível a realização de metanálise. Não foram encontradas diferenças na redução da circunferência da cintura e da

massa de gordura abdominal entre as dietas para a maioria dos estudos. No entanto, dietas com objetivo de redução de peso corporal, independentemente da composição nutricional podem diminuir a massa de gordura abdominal em mulheres de meia-idade.

As recomendações de ingestão (DRI) de proteína para idosos, homens e mulheres, continuam sendo as mesmas de adultos jovens saudáveis. A estimativa média de requerimento (EAR) e a recomendação diária de ingestão (RDA) são de 0,66 e 0,8 g/kg de peso corporal, respectivamente, sendo que a RDA teoricamente satisfaz as necessidades de 97,5 % da população. No entanto, em dois estudos recentes que avaliaram as recomendações de proteína para mulheres com mais de 65 e 80 anos de idade, encontraram uma EAR de 0,85 e 0,96 e uma RDA de 1,15 g e 1,29 g/kg de peso corporal, respectivamente. Dessa forma, o objetivo do segundo estudo foi investigar a associação entre massa muscular esquelética e ingestão de proteína, atividade física habitual, composição corporal e variáveis metabólicas em mulheres na pós-menopausa, encaminhadas através de chamamento na mídia e atendidas pelo grupo de endocrinologia ginecológica do Hospital de Clínicas de Porto Alegre. A prevalência de baixa massa muscular foi de apenas 7%, visto que nossas mulheres eram jovens e sem doenças clínicas evidentes. O índice de massa muscular foi positivamente associado com ingestão de proteína e negativamente associado com % gordura corporal. As participantes com ingestão proteica no tercil superior apresentaram melhor perfil metabólico e padrão alimentar mais saudável. Este grupo também apresentou menor IMC, circunferência da cintura e massa de gordura do tronco.

O declínio nos níveis de 17-β-estradiol está associado com o aumento na perda de DMO. Esse declínio de DMO também pode ser atribuído a diversos outros fatores, incluindo: idade, carga genética, nutrição, estilo de vida e uso prolongado de algumas medicações. Portanto, o objetivo do terceiro trabalho foi investigar como a composição corporal, padrão alimentar e atividade física habitual são associados com DMO de acordo com o tempo de menopausa em mulheres do sul do Brasil, sem doença clínica evidente. Nesse estudo transversal, o tempo de menopausa, menor massa magra e de gordura, além de ingestão de vitamina A < 700 µg/dia foram associados com baixa massa óssea. Ingestão calórica e de macronutrientes, bem como atividade física habitual, não interferiram na DMO, no entanto a maior parte das participantes eram sedentárias.

ABSTRACT

Menopause is defined as the permanent absence of periods, due to the decline in ovarian follicular function or ovaries surgical removal. The decline in endogenous estrogen production during the menopausal transition has been associated with loss of bone mineral density (BMD) and skeletal muscle mass. Furthermore, not only the menopausal status, but also aging promotes other changes in body composition, resulting in increases visceral fat mass. These body composition changes have potential impact on health status in women, increasing the risk of cardiovascular disease, fracture and mortality.

Intervention studies in the general population have provided evidence that high protein (HP) compared with low protein diets improve loss of waist circumference and abdominal fat during follow-up. The Nurses' Health Study observed a 26% reduction in the risk of cardiovascular disease in the group of women with higher protein intake compared to the lowest intake group. Specifically in women, there are no reviews on the effect of HP diets on abdominal obesity. Therefore, the aim of introduction and Chapter 1 of this thesis was to conduct a systematic review of randomized clinical trials to identify the effects of HP diets compared to control diets on abdominal obesity in middle-aged women. The limited evidence identified in this systematic review does not show a HP diet conferring benefit over control diet in middle-aged women. In conclusion, a diet aimed at reducing weight improved abdominal fat in middle-aged women regardless of the diet composition.

The dietary recommend intake (DRI) of protein for the elderly, men and women, are still the same for healthy young adults. The estimated average requirement (EAR) and the recommended daily intake (RDA) are 0.66 and 0.8 g/ kg of body weight, respectively, and the RDA theoretically meets the needs of 97.5% of the population. However, two recent studies evaluating the protein recommendations for women aged 65 to 80, found a RDA of 0.85 and 0.96 and a EAR of 1.15 and 1.29 g / kg of body weight, respectively. Thus, the purpose of the second study was to investigate the associations between skeletal muscle mass and protein intake, habitual physical activity, body composition and metabolic variables in postmenopausal women, attended by gynecological endocrinology group from a Porto Alegre University Hospital. Probably because of high mean protein intake, along with the mean age (55.2 ± 4.9 years) of our postmenopausal women, the prevalence of low muscle mass in our sample was only 7%. The body mass index was positively associated with protein intake and negatively associated with percentage body fat. Participants with protein intake in the highest tertile had a better metabolic profile and healthier eating pattern. This group also had lower BMI, waist and trunk fat mass.

Falling levels of 17-β-estradiol are thought to accelerate the decline in BMD, which remains the single best predictor of primary osteoporotic fracture. This decline can also be attributed to a number of factors: age, genetics, nutrition, lifestyle factors, or the prolonged use of certain medication. Therefore, the aim of the third study was to investigate whether body composition, dietary pattern and habitual physical activity are associated with BMD according to time since menopause in women from Southern Brazil with no clinical evidence of disease.

In this cross-sectional study with postmenopausal women with no clinical evidence of disease, time since menopause, low lean and fat mass were associated with low bone mass. Calories and macronutrients intake as well as habitual physical activity did not interfere with BMD, but participants were mostly sedentary.

CAPÍTULO 1

Effects of high protein diet on abdominal obesity in middle-aged women: systematic review of randomized controlled studies

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ABSTRACT

Aging in women is associated with a natural decline in estrogen that increases visceral fat mass. Clinical trials have provided some evidence that higher- versus lower-protein diets shows a greater waist circumference loss. We conducted a systematic review of randomized controlled studies to identify the effects of HP compared with control diets on abdominal obesity in middle-aged women. Two electronic databases (Medline and EMBASE) were searched until June 16 to identify relevant studies (MeSH terms: 'high protein' and 'abdominal fat'). Inclusion criteria were randomized clinical trials in middle-aged women, considering mean age > 45 years old analyzing the effect of high dietary protein intake on abdominal fat. Among the 1,237 initially identified references, six studies were selected, including 288 middle-aged women. A meta-analysis was not performed due to clinical heterogeneity for factors including participants, dietary intervention composition and follow-up. There were no differences for WC and abdominal fat mass (AFM) loss between HP and CD assessed in the majority of studies, with only one study reporting a low-fat, HP diet associated with a greater abdominal fat mass loss compared with a high-fat, standard protein diet. The limited evidence identified in this systematic review does not show a HP diet conferring benefit over control diet in middle-aged women. In conclusion, a diet aimed at reducing weight improved abdominal fat in middle-aged women regardless of the diet composition.

INTRODUCTION

Aging in women is associated with a natural decline in estrogen that increases visceral fat mass, decreases muscle mass, and strength in women (1-3). The *Study of Women's Health Across the Nation* (SWAN) demonstrated in 543 pre- or early perimenopausal women aged 42–52 years at baseline examination, an absolute cumulative six-year increase in fat mass of 3.4 kg and six-year increase of ~5.7 cm in waist circumference (WC), and the FSH change was positively correlated with fat mass change (4). These body composition changes, especially increased in abdominal fat mass, have potential impact on health status in women (5).

Clinical intervention studies in general population, have provided some evidence that higher- versus lower-protein diets shows a greater WC loss over three months of follow-up (6). In women (44.0 ± 9.08 years), a recent six-month, randomized study reported that an energy-restricted diet with 35% protein, more effectively impacted cardiovascular and metabolic profile, decreased fat mass, lipids and insulin resistance than an energy-restricted diet with lower protein (20%) intake (7). Additionally, a positive health benefit from a high protein (HP) intake was also observed in the Nurses' Health Study (80082 women aged 34–59 years) which found a 26% lower rate of cardiovascular disease in those women in the highest protein intake group (8). On the other hand, in obese women (49 ± 9 years), another study showed both the HP and high carbohydrate (HC) hypocaloric diets resulted in significant improvements in markers of cardiovascular disease risk (9).

In the menopause transition, it remains unclear if HP diets improve the fat distribution and protect for metabolic and cardiovascular diseases. Therefore, we

conducted a systematic review of randomized controlled studies to identify the effects of HP compared with control diets on abdominal obesity in middle-aged women.

METHODS

Data Sources, Search Strategy and Eligibility Criteria

A systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (10) (Appendix 1). Two independent authors (FMM and TRS), in duplication, sought studies published before June 16, 2016 (date last searched) using Medline and EMBASE databases, to identify the effect of high dietary protein intake on abdominal obesity in middle-aged women, considering mean age > 45 years. The initial search included the key search terms ‘high protein’ and ‘abdominal fat’ without any language restriction. The complete ‘Medline’ search strategy is provided in the Appendix 2.

Study Selection

Randomized intervention studies were eligible for inclusion if they reported relevant estimates on the abdominal fat or body composition measurements in middle-aged women. Two independent reviewers, working in pairs, screened the titles and abstracts of all initially identified studies according to the selection criteria. In case of disagreement, decision was reached through consensus or consultation with a third independent author (PMS). Full texts were retrieved from studies that satisfied all selection criteria.

Data Extraction

Data were extracted by two independent authors (TRS and FMM). A predesigned data extraction form was used to collect relevant information. This included questions on study size; study design; baseline population; location; age at baseline; duration of follow-up; anthropometric and body composition characteristics (weight, BMI, waist circumference, total or percentage of body fat and total abdominal fat) and method of measurement of body composition. Data on total energy, macronutrients (type and amount) and dietary compliance were collected from the description of the intervention and control diets. Data extracted for dietary protein included the following: total in grams, g/kg body weight (BW) or percentage of total energy intake. Data on means and statistical dispersion for anthropometric and body composition variables at baseline and at the end of the study were extracted. Percentage changes in anthropometric and body composition at the end of each study were calculated for all studies that presented baseline anthropometric and body composition values. Change of body weight (kg), waist circumference (cm), abdominal fat (kg) and fat mass (kg or %) were the outcomes of interest in this systematic review. Additionally, in the case of multiple publications, the most up-to-date or comprehensive information was included. If data necessary for the review were missing, we contacted the authors by e-mail.

Assessing the Risk of Bias

Bias within each individual study was assessed by two independent reviewers (FMM and TRS), and was classified into six domains: selection;

performance; detection; attrition; reporting; other (10, 11). The ‘other’ domain included the assessment of dietary compliance. The risk of bias in each domain was classified as high, low or unclear. Regarding dietary compliance, the risk of bias was classified as ‘low’ if the study described the method for the assessment of dietary compliance.

RESULTS

The search strategy identified 1,237 unique citations. Following initial screening, based on titles and abstracts, 1026 records were excluded and 211 articles were retrieved and evaluated further. From these articles, 205 were excluded for different reasons shown in Figure 1. The remaining six randomized controlled articles (12-17) were included in the systematic review (Table 1). These studies included data from 288 middle-aged women, 154 on HP and 149 on control diet (CD). All studies were parallel design, with the exception of van Nielen and colleagues (14) which was an acute (4-week) crossover trial. Two studies included women from Australia, whilst the remaining study included participants from the USA, Italy, Holland and Sweden. The age of women ranged from 20 to 70 years old. Participants were not blinded to their assignment because of the nature of the intervention. Study duration ranged from 4 weeks to 2 years. All trials were conducted among overweight or obese women, except for one study that included women with WC higher than 80 cm, BMI data not shown (14). Body composition was analyzed by dual-energy X-ray absorptiometry (DXA), only Muscariello and colleagues (12) performed by electrical bioimpedance (BIA). Physical activity (walking and/or aerobic activity) was recommended for 30 minutes at least five times a week in one study (12).

Participants were asked to keep physical activity during the follow-up in three studies (14, 16, 17). There was no specific physical activity advice provided in other two trials (13, 15). The baseline characteristics of HP groups, including body weight, WC and abdominal fat mass, were similar to those of control diet groups (Table 2).

Differences in methodological characteristics were found among the studies, such as study duration, dietary protocol, and physical activity advice, with considerable clinical and methodological heterogeneity. In consequence, a meta-analysis could not be performed, as it would not have yielded clinically meaningful results. The risk of bias of the randomized trials included in the systematic review is shown in Table 3. All available studies were judged as medium or high quality, with few potential source of bias coming from participant selection.

Dietary composition of included studies

The goal dietary nutritional composition varied across the 6 studies, with protein consumption ranging from 1.2 to 1.7g/kg BW or 22 to 40% of total energy intake. Three studies (13, 15, 17) offered an HP diet with low carbohydrate (30-45% of total energy intake). On the other hand, Luscombe and colleagues (16), compared an HP diet with low fat diet (40% of energy as protein, 30% of energy as fat) to standard-protein high fat diet (20% of energy as protein and 50% of energy as fat). van Nielen and colleagues (14), compared two diets, one containing protein of mixed origin (mainly meat, dairy, and bread), and the other diet partly replaced meat with soy meat analogues and soy nuts. However, both diets had the same macronutrients dietary composition (22% protein, 27% fat, and 50% carbohydrate). Only one study did not present the dietary composition

of carbohydrates and fats (12) (Table 4). In two trials (12, 15) participants were assigned to reduce caloric intake, in two other studies (16, 17) both dietary groups underwent 12 weeks of energy restriction and 4 weeks of energy balance, in one study (14) energy intake was calculated to keep body weight stable, and another study (13) diets were consumed ad libitum.

Abdominal fat

Of the total selected studies, only two (12, 13) investigated the effects of a diet with a high protein content (1.2 g/kg BW or 30% of total energy) on WC compared with a control diet with a conventional protein content (0.8g/kg BW or 15% of total energy) in postmenopausal women. Mean change of WC in HP was -1.9 to -12.4 cm and in control diet was -2.8 to -10.0 cm. Participants from both diets combined having improvements in WC, and there was no statistically difference between HP diets or control diets (Table 5). The change in abdominal fat mass was assessed for three studies (14, 16, 17). Therefore, only one study showed a significant difference between two different diet groups (16), with a low-fat, HP diet over 12 weeks of energy restriction and 4 weeks of energy balance resulting in a greater abdominal fat mass loss compared with a high-fat, standard protein diet. For the other two studies (14, 17), similar decreases in abdominal fat mass occurred independently of diet composition (Table 5).

Other body composition variables

Five studies measured body weight outcomes. Stomby and colleagues (13) reported a greater weight loss over 6 months of ad libitum Paleolithic diet (30% of total energy as protein) compared with ad libitum Nordic nutrition

recommendations diet (15% of total energy as protein), besides that no difference between diet groups was observed over 24 months. On the other hand, Tang and colleagues (15) reported that standard protein in a lactovegetarian diet improved the weight loss in comparison to HP omnivorous diet in 12 weeks (-8.0 ± 6.9 vs 9.6 ± 10.6 respectively). Three other clinical trials (14, 16, 17) observed weight loss in middle-aged women independent of diet composition (Table 5).

Total fat mass was reported in all studies (Table 5), and decreases were demonstrated independent of diet composition, except for Stomby and collegues (13). In this study there was a greater fat mass loss over 6 months of ad libitum Paleolithic diet compared with ad libitum Nordic nutrition recommendations diet, and as we observed in body weight measurements, no difference between the two diet groups occurred over 24 months in fat mass loss.

DISCUSSION

To the best of our knowledge, we report for the first time a systematic review evaluating the effect of high protein diet on abdominal fat in middle-aged women. There were no differences for WC and abdominal fat mass (AFM) loss between HP and CD assessed in the majority of the studies, with only one study reporting a low-fat, HP diet associated with a greater abdominal fat mass loss compared with a high-fat, standard protein diet. More importantly, a diet aimed at reducing weight improved abdominal fat (WC and AFM), in middle-aged women regardless of diet composition.

Menopausal transition has been associated with changes in body composition (5, 18). Our group has reported that postmenopausal women had a significantly greater waist circumference and waist-to-hip ratio than

premenopausal women, independent of age, BMI, educational attainment, and hormone therapy use (3). The presence of abdominal obesity further aggravates the cardiovascular risk imposed by menopause (19). Limited research is conducted assessing the impact of dietary protein in fat distribution in postmenopausal women. In the present review including middle-aged women, only one study showed a significant difference between two different diet groups (16), with a low-fat, HP diet over 12 weeks of energy restriction and 4 weeks of energy balance resulting in a greater abdominal fat mass loss compared with a high-fat, standard protein diet. In the general population, the effects of higher- versus lower-protein diets on health outcomes were observed in a systematic review and meta-analysis (6). Fifteen studies reported WC measurements (1214 participants), at 3 months, the meta-analysis shows a greater WC loss with HP diets (-1.66 cm, CI -2.66 to -0.62), statistically significant and represented small to moderate effects, with heterogeneity across trials (I^2 75%). In other previously study (20), subjects with high triglycerides level lost more abdominal fat when consuming a HP diet (HP 1.92 ± 0.17 kg; SP 1.23 ± 0.19 kg, *P* = 0.005), the authors of this no systematic review included two of our studies (16, 17), but women and men were analyzed together.

Age-related changes in the content and distribution of adipose tissue is purported to strongly reduction in insulin sensitivity (21-23). HP diets could uncover a significant reduction in fasting insulin concentration, as shown in a meta-analysis of 10 studies that included 718 participants that also observed moderate reductions in triglycerides (6). In one study (24), with the same population that Stomby and colleagues (13), included in our review, a Paleolithic-type diet led to a 49% reduction in liver triglyceride levels during a 5-week

intervention, and this was associated with improved hepatic insulin sensitivity. The benefits in metabolic profile of HP diets have been explained by reductions in dietary carbohydrate intake and by the greater preservation of fat-free mass (7, 25).

In relation to body weight loss, several meta-analyses showed that LCD is effective for weight loss (26, 27). However HP diets resulting in weight loss studies have led to various and often contradictory conclusions (6, 28). Thereby, a meta-analysis that investigated low-carbohydrate and HP diets, shows favorably affect body weight loss dependent of the energy intake (29). Protein has a greater effect on satiety (30, 31), and high-protein diet, appear to be greater under conditions of ad libitum energy intake than under conditions of isoenergetic diets (32, 33). There may also have been a small increase in energy expenditure as the thermic effect of feeding has been shown following a high protein meal (34). Preventing a weight cycling effect a dietary protein contributes to the treatment of obesity (35). In this present systematic review, diets result in clinically meaningful weight loss regardless of which macronutrients they emphasize, probably because majority studies included isoenergetic diets. The only study that recommended ad libitum diets shows significant body weight and fat mass loss with HP diet. HP diets may be useful in regulating postprandial glucose, whereas a significantly higher preprandial glucose combined with a lower concentration of ghrelin may contribute to the decrease in ad libitum caloric intake (36). In addition, a recent clinical trial demonstrated that the glucose concentration was suppressed and glucagon-like peptide 1 increased more after intake of the HP (40%) than after the other preloads breakfast ($P < 0.001$) in 36

adults, therefore protein had a more pronounced effect on suppressing appetite than did carbohydrates and fat (37).

Kidney function and protein intake have become a reason for concern in the past years, but recent data (mean follow-up 6.4 ± 1.2 years) suggest that higher protein intake does not have a major effect on kidney function decline among elderly men and women (38).

Vissers and colleagues (39) show in a systematic review and meta-analysis, that aerobic training of moderate or high intensity has the highest potential to reduce visceral adipose tissue in overweight males and females. These results suggest that an aerobic exercise program, without hypocaloric diet, can show beneficial effects to reduce visceral adipose tissue even after 12 weeks. However, none studies in this present systematic review included structured physical activity protocol. Physical activity (walking and/or aerobic activity) was recommended for 30 minutes at least five times a week in one study (12) but the authors did not observe differences between HP and CD for WC.

Limitations of this systematic review include a considerable clinical heterogeneity and the small sample size of the studies, which limits the interpretation of data and suggests similar effects of HP and CD diets. Overall, the body of evidence was not sufficient to draw reliable conclusions about the optimal dietary composition for reduction in abdominal fat in middle-aged women. This lack of consensus limits the ability to develop dietary guidelines about protein intake in middle-aged women, and apply the evidence in clinical practice.

CONCLUSION

The limited evidence identified in this systematic review does not show a HP diet conferring benefit over CD in middle-aged women. The implications of this systematic review suggest that there were no differences for WC and abdominal fat mass between HP diet and CD assessed in the majority of studies, and a diet aimed at reducing weight, improved abdominal fat in middle-age women regardless of the diet composition. Further research is needed, including high quality, long-term randomized clinical trials that assess the effect of high protein diets in middle-aged women.

References

1. Rolland YM, Perry HM, 3rd, Patrick P, Banks WA, Morley JE. Loss of appendicular muscle mass and loss of muscle strength in young postmenopausal women. The journals of gerontology Series A, Biological sciences and medical sciences. 2007;62(3):330-5.

2. Maltais ML, Desroches J, Dionne IJ. Changes in muscle mass and strength after menopause. Journal of musculoskeletal & neuronal interactions. 2009;9(4):186-97.

3. Donato GB, Fuchs SC, Oppermann K, Bastos C, Spritzer PM. Association between menopause status and central adiposity measured at different cutoffs of waist circumference and waist-to-hip ratio. Menopause. 2006;13(2):280-5.

4. Sowers M, Zheng H, Tomey K, Karvonen-Gutierrez C, Jannausch M, Li X, et al. Changes in body composition in women over six years at midlife: ovarian and chronological aging. J Clin Endocrinol Metab. 2007;92(3):895-901.

5. Wilson PW, D'Agostino RB, Sullivan L, Parise H, Kannel WB. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. Arch Intern Med. 2002;162(16):1867-72.

6. Santesso N, Akl EA, Bianchi M, Mente A, Mustafa R, Heels-Ansdell D, et al. Effects of higher- versus lower-protein diets on health outcomes: a systematic review and meta-analysis. Eur J Clin Nutr. 2012;66(7):780-8.

7. Mateo-Gallego R, Marco-Benedi V, Perez-Calahorra S, Bea AM, Baila-Rueda L, Lamiquiz-Moneo I, et al. Energy-restricted, high-protein diets more effectively impact cardiometabolic profile in overweight and obese women than lower-protein diets. Clin Nutr. 2016.

8. Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Speizer FE, et al. Dietary protein and risk of ischemic heart disease in women. Am J Clin Nutr. 1999;70(2):221-7.

9. Noakes M, Keogh JB, Foster PR, Clifton PM. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. Am J Clin Nutr. 2005;81(6):1298-306.

10. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. Journal of clinical epidemiology. 2009;62(10):e1-34.

11. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:d5928.

12. Muscariello E, Nasti G, Siervo M, Di Maro M, Lapi D, D'Addio G, et al. Dietary protein intake in sarcopenic obese older women. Clin Interv Aging. 2016;11:133-40.

13. Stomby A, Simonyte K, Mellberg C, Ryberg M, Stimson RH, Larsson C, et al. Diet-induced weight loss has chronic tissue-specific effects on glucocorticoid metabolism in overweight postmenopausal women. Int J Obes (Lond). 2015;39(5):814-9.

14. van Nielen M, Feskens EJ, Rietman A, Siebelink E, Mensink M. Partly replacing meat protein with soy protein alters insulin resistance and blood lipids in postmenopausal women with abdominal obesity. J Nutr. 2014;144(9):1423-9.

15. Tang M, Leidy HJ, Campbell WW. Regional, but not total, body composition changes in overweight and obese adults consuming a higher protein, energy-restricted diet are sex specific. Nutr Res. 2013;33(8):629-35.

16. Luscombe-Marsh ND, Noakes M, Wittert GA, Keogh JB, Foster P, Clifton PM. Carbohydrate-restricted diets high in either monounsaturated fat or protein are equally effective at promoting fat loss and improving blood lipids. Am J Clin Nutr. 2005;81(4):762-72.

17. Farnsworth E, Luscombe ND, Noakes M, Wittert G, Argyiou E, Clifton PM. Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. Am J Clin Nutr. 2003;78(1):31-9.

18. Douchi T, Yamamoto S, Nakamura S, Ijuin T, Oki T, Maruta K, et al. The effect of menopause on regional and total body lean mass. Maturitas. 1998;29(3):247-52.

19. Creatsas G, Christodoulakos G, Lambrinoudaki I. Cardiovascular disease: screening and management of the a-symptomatic high-risk post-menopausal woman. Maturitas. 2005;52 Suppl 1:S32-7.

20. Clifton PM, Bastiaans K, Keogh JB. High protein diets decrease total and abdominal fat and improve CVD risk profile in overweight and obese men and women with elevated triacylglycerol. Nutr Metab Cardiovasc Dis. 2009;19(8):548-54.

21. Kelley DE, Thaete FL, Troost F, Huwe T, Goodpaster BH. Subdivisions of subcutaneous abdominal adipose tissue and insulin resistance. Am J Physiol Endocrinol Metab. 2000;278(5):E941-8.

22. Goodpaster BH, Krishnaswami S, Resnick H, Kelley DE, Haggerty C, Harris TB, et al. Association between regional adipose tissue distribution and both type 2 diabetes and impaired glucose tolerance in elderly men and women. Diabetes Care. 2003;26(2):372-9.

23. Lalia AZ, Dasari S, Johnson ML, Robinson MM, Konopka AR, Distelmaier K, et al. Predictors of Whole-Body Insulin Sensitivity Across Ages and Adiposity in Adult Humans. J Clin Endocrinol Metab. 2016;101(2):626-34.

24. Ryberg M, Sandberg S, Mellberg C, Stegle O, Lindahl B, Larsson C, et al. A Palaeolithic-type diet causes strong tissue-specific effects on ectopic fat deposition in obese postmenopausal women. J Intern Med. 2013;274(1):67-76.

25. Soenen S, Martens EA, Hochstenbach-Waelen A, Lemmens SG, Westerterp-Plantenga MS. Normal protein intake is required for body weight loss and weight maintenance, and elevated protein intake for additional preservation of resting energy expenditure and fat free mass. J Nutr. 2013;143(5):591-6.

26. Nordmann AJ, Nordmann A, Briel M, Keller U, Yancy WS, Jr., Brehm BJ, et al. Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. Arch Intern Med. 2006;166(3):285-93.

27. Bueno NB, de Melo IS, de Oliveira SL, da Rocha Ataide T. Very-low-carbohydrate ketogenic diet v. low-fat diet for long-term weight loss: a meta-analysis of randomised controlled trials. Br J Nutr. 2013;110(7):1178-87.

28. Sacks FM, Bray GA, Carey VJ, Smith SR, Ryan DH, Anton SD, et al. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. N Engl J Med. 2009;360(9):859-73.

29. Krieger JW, Sitren HS, Daniels MJ, Langkamp-Henken B. Effects of variation in protein and carbohydrate intake on body mass and composition during energy restriction: a meta-regression 1. Am J Clin Nutr. 2006;83(2):260-74.

30. Hochstenbach-Waelen A, Veldhorst MA, Nieuwenhuizen AG, Westerterp-Plantenga MS, Westerterp KR. Comparison of 2 diets with either 25% or 10% of energy as casein on energy expenditure, substrate balance, and appetite profile. Am J Clin Nutr. 2009;89(3):831-8.

31. Lejeune MP, Westerterp KR, Adam TC, Luscombe-Marsh ND, Westerterp-Plantenga MS. Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety, and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber. Am J Clin Nutr. 2006;83(1):89-94.

32. Weigle DS, Breen PA, Matthys CC, Callahan HS, Meeuws KE, Burden VR, et al. A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. Am J Clin Nutr. 2005;82(1):41-8.

33. Westerterp-Plantenga MS, Nieuwenhuizen A, Tome D, Soenen S, Westerterp KR. Dietary protein, weight loss, and weight maintenance. Annu Rev Nutr. 2009;29:21-41.

34. Luscombe ND, Clifton PM, Noakes M, Parker B, Wittert G. Effects of energy-restricted diets containing increased protein on weight loss, resting energy expenditure, and the thermic effect of feeding in type 2 diabetes. Diabetes Care. 2002;25(4):652-7.

35. Westerterp-Plantenga MS, Lemmens SG, Westerterp KR. Dietary protein - its role in satiety, energetics, weight loss and health. Br J Nutr. 2012;108 Suppl 2:S105-12.

36. Yang D, Liu Z, Yang H, Jue Y. Acute effects of high-protein versus normal-protein isocaloric meals on satiety and ghrelin. Eur J Nutr. 2014;53(2):493-500.

37. Dougkas A, Ostman E. Protein-Enriched Liquid Preloads Varying in Macronutrient Content Modulate Appetite and Appetite-Regulating Hormones in Healthy Adults. J Nutr. 2016;146(3):637-45.

38. Beasley JM, Katz R, Shlipak M, Rifkin DE, Siscovick D, Kaplan R. Dietary protein intake and change in estimated GFR in the Cardiovascular Health Study. Nutrition. 2014;30(7-8):794-9.

39. Vissers D, Hens W, Taeymans J, Baeyens JP, Poortmans J, Van Gaal L. The effect of exercise on visceral adipose tissue in overweight adults: a systematic review and meta-analysis. PLoS One. 2013;8(2):e56415.

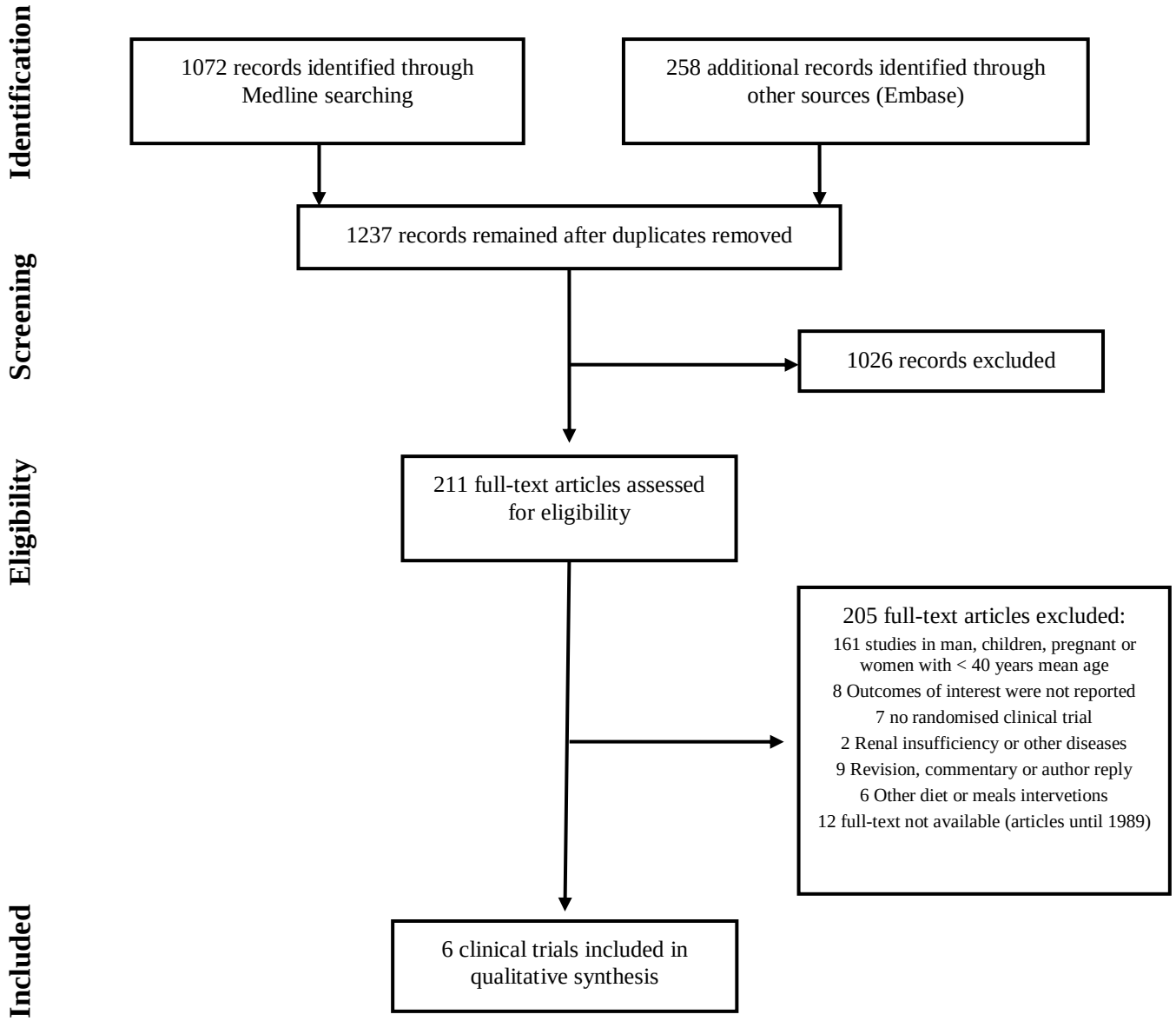

Fig. 1. Flow chart of the literature search and the study selection process

Table 1. Characteristics of randomized controlled clinical trials of abdominal obesity outcomes included in review

Author Year Reference	Country	Study design Follow-up	Baseline Sample	Final sample	Inclusion criteria	Mean age HP diet (year ± SD)	Mean Age Control diet (year ± SD)	Measurement of body composition
Muscariello (2016) (12)	Italy	Parallel 12 weeks	HP: 54 CD: 50	HP: 54 CD: 50	BMI >30 kg/m^2 >65 years	66.9 ± 5.2	66.4 ± 4.5	BIA
Stomby (2015) (13)	Sweden	Parallel 2 years	70	HP: 27 CD: 22	BMI >27 kg/m^2 postmenopausal women	59.5 ± 5.5	60.3 ± 5.9	DXA
van Nielen (2014) (14)	Holland	Crossover 4 weeks Washout 4 weeks	15	15	WC >80 cm postmenopausal women	61.0 ± 5.0	61.0 ± 5.0	DXA
Tang (2013) (15)	USA	Parallel 12 weeks	54	HP: 22 CD: 23	BMI 25-39.9 kg/m^2 Women >21 years	46.0 ± 2.0	53.0 ± 3.0	DXA
Luscombe (2005) (16)	Australia	Parallel 16 weeks	HP: 23 CD: 24	HP: 15 CD: 17	BMI 27-40 kg/m^2 Women, 20-60 years	53.0 ± 2.0	48.0 ± 3.0	DXA
Farnsworth (2003) (17)	Australia	Parallel 16 weeks	No data	HP: 21 CD: 22	BMI 27-43 kg/m^2 Women, 20-65 years	50.6 ± 2.1	50.6 ± 2.1	DXA

HP: high protein diet; CD: Control diet; BMI: body mass index; WC: waist circumference; BIA: electrical bioimpedance; DXA: dual-energy X-ray absorptiometry.

Table 2. Baseline weight, waist circumference, abdominal fat mass and fat mass with high protein diet vs. control diet in middle-aged women

Author Year Reference	Body Weigh (kg)		WC (cm)		AFM (kg)		Fat Mass	
	HP diet	Control Diet	HP diet	Control Diet	HP diet	Control Diet	HP diet	Control Diet
Muscariello (2016) (12)	No data		97.5 ± 4.8	98.5 ± 5.0	No data		34.2 ± 4.3 kg	34.8 ± 4.3 kg
Stomby (2015) (13)	87.8 ± 10.6	86.8 ± 10.0	105.4 ± 10.0	104.7 ± 10.4	No data		39.8 ± 7.2 kg	40.9 ± 8.6 kg
van Nielen (2014) (14)	69.1 ± 12.3	69.7 ± 12.9	90 ± 10		10.2 ± 2.4	9.7 ± 2.9	35.0 ± 7.0 %	35.0 ± 7.0 %
Tang (2013) (15)	84 ± 2	83 ± 2	No data		No data		37.0 ± 1.3 kg	37.0 ± 1.3 kg
Luscombe (2005) (16)	90.5 ± 2.8	90.0 ± 2.4	No data		10.1 ± 0.5	10.4 ± 0.3	42.5 ± 2.0 kg	42.4 ± 2.0 kg
Farnsworth (2003) (17)	89.1 ± 2.2	88.1 ± 2.3	No data		15.4 ± 0.5	15.6 ± 0.6	42.7 ± 2.0 kg	43.2 ± 1.3 kg

WC: waist circumference; AFM: abdominal fat mass; HP: high protein diet; CD: Control diet; BIA: electrical bioimpedance; DXA: dual-energy X-ray absorptiometry; BMI: body mass index.

Table 3. Assessment of risk of bias

	Selection bias	Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias	
	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Dietary compliance assessed
Muscariello et al., 2016	Low	Low	Unclear	Low	Low	Low	Low
Stomby et al., 2015	Low	Unclear	Unclear	Low	Low	Low	Low
van Nielen et al., 2014	Low	Low	Unclear	Low	Low	Low	Low
Tang et al., 2013	Low	Unclear	High	Unclear	Low	Low	Low
Luscombe et al., 2005	Low	Unclear	High	Unclear	Low	Low	Low
Farnsworth et al., 2003	Low	Unclear	High	Unclear	Low	Low	Low

Table 4. Dietary and nutrition intake of study participants

Author Year Reference	Definition of HP diet	Definition of Control diet	Nutrition intake at endpoint HP	Nutrition intake at endpoint CD
Muscariello (2016) (12)	Pro: 1.2 g/kg BW 20–25 kcal/kg BW, good sources of proteins low in fat, including lean meat, poultry, and fish, were recommended	Pro: 0.8 g/kg BW	No data	No data
Stomby (2015) (13)	Pro: 30 % Carb: 30 % Fat: 40 % ad libitum Paleolithic Diet	Pro: 15 % Carb: 55-60 % Fat: 25-30 % ad libitum NNR	No data	No data
van Nielen (2014) (14)	HP diet of mixed, non-soy sources Energy intake to keep body weight stable	HP diet of mixed sources including soy (30 g/day)	Pro: 1.7 ± 0.3 g/kg BW Carb: 263 ± 57g Fat: 67 ± 14g 2174.9 ± 430.0 Kcal	Pro: 1.6 ± 0.3 g/kg BW Carb: 274 ± 55g Fat: 63 ± 12g 2152.0 ± 406.3 Kcal
Tang (2013) (15)	Pro: 1.4 g/kg BW 750 kcal/day less than energy needs, 1 multivitamin/mineral supplement and 2 calcium citrate tablets (800 mg/day)	Pro: 0.8 g/kg BW Lactovegetarian	Pro: 1.52 ± 0.02 g/kg BW Carb: 172 ± 6 g Fat: 42 ± 2 g 1540 ± 60 Kcal	Pro: 0.92 ± 0.02 g/kg BW Carb: 214 ± 6g Fat: 36 ± 2g 1500 ± 40 Kcal
Luscombe (2005) (16)	Pro: 40 % (136 g/day) Carb: 30 % Fat: 30 % LF-HP diet 12 weeks of energy restriction (1400 ± 23 kcal) and 4 weeks of energy balance (1800 ± 72 kcal)	Pro: 20 % (67 g/day) Carb: 30 % Fat: 50 % HF-SP diet	Pro: 33 ± 0.5 % Carb: 36 ± 1 % Fat: 29 ± 1 % 1806.9 ± 64 Kcal	Pro: 18 ± 0.4 % Carb: 45 ± 1 % Fat: 36 ± 1 % 1714.9 ± 78.1 Kcal
Farnsworth (2003) (17)	Pro: 30 % (110 g/day) Carb: 40 % Fat: 30 % 12 weeks of energy restriction (1400 to 1500 kcal) and 4 weeks of energy balance (2000 kcal)	Pro: 15 % (60 g/day) Carb: 55 % Fat: 30 %	Pro: 27.3 ± 0.3 % Carb: 44.6 ± 0.5 % Fat: 26.9 ± 0.5 % 1912.0 ± 48 Kcal	Pro: 15.4 ± 0.3 % Carb: 56.9 ± 0.3 % Fat: 27.5 ± 0.4 % 1959.8 ± 48 Kcal

HP: high protein diet; CD: Control diet; Pro: protein intake; Carb: Carbohydrate intake, Fat: Fat intake; NNR: Nordic nutrition recommendations; BW: body weight; LF-HP: low-fat, high-protein; HF-SP: high-fat, standard-protein.

Table 5. Changes in weight, waist circumference, abdominal fat mass and fat mass with high protein diet vs. control diet in middle-aged women

	Body Weigh (kg)			WC (cm)			Fat Mass (kg)			AFM (kg)		
Author Year Reference	**HP (delta ± SD)**	**CD (delta ± SD)**	***P****	**HP (delta ± SD)**	**CD (delta ± SD)**	***P****	**HP (delta ± SD)**	**CD (delta ± SD)**	***P****	**HP (delta ± SD)**	**CD (delta ± SD)**	***P****
Muscariello (2016) (12)	No date			-1.9 ± 4.1†	-2.8 ± 4.3†	>0.05	-2.4 ± 3.4†	-2.2 ± 3.4†	>0.05	No data		
Stomby (2015) (13)	6 months: -9.0 ± 11.6†	6 months: -4.7 ± 8.4†	**≤0.01**	6 months: -12.4 ± 11.8†	6 months: -7.6 ± 9.5†	>0.05	6 months: -7.6 ± 5.6†	6 months: -4.5 ± 3.7†	**<0.05**	No data		
	24 months: -7.9 ± 11.8†	24 months: -4.7 ± 8.7†	>0.05	24 months: -12.0 ± 12.5†	24 months: -10.0 ± 9.0†	>0.05	24 months: -7.7 ± 5.6†	24 months: -4.7 ± 3.9†	>0.05			
van Nielen (2014) (14)	-0.5 ± 12.3†	-0.5 ± 12.8†	0.610	No data			-0.8 ± 4.8†	-0.8 ± 4.9†	0.100	-0.9 ± 2.5†	-0.5 ± 2.8†	0.130
Tang (2013) (15)	-8.0 ± 6.9†	-9.6 ± 10.6†	**<0.05**	No data			-6.5 ± 5.5†	-6.7 ± 10.5†	>0.05	No data		
Luscombe (2005) (16)	-7.8 ± 4.9†	-7.9 ± 4.7†	>0.05	No data			-4.3 ± 3.0†	-4.8 ± 3.1†	>0.05	-1.2 ± 0.2†	-1.1 ± 0.4†	**<0.05**
Farnsworth (2003) (17)	-6.6 ± 3.6†	-7.4 ± 4.4†	>0.05	No data			-6.6 ± 3.6†	-7.1 ± 4.0†	>0.05	-2.9 ± 1.5†	-2.9 ± 1.6†	>0.05

WC: waist circumference; AFM: abdominal fat mass; HP: high protein diet; CD: Control diet;
†Significant change from baseline (*P*<0.05);
**P-values* between the HP diet and control

Appendix 1

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	16
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	17
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	18
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	18
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	19
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	19
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	19-20
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Appendix 2
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	19-20
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	19-20

Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	19-20
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	20, Table 2
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	19-20
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	19-20

Appendix 2

Relevant studies, published before June 16, 2016 (date last searched), were identified through electronic searches not limited to the English language using MEDLINE and EMBASE. Electronic searches were supplemented by scanning reference lists of articles identified for all relevant studies (including review articles), by hand searching of relevant journals and by correspondence with study investigators. The computer-based searches combined search terms to high protein diet and abdominal obesity.

("Diet, Paleolithic"[Mesh] OR "paleolithic diet" OR "high protein" OR “low-carb” OR “carbohydrate restricted diet” OR “carbohydrate restricted diets”)

AND

("Abdominal Fat"[Mesh] OR "Waist-Hip Ratio"[Mesh] OR "Waist Circumference"[Mesh] OR "Obesity, Abdominal"[Mesh] OR “abdominal obesity” OR “waist-circumference” OR “waist-hip-ratio” OR obes* OR waist*)

CAPÍTULO 2

Skeletal muscle mass is associated with higher dietary protein intake and lower body fat in postmenopausal women: a cross-sectional study

Running title: Skeletal muscle mass in post menopause

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ABSTRACT:

Objective: Declines in endogenous estrogen production during the menopausal transition have been associated with skeletal muscle mass loss. Evidence suggests that older people may require more dietary protein than younger people to prevent this loss. We investigated the association between skeletal muscle mass and dietary protein intake, habitual physical activity, body composition, and metabolic variables.

Methods: One hundred and three healthy postmenopausal women (age 55.2 ± 4.9 years, time since menopause 6.8 ± 1.0 years, BMI 27.2 ± 4.6 kg/m^2) were enrolled. Protein intake was measured by validated food frequency questionnaire and categorized into tertiles: ≤0.93 g/kg body weight (BW), 0.94–1.29 g/kg BW, and ≥1.3 g protein/kg BW.

Results: The prevalence of low lean mass, defined as skeletal muscle mass index (SMI, appendicular lean mass standardized to BMI) <0.512, was 7%. Waist circumference, % body fat, trunk fat mass, and diastolic blood pressure were higher, whereas SMI and mean daily steps were lower in women with protein intake lower than 0.93 g/kg BW. SMI was positively correlated with physical activity (r=0.205, *P*=0.038) and protein intake (r=0.334, *P*=0.001), and negatively correlated with waist circumference (r=-0.505, *P*<0.001) and % body fat (r=-0.808, *P*<0.001). In linear regression analysis, adjusted for age, time since menopause, previous smoking behavior and energy intake, there was an independent, positive contribution of protein intake (*P*=0.017) and an independent, negative contribution of % body fat (*P*<0.001) to SMI.

Conclusion: In our healthy postmenopausal women, SMI was positively associated with protein intake, and negatively associated with % body fat.

Keywords: menopause; dietary protein, skeletal muscle mass index; body composition.

INTRODUCTION

Declines in endogenous estrogen production during the menopausal transition have been associated with skeletal muscle mass loss in postmenopausal women.[1,2] An absolute cumulative six-year decrease in skeletal muscle mass of about 0.23 kg has been described for perimenopausal African-American and Caucasian women.[3]

The most usual method to estimate muscle mass is whole-body dual-energy X-ray absorptiometry (DXA). Appendicular lean mass (ALM) derived from DXA scans, which represents the sum of lean tissue in the arms and legs, may be scaled to height squared or body mass index (BMI), and is often used to estimate muscle mass in sarcopenia research.[4] The age-related loss of skeletal muscle mass is associated with the prevalence of sarcopenia, which may be as high as 30% after 60 years of age, and continues to progress.[5]

Recently, low ALM/BMI ratio has been recommended as a potential criterion to indicate clinically relevant low lean mass in older men and women. While a cut-off value of <0.512 for women has been suggested as a predictor of incident mobility impairment,[6] additional studies are needed on different populations in order to confirm these findings.

Many different strategies have been proposed to maintain and regain muscle mass in older people and specifically in postmenopausal women, such as physical activity, diet, and pharmacological interventions. Exercise alone may improve body composition, especially skeletal muscle mass in postmenopausal women.[7,8] Resistance exercise and adequate protein intake are the current most effective means to limit the loss of muscle mass with aging.[9]

In this sense, evidence suggests that older people may require more dietary protein than do younger people.[10] The impaired ability of aging muscle to activate protein synthesis in response to anabolic stimuli, such as exercise and protein intake, could be associated with insulin resistance.[11-13] The current protein recommended dietary allowance (RDA) is 0.8 g/kg body weight (BW);[14] however, the adequacy of this recommendation has been recently questioned, since this amount might not be enough to maintain lean mass and to prevent functional decline among the elderly.[15,16] The PROT-AGE study group has recommended an average daily protein intake in the range of 1.0 to 1.2 g/kg body weight (BW) for older people.[10] Specifically in postmenopausal women, higher protein intake (1.2 g/kg BW, or about 16% of total energy) was associated with a 32% lower risk of frailty and better physical function in the Women's Health Initiative.[17,18]

Despite the recognized importance of preventing muscle mass loss during senescence, only a few studies have focused on the influence of diet on muscle mass in postmenopausal women. Therefore, the aim of this study was to investigate the association between skeletal muscle mass and dietary protein intake, habitual physical activity, body composition, and metabolic variables in healthy postmenopausal women.

METHODS

Participants and design

In this cross-sectional study, participants were invited by advertisement in local newspapers and radio stations to come to the Gynecological Endocrinology Unit

at Hospital de Clínicas de Porto Alegre, Brazil, from October 2010 to February 2012. Inclusion criteria were as follows: 1) menopause, defined as last menstrual period at least 1 year before the beginning of the study plus follicle stimulating hormone (FSH) levels > 35 IU/L; 2) age between 45 and 65 years; and 3) no use of hormone therapy in the past 3 months. Individuals with diabetes or previous diagnosis of heart disease and current smokers were excluded. One hundred and nineteen postmenopausal women fulfilling all the inclusion criteria were consecutively enrolled, but 103 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 five participants dropped out because they were unable to commit to the study (no time for blood collection and indirect calorimetry). The local Ethics Committee approved the study protocol, and written informed consent was obtained from every participant.

Anthropometric measurements, body composition and resting metabolic rate

Anthropometric measurements were performed in duplicate and included body weight, height, and waist circumference, measured in the standing position. Waist circumference was measured at the midpoint between the lower rib margin and

the iliac crest, perpendicular to the long axis of the body.[19] BMI was calculated by dividing body weight (kg) by the square of the height (m).

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). BMD was measured in lumbar spine (L1-L4), and femoral neck, and expressed as g/cm^2.[20] Skeletal muscle mass index (SMI) is ALM standardized to BMI (ALM/BMI). The cut-off value for low lean mass was <0.512, as described in the FNIH study.[6] Resting metabolic rate (RMR) was obtained by indirect calorimetry (Fitmate®, Cosmed, Rome, Italy).

Dietary assessment

Dietary intake in the previous month was assessed with a validated food frequency questionnaire consisting of 120 items.[21] Nutritional composition was calculated using the Brazilian Table of Food Composition.[22] Vitamin D, E, and A were assessed using the United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference.[23] Food group portions were calculated according to the Brazilian Nutrition Guide,[24] including fruits, vegetables, beans, whole grain, refined grain, processed meats, meat and eggs, sweets and desserts, and dairy foods.

Protein intake was measured as g/kg BW and categorized into tertiles according to the following cut-off points: ≤0.93 g/kg BW, 0.94–1.29 g/kg BW, and ≥1.3 g protein/kg BW.

Physical activity assessment

Assessment of habitual physical activity was performed with a digital pedometer (BP 148, Tech Line, São Paulo, Brazil).[25-27] The device was configured individually according to weight (kg) and individual step length. The equipment was used for six consecutive days, providing the weekly average number of steps. Subjects were encouraged not to change their physical activity habits during the study.

Blood pressure and biochemical and hormone tests

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). Blood samples were collected after a 12-hour fast. All samples were obtained between 8 AM and 10 AM. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were determined by colorimetric–enzymatic methods (Bayer 1800 Advia System, Deerfield, IL, USA), with intra- and interassay coefficients of variation (CVs) < 3%. Glucose was determined by the hexokinase method (Advia 1800) with intra-assay CV <3.4% and interassay CV < 2.1%. High-sensitivity C-reactive protein (hs-CRP) was assayed using stored specimens. A validated high-sensitivity nephelometric method (Dade Behring Marburg, Marburg, Germany) was used for us-CRP analysis, with sensitivity of 0.17 mg/L and intra and interassay CVs of 4.4% and 5.7% respectively. Individual results below the limit of sensitivity were considered as equal to 0.17 mg/L. FSH was measured by chemiluminescence

immunoassay (CentaurXP, Roche Diagnostics, Mannheim, Germany), with sensitivity of 0.3 IU/L and intra- and interassay CVs of 2.9% and 2.7% respectively. Total testosterone levels were also measured by chemiluminescence immunoassay (Centaur XP) with sensitivity of 10 ng/mL and intra- and interassay CVs of 3.3% and 7.5% respectively. SHBG was measured by chemiluminescence enzyme immunoassay (Immulite 2000, Centaur XP, Roche Diagnostics, Mannheim, Germany), with sensitivity of 0.02 nmol/L and intra- and interassay CVs of 5.3% and 6.6% respectively. Serum insulin levels were measured using chemiluminescence immunoassays (Centaur XP), with a sensitivity of 0.200 *μ*IU/mL and intra- and interassay CVs of 2.0% and 4.3% respectively. Free androgen index was estimated by dividing total testosterone (in nanomoles per liter) by SHBG (in nanomoles per liter) × 100. Low-density lipoprotein (LDL) cholesterol was determined indirectly using the Friedewald formula: LDL = total cholesterol − HDL − (triglycerides/5). Homeostasis model assessment (HOMA) was calculated by multiplying insulin (*μ*IU/mL) by glucose (mmol/L) and dividing this product by 22.5, as previously described.[28] Lipid accumulation product (LAP) was also calculated (waist-58 × triglycerides [nmol/L]).[29]

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. The Kolmogorov-Smirnov test was used to confirm the normal distribution of the key variables. Analysis of variance (ANOVA) was used to

compare the differences between means of parametric data. Variables with non-Gaussian distribution were log-transformed for statistical analysis and back-transformed in their original units for data presentation. Pairwise comparisons of group means were performed with Bonferroni's post hoc test. Difference in micronutrients intake was measured controlling for the effects of total energy intake by analysis of covariance (ANCOVA). χ^2 test was used for comparisons of dichotomous variables. The degree of linear dependence between protein intake (g/Kg BW) body composition, and physical activity was analyzed by Pearson's correlation coefficient. A multiple linear regression model was also developed to explore the relationship between SMI as a dependent variable and protein intake, % of body fat, and physical activity as independent variables, adjusted for age, time since menopause, previous smoking behavior and energy intake. Collinearity was estimated for the model using variance inflation factors and tolerances for individual variables. No collinearity was found. 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 $P < 0.05$.

Results

The mean age of participants was 55.2 ± 4.9 years, the mean time since menopause was 6.8 ± 1.0 years, and mean BMI was 27.2 ± 4.6 kg/m^2. The prevalence of low lean mass, defined as SMI <0.512, was 7%. Mean energy intake was 1,865 ± 622 kcal/day; total protein intake was 76.9 ± 26.6 g, which constituted 17% of total energy intake and corresponded to 1.2 g/kg BW. The minimum protein intake reported was 0.38, and the maximum was 2.46 g/kg BW.

Table 1 presents demographic and clinical characteristics and body composition of participants according to tertiles of protein intake (≤ 0.93, 0.94-1.29, and ≥ 1.3 g/kg BW). No differences were observed between the groups regarding age, time since menopause, skin color, and BMD in all sites. However, waist circumference, % body fat, % trunk fat, RMR and diastolic blood pressure were higher, whereas years at school, SMI and mean daily steps were lower in women with protein intake lower than 0.93 g/kg BW.

Table 2 shows metabolic and hormonal variables according to protein intake tertiles. Lipid profile, glucose, insulin, HOMA values and prevalence of metabolic syndrome did not differ significantly between the groups and hs-CRP and LAP were higher in the lowest tertile. Androgens, estradiol, and FSH levels, as well as the proportion of previous users of hormone therapy were also similar in the three groups.

There were no differences in carbohydrate and fat intake between the three groups. Women in the higher protein intake tertile consumed a diet with higher total energy. After adjusted for total kcal intake, the highest protein intake tertile group consumed more cholesterol, calcium, iron, zinc, selenium, folato and vitamin B-12. Consumption of fruits, vegetables, beans, whole grain, dairy foods, eggs and meat, especially chicken and fish, were also higher in the group with protein intake ≥1.3 g/kg BW (Table 3).

SMI was positively correlated with physical activity (r=0.205, *P*=0.038) and protein intake (r=0.334 and *P*=0.001). Negative correlations were observed between SMI and waist circumference (r=-0.505, *P*<0.001) and % body fat (r=-0.808, *P*<0·001) (Figure 1).

A multiple linear regression model was set up with SMI as the dependent variable to test the hypothesis that protein intake, % body fat, and mean steps a day might be influencing muscle mass. As shown in Table 4, there was an independent, positive contribution of protein intake (P=0.017) and an independent, negative contribution of % body fat to SMI (P<0.001), the model was adjusted for age, time since menopause, previous smoking behavior and energy intake.

Discussion

In the present study, SMI was positively associated with dietary protein intake, and negatively associated with % body fat in apparently healthy postmenopausal women, most of them not presenting impaired muscle mass or osteoporosis. Moreover, women in the higher tertile of dietary protein intake presented better metabolic profile and healthier nutritional choices. In addition, these postmenopausal women also had lower BMI, waist circumference, and trunk fat mass. Only a few studies have examined the association of body composition variables, BMD, and dietary protein intake with muscle mass specifically in postmenopausal women.[30,31] A previous cohort study[32] has also reported positive associations between high protein intake and skeletal muscle mass and muscle strength. However, the sample included in that study was older than our sample, and many of the participants had sarcopenia. Tyrovolas and co-workers[33] investigated the role of various determinants on low skeletal muscle mass, sarcopenia, and sarcopenic obesity among older populations in countries at different stages of the socio-economic, nutritional, and epidemiological transition. Lower socio-economic status and higher % body fat were associated with low

muscle mass and sarcopenia in almost all the countries studied, which are in agreement with our study.

Interestingly, in the present study, participants in the higher protein intake group were found to have a lower RMR, possibly due to their lower BMI in comparison to lowest tertile. Evidence suggests that increased adipose tissue and the consequent increase in leptin secretion may translate into higher energy expenditure and curtail hunger mechanisms, leading to weight control.[34]

The mechanisms involved in the progressive loss of muscle mass during senescence are not entirely known. It is believed that reduced response to anabolic stimuli is responsible for the age-related decline in skeletal muscle mass. A recent report has shown that the activation of the mammalian target of rapamycin complex 1 (mTORC1), an essential site of integration for anabolic signals that stimulate muscle protein synthesis, is reduced in skeletal muscle of older individuals after protein ingestion compared with young subjects, which could be due to an altered anabolic sensitivity.[13] Furthermore, it has been suggested that an accelerated loss of muscle mass and strength occurs at an earlier age in women than in men, around the time of menopause.[35] Menopause-associated decline in hormonal levels is thought to be implicated in this process.[2] However, in our population, estradiol at menopausal levels did not seem to influence the differences we found in skeletal muscle mass according to protein intake tertiles, since no differences in estradiol levels were found among groups.

Considering androgens exert a well-known anabolic effect on muscle mass,[36] we assessed whether DHEAS, total testosterone levels or free androgen index could be influencing on SMI in the postmenopausal participants. However, no

differences on androgen levels were found between groups, in agreement with the notion of progressive decrease of the androgen secretion across the postmenopausal years.[37]

Protein intake higher than the current RDA (0.8 g/kg BW) has been associated with better SMI among postmenopausal women.[32] There is increasing evidence that the current daily allowance (0.8 g/kg BW) of protein is insufficient to promote and maintain muscle mass in the elderly.[10,16,38] In the Health, Aging, and Body Composition study, dietary protein was inversely associated with loss of lean mass.[39] Hence, dietary interventions targeting protein intake above the current RDA are a viable approach to treating sarcopenia.[40] The mean protein intake in our sample was 1.2 g/kg BW, representing a 50% increase over the current RDA. Probably as a result of this high mean intake, along with the mean age (55.2 ± 4.9 years) of our postmenopausal women, the prevalence of low muscle mass in our sample was only 7%. However, loss of muscle mass has been shown to be accelerated throughout the postmenopausal period, and women in this stage may benefit from preventive interventions.[41] Further, in our cross-sectional findings, women in the higher protein intake tertile had better body composition, waist circumference, % body fat, and trunk fat mass, even if they consumed more calories. A systematic review with meta-regression showed that low-carbohydrate, high-protein diets favorably affect body mass and composition independent of energy intake, which in part supports the proposed metabolic advantage of these diets.[42] Kidney function and protein intake have become a reason for concern in the past years, but recent data (mean follow-up 6.4 ± 1.2

years) suggest that higher protein intake does not have a major effect on kidney function decline among elderly men and women.[43]

Other nutrients associated with higher protein intake which could be associated with SMI were magnesium,[44] and selenium,[45] but in the simple linear regression analysis (data not shown) only protein intake remained associated with SMI. Therefore, as previously observed, protein intake is the main predictor of muscle protein synthesis.[46] Essential amino acids are especially apt to stimulate muscle protein synthesis.[47] Food sources of these amino acids include meat, eggs, and dairy foods, which, as expected, were consumed in higher amounts by women in the higher protein intake category. However, an interesting finding was that the consumption of beans, fruits, vegetables, and whole foods was also higher in this group, which overall had a healthier diet.

Diastolic blood pressure, LAP and hsCRP were lower in women in the higher protein intake tertile. Indeed, these women also present lower BMI and waist circumference and higher mean steps/day reflecting a better metabolic *status* and general healthier lifestyle preferences. Additionally, in a systematic review, higher protein diets were found to improve blood pressure, but a meta-regression analysis showed no significant dose response with higher protein intake. Therefore, the mechanism underlying the specific association between higher protein intake and blood pressure remains unclear.[48]

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 SMI, higher protein intake and more favorable body composition and metabolic parameters. Limitations include the

cross-sectional design, which precludes conclusions regarding the direction of cause and effect. Also, although pedometers are increasingly used to estimate habitual physical activity, they are not sensitive to the intensity or the type of the activity performed, and therefore may not accurately depict the loading forces of the activities performed, an important aspect for skeletal muscle mass improvement. Lean mass is a poor predictor of functional outcomes compared with low grip strength.[6]

CONCLUSIONS

In conclusion, in our healthy postmenopausal women, SMI was positively associated with protein intake, and negatively associated with % body fat. Dietary intervention studies are necessary to clarify the effect of higher protein intake on body composition and functional outcomes throughout the postmenopausal period.

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REFERENCES

1. Rolland YM, Perry HM, 3rd, Patrick P, Banks WA, Morley JE. Loss of appendicular muscle mass and loss of muscle strength in young postmenopausal women. *J Gerontol A Biol Sci Med Sci.* Mar 2007;62(3):330-335.
2. Maltais ML, Desroches J, Dionne IJ. Changes in muscle mass and strength after menopause. *J Musculoskelet Neuronal Interact.* Oct-Dec 2009;9(4):186-197.
3. Sowers M, Zheng H, Tomey K, et al. Changes in body composition in women over six years at midlife: ovarian and chronological aging. *J Clin Endocrinol Metab.* Mar 2007;92(3):895-901.
4. Cawthon PM. Assessment of Lean Mass and Physical Performance in Sarcopenia. *Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry.* Oct-Dec 2015;18(4):467-471.
5. Doherty TJ. Invited review: Aging and sarcopenia. *Journal of applied physiology.* Oct 2003;95(4):1717-1727.
6. McLean RR, Shardell MD, Alley DE, et al. Criteria for clinically relevant weakness and low lean mass and their longitudinal association with incident mobility impairment and mortality: the foundation for the National Institutes of Health (FNIH) sarcopenia project. *J Gerontol A Biol Sci Med Sci.* May 2014;69(5):576-583.
7. Figueroa A, Going SB, Milliken LA, et al. Effects of exercise training and hormone replacement therapy on lean and fat mass in postmenopausal women. *J Gerontol A Biol Sci Med Sci.* Mar 2003;58(3):266-270.

8. Teixeira PJ, Going SB, Houtkooper LB, et al. Resistance training in postmenopausal women with and without hormone therapy. *Medicine and science in sports and exercise.* Apr 2003;35(4):555-562.

9. Breen L, Phillips SM. Interactions between exercise and nutrition to prevent muscle waste during ageing. *British journal of clinical pharmacology.* Mar 2013;75(3):708-715.

10. Bauer J, Biolo G, Cederholm T, et al. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. *J Am Med Dir Assoc.* Aug 2013;14(8):542-559.

11. Walrand S, Guillet C, Salles J, Cano N, Boirie Y. Physiopathological mechanism of sarcopenia. *Clinics in geriatric medicine.* Aug 2011;27(3):365-385.

12. Markofski MM, Dickinson JM, Drummond MJ, et al. Effect of age on basal muscle protein synthesis and mTORC1 signaling in a large cohort of young and older men and women. *Experimental gerontology.* May 2015;65:1-7.

13. Francaux M, Demeulder B, Naslain D, et al. Aging Reduces the Activation of the mTORC1 Pathway after Resistance Exercise and Protein Intake in Human Skeletal Muscle: Potential Role of REDD1 and Impaired Anabolic Sensitivity. *Nutrients.* 2016;8(1).

14. Trumbo P, Schlicker S, Yates AA, Poos M, Food, Nutrition Board of the Institute of Medicine TNA. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *J Am Diet Assoc.* Nov 2002;102(11):1621-1630.

15. Fulgoni VL, 3rd. Current protein intake in America: analysis of the National Health and Nutrition Examination Survey, 2003-2004. *Am J Clin Nutr.* May 2008;87(5):1554S-1557S.

16. Nowson C, O'Connell S. Protein Requirements and Recommendations for Older People: A Review. *Nutrients.* Aug 2015;7(8):6874-6899.

17. Beasley JM, LaCroix AZ, Neuhouser ML, et al. Protein intake and incident frailty in the Women's Health Initiative observational study. *J Am Geriatr Soc.* Jun 2010;58(6):1063-1071.

18. Beasley JM, Wertheim BC, LaCroix AZ, et al. Biomarker-calibrated protein intake and physical function in the Women's Health Initiative. *J Am Geriatr Soc.* Nov 2013;61(11):1863-1871.

19. Donato GB, Fuchs SC, Oppermann K, Bastos C, Spritzer PM. Association between menopause status and central adiposity measured at different cutoffs of waist circumference and waist-to-hip ratio. *Menopause.* Mar-Apr 2006;13(2):280-285.

20. Silva TR, Franz R, Maturana MA, Spritzer PM. Associations between body composition and lifestyle factors with bone mineral density according to time since menopause in women from Southern Brazil: a cross-sectional study. *BMC endocrine disorders.* 2015;15:71.

21. Zanolla AF, Olinto MT, Henn RL, Wahrlich V, Anjos LA. [Assessment of reproducibility and validity of a food frequency questionnaire in a sample of adults living in Porto Alegre, Rio Grande do Sul State, Brazil]. *Cadernos de saude publica.* Apr 2009;25(4):840-848.

22. NEPA-UNICAMP. Center for Studies and Research in Food: Brazilian table of food composition. Campinas. Avaiable at:

http://www.unicamp.br/nepa/taco/contar/taco_4_edicao_ampliada_e_revisada. Accessed April 04, 2016.

23. National Nutrient Database for Standard Reference Release 28. Avaiable at: https://ndb.nal.usda.gov/ndb/search/list. Accessed April 08, 2016.

24. GUIA ALIMENTAR PARA A POPULAÇÃO BRASILEIRA: Promovendo a Alimentação Saudável. In: SAÚDE MD, ed. Brasília, DF.2005.

25. Graff SK, Alves BC, Toscani MK, Spritzer PM. Benefits of pedometer-measured habitual physical activity in healthy women. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme.* Feb 2012;37(1):149-156.

26. Silva TR, Alves BC, Maturana MA, Spritzer PM. Healthier dietary pattern and lower risk of metabolic syndrome in physically active postmenopausal women. *J Am Coll Nutr.* 2013;32(5):287-295.

27. Colpani V, Oppermann K, Spritzer PM. Association between habitual physical activity and lower cardiovascular risk in premenopausal, perimenopausal, and postmenopausal women: a population-based study. *Menopause.* May 2013;20(5):525-531.

28. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* Jul 1985;28(7):412-419.

29. Maturana MA, Moreira RM, Spritzer PM. Lipid accumulation product (LAP) is related to androgenicity and cardiovascular risk factors in postmenopausal women. *Maturitas.* Dec 2011;70(4):395-399.

30. Rizzoli R, Stevenson JC, Bauer JM, et al. The role of dietary protein and vitamin D in maintaining musculoskeletal health in postmenopausal women: a consensus statement from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). *Maturitas.* Sep 2014;79(1):122-132.

31. Genaro Pde S, Pinheiro Mde M, Szejnfeld VL, Martini LA. Dietary protein intake in elderly women: association with muscle and bone mass. *Nutr Clin Pract.* Apr 2015;30(2):283-289.

32. Isanejad M, Mursu J, Sirola J, et al. Dietary protein intake is associated with better physical function and muscle strength among elderly women. *The British journal of nutrition.* Apr 2016;115(7):1281-1291.

33. Tyrovolas S, Koyanagi A, Olaya B, et al. Factors associated with skeletal muscle mass, sarcopenia, and sarcopenic obesity in older adults: a multi-continent study. *Journal of cachexia, sarcopenia and muscle.* Oct 7 2015.

34. Lazzer S, Meyer F, Meyer M, Boirie Y, Vermoret M. [Assessment on the usual physical activity in overweight and obese adolescents]. *Presse medicale.* Oct 23 2004;33(18):1255-1259.

35. Lindle RS, Metter EJ, Lynch NA, et al. Age and gender comparisons of muscle strength in 654 women and men aged 20-93 yr. *Journal of applied physiology.* Nov 1997;83(5):1581-1587.

36. Bann D, Wu FC, Keevil B, et al. Changes in testosterone related to body composition in late midlife: Findings from the 1946 British birth cohort study. *Obesity.* Jul 2015;23(7):1486-1492.

37. Bell RJ, Davison SL, Papalia MA, McKenzie DP, Davis SR. Endogenous androgen levels and cardiovascular risk profile in women across the adult life span. *Menopause.* Jul-Aug 2007;14(4):630-638.

38. Nordic Nutrition Recommendations 2012. Integrating Nutrition and Physical Activity, Nord 2014:002. In: editor NCoM, ed. 5th ed. ed. Copenhagen: Nordic Council of Ministers 20142014.

39. Houston DK, Nicklas BJ, Ding J, et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. *Am J Clin Nutr.* Jan 2008;87(1):150-155.

40. Beasley JM, Shikany JM, Thomson CA. The role of dietary protein intake in the prevention of sarcopenia of aging. *Nutr Clin Pract.* Dec 2013;28(6):684-690.

41. Dionne IJ, Kinaman KA, Poehlman ET. Sarcopenia and muscle function during menopause and hormone-replacement therapy. *J Nutr Health Aging.* 2000;4(3):156-161.

42. Krieger JW, Sitren HS, Daniels MJ, Langkamp-Henken B. Effects of variation in protein and carbohydrate intake on body mass and composition during energy restriction: a meta-regression 1. *Am J Clin Nutr.* Feb 2006;83(2):260-274.

43. Beasley JM, Katz R, Shlipak M, Rifkin DE, Siscovick D, Kaplan R. Dietary protein intake and change in estimated GFR in the Cardiovascular Health Study. *Nutrition.* Jul-Aug 2014;30(7-8):794-799.

44. Welch AA, Kelaiditi E, Jennings A, Steves CJ, Spector TD, MacGregor A. Dietary Magnesium Is Positively Associated With Skeletal Muscle Power

and Indices of Muscle Mass and May Attenuate the Association Between Circulating C-Reactive Protein and Muscle Mass in Women. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research.* Feb 2016;31(2):317-325.

45. Lauretani F, Semba RD, Bandinelli S, Ray AL, Guralnik JM, Ferrucci L. Association of low plasma selenium concentrations with poor muscle strength in older community-dwelling adults: the InCHIANTI Study. *Am J Clin Nutr.* Aug 2007;86(2):347-352.
46. Yanai H. Nutrition for Sarcopenia. *Journal of clinical medicine research.* Dec 2015;7(12):926-931.
47. Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, Wolfe RR. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. *Am J Clin Nutr.* Aug 2003;78(2):250-258.
48. Santesso N, Akl EA, Bianchi M, et al. Effects of higher- versus lower-protein diets on health outcomes: a systematic review and meta-analysis. *Eur J Clin Nutr.* Jul 2012;66(7):780-788.

Table 1. Characteristics of postmenopausal women stratified by protein intake tertiles

	≤0.93 g/kg BW (n = 34)	**0.94–1.29 g/kg BW (n = 34)**	**≥1.3 g/kg BW (n = 35)**	***P*-value**
Age (years)	54.5 ± 5.2	56.0 ± 4.7	55.0 ± 4.8	0.463
Time since menopause (years)[c]	6 (3 – 9)	7.5 (3 – 11)	5 (3 – 10)	0.864
Years at school (years)[c]	5 (4 – 11)[a]	8 (5 – 11) [ab]	11 (5 – 14)[b]	**0.042**
White, n (%)[d]	28 (82)	33 (94)	29 (85)	0.422
BMI (kg/m^2)	29.4 ± 5.6[a]	26.9 ± 3.4 [ab]	25.3 ± 3.6[b]	**0.001**
Waist circumference (cm)	92.2 ± 13.5[a]	86.6 ± 10.1[ab]	81.2 ± 9.7[b]	**<0.001**
Body fat %	43.7 ± 6.5[a]	41.1 ± 5.1[a]	36.7 ± 7.5[b]	**<0.001**
Trunk fat mass (%)	46.0 ± 7.2[a]	43.0 ± 5.9[a]	38.0 ± 8.3[b]	**<0.001**
Skeletal muscle mass index	0.58 ± 0.07[a]	0.60 ± 0.07[a]	0.65 ± 0.09[b]	**0.001**
Lumbar spine BMD (g/cm^2)	1.08 ± 0.14	1.03 ± 0.17	1.01 ± 0.14	0.191
Femoral neck BMD (g/cm^2)	0.92 ± 0.13	0.88 ± 0.10	0.85 ± 0.11	0.050
Osteoporosis, n (%)[d]	4 (12)	9 (27)	7 (20)	0.316
Mean steps/day	4724.7 ± 2269.7[a]	5330.6 ± 2657.9[a]	7600.9 ± 3227.0[b]	**<0.001**
Sedentary, n (%)[d]	26 (76)	20 (59)	13 (37)	**0.003**
RMR (Kcal/day)	1341.2 ± 245.3[a]	1232.1 ± 176.4[ab]	1216.0 ± 131.2[b]	**0.014**
SBP (mmHg)[c]	125 (120 – 144)	120 (117 – 134)	120 (110 – 130)	0.060
DBP (mmHg)[c]	80 (80 – 80)[a]	80 (70 – 80)[ab]	80 (70 – 80)[b]	**0.019**
Previous smoking behavior (%)[d]	13 (38)	12 (35)	12 (34)	**0.933**

BMI: body mass index; BMD: bone mineral density; RMR: resting metabolic rate; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Different superscript letter in a row indicate significant differences (P<0.05, ANOVA and Bonferroni post hoc tests).

[c]variables analyzed after log transformation; [d]$\chi 2$ test

Table 2. Metabolic and hormonal variables of postmenopausal women stratified by protein intake tertiles

	≤0.93 g/kg BW (n = 34)	**0.94–1.29 g/kg BW (n = 34)**	**≥1.3 g/kg BW (n = 35)**	***P*-value**
Total cholesterol (mg/dL)	220.0 ± 31.1	212.7 ± 33.2	215.4 ± 34.6	0.681
LDL cholesterol (mg/dL)	142.0 ± 31.1	136.3 ± 28.8	138.6 ± 29.9	0.732
HDL cholesterol (mg/dL)	51.5 ± 10.2	53.5 ± 14.9	56.2 ± 12.7	0.314
Triglycerides (mg/dL)[c]	114.0 (73.2 – 145.2)	89.0 (70.0 – 134.5)	84.0 (67.0 – 138.0)	0.131
Fasting glucose (mg/dL)	94.8 ± 8.1	93.3 ± 9.1	92.1 ± 8.8	0.429
Fasting insulin (µUI/mL)[c]	9.0 (7.2 – 14.2)	7.9 (5.7 – 12.8)	8.7 (5.5 – 11.2)	0.361
HOMA IR	2.7 ± 1.4	2.4 ± 1.5	2.6 ± 2.4	0.710
LAP	39.6 (26.6 – 54.5)[a]	29.8 (20.1 – 46.9)[ab]	23.1 (15.6 – 38.0)[b]	**0.010**
hs-CRP(mg/L)[c]	1.9 (0.4 – 4.8)[a]	1.8 (0.7 – 7.3)[ab]	0.6 (0.2 – 2.3)[b]	**0.006**
Metabolic Syndrome n (%)[d]	9 (26.5)	10 (29.4)	3 (8.6)	0.084
Estradiol (pg/mL)[c]	17.6 (11.2 – 31.5)	20.5 (10.3 – 31.0)	19.4 (11.7 – 25.5)	0.962
FSH (mUI/mL)	78.0 ± 30.1	79.6 ± 30.9	88.9 ± 29.7	0.271
SHBG (nm/L)[c]	44.7 (31.1 – 59.1)	41.8 (29.5 – 60.1)	51.6 (38.4 – 68.1)	0.092
DHEA-S (µg/dL)[c]	46.9 (34.6 – 88.7)	64.8 (30.4 – 90.4)	48.0 (29.3 – 85.2)	0.357
FAI[c]	3.0 (1.7 – 4.2)	2.1 (1.5 – 4.9)	2.1 (1.4 – 3.8)	0.301
Total testosterone (ng/mL)	0.39 ± 0.17	0.39 ± 0.20	0.33 ± 0.16	0.276
Previous hormone therapy n (%)[d]	11 (32)	12 (35)	10 (29)	0.747

LDL: low-density lipoprotein, HDL: high-density lipoprotein; HOMA IR: homeostatic model assessment of insulin resistance; LAP: lipid accumulation product; hs-CRP: high-sensitive C-reactive protein; FSH: follicle-stimulating hormone; SHBG: sex hormone–binding globulin; DHEA-S: dehydroepiandrosterone sulfate; FAI: free androgen index.

Different superscript letter in a row indicate significant differences ($P<0.05$, ANOVA and Bonferroni post hoc tests).

[c]variables analyzed after log transformation; [d]$\chi 2$ test.

Table 3. Dietary variables of postmenopausal women stratified by protein intake tertiles

	≤0.93 g/kg BW (n = 34)	**0.94–1.29 g/kg BW (n = 34)**	**≥1.3 g/kg BW (n = 35)**	***P*-value**	***P*-value[d]**
Macro/micronutrients					
Kcal	1433.5 ± 464.3[a]	1800.3 ± 449.1[b]	2331.7 ± 589.9[c]	**<0.001**	
Protein (%)	15.5 ± 3.2[a]	16.9 ± 3.2[b]	18.0 ± 2.5[c]	**0.002**	**<0.001**
Fat (%)	23.5 ± 5.7	24.2 ± 5.7	24.5 ± 4.5	0.728	
Carbohydrate (%)	60.2 ± 7.2	58.1 ± 7.8	56.5 ± 5.8	0.094	
Glycemic index (%)	56.6 ± 5.0	56.2 ± 4.1	54.3 ± 4.4	0.092	
Ethanol (g)[e]	0.0 (0.0 – 3.0)	0.5 (0.0 – 2.0)	0.0 (0.0 – 1.4)	0.492	
Cholesterol (mg)	137.1 ± 63.3[a]	203.8 ± 82.2[b]	301.6 ± 128.7[c]	**<0.001**	**0.008**
Fiber (g)	22.5 ± 8.6[a]	27.7 ± 11.5[ab]	38.1 ± 14.2[b]	**<0.001**	0.215
Calcium (mg)	534.3 ± 251.7[a]	752.7 ± 260.3[b]	1041.5 ± 406.0[c]	**<0.001**	**0.024**
Magnesium (mg)	200.6 ± 72.7[a]	258.8 ± 77.1[b]	350.5 ± 110.4[c]	**<0.001**	0.054
Iron (mg)	6.6 ± 2.1[a]	9.1 ± 3.0[b]	12.1 ± 3.7[c]	**<0.001**	**0.014**
Zinc (mg)	6.0 ± 2.2[a]	8.0 ± 1.8[b]	10.6 ± 3.6[c]	**<0.001**	**0.001**
Sodium (mg)	1478.6 ± 342.5[a]	1868.1 ± 501.9[b]	2398.5 ± 673.1[c]	**<0.001**	**0.027**
Selenium (µg)	64.7 ± 21.7[a]	93.1 ± 18.4[b]	119.1 ± 32.4[c]	**<0.001**	**<0.001**
Folate (µg)	409.7 ± 141.2[a]	522.4 ± 211.9[a]	715.2 ± 273.3[b]	**<0.001**	**0.043**
Vitamin B-12 (µg)[e]	2.8 (1.5 – 3.7)[a]	4.1 (3.0 – 5.9)[b]	5.4 (4.2 – 6.6)[b]	**<0.001**	**0.022**
Vitamin D‡ (µg)[e]	4.6 (1.5 – 9.5)	2.7 (2.4 – 8.3)	5.2 (3.7 – 11.0)	0.114	
Vitamin E‡ (mg)[e]	2.6 (1.9 – 3.6)[a]	3.9 (2.8 – 4.8)[b]	4.8 (3.2 – 6.3)[b]	**<0.001**	0.219
Vitamin C (mg)[e]	122.9 (49.3 – 207.6)[a]	188.4 (117.4 – 253.6)[b]	272.2 (138.4 – 422.7)[b]	**<0.001**	0.420
Vitamin A (mg)[e]	487.2 (264.9 – 958.6)[a]	791.0 (451.3 – 1654.4)[b]	1045.0 (646.6 – 1514.4)[b]	**<0.001**	0.393
Food groups (portions/day)					
Fruits[e]	2.5 (1.1 – 4.7)[a]	3.5 (2.3 – 5.6)[ab]	5.4 (2.0 – 8.9)[b]	**0.026**	
Vegetables[e]	1.9 (0.8 – 4.0)[a]	2.3 (1.5 – 5.0)[ab]	4.0 (3.1 – 8.1)[b]	**<0.001**	
Beans[e]	1.4 (0.7 – 1.9)	1.0 (0.4 – 1.9)	1.9 (1.0 – 2.1)	**0.004**	

Whole grain[e]	0.4 (0.1 – 0.9)[a]	1.1 (0.5 – 1.9)[b]	1.1 (0.6 – 2.1)[b]	**<0.001**
Refined grain	2.2 ± 1.2	2.2 ± 1.5	2.6 ± 1.2	0.443
Eggs and meat	0.8 ± 0.4[a]	1.1 ± 0.5[a]	1.7 ± 0.7[b]	**<0.001**
Red meat (g/d)[e]	45.1 (32.7 – 85.7)	56.0 (34.2 – 81.7)	50.7 (19.0 – 74.3)	0.107
Fish and chicken (g/d)[e]	14.3 (6.3 – 31.8)[a]	27.1 (12.7 – 49.4)[ab]	45.4 (13.6 – 77.1)[b]	**0.001**
Sweets and desserts[e]	1.1 (0.6 – 1.7)	0.9 (0.6 – 2.1)	1.8 (0.7 – 2.8)	0.238
Dairy foods[e]	0.9 (0.5 – 1.8)[a]	1.6 (0.9 – 2.0)[ab]	2.0 (1.0 – 3.2)[b]	**0.003**

Different superscript letter in a row indicate significant differences ($P<0.05$, ANOVA and Bonferroni post hoc tests). [d]ANCOVA, controlling for energy intake; [e]variables analyzed after log transformation.

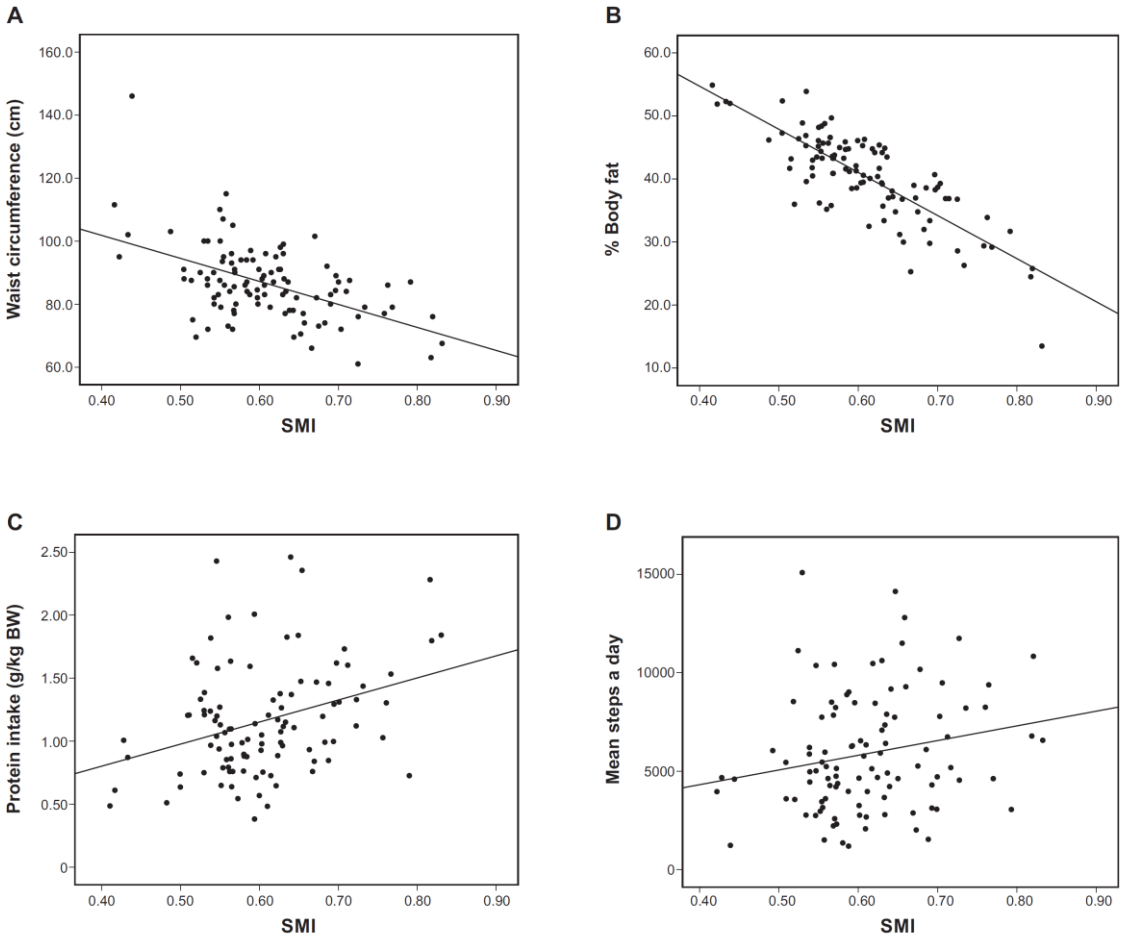

Figure 1. Pearson's correlation coefficient between Skeletal muscle mass index (SMI) and **a**. Waist circumference (cm), **b**. % body fat, **c**. Protein intake (g/kg BW), **d**. Mean steps a day. All analysis $P<0.05$.

Table 4. Multiple linear regression analysis with skeletal muscle mass index as the dependent variable

	Skeletal muscle mass index	
Variables	**Unstandardized β (95%IC)**	***P*-value***
% Body fat	**-0.010 (-0.011 – -0.008)**	**<0.001**
Protein intake (per 10-g increase)	**0.007 (0.000 – 0.014)**	**0.044**
Mean steps a day (per 2000-step increase)	-0.005 (-0.012 – 0.003)	0.228

*Model adjusted for age, time since menopause, previous smoking behavior and energy intake, $R^2 = 0.70$

CAPÍTULO 3

Associations between body composition and lifestyle factors with bone mineral density according to time since menopause in women from Southern Brazil: a cross-sectional study

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ABSTRACT

Background: The aim of this study was to investigate whether body composition, dietary pattern and habitual physical activity are associated with BMD according to time since menopause in women from Southern Brazil with no clinical evidence of disease.

Methods: 99 participants were enrolled and anthropometry, body composition and BMD by dual energy x-ray absorptiometry, rest metabolic rate by indirect calorimetry, dietary pattern by semi quantitative food frequency questionnaire and habitual physical activity by pedometer were performed.

Results: Mean age was 55.2±4.9 years and mean time since menopause was 6.8±1.0 years. Weight, BMI, lean and fat mass and RMR were higher in women with less than 5 years since menopause with normal *versus* low bone mass. No differences were found in the studied variables between participants with normal or low bone mass and more than 5 years of menopause. Women with > 5 years since menopause had higher prevalence of osteoporosis, as well as lower BMD in all sites when compared to those with less time since menopause. Calories, carbohydrate, protein, fat and micronutrients intake were similar between groups. When the sample was adjusted for time since menopause, the odds ratio (OR) for low bone mass was 5.21 (95% CI 1.57-17.25, *P*=0.004) for BMI <25 kg/m^2, for lean mass <37.5 Kg an OR of 4.4 (95% CI 1.64-11.80, *P*=0.004, for fat mass <26.0 Kg an OR of 3.39 (95% CI 1.29-8.85, *P*=0.010) and for the intake of vitamin A < 700mcg/day an OR of 3.00 (95% CI 1.13-7.94, *P*=0.012). Low meat and eggs intake or low protein intake did not influence the odds ratio for low bone mass

Conclusion: In this cross-sectional study with postmenopausal women with no clinical evidence of disease, time since menopause, low lean and fat mass were associated with low bone mass. Calories and macronutrients intake as well as habitual physical activity did not interfere with BMD, but participants were mostly sedentary. Further studies are needed in order to determine whether the adequate intake of specific food groups and the type of physical activity could attenuate the time since menopause impact on BMD.

Key words: Menopause; diet; bone mass density; lean mass; osteoporosis; lifestyle

BACKGROUND

Bone mineral density (BMD) declines with increasing age, and the rate of decline is more pronounced after menopause [1]. Falling levels of 17-β-estradiol are thought to accelerate the decline in BMD, which remains the single best predictor of primary osteoporotic fracture [2]. This decline can also be attributed to a number of factors: age, genetics, nutrition, lifestyle factors, or the prolonged use of certain medication [3].

Body mass index (BMI) is known to be positively correlated with BMD, and low BMI (< 19 kg/m^2) significantly increases the risk of osteoporosis in postmenopausal women as compared to normal range BMI [4]. However, the contributions of lean and fat body mass to BMD, related to BMI *stratus*, are still not completely understood in different populations [5].

Lifestyle factors, such as physical activity (PA) and diet may exert influence on BMD in both pre- and postmenopausal women. PA plays a major role in minimizing bone loss as we age [6]. In addition, adequate dietary behavior seems to also influence on bone loss in 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. [7-10] Besides that, studies have shown that diets with high content in vegetables, fruit, and whole grains may be associated with lower premenopausal bone loss in menopausal transition and lower risk of low-trauma fracture, particularly in older women [11, 12].

Therefore, the aim of this study was to investigate whether body composition, dietary pattern and habitual physical activity are associated with BMD according to time since menopause in women from Southern Brazil with no clinical evidence of disease.

METHODS

Subjects

This cross-sectional study was carried out at the Gynecological Endocrinology Unit at Hospital de Clínicas de Porto Alegre, Brazil, from October 2010 to February 2012. Participants were recruited by advertisement in a local newspaper and radio station. Inclusion criteria were as follows: 1) menopause, defined as last menstrual period at least 1 year before the beginning of the study plus follicle stimulating hormone (FSH) levels > 35 IU/L; 2) age between 45 and 65 years; and 3) no use of hormone therapy in the past 3 months. Diabetic patients, patients with prior diagnosis of heart disease, and current smokers were excluded. These criteria were chosen because of the interest to study women with no clinically established systemic diseases. One hundred and nineteen postmenopausal women fulfilling all the inclusion criteria were consecutively enrolled. They were stratified by time since menopause (≤ 5 or > 5 years) and BMD (low or normal bone mass). The study protocol was approved by the local Research Ethics Committee from Hospital de Clinicas de Porto Alegre, and written informed consent was obtained from every participant.

Design

All participants completed a questionnaire about their sociodemographic characteristics (e.g., age, education, household income, and marital status) and medical history (including current medications). The variable skin color was defined by auto-reference: participants were asked about their skin color and were stratified in white and no white. Anthropometric measurements were performed in duplicate and included body weight, height, and waist circumference [13]. BMI (kg/m^2) was calculated. Resting metabolic rate (RMR) was obtained by indirect calorimetry (Fitmate®, Cosmed, Rome, Italy).

Blood pressure was measured after resting for 10 minutes, in the sitting position. Two measurements were performed at a 10-min interval, using an automatic blood pressure monitor (Omron HEM 742, Rio de Janeiro, Brazil) with an appropriate cuff for the arm diameter. FSH, estradiol, total testosterone, sex-hormone binding globulin (SHBG), ultrasensitive C-reactive protein (us-CRP), total and high-density lipoprotein (HDL) cholesterol, triglycerides, fasting glucose and insulin were determined using the 12h fasting blood sample. All samples were obtained between 8AM and 10AM, and were run immediately after collection. The methods of analysis did not change during the study.

Assays

Total cholesterol, HDL cholesterol and triglycerides were determined by colorimetric-enzymatic methods (Bayer 1800 Advia System), with intra and interassay coefficients of variation (CV) < 3%. Glucose was determined by the

hexokinase method (Advia 1800) with intra-assay CV < 3.4% and interassay CV < 2.1%. FSH was measured by chemiluminescence immunoassay (CLIA) (Centaur XP), with sensitivity of 0.3 IU/L and intra and interassay CV of 2.9% and 2.7% respectively. Total testosterone levels were also measured by CLIA (Centaur XP) with sensitivity of 10 ng/mL and intra and interassay CV of 3.3% and 7.5% respectively. SHBG was measured by CLIA (Immulite 2000), with sensitivity of 0.02 nmol/L and intra- and interassay CV of 5.3% and 6.6% respectively. Serum insulin levels were measured using CLIA (Centaur XP), with a sensitivity of 0.200 µIU/mL and intra- and interassay CV of 2.0% and 4.3% respectively. FAI was estimated by dividing TT (in nanomoles per liter) by SHBG (in nanomoles per liter) × 100. Low-density lipoprotein (LDL) cholesterol was determined indirectly using the Friedewald formula LDL = total cholesterol – HDL – (triglycerides / 5). Homeostasis model assessment (HOMA) was calculated by multiplying insulin (µIU/ml) by glucose (mmol/l) and dividing this product by 22.5.

Bone mass and body composition assessments

BMD was assessed in lumbar spine (L1-L4), femoral neck and proximal total femur by dual-energy X-ray absorptiometry (DXA) (GE Lunar Prodigy, Radiation Corporation, Madison, WI, USA). BMD was expressed by g/cm^2 and T-scores. Normal bone mass was defined as a T score above -1 standard deviations (SD) and low bone mass was defined as the presence of at least one site of osteopenia or osteoporosis, according to the World Health Organization (WHO) [14].

A whole body scan was also performed by DXA to assess body composition. Lean mass and fat mass were determined for the whole body, with a CV lower than 2%.

Dietary assessment

Usual dietary intake was assessed with a validated food frequency questionnaire consisting of 120 items [15]. Nutritional composition was calculated using the Brazilian Table of Food Composition [16] except for vitamin D, E, and A, which were assessed using the United States Department of Agriculture (USDA) National Standard Reference Database. Reference values for daily dietary intake were based on national [17] and international guidelines [18].

Physical activity assessment

Assessment of habitual PA was performed with a digital pedometer (BP 148, Tech Line, São Paulo, Brazil). The device was configured individually according to weight (kg) and individual step length. The equipment was used for six consecutive days, providing a weekly average number of steps. Participants were stratified in active >6,000 steps per day) or sedentary ≤ 6,000 steps per day), according to previously reported [15, 19, 20]. Subjects were encouraged not to change their physical activity habits during the study.

Sample size estimation and statistical analyses

Sample size was estimated based on a previous study [21], considering a power of 80% and alpha of 5%. One hundred women were required to detect a difference of 4.3 in BMI between women with normal and low bone mass.

Results are presented as mean ± standard deviation (SD), or median and inter-quartile range, depending on the Gaussian or non-Gaussian distribution of variables. Two-way ANOVA was used to assess the simultaneous effects of time since menopause and BMD. χ^2 was calculated for comparisons of dichotomous variables. A logistic regression model was used to estimate the odds ratio of different variables for–low bone mass, which was considered as the dependent variable. 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 $p < 0.05$.

RESULTS

Of 119 volunteers, 13 were excluded (five with diabetes, one with hyperthyroidism, two with untreated hypothyroidism, two with breast cancer, one who was premenopausal and two with spinal disc prosthesis). An additional seven participants dropped out because they were unable to commit to the study (no time for blood collection, DXA and indirect calorimetry). Thus, 99 women were enrolled. Mean age was 55.2±4.9 years and mean time since menopause was 6.8±1.0 years. Participants had attended school for a mean of 8.5±4.2 years, and

87% were white. Forty participants were on antihypertensive drugs, two women were on statins, and one was taking aspirin.

Table 1 presents the demographic, hormonal and body composition characteristics of participants according to time since menopause (≤ 5 or > 5 years) and BMD (low or normal). The groups were similar regarding years at school, skin color, estradiol and free estradiol index. Number of steps per day, as an index of habitual physical activity was low and similar between groups and the prevalence of sedentary was around 50 and 70% among groups. In turn, participants with ≤ 5 years since menopause presented higher weight, BMI, body fat %, lean mass, fat mass and RMR in the normal bone mass sub-group as compared to the group with low bone mass. Lumbar spine, femoral neck, and total femoral BMD were lower in both subgroups of low bone mass. Women with > 5 years since menopause and with low bone mass presented the lowest BMD in all sites in comparison with women with low bone mass and with ≤ 5 years. In addition, as shown in Figure 1, women with > 5 years since menopause had also higher prevalence of osteoporosis.

Calories, carbohydrate, protein, fat and micronutrients intake were similar between groups (data not presented). Vitamin A intake was greater in the groups with normal bone mass compared to groups with low bone mass, with a borderline significance (1239.7 ± 778.6 *vs* 926.8 ± 819.0 for groups with ≤ 5 years since menopause and 1363.4 ± 1199.4 *vs* 895.1 ± 871.0 for those with >5 years of menopause; *P*=0.051).

Table 2 shows lumbar spine, femoral neck and total femoral BMD in postmenopausal women according to different factors, stratified by tertiles. Age,

time since menopause, fat mass, and RMR were associated with BMD in all sites. BMI was associated with BMD on femoral neck and total femoral but not in the lumbar spine. None dietary or hormonal variables were associated with BMD (data not presented).

Compared with women who underwent menopause ≤5 years, those who underwent menopause >5 years ago had a 3-fold increase in odds ratio for low bone mass (95% CI 1.27-7.34, *P*=0.016). However, being older than 55 years (mean age of participants in this sample) did not increase the odds ratio for low bone mass. Therefore, when adjusted for time since menopause and previous hormone therapy the odds ratio (OR) for low bone mass was 5.21 (95% CI 1.57-17.25, *P*=0.004), for BMI <25 kg/m^2, for lean mass <37.5 Kg an OR of 4.4 (95% CI 1.64-11.80, *P*=0.004, for fat mass <26.0 Kg an OR of 3.39 (95% CI 1.29-8.85, *P*=0.010) and for the intake of vitamin A < 700mcg/day an OR of 3.00 (95% CI 1.13-7.94, *P*=0.012). Low meat and eggs intake or low protein intake (defined as the median consumption of participants in this sample) did not influence the odds ratio for low bone mass (Table 3).

DISCUSSION

In the present study, weight, BMI, lean and fat mass and RMR were higher in postmenopausal women with less than 5 years since menopause with normal *versus* low bone mass. These variables did not differ significantly between women with normal and low bone mass and more than 5 years since menopause. In fact, evidence suggests that BMD is better correlated with percentage of body

fat in pre- and perimenopausal than in postmenopausal women [22]. In addition, while it is known that total BMD is associated with higher BMI and fat mass, trabecular and cortical volumetric BMD (vBMD) show a different profile according to BMI. Indeed, obese adults present higher trabecular vBMD but lower cortical vBMD [23]. Therefore, we hypothesized that the association of adiposity and BMD was more evident in recent postmenopausal women, having more trabecular bone than in those in the later postmenopausal life (in which trabecular bone is lost due to the high bone turnover occurring throughout the postmenopausal years). Further studies assessing volumetric bone density and microarchitecture by high-resolution peripheral quantitative computed tomography in postmenopausal populations is needed to confirm this hypothesis.

When all sample was analyzed age, time since menopause, fat mass and RMR were associated with BMD in all three sites. Menopause is associated with a few years of rapid bone loss attributed to lower circulating levels of 17β-estradiol, related primarily to the decline in estrogen-mediated inhibition of bone resorption without a fully compensatory increase in bone formation [2]. For an interval of few years around the menopause, women lose 2% of bone annually. Afterward, bone loss slows to about 1 to 1.5% per year [24, 25]. Recker et al., [25] found that menopausal bone loss is a composite of loss caused by estrogen deprivation and age *per se* for the hip and total body, but is caused by estrogen deprivation alone for the spine. In the Study of Women's Health Across the Nation (SWAN), women who transitioned through menopause experienced a significantly higher rate of bone loss than women who remained premenopausal, independent of age [26]. In turn, a recent study suggests that

time since menopause may have a stronger predictive value for low BMD in the lumbar and hip areas than did serum FSH or estradiol levels [27]. Data from the present study reinforces that idea, showing that women with more than 5 years since menopause had higher prevalence of osteoporosis, as well as lower BMD in all sites when compared to those with less time since menopause. In addition, women who underwent menopause more than 5 years ago had a 3-fold increased odds ratio for low bone mass.

Regarding the association between BMD and BMI we found in our study, the odds ratio for low bone mass was five times for a BMI lower than 25 kg/m^2, being this cut-off the lowest tertile of our sample. In this sense, low weight or low BMI is a well-documented risk factor for future fracture [1]. Zhu and coworkers have recently reported in a Western Australian population that the associations of BMI with BMD measures were attenuated in those with high BMI [5], suggesting that low body weight should be considered as a risk factor for osteoporosis and related fracture, rather than obesity being a protective factor.

In the present study fat mass was associated with BMD in all sites and with reduced odds ratio for low bone mass. However, the influence of fat mass on BMD is a debatable issue and seems to be related to menopausal status [28]. In postmenopausal women, adipose tissue is the major sources of estrogen from aromatization [29]. Therefore, it has been suggested that subcutaneous adipose tissue have higher aromatase activity in comparison to visceral adipose tissue, and could exert a more beneficial effect than visceral fat in bone health after menopause. In this sense, body composition analysis by DXA does not allow to discriminate subcutaneous and visceral fat, which is a limitation of the present

study. Further studies using other methodologies are needed in order to clarify this issue.

While in our study BMD measures in all three sites did not differ according to lean mass tertiles, lean mass was lower in participants with low bone mass *versus* normal bone mass and less than 5 years since menopause. Lima and coworkers showed that in older women, lean mass was significantly correlated with BMD independently of height and fat mass [28]. Some other cross sectional studies with postmenopausal women suggests that lean mass is not an independent correlate of BMD [30, 31]. In turn, in pre- and peri-menopausal women lean mass has been reported to be a main predictor of BMD [32, 33]. The stronger association between lean mass and BMD may be attributed to differences in determinants of lean mass, such as exercise, lifestyle factors, serum estrogen concentrations or a combination of these factors [32].

Concerning RMR, studies in different populations have also reported a strong relationship between BMD and RMR [34, 35]. We found that participants with low bone mass have lower RMR, in line with a previous research that have also shown that a lower lean mass in postmenopausal women is associated with a lower RMR [36]. Taken together these data suggest that interventions aiming to increase lean mass, which increases RMR, could represent a simple and useful strategy to prevent osteoporosis in women, especially in recent postmenopausal women, such as physical activity (PA) practice. In fact, intervention studies have reported positive effects or associations between PA, BMD and markers of bone metabolism in pre- and postmenopausal women [37, 38]. However, walking may not be enough as a stimulus to increase lean mass in

postmenopausal women [39], and these women should be encouraged to participate in regular programs of moderate physical activity [40]. Indeed, in the present study, participants were mostly sedentary, as objectively estimated by a pedometer, and this could have influenced on the association between lean mass and BMD that was independent of habitual PA.

In the specific context of osteoporosis prevention and management, a discussion of nutrition appropriately focuses on vitamin D, and protein in addition to calcium. According to our data the mean calcium intake was 799 mg/day meaning 69% of dietary reference intakes for American women (1200mg/day). However, in a longitudinal and prospective cohort study, based on the Swedish Mammography-Cohort [39] only calcium intake lower than 700mg/day increased risk of fractures and of osteoporosis, high levels of intake did not further decrease the rate of fracture. In the same cohort although not reflected in the fracture rate, women with high vitamin D intake (>5.4µg/day) tended to have a slightly higher BMD, but in the present study vitamin D (mean of intake 4µg/day) did not influence the BMD. In a recently published reanalysis of randomized vitamin D supplementation trials, it was concluded that an intake of 20 µg/day is needed to prevent nonvertebral fractures in women and men aged 65 years or older [6].

Regarding calories intake, a previous study showed higher cortical BMD in elderly women (mean age of 75 years) eating a diet exceeding the RDA for macronutrients (44 kcal/kg of ideal body weight) [42]. Interestingly, we did not find any association between calories intake and BMD, probably because our participants were in early postmenopausal. Dietary protein is positively linked to the maintenance of bone and muscle health and some experts suggest that the

current recommended protein intake (≈ 70g/day) may be inadequate for optimum skeletal and muscle health [43]. Our postmenopausal women had an average protein intake of 77g/day, which may not be sufficient for interfering with the risk for low bone mass, as shown by the neutral OR related to meat and eggs food intake.

In turn, when micronutrients intake was analyzed, only vitamin A appears to be less consumed among women with lower bone mass. Considering all sample, vitamin A intake lower than 700mcg a day, that is the recommended amount for dietary reference intakes [18], was related to a higher OR for low bone mass. Indeed, vitamin A, retinol, beta-carotene, and its metabolites are involved in bone metabolism and Wattanapenpaiboon *et al* have shown a positive correlation of lumbar BMD with β -carotene levels in postmenopausal women [44]. A recent meta-analysis, also reported an U-shaped relationship between serum retinol levels and hip fracture risk [45].

One limitation of the present study is the cross-sectional design that does not allow conclusions regarding the direction of cause and effect. Other limitation is the relatively small sample size of 99 participants and a moderate enrollment rate (16% excluded participants), which could affect the external validity. However, the results observed in our sample of Southern Brazilian postmenopausal women are consistent and in line to those reported in other populations. Another limitation is that although pedometers are increasingly used to estimate habitual physical activity, they are not sensitive to the intensity or the type of the activity performed, and therefore may not accurately depict the loading

forces of the activities performed, which are important for bone maintenance and/or development [38].

CONCLUSIONS

In postmenopausal women from Southern Brazil, with no clinical evidence of disease, time since menopause, low lean and fat mass were associated with low bone mass. Calories and macronutrients intake as well as habitual physical activity did not interfere with BMD, but participants were mostly sedentary. Further studies are needed in order to determine whether the adequate intake of specific food groups and the type of physical activity could attenuate the aging and time since menopause impact on BMD.

COMPETING INTERESTS

The authors declare that they have no competing interests

AUTHORS' CONTRIBUTIONS

TRS participated in the design of the study and was involved in the data collection and analysis, and drafted the article.

RF and MAM were involved in the data collection.

PMS conceived the study, participated in its design and coordination, was involved in the data analysis and helped to draft the manuscript.

All authors read and approved the final manuscript.

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REFERENCES

[1] Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, Cauley J, Black D, Vogt TM: **Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group**. *N Engl J Med* 1995, **23**;332:767-73

[2] Riggs BL, Khosla S, Melton LJ, 3rd: **A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men**. *J Bone Miner Res* 1998, **13**:763-73.

[3] Guthrie JR, Ebeling PR, Hopper JL, Barrett-Connor E, Dennerstein L, Dudley EC, Burger HG, Wark JD: **A prospective study of bone loss in menopausal Australian-born women.** *Osteoporos Int* 1998, **8**:282-90.

[4] The North American Menopause Society: Management of osteoporosis in postmenopausal women. *Menopause* 2010, **17**:25-54.

[5] Zhu K, Hunter M, James A, Lim EM, Walsh JP: **Associations between body mass index, lean and fat body mass and bone mineral density in middle-aged Australians: The Busselton Healthy Ageing Study**. *Bone* 2015, **74**: 146–152.

[6] Kohrt WM, Bloomfield SA, Little KD, Nelson ME, Yingling VR: **American College of Sports Medicine Position Stand: physical activity and bone health**. *Med Sci Sports Exerc* 2004, **36**:1985-96.

[7] DIPART (Vitamin D Individual Patient Analysis of Randomized Trials) Group. Patient level pooled analysis of 68500 patientes from seven major vitamin D fracture trials in US and Europe. *BMJ* 2010, **340**:b5463.

[8] Avenell A, Gillespie WJ, Gillespie LD, O'Connell D: **Vitamin D and vitamin D analogues for preventing fractures associated with involutional and post-menopausal osteoporosis**. *Cochrane Dataset Syst Rev* 2009, **2**:CD000227.

[9] Tang BM, Eslick GD, Nowson C, Smith C, Bensoussan A: **Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis**. *Lancet* 2007, **370**:657-66.

[10] Bischoff-Ferrari HA, Dawson-Hughes B, Baron JA, Burckhardt P, Li R, Spiegelman D, et al. **Calcium intake and hip fracture risk in men and women: a meta-analysis of prospective cohort studies and randomized controlled trials**. *Am J Clin Nutr* 2007, **86**:1780-90.

[11] Macdonald HM, New SA, Golden MH, Campbell MK, Reid DM: **Nutritional associations with bone loss during the menopausal transition: evidence of a beneficial effect of calcium, alcohol, and fruit and vegetable nutrients and of a detrimental effect of fatty acids.** *Am J Clin Nutr* 2004, **79**:155-65.

[12] Langsetmo L, Hanley DA, Prior JC, Barr SI, Anastassiades T, Towheed T

Goltzman D, Morin S, Poliquin S, Kreiger N: **Dietary patterns and incident low-trauma fractures in postmenopausal women and men aged ≥ 50 y: a population-based cohort study**. *Am J Clin Nutr* 2011, **93**:192-9.

[13] Donato GB, Fuchs SC, Oppermann K, Bastos C, Spritzer PM: **Association between menopause status and central adiposity measured at different cutoffs of waist circumference and waist-to-hip ratio**. *Menopause* 2006, **13**(2):280-5.

[14] Kanis JA, Melton LJ III, Christiansen C, Johnston CC, Khaltaev N: **The diagnosis of osteoporosis.** *J Bone Miner Res* 1994, **9**:1137-1141.

[15] Silva TR, Maturana M, Spritzer PM: **Healthier dietary pattern and lower risk of metabolic syndrome in physically active postmenopausal women**. *J Am Coll Nutr* 2013, **32**:287-95.

[16] Center for Studies and Research in Food: Brazilian table of food composition. Campinas: NEPA-UNICAMP 2011, São Paulo.

[17] Fernandes CE, Pinho-Neto JSL, Gebara OCE, Santos Filho RD, Pinto Neto AM: **I Diretriz Brasileira sobre Prevenção de Doenças Cardiovasculares em Mulheres Climatéricas e a Influência da Terapia de Reposição Hormonal (TRH) da Sociedade Brasileira de Cardiologia (SBC) e da Associação Brasileira do Climatério (SOBRAC).** *Arq Bras Cardiol* 2008, **91**(Suppl 1):1-23.

[18] Trumbo P, Schlicker S, Yates AA, Poos M: **Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids.** *J Am Diet Assoc* 2002, **102**:1621-30.

[19] Graff SK, Alves BC, Toscani MK, Spritzer PM: **Benefits of pedometer-measured habitual physical activity in healthy women**. *Appl Physiol Nutr Metab* 2012, **37**:149-56.

[20] Colpani V, Oppermann K, Spritzer PM: **Association between habitual physical activity and lower cardiovascular risk in premenopausal, perimenopausal, and postmenopausal women: a population-based study**. *Menopause* 2013, **20**:525-31.

[21] Sheng Z, Xu K, Ou Y, Dai R, Luo X, Liu S, et al. **Relationship of body composition with prevalence of osteoporosis in central south Chinese postmenopausal women.** *Clin Endocrinol* 2011, **74**:319–324.

[22] Lindsay R, Cosman F, Herrington BS, Himmelstein S. **Bone mass and body composition in normal women**. *J Bone Miner Res* 1992, **7**:55–63.

[23] Sukumar D, Schlussel Y, Riedt CS, Gordon C, Stahl T, Shapses SA et al. **Obesity alters cortical and trabecular bone density and geometry in women.** Osteoporos Int 2011, **22**: 635–645.

[24] Pouilles JM, Tremollieres F, Ribot C: **Vertebral bone loss in perimenopause: results of a 7-year longitudinal study.** *Presse Med* 1996, **25**:277-280.

[25] Recker RR, Lappe J, Davies K, Heaney R. **Characterization of perimenopausal bone loss: a prospective study.** *J Bone Miner Res* 2000, **15**:1965-73.

[26] Finkelstein JS, Brockwell SE, Mehta V, Greendale GA, Sowers MR, Ettinger B, et al. **Bone Mineral Density Changes during the Menopause**

Transition in a Multiethnic Cohort of Women. J *Clin Endocrinol Metab* 2008, **93**(3):861-8.

[27] Yoldemir T, Erenus M, Durmusoglu F: **The impact of serum FSH and estradiol**

on postmenopausal osteoporosis related to time since menopause. *Gynecol Endocrinol* 2012, **28**(11):884-8.

[28] Lima RM, Bezerra LM, Rabelo HT, Silva MA, Silva AJ, Bottaro M, de Oliveira RJ**. Fat-free mass, strength, and sarcopenia are related to bone mineral density in older women**. *J Clin Densitom* 2009, **12**(1):35-41.

[29] Simpson ER: **Sources of estrogen and their importance.** *J Steroid Biochem Mol Biol* 2003, **86**(3–5):225–230.

[30] Reid IR, Ames R, Evans MC, Sharpe S, Gamble G, France JT, Lim TM, Cundy TF: **Determinants of total body and regional bone mineral density in normal postmenopausal women-a key role for fat mass**. *J Clin Endocrinol Metab,* 1992, **75**(1):45-51.

[31] Nur H, Toraman NF, Arica Z, Sarier N, Samur A: **The relationship between body composition and bone mineral density in postmenopausal Turkish women.** *Rheumatol Int* 2013, **33**(3):607-12.

[32] Salamone LM, Glynn N, Black D, Epstein RS, Palermo L, Meilahn E, Kuller LH, Cauley JA: **Body composition and bone mineral density in premenopausal and early perimenopausal women**. *J Bone Miner Res* 1995, **10**(11):1762-8.

[33] Li S, Wagner R, Holm K, Lehotsky J, Zinaman MJ: **Relationship between soft tissue body composition and bone mass in perimenopausal women.** *Maturitas* 2004, **47**(2):99-105.

[34] Kaufman BA, Warren MP, Dominguez JE, Wang J, Heymsfield SB, Pierson RN: **Bone density and amenorrhea in ballet dancers are related to a decreased resting metabolic rate and lower leptin levels.** *J Clin Endocrinol Metab* 2002, **87**:2777-83.

[35] Doyle-Lucas AF, Akers JD, Davy BM: **Energetic efficiency, menstrual irregularity, and bone mineral density in elite professional female ballet dancers.** *J Dance Med Sci* 2010, **14**:146-54.

[36] Hodson L, Harnden K, Banerjee R, Real B, Marinou K, Karpe K et al. **Lower resting and total energy expenditure in postmenopausal compared with premenopausal women matched for abdominal obesity.** *J Nutr Sci*, 2014, **3**(e3):1-9

[37] Heinonen A, Oja P, Sievanen H, Pasanen M, Vuori I: **Effect of two training regimens on bone mineral density in healthy perimenopausal women: a randomized controlled trial.** *J Bone Miner Res* 1998, **13**:483-90.

[38] Yamazaki S, Ichimura S, Iwamoto J, Takeda T, Toyama Y: **Effect of walking exercise on bone metabolism in postmenopausal women with osteopenia/osteoporosis**. *J Bone Miner Metab* 2004, **22**:500-8.

[39] Hinriksdottir G, Arngrimsson SA, Misic MM, Evans EM: **Lean soft tissue contributes more to bone health than fat mass independent of physical activity in women across the lifespan**. *Maturitas* 2013, **74**:264-269.

[40]Wee J, Sng BY, Shen L, Lim CT, Singh G, Das De S: **The relationship between body mass index and physical activity levels in relation to bone mineral density in premenopausal and postmenopausal women.** *Arch Osteoporos* 2013, **8**(1-2):162.

[41] Warensjo E, Byberg L, Melhus H, Gedeborg R, Mallmin H, Wolk A, Michaelsson K: **Dietary calcium intake and risk of fracture and osteoporosis: prospective longitudinal cohort study**. *BMJ* 2011, **342**:d1473.o

[42] Pedone C, Napoli N, Pozzilli P, Rossi FF, Lauretani F, Bandinelli S et al. **Dietary pattern and bone density changes in elderly women: a longitudinal study.** *J Am Coll Nutr* 2011, **30**(2):149-54.

[43] Heaney RP, Layman DK: **Amount and type of protein influences bone health**. *Am J Clin Nutr 2008*, **87**(Suppl 5):1567-70.

[44] Wattanapenpaiboon N, Lukito W, Wahlqvist ML, Strauss BJ**: Dietary carotenoid intake as a predictor of bone mineral density**. *Asia Pac J Clin Nutr* 2003**, 12**(4):467-73.

[45] Wu AM, Huang CQ, Lin ZK, Tian NF, Ni WF, Wang XY et al. **The relationship between vitamin A and risk of fracture: meta-analysis of prospective studies**. *J Bone Miner Res* 2014, **29**(9):2032-9.

Table 1. Characteristics of postmenopausal women according to time since menopause and bone mass *status*

	≤ 5 years since menopause		> 5 years since menopause				
	Normal Bone Mass	Low Bone Mass	Normal Bone Mass	Low Bone Mass	*P value*		
	(n = 21)	(n = 28)	(n = 10)	(n = 40)	Time since menopause	BMD	T x B[1]
Age (years)	52.2 ± 4.2[a]	52.1 ± 3.3[a]	55.9 ± 4.4[b]	58.9 ± 3.8[c]	**<0.001**	0.096	0.076
Years at school (years)	9.5 ± 3.4	8.3 ± 4.5	6.2 ± 3.3	8.5 ± 4.7	0.120	0.535	0.079
White, n (%)*	19 (90)	24 (86)	8 (80)	35 (87)		0.688	
Weight (kg)	77.9 ± 15.2[b]	62.7 ± 8.6[a]	69.5 ± 9.4[b]	65.5 ± 11.0[ba]	0.282	**<0.001**	**0.033**
BMI (kg/m^2)	30.6 ± 5.8[b]	25.6 ± 2.9[a]	27.4 ± 3.5[b]	26.3 ± 4.3[ba]	0.189	**0.002**	**0.050**
Waist circumference (cm)	95.4 ± 14.8[b]	82.5 ± 7.9[a]	87.6 ± 8.9[b]	84.4 ± 10.7[ba]	0.239	**0.002**	0.054
Body fat %	44.2 ± 5.6[b]	39.5 ± 6.2[a]	41.3 ± 4.8[b]	38.9 ± 6.2[ba]	0.262	**0.025**	0.453
Lean mass (kg)	41.3 ± 5.9[b]	35.6 ± 4.2[a]	38.7 ± 5.3[b]	37.7 ± 3.8[ba]	0.797	**0.002**	**0.027**
Fat mass (kg)	33.7 ± 10.3[b]	25.2 ± 9.6[a]	27.5 ± 6.2[b]	25.0 ± 8.7[ba]	0.131	**0.010**	0.159
RMR (Kcal/day)	1358.4 ± 285.8[b]	1191.8 ± 134.8[a]	1302.0 ± 189.8[b]	1242.5 ± 165.3[ba]	0.949	**0.012**	0.227
Mean steps/day	3681.6 ± 208.8	3718.9 ± 262.3	3659.1 ± 225.7	3731.0 ± 243.8	0.925	0.323	0.754
Sedentary, n (%)*	14 (67)	16 (57)	7 (70)	21 (52)		0.564	
Lumbar spine BMD (g/cm^2)	1.20 ± 0.08[c]	1.00 ± 0.08[b]	1.19 ± 0.09[c]	0.94 ± 0.13[a]	0.139	**<0.001**	0.352
Femoral neck BMD (g/cm^2)	1.01 ± 0.08[c]	0.88 ± 0.08[b]	0.98 ± 0.07[c]	0.80 ± 0.09[a]	**0.027**	**<0.001**	0.344
Total femoral BMD (g/cm^2)	1.07 ± 0.09[c]	0.92 ± 0.09[b]	1.02 ± 0.08[c]	0.86 ± 0.10[a]	**0.015**	**<0.001**	0.744
Estradiol (pg/mL)	27.6 ± 15.7	22.2 ± 12.6	20.9 ± 10.4	20.1 ± 11.9	0.178	0.191	0.583
Free estradiol index (pg/mL)	0.4 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.2	0.178	0.191	0.583

Values are expressed as mean ± SD, median and 25–75 inter-quartile range or absolute and percentage number.
BMI: body mass index; RMR: resting metabolic rate; BMD: bone mineral density. [1]T × B = Time since menopause × Bone Mineral Density interaction effect. [a-c] Means in a row without a common superscript letter differ ($P < 0.05$), as analyzed by two-way ANOVA and Bonferroni test. *Qui-square Test

Figure 1. Prevalence of osteoporosis, osteopenia, and normal bone mass in women grouped according to time since menopause (≤ 5 and > 5 years).

Table 2. Lumbar spine, femoral neck and total femoral bone mineral density in postmenopausal women according to factors

Factor	Tertile	N	Lumbar spine BMD (g/cm^2)	*P**	Femoral neck BMD (g/cm^2)	*P**	Total femoral BMD (g/cm^2)	*P**
Age (years)	≤53	37	1.11 ± 0.13[a]		0.94 ± 0.11[a]		1.00 ± 0.11[a]	
	53-58	31	1.00 ± 0.15[b]	**0.002**	0.88 ± 0.11[a]	**<0.001**	0.93 ± 0.11[b]	**<0.001**
	≥58	31	0.98 ± 0.14[b]		0.81 ± 0.09[b]		0.88 ± 0.11[b]	
Time since menopause (years)	≤3	34	1.08 ± 0.13[a]		0.91 ± 0.11[a]		0.98 ± 0.12[a]	
	3-8	32	1.07 ± 0.15[a]	**0.002**	0.90 ± 0.11[a]	**0.008**	0.96 ± 0.11[a]	**0.002**
	≥8	33	0.96 ± 0.15[b]		0.83 ± 0.11[b]		0.88 ± 0.12[b]	
BMI (kg/m^2)	≤25	33	1.00 ± 0.13		0.82 ± 0.10[a]		0.87 ± 0.10[a]	
	25-28.2	34	1.04 ± 0.14	0.086	0.91 ± 0.10[b]	**0.001**	0.97 ± 0.10[b]	**<0.001**
	≥28.2	32	1.08 ± 0.17		0.91 ± 0.12[b]		0.98 ± 0.13[b]	
Fat Mass (kg)	≤23.4	33	1.01 ± 0.13[a]		0.84 ± 0.12[a]		0.89 ± 0.1 [a]	
	23.4-29.8	34	1.01 ± 0.17[a]	**0.005**	0.89 ± 0.12[ab]	**0.016**	0.94 ± 0.13[ab]	**0.003**
	≥29.8	32	1.11 ± 0.13[b]		0.92 ± 0.10[b]		0.99 ± 0.110[b]	
Lean Mass (kg)	≤36.0	34	1.02 ± 0.15		0.86 ± 0.10		0.92 ± 0.10	
	36.0-39.2	33	1.02 ± 0.14	0.108	0.87 ± 0.12	0.086	0.93 ± 0.13	0.130
	≥39.2	32	1.08 ± 0.15		0.92 ± 0.12		0.98 ± 0.12	
Estradiol (pg/mL)	≤15	34	1.00 ± 0.14		0.85 ± 0.12		0.91 ± 0.13	
	15-26	33	1.04 ± 0.16	0.252	0.88 ± 0.12	0.069	0.94 ± 0.13	0.102
	≥26	32	1.07 ± 0.15		0.91 ± 0.10		0.97 ± 0.10	
Free estradiol (pg/mL)	≤0.20	33	0.99 ± 0.14		0.84 ± 0.12[a]		0.91 ± 0.13	
	0.2-0.35	32	1.04 ± 0.15	0.207	0.89 ± 0.12[ab]	0.053	0.94 ± 0.13	0.093
	≥0.35	34	1.07 ± 0.15		0.91 ± 0.10[b]		0.97 ± 0.10	
RMR (Kcal)	≤1175	33	1.01 ± 0.15[a]		0.85 ± 0.11[a]		0.90 ± 0.10	
	1175-1324	35	1.00 ± 0.14[a]	**0.005**	0.89 ± 0.11[ab]	**0.046**	0.95 ± 0.13	**0.028**
	≥6564	31	1.01 ± 0.13		0.91 ± 0.11[b]		0.98 ± 0.12	

[a-b]Means in a column without a common superscript letter differ ($P < 0.05$), as analyzed by one-way ANOVA and the Bonferroni test.

Table. 3 Odds ratio for Low Bone Mass

Variables	**OR**	**95% CI**	***P*** *
BMI (< 25 kg/m^2)[a]	**5.21**	**1.57 – 17.25**	**0.004**
Lean Mass (< 37.5 kg)[b]	**4.40**	**1.64 – 11.80**	**0.004**
Fat Mass (< 26.0 kg)[b]	**3.39**	**1.29 – 8.85**	**0.010**
Vitamin A (< 700mcg/day)[c]	**3.00**	**1.13 – 7.94**	**0.012**
Meat and eggs (< 96g/day)[b]	2.30	0.90 – 5.86	0.081

BMI: body mass index. *Logistic regression adjusted for time since menopause and previous hormonal therapy. [a]Defined as the first tertile of the studied sample; [b]defined as the median of participants in this sample; [c]Dietary Reference Intake (2002).

CAPÍTULO 4

CONSIDERAÇÕES FINAIS

Com base nos achados da revisão sistemática, não foram encontradas diferenças entre as dietas hiperproteicas em relação às dietas controles em ensaios clínicos randomizados que avaliaram os parâmetros de gordura abdominal em mulheres de meia-idade. No entanto, devido ao número limitado de evidências não podemos concluir com propriedade sobre o tema. Ademais, dietas com objetivo de redução de peso corporal, independentemente da composição nutricional podem diminuir obesidade abdominal em mulheres de meia-idade.

Em nossa amostra de mulheres na pós-menopausa recente e sem doenças clínicas evidentes, a prevalência de baixa massa muscular foi de apenas 7%. O índice de massa muscular foi positivamente associado com ingestão de proteína e negativamente associado com % gordura corporal. As participantes com ingestão proteica no tercil superior apresentaram melhor perfil metabólico e padrão alimentar mais saudável. Este grupo também apresentou menor IMC, circunferência da cintura e massa gorda do tronco.

Por fim, em outro trabalho com a mesma população do estudo anterior, o tempo de menopausa, a menor massa magra e de gordura corporal, além de ingestão de vitamina A < 700 µg/dia foram associados com baixa massa óssea. Ingestão calórica e de macronutrientes, bem como atividade física habitual, não

interferiram na DMO, no entanto a maior parte das participantes eram sedentárias.