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EFEITOS DA INGESTÃO PROTEICA SUPERIOR À RECOMENDAÇÃO DA RDA NA FUNÇÃO MUSCULAR DE MULHERES PÓS-MENOPAUSADAS PRATICANTES DE EXERCÍCIO DE FORÇA

PAULA CÂNDIDO NAHAS

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Faculdade de Medicina da Universidade Federal de Uberlândia, como requisito parcial para a obtenção do título de Mestre em Ciências da Saúde.

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Paula Cândido Nahas

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"Que os vossos esforços desafiem as impossibilidades, lembrai-vos de que as grandes coisas do homem foram conquistadas do que parecia impossível".

Charles Chaplin

RESUMO

Introdução: O período de pós-menopausa propicia mudanças na composição corporal da mulher, principalmente no que se diz respeito à redução da massa muscular, força e capacidade funcional. Tais alterações se relacionam com o comprometimento funcional, já que há maior risco de quedas, fraqueza e perda da independência, impactando negativamente na qualidade de vida da mulher. **Objetivo:** Avaliar o efeito da ingestão de proteína recomendada pela *Recommended Dietary Allowance* (RDA) com novas propostas de consumo e o impacto na força e capacidade funcional de mulheres pós-menopausadas após protocolo de exercício de força. **Material e métodos:** Foi realizado um estudo cego, randomizado, paralelo e prospectivo. As mulheres pós-menopausadas foram divididas em dois grupos: Grupo RDA (NP), que recebeu um plano alimentar contendo ~0,8g de proteína/kg/dia ou Grupo Novas Propostas (HP), que recebeu ~1,2g de proteína/kg/dia. O protocolo de treinamento de força foi realizado 3 vezes/semana, com intensidade de 10-12 repetições máximas. Força (uma repetição máxima - 1-RM e força de preensão manual) e capacidade funcional (*Short Physical Performance Battery* - *S*PPB, teste de caminhada de 6 minutos, teste de caminhada de 400 metros, teste de caminhada de 10 metros e *Timed Up and Go test* - TUG) foram avaliados antes e após a intervenção. A dieta foi avaliada por nove recordatórios de 24 horas. A intervenção dietética e o treinamento de força duraram 10 semanas. **Resultados:** O Grupo HP apresentou maior consumo proteico (1,18±0,3 vs 0,87±0,2 g/kg/dia, p=0,008) comparado ao grupo NP, respectivamente, durante o estudo. Após a intervenção, apenas o grupo HP aumentou o 1-RM do supino, 1-RM da extensora e força de preensão manual direita e esquerda, porém, não foi encontrado diferenças analisando o delta (Δ) dessas variáveis quando comparado com o grupo NP (Δ1-RM supino: 2,75±3,01 vs 1,09±2,25 kg, p=0,153; Δ1-RM extensora: 6,75±12,46 vs 9,45±7,03 kg, p=0,533; ΔHandgrip direita: 1,66±3,20 vs 1,90±1,64 kg, p=0,823 e ΔHandgrip esquerda: 1,83±2,62 vs 2,81±2,56 kg, p=0,373, grupo NP e HP, respectivamente. Para os demais testes não foram observadas diferenças entre grupos e/ou momentos. **Conclusão:** A ingestão de proteína de acordo com novas propostas não promoveu melhora de força e capacidade funcional de mulheres pós-menopausadas quando comparado a recomendação da RDA após protocolo de exercício de força.

Palavras-chave: Pós-Menopausa. Função Muscular. Massa Muscular. Intervenção Dietética.

ABSTRACT

Introduction: Postmenopausal period leads to changes in women body composition, especially in regard to the reduction of muscle mass, strength and functional capacity. Such changes are totally related to functional impairment, since there is a greater risk of falls, weakness and loss of independence, negatively impacting women's quality of life. **Objective:** To evaluate the effect of protein intake recommended by Recommended Dietary Allowance (RDA) with new consumption proposals and the impact on strength and functional capacity of postmenopausal women after exercise protocol. **Material and methods:** A blind, randomized, parallel and prospective study was performed. Postmenopausal women were divided into two groups: RDA group (NP), which received a diet plan containing ~0.8g protein$\cdot$kg$^{-1}\cdot$d^{-1} or New Proposals Group (HP), which received ~1.2g protein$\cdot$kg$^{-1}\cdot$d^{-1}. Resistance training protocol was performed 3 times/week, with intensity of 10-12 maximal repetitions. Strength (maximum repetition - 1-RM and handgrip strength) and functional capacity (Short Physical Performance Battery - SPPB, 6-minute walk test, 400-meter walk test, 10-meter walk test and Timed Up and Go test - TUG) were evaluated before and after the intervention. The diet was evaluated by nine 24-hour food recalls. Dietary intervention and resistance training protocol lasted 10 weeks. **Results:** HP group presented higher protein intake (1.18±0.3 vs 0.87±0.2g protein$\cdot$kg$^{-1}\cdot$d^{-1}, $p = 0.008$) than NP group, respectively, during the study. After intervention, only HP increased bench press RM, leg extension RM and right/left handgrip, however, no differences were found by analyzing the delta (Δ) of these variables when compared to NP group (ΔBench press RM: 2.75±3.01 vs 1.09±2.25 kg, $p = 0.153$; ΔLeg extension RM: 6.75±12.46 vs 9.45±7.03 kg, $p = 0.533$; ΔRight handgrip: 1.66±3.20 vs 1.90±1.64 kg, $p = 0.823$ and ΔLeft handgrip: 1.83±2.62 vs 2.81±2.56 kg, $p = 0.373$), NP and HP group respectively. For the other tests, no differences between groups and/or moments were observed. **Conclusion:** A protein intake according to new proposals did not improve strength and functional capacity of postmenopausal women when compared to RDA recommendation after resistance training protocol.

Key Words: Postmenopausal. Muscle Function. Muscle Mass. Diet Intervention.

LISTA DE ABREVIATURAS E SÍMBOLOS

AVD	Atividades de Vida Diária
BIA	Bioimpedância elétrica
DMO	Densidade Mineral Óssea
DEXA	Absorciometria de raios X de dupla energia
EWGSOP	Grupo de Pesquisa Europeu em Estudo da Sarcopenia
FPM	Força de Preensão Manual
FSH	Hormônio Folículo-Estimulante
HP	Hiperproteico
LH	Hormônio Luteinizante
NP	Normoproteico
RDA	*Recommended Dietary Allowance*
RM	Ressonância Magnética
SPPB	*Short Physical Performance Battery*
TC	Tomografia Computadorizada
TUG	*Timed get-Up-and-Go*
1-RM	Uma repetição máxima

SUMÁRIO

1 INTRODUÇÃO

A proporção de pessoas idosas é crescente, sendo que o aumento da expectativa de vida nos últimos anos despertou maior interesse e preocupação com a saúde e a qualidade de vida dos indivíduo (WORLD HEALTH ORGANIZATION, 2005). Considerando que as mulheres podem viver mais de um terço de suas vidas após o período da menopausa, surge a necessidade de buscar maiores conhecimentos sobre o processo fisiológico desta fase de vida (BRASIL, 2004).

Alterações provenientes do período da pós-menopausa, tais como mudanças na composição corporal e redução da densidade mineral óssea, as quais coincidem com alterações provenientes do próprio envelhecimento, são importantes e merecem atenção (LEBRUN et al., 2006; RIGGS; KHOSLA; MELTON, 1998). Mulheres pós-menopausadas que apresentam redução da funcionalidade muscular, geralmente acompanhada ou não da diminuição da massa muscular, estão mais propícias à quedas, justamente porque com o avanço da idade ocorre redução da velocidade de marcha, baixa resistência física e diminuição da mobilidade (ANJOS et al., 2016). Dentre as estratégias que devem ser utilizadas para atenuar essa condição, merece destaque o treinamento de força e a ingestão proteica adequada (BREEN; PHILLIPS, 2012; LANDI et al., 2013).

Sendo assim, este trabalho justifica-se pela necessidade de investigar se a dieta hiperproteica, associada ao treinamento de força, é importante para a melhora da função muscular (força e capacidade funcional) de mulheres pós-menopausadas. Caso a hipótese seja verdadeira, o intuito é estabelecer condutas dietéticas eficientes para melhora da função muscular nessa população.

2 FUNDAMENTAÇÃO TEÓRICA

2.1 Período pós-menopausa

2.1.1 Definição

Após o período fértil, por volta dos 40 anos de idade da mulher, inicia-se uma fase de irregularidades do ciclo menstrual, denominada climatério (FREEMAN; SHERIF, 2007). Tais oscilações são resultantes das alterações endócrinas que iniciam nesse período, principalmente pela atenuação da atividade folicular ovariana, redução da produção de estrógeno, da inibina B e do hormônio anti-mülleriano, marcadores da atividade ovariana. A redução de inibina B permite que os níveis do hormônio folículo-estimulante (FSH) e do hormônio luteinizante (LH) permaneçam aumentados, fator importante para a manutenção da concentração de estradiol até o final da vida reprodutiva (BURGER et al., 2007). Já a menopausa corresponde ao último episódio de menstruação da mulher seguido pelo ano subsequente de amenorreia, período marcado também pela instabilidade hormonal. Após essa fase, inicia-se o período de pós-menopausa (BURGER et al., 2007; PALACIOS et al., 2010).

O período de pós-menopausa inicia-se após a fase em que ocorre a cessação espontânea e permanente da menstruação da mulher por pelo menos 12 meses. Esse acontecimento marca o fim da vida reprodutiva e se estende até a morte (PALACIOS et al., 2010). O término do período menstrual na pós-menopausa ocorre principalmente por conta de diversas alterações na secreção de hormônios ovarianos (estrógeno e progesterona), hormônios hipofisários (FSH e LH) e consequente alterações na produção de folículos ovarianos (BURGER et al., 2007).

2.1.2 Principais alterações provenientes da pós-menopausa

Uma das principais alterações geradas pela pós-menopausa é a modificação da composição corporal. A redução da produção ovariana de estrógeno, que se inicia no período de irregularidade menstrual na mulher, é um dos fatores que promove tais mudanças. Ocorre aumento de gordura corporal, principalmente na região abdominal, e progressiva redução da massa muscular esquelética (LANDI et al., 2013; SOWERS et al., 2007), que são potencializadas pelo envelhecimento. A redução progressiva e generalizada da massa muscular esquelética, acompanhada da diminuição da função muscular (força e/ou capacidade funcional) denomina-se sarcopenia (CRUZ-JENTOFT et al., 2010).

A redução da produção de estrógeno também propicia a diminuição da integridade estrutural e da densidade mineral óssea (DMO) da mulher pós-menopausada, levando à rápida perda de massa óssea (RIGGS et al., 1998). Tal condição ocasiona aumento do risco de desenvolvimento da osteoporose (CUMMINGS; MELTON, 2002), sendo que sua principal consequência é a fratura óssea, perda da funcionalidade e da independência (SUOMINEN, 2006). É importante ressaltar que a sarcopenia se correlaciona positivamente com a redução da DMO (ORSATTI et al., 2011).

2.2 Massa muscular e função muscular

Uma vez que mulheres pós-menopausadas tendem a apresentar quantidades de massa muscular e funcionalidade muscular reduzidas, tanto pelo envelhecimento, quanto pela condição da pós-menopausa, é importante realizar a avaliação destas duas variáveis como forma de prevenção e de tratamento. Dessa forma, o Grupo de Pesquisa Europeu em Estudo da Sarcopenia (EWGSOP) descreve as principais técnicas de avaliação da massa muscular e função muscular que são mais adequadas para a prática clínica e/ou pesquisas, salientando que tais medidas devem ser repetidas ao longo do tempo para observar possíveis alterações nestes parâmetros (CRUZ-JENTOFT et al., 2010).

2.2.1 Métodos de avaliação da massa muscular

De acordo com o EWGSOP, diversos métodos podem ser utilizados para a avaliação da massa muscular, tais como, técnicas de imagem (tomografia computadorizada – TC, ressonância magnética – RM, absorciometria de raios X de dupla energia – DEXA), análise de bioimpedância (BIA) e medidas antropométricas.

A TC e a RM são métodos extremamente precisos, sendo considerados padrão ouro para estimar massa muscular em pesquisas (MITSIOPOULOS et al., 1998), enquanto o DEXA (CHEN et al., 2007) apresenta-se como um método alternativo para pesquisas e utilização clínica, também com grande fidedignidade. Técnicas de imagem ainda são pouco utilizadas principalmente por conta do custo e da disponibilidade, porém, são as mais recomendadas para pesquisas (CRUZ-JENTOFT et al., 2010). A BIA é um método que estima a água e a massa magra corporal apresentando-se como alternativa ao DEXA, justamente pelo seu menor custo e por ser portátil (OSHIMA et al., 2010). Existem equações preditivas e validadas para adultos que correlacionam a BIA com RM (JANSSEN et al., 2000), como forma de aproximá-la ao padrão ouro. Por fim, medidas antropométricas são frequentemente utilizadas em fórmulas de predição de massa muscular, principalmente circunferências e dobras cutâneas (braço, coxa e panturrilha) (LEE et al., 2000; MARTIN et al., 1990). Apesar de se correlacionarem com a massa muscular, poucos são os estudos que validam essas medidas em populações e condições específicas, além de serem mais suscetíveis a erros (CRUZ-JENTOFT et al., 2010).

2.2.2 Métodos de avaliação da função muscular

Para a função muscular, existem inúmeros testes específicos de avaliação, tanto para a força quanto para a capacidade funcional. Para a força recomenda-se os testes de força de preensão manual (FPM), teste de flexão/extensão do joelho e teste de uma repetição máxima (1-RM). Já para a capacidade funcional, os testes do *Short Physical Performance Battery* (SPPB), teste de velocidade de marcha habitual, teste de caminhada de 6 minutos, teste de potência de subida de escada e teste *Timed get-Up-and-Go* (TUG) são os mais utilizados na prática clínica e em pesquisas. É importante ressaltar que existe menor quantidade de técnicas que foram bem validadas para avaliar a função muscular em comparação com os métodos de avaliação da massa muscular.

Em relação aos testes de força, a FPM é um teste isométrico simples e que apresenta forte correlação com a força muscular de membros inferiores (NORMAN et al., 2011). Pode ser utilizado para pacientes a nível ambulatorial e acamados e está associado com a mobilidade (RIJK et al., 2016) e mortalidade (MENDES; AZEVEDO; AMARAL, 2014). O teste de flexão/extensão do joelho assemelha-se com o teste de FPM, já que também é uma medida de força isométrica, além de predizer também força de membro inferior (EDWARDS et al., 1977). Este teste é mais utilizado em pesquisas, uma vez que sua utilização na prática clínica é limitada pela necessidade de equipamento e treinamento. Finalmente, o teste de 1-RM corresponde a carga máxima que o indivíduo consegue suportar para realizar apenas uma repetição completa de um exercício. Este teste correlaciona-se fortemente com a força das pernas (VERDIJK et al., 2009).

No que diz respeito aos testes de capacidade funcional, o SPPB consiste em uma bateria de testes de desempenho físico relacionado a membros inferiores, avaliando o equilíbrio, a marcha habitual, a força e a resistência (GURALNIK et al., 1994). Usualmente é utilizado na prática clínica e em pesquisas. A velocidade de marcha habitual avaliada pelo SPPB também pode ser utilizada isoladamente como um parâmetro de capacidade funcional (GURALNIK et al., 2000). O teste de caminhada de 6 minutos avalia a capacidade aeróbia do indivíduo, com o intuito de representar as atividades diárias normalmente realizadas, além de ser de fácil execução. É utilizado também para prever a morbidade e mortalidade de pacientes com doença cardiovascular ou pulmonar (CAHALIN et al., 1995). O teste de potência de subida de escada avalia a força de membro inferior, é de simples execução e apropriada principalmente para avaliação de desempenho de idosos (BEAN et al., 2007). Por fim, o TUG representa um teste de agilidade e também de força de membros inferiores, utilizado principalmente para avaliação do desempenho de adultos mais velhos e idosos (BEAUCHET et al., 2011; STEFFEN; HACKER; MOLLINGER, 2002).

2.2.3 Consequências da redução da funcionalidade muscular

Sabe-se que o sistema locomotor (composto por músculos, ossos e tendões) é responsável pelo movimento do corpo (SALTIN, 2011). Portanto, alterações fisiológicas provenientes do envelhecimento e relacionadas à funcionalidade desses tecidos, como a perda de massa e função muscular (sarcopenia) e/ou redução da DMO, impactam de forma prejudicial sobre o desempenho motor, o qual é determinante da capacidade funcional (LANDI et al., 2013).

O desencadeamento destas alterações funcionais, consequentemente, acarreta em redução da velocidade de marcha, baixa resistência física e diminuição da mobilidade (ROBERTS et al., 2011), aumentando o risco de quedas em adultos mais velhos e idosos. Entende-se por queda um deslocamento involuntário do corpo para um nível inferior à posição inicial, sem que o indivíduo consiga regredir em tempo hábil, causado por uma série de fatores, comprometendo a estabilidade (SIROLA; RIKKONEN, 2005). Tal evento é importante causador de lesões e de diminuição da qualidade de vida, além de propiciar maiores gastos na saúde (HASHEMI et al., 2015) e um maior risco de morte nessa população (SIROLA; RIKKONEN, 2005). A queda apresenta-se como um importante sinalizador do declínio da capacidade funcional e é o mais sério e frequente acidente doméstico que ocorre em pessoas de maior idade, sendo considerada a principal causa de morte acidental em indivíduos com idade superior a 65 anos (ANJOS et al., 2016). Além disso, os indivíduos ficam mais dependentes para realizar atividades de vida diária (AVD), necessitando de ajuda para cumprir tarefas básicas, tais como se alimentar, vestir, fazer higiene pessoal e realizar trabalhos *domésticas* (JANSSEN et al., 2004).

Dados de estudos prospectivos (PERRACINI; RAMOS, 2002; REYES-ORTIZ; AL SNIH; MARKIDES, 2005) indicam que de 30 a 60% dos indivíduos idosos caem anualmente, sendo que metade destes apresentam quedas múltiplas no ano. Aproximadamente 50% desses acontecimentos levam a lesões, sendo 5% corresponde a danos mais graves e 5% a fraturas, principalmente de fêmur, úmero, rádio e vertebral. Outro aspecto importante é que quanto maior a idade do indivíduo, maior é a tendência de queda por ano (SALTIN, 2011). Além disso, a queda que ocasiona hospitalização pode acarretar em complicações cardiovasculares, pulmonares e levar até a morte do indivíduo (PALMER, 2001).

Portanto, a manutenção da massa e função muscular, juntamente com uma boa saúde óssea no envelhecimento certamente são fatores protetores contra quedas e consequentemente previne a ocorrência de lesões, diminui gastos hospitalares, reduz a morbidade e a mortalidade e promove melhor qualidade de vida para um envelhecimento saudável (SIROLA; RIKKONEN, 2005).

2.2.4 Função muscular no período da pós-menopausa

Como dito anteriormente, a redução da massa muscular esquelética e função muscular (LANDI et al., 2013) somado à acentuada perda óssea (RIGGS et al., 1998), ambos decorrentes do próprio envelhecimento, aumenta a exposição do indivíduo para quedas e fraturas ósseas (CUMMINGS; MELTON, 2002).

O período da pós-menopausa coincide com o envelhecimento (~45-50 anos de idade na mulher). Sendo assim, mulheres pós-menopausadas também estão mais propícias a redução da funcionalidade muscular (LANDI et al., 2013). Isso porque, nesse período de vida a mulher apresenta redução na produção de estrógeno e progesterona, hormônios anabólicos e relacionados também com a composição corporal, que estão em altas concentrações na idade fértil. Sendo assim, a redução da quantidade de massa muscular e da função muscular aumenta o risco para quedas, fraturas, redução da mobilidade e diminuição da qualidade de vida principalmente nessa população (BURGER et al., 2007).

A manutenção ou até melhora da função muscular das mulheres durante o envelhecimento pode reduzir o risco de quedas e fraturas devido à fragilidade, diminuindo o peso da osteoporose e outras complicações (KOHRT et al., 2004).

2.3 Intervenções para melhora da função muscular de mulheres pós-menopausadas

2.3.1 Treinamento de força

O exercício físico é considerado uma das intervenções eficazes para neutralizar o declínio da massa e função em indivíduos mais velhos (BRASIL, 2004). Dentre os tipos de exercícios, o destaque é para o treinamento de força, bem estabelecido como uma estratégia efetiva para combater a perda de massa muscular e de força em idosos (FIATARONE et al., 1990; KOSEK et al., 2006). Leenders e colaboradores (2013) (LEENDERS et al., 2013) demonstraram que o treinamento de força realizado durante um período de 6 meses promoveu benefícios em relação à perda de massa muscular e força provenientes do envelhecimento, para homens e mulheres idosos.

Mulheres pós-menopausadas podem se beneficiar do treinamento de força, uma vez que nesse período ocorre alterações na composição corporal, que se caracterizam por um aumento da gordura corporal, especialmente na região abdominal, e uma progressiva redução de massa muscular e força (LANDI et al., 2013; MESSIER et al., 2011). Nunes e colaboradores (2016) (NUNES et al., 2016) observaram que o treinamento de força com baixo volume (3 séries) melhorou a força muscular de mulheres pós-menopausadas.

Além de promover a manutenção e até mesmo o ganho de massa muscular e melhora da função muscular, o treinamento de força também é uma das principais intervenções para doenças crônico degenerativas (BOOTH; ROBERTS; LAYE, 2012) e para melhora da DMO (WATSON et al., 2015). Associada ao treinamento de força, atualmente há um número crescente de evidências a respeito de estratégias nutricionais, sendo que também podem ser eficazes nessa população de mulheres pós-menopausadas. Destacamos o papel da proteína, da creatina e do ômega-3 em relação à massa e a função muscular.

2.3.2 Proteína

Uma estratégia eficaz para atenuar as perdas ou até mesmo para ganho de massa muscular e melhora da função muscular consiste em associar o treinamento de força com a ingestão adequada de proteínas (BREEN; PHILLIPS, 2012).

A quantidade diária recomendada para adultos e idosos, incluindo mulheres pós-menopausadas, de acordo com a Ingestão Dietética Recomendada (RDA) é de 0,8g de proteína/kg de peso corporal/dia (IOM, 2005). Porém, evidências atuais indicam que o envelhecimento promove "resistência ao anabolismo", sugerindo que pessoas de maior idade, incluindo também mulheres pós-menopausadas, necessitam de ingestão proteica superior à quantidade proposta pela RDA para estimular adequadamente síntese proteica muscular (CHURCHWARD-VENNE et al., 2013). Estudos apontam que pessoas de maior idade necessitam ingerir uma quantidade de 1,0 a 1,3g de proteína/kg de peso corporal/dia (BAUER et al., 2013; PADDON-JONES et al., 2015; PHILLIPS; CHEVALIER; LEIDY, 2016).

Estudos associativos indicam que mulheres pós-menopausadas que consumiam quantidades maiores de proteína do que é recomendado pela RDA apresentaram melhora da função muscular (GREGORIO et al., 2014) e que o consumo ≥1,2g/kg/dia por mulheres idosas também se correlaciona positivamente com melhor função muscular (ISANEJAD et al., 2016).

Outro aspecto que deve ser considerado é a qualidade proteica, sendo que deve-se estimular o consumo de proteínas ricas em leucina, aminoácido capaz de estimular a via de síntese proteica (BREEN; PHILLIPS, 2012).

Além disso, vários pesquisadores recomendam que o consumo proteico seja igualmente distribuído ao longo do dia, de modo que todas as refeições contenham quantidades suficientes de proteína para estimular satisfatoriamente o anabolismo proteico (ARETA et al., 2013; CHURCHWARD-VENNE et al., 2013). Atualmente recomenda-se uma quantidade aproximada de 0,4g de proteína/kg de peso corporal/ refeição, cerca de 30g por refeição (PADDON-JONES et al., 2015). Outro ponto que deve ser considerado é que com o envelhecimento os indivíduos apresentam inapetência, dificilmente conseguindo atingir tais quantidades de proteína propostas acima, por meio apenas de alimentos fontes (LANDI et al., 2016). Por isso, uma alternativa eficaz é a utilização de suplemento proteico, com destaque para a proteína do soro do leite (*whey protein*), uma vez que possui alto valor nutricional por conter grandes concentrações de aminoácidos de cadeia ramificada, principalmente a leucina (SOUSA et al., 2012).

3 OBJETIVOS

3.1 Objetivo Geral

Avaliar o efeito da ingestão proteica recomendada pela *Recommended Dietary Allowance* (RDA) com novas propostas de consumo e seus efeitos na função muscular de mulheres pós-menopausadas praticantes de exercício de força.

3.2 Objetivos Específicos

- Avaliar a ingestão dietética de mulheres pós-menopausadas antes, durante e após a intervenção;
- Avaliar a força muscular e a capacidade funcional de mulheres pós-menopausadas antes e após a intervenção;
- Mensurar parâmetros antropométricos e de composição corporal de mulheres pós-menopausadas antes e após a intervenção.

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4. ARTIGO CIENTÍFICO

“Protein intake higher than RDA recommendation does not improve muscle function in postmenopausal women after resistance training protocol”

Original Article

Protein intake higher than RDA recommendation does not improve muscle function in postmenopausal women after resistance training protocol

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ABSTRACT

Objectives: To evaluate the effect of new proposal of protein intake, compared to RDA recommendation, on muscle function improvements in postmenopausal women (PW) after resistance training protocol. **Design:** A single-blind, randomized, parallel and prospective study. **Participants:** Twenty-three PW. **Intervention:** PW were randomized in two groups: RDA (NP) (n=12), who received a dietary plan containing ~0.8 g protein·kg^{-1}·d^{-1}, and new proposals (HP) (n=11), whereas ~1.2 g protein·kg^{-1}·d^{-1} was recommended. Resistance training was performed during 10 weeks, 3 times/week. **Measurements:** Strength (one repetition maximum test - 1-RM and handgrip strength) and functional capacity tests (Short Physical Performance Battery - SPPB, 6-minute walk, 400-meter walk, 10-meter walk and Timed Up and Go test - TUG) was evaluated before and after intervention. Dietary intake was assessed by nine 24-hour food recall. **Results:** HP group presented higher protein intake during the study (1.18±0.3 vs 0.87±0.2 g·kg^{-1}·d^{-1}, p=0.008) than NP group, respectively. After intervention, only HP increased bench press RM, leg extension RM and handgrip, however, no differences were found between groups. Analyzing the delta (Δ) of these variables, no differences were found for Δ bench press RM (2.75±3.01 vs 1.09±2.25 kg, p=0.153), Δ leg extension RM (6.75±12.46 vs 9.45±7.03 kg, p=0.533), and Δ handgrip: 1.66±3.20 vs 1.90±1.64 kg, p=0.823) in NP and HP, respectively. No difference was observed between groups and/or moments for other variables. **Conclusion:** Protein intake close to new proposals did not lead to higher increase in strength and functional capacity when compared to RDA recommendations after resistance exercise protocol in PW. Clinical trial: NCT03024125.

Key Words: Muscle strength, Diet Intervention, Functionality, Muscle Mass.

INTRODUCTION

Postmenopausal period results in loss of ovarian follicular activity and reduction in estrogen production (1), which may lead to muscle mass loss concomitantly with increase in body fat (2, 3). Additionally, postmenopausal women (PW) have decline in muscle strength and function (3), which are positively correlated with functional impairment, such as, difficulties in carrying out daily activities, weakness, falls, loss of independence, and consequently decrease in quality of life (4, 5).

Muscle mass loss and the decline in strength and muscle function, in consequence of postmenopausal period and aging, are caused by a combination of several factors, among them, sedentary lifestyle (6) and low protein intake (7). In this way, resistance training (RT) is a known intervention that promotes muscle mass and strength gains in adults (8), older adults (9) and PW (10). Indeed, associating RT with adequate protein intake higher increases in muscle mass and function can be found (11).

The Recommended Dietary Allowance (RDA) of protein intake for adults and older individuals, including PW, is 0.8 $g \cdot kg^{-1} \cdot d^{-1}$ (12). However, current evidences suggest that aging leads to "resistance anabolism" (13), showing that older individuals probably need to ingest a greater amount of protein to stimulate adequately protein synthesis (11, 14). Therefore, RDA recommendations seems to be not sufficient since recent evidences show that older adults might ingest an amount between 1.0 to 1.3 g of protein$\cdot kg^{-1} \cdot d^{-1}$ (15-17). A recent study showed that older women ingesting ~1.2$g \cdot kg^{-1} \cdot d^{-1}$ of protein is associated with better physical function and muscle strength when compared to smaller amount (~0.8$g \cdot kg^{-1} \cdot d^{-1}$ or less) (18). However, studies comparing these protein intake recommendations (~0.8 vs ~1.2 $g \cdot kg^{-1} \cdot d^{-1}$) and their impact on increases in muscle function in PW are lacking. Therefore, we aimed to evaluate the effect of new proposal of protein intake, compared to RDA recommendation, on strength and functional capacity gains in PW after resistance training protocol.

METHODS

Subjects and randomization

The volunteers of the study were recruited and selected at a neighborhood association near from Federal University of Triangulo Mineiro, Brazil. The study sample contained only women in postmenopausal period, i.e., those who had spontaneous amenorrhea for at least 12 months (self-report), with good overall health, who agreed to participate and signed the written informed consent. Those who did not provided the necessary information for development of study, used hormone therapy or phytoestrogens, alcoholics, presented orthopedic limitations and any stage of kidney disease were excluded.

In total, 48 women were initially recruited. Once consent was obtained, all the participants were randomly assigned by MedCalc® software (version 11.1) and allocated into two groups: RDA group (NP), which received ~0.8g protein$\cdot$kg$^{-1}\cdot$d^{-1}, as recommended by RDA, or new proposals group (HP), which received ~1.2g protein$\cdot$kg$^{-1}\cdot$d^{-1}, according to new protein intake proposals. After losses incurred during the intervention, 12 volunteers were part of NP group and 11 subjects in HP group (**Figure 1**). During the 10-week intervention period there was a significant loss of ~50% of the sample, ending with a total of 23 women.

All subjects were informed about the possible risks and the nature of the experimental procedures before their written informed consents were obtained. This study was approved by the Research Ethics Committee of the Federal University of Uberlandia (1.733.512/2016) and Federal University of Triangulo Mineiro (1.090.676/2015) and registered in clinical trials (NCT03024125).

Study Design

This clinical trial was a single-blind, randomized and parallel prospective. At first, a sample calculation was performed as: $n = [(Z\alpha_{/2} * \sigma) / E)]^2$, where, n, number of individuals in the sample; $Z\alpha_{/2}$, critical value that corresponds to the degree of confidence; σ, population standard deviation of the studied variable and E, maximum estimation error. For the $Z\alpha_{/2}$ value, was used 1.96; for σ, 6 (standard deviation found in Tieland *et al.,* 2012) (19) and for E, was used the value 3, referring to 3 kg of strength acceptable as an error for more or for less. Thus, $n = [(1.96 * 6) / 3)]^2 = 15.37 = 16$ total individuals.

Before the beginning of the study, anthropometric measurements, body composition, functional capacity tests, strength, resting energy expenditure (REE), and dietary intake (three food recall) were assessed. These analyzes were performed during two weeks. Volunteers also proceeded for a period of adaptation training, which lasted two weeks, considering that in the first week was the familiarization period and in the second week was performed one maximal repetition test (1-RM). These sessions of adaptation training occurred 3 times a week, in non-consecutive days, and after this period the RT protocol was began. At sixth week of RT, the load was adjusted to keep the relative load. RT and diet intervention were performed during 10 weeks. Dietary intake was assessed at 5th and 6th weeks and at 9th and 10th weeks, being applied six food recall. At the end of the study, anthropometric measurements, body composition, functional capacity tests and strength were evaluated again. The general protocol is described in **Figure 2**.

Assessments

Anthropometric parameters

Body mass was measured in a scale and height with a stadiometer coupled to scale (Lider®). Both measures were performed according to protocol proposed by Lohman (20).

After measures, BMI was calculated (body mass in kilograms divided by height in square meter). PW under 60 years old were classified according to the proposal by World Health Organization (21) and over 60 years by Lipschitz classification (22).

Body composition

The body composition was measured using dual-energy x-ray absorptiometry scanning (DEXA) (Lunar iDXA, GE Healthcare, USA) and analyzed by Encore software (version 14.10). Twenty-four hours before the evaluation, the volunteers were instructed to drink two liters of water to standardize the level of muscle hydration and were oriented to perform 8 to 10 hours fasting. As a criteria evaluation, the volunteers wore light and comfortable clothes without the presence of metal objects. It was evaluated the lean mass (whole-body and legs).

The Muscle Mass Index (MMI) was calculated from appendicular muscle mass (AMM) (kg) divided by the height square ($MMI = AMM / height^2$). The results were evaluated according to the recommendations provided by European Consensus on Definition and Diagnosis (23).

Functional Capacity

Short Physical Performance Battery (SPPB)

The SPPB test (24) included three tests performed in following order: balance test, four-meter walk test and five-time-sit-to-stand test. Each test score varied from zero to four points, and the SPPB score varied from zero to 12 points (the sum of the scores from the three tests).

For tests of balance, PW were asked to attempt to maintain their feet in the side-by-side, *semi-tandem* (heel of one foot beside the big toe of the other foot), and *tandem* (heel of one foot directly in front of the other foot) positions for 10 seconds each. Four-meter walk test was assessed by the time walked in a distance of 4 meters in habitual gait speed. Two measures (go and come back) were recorded after the volunteer completed the test and the shortest time was considered as the valid measure. Five-time-sit-to-stand test, subjects were instructed to fold their arms across their chests and was evaluated the time spent in five maximum velocity squats in a chair. The technique consisted of a full sit and stand position and the volunteer started in the sitting position.

Six-Minute Walk Test (6MWT)

The six-minute walk test (25) was performed in an indoor sports court. The walking course was 114 m long and it was marked every 3 m. A starting line, which marked the beginning and end of each 114 m lap, was marked on the floor using brightly colored tape. All volunteers were advised to walk as fast as possible in the six minutes of the test. The distance was recorded after the volunteer completed the test.

400-Meter Walk Test (400MWT)

The 400-meter walk test (26) was performed in an indoor sports court. The volunteer was instructed to walk 400 m in fast gait speed. A starting line, which marked the beginning and end the course was marked on the floor using brightly colored tape. The time was recorded after the volunteer completes the test.

10-Meter Walk Test (10MWT)

The 10-meter walk test(27) was performed in an indoor sports court. The volunteer was instructed to walk 10 m in habitual gait speed. The line marked the beginning and end the course was marked on the floor using brightly colored tape. The time was recorded after the volunteer completes the test.

Time Up and Go Test (TUG)

The TUG test (25) was measured the time that lasted a subject to stand up from a chair (without help of hands), walk a distance of 3 m, turn around in a cone, walk back to the chair, and sit down. The volunteer started sitting on a chair (with feet on the floor and the back against the chair) and started the test after voice command "Go". The volunteer was instructed to walk in fast gait speed and the time was recorded after completes the test.

Strength measurement

Muscle strength was analyzed by one repetition maximum test (1-RM) and handgrip strength test (HGS). Before 1-RM test, all women participated in 1-week familiarization (three sessions in a week, in non-consecutive days), period with low loads in order to learn the exercise techniques. On the next week, three sessions in non-consecutive days of the 1-RM test were performed. In the first session, PW started with warm-up approximately 70% 1-RM and were instructed to perform 8 to 10 repetitions with this load. After 1 minute of rest, the load was increased to approximately 85% of 1-RM, performing 3 to 5 repetitions. Again, after 1 minute of rest the first attempt of the test with a new increase of load was performed. If PW performed only one complete movement, the 1-RM was determined. If could not move or perform more than one repetition, a new attempt was made. A rest of 3 to 5 minutes was determined and after, that the load was increased or decreased. 1-RM was determined with a maximum of 5 trials. The load used as the maximum weight was the weight of the individual last exercise successfully performed (full range of motion). 1-RM test was realized in bench press and leg extension, being that such equipment served as indicator of the muscle strength gain. An experienced examiner performed all 1-RM measures.

Handgrip strength (HGS) test was measured with a manual dynamometer (Jamar®), adopting the unit of measure in kilograms (kg). The HGS scale was from 0 up to 90 kg and progressed each two kilograms. PW were standing with the arm adducted and neutral rotation, with forearm and wrist in neutral rotation. The unit's rod was placed between the second phalanges of the fingers (index, middle and ring). In the test, the pointer was placed in the neutral position (zero). At the evaluator's voice command, PW should hold the power utmost to bring the two device rods. Three measures were taken of each hand and the highest value in each hand was considered.

Resting Energy Expenditure (REE) and Total Energy Expenditure (TEE)

The REE was performed by indirect calorimetry by analyzer VO2000 (Imbrasport®), which analyzes VO_2 (oxygen consumption in $mL \cdot kg^{-1} \cdot min^{-1}$), VCO_2 (carbon dioxide production in $mL \cdot kg^{-1} \cdot min^{-1}$) and QR (respiratory quotient = VCO_2/VO_2). The equipment was turned on 30 minutes before the test for proper stabilization and calibration of the analyzers of O_2 and CO_2 with a gas of known concentration (28). The volunteers were at overnight fasting during 12 hours, 6 to 8 hours of sleep, without intense physical activity in the previous 48 hours to the examination and without 24 hours' caffeine consumption before the test. Test was conducted in a quiet room with low light and controlled temperature (29). The test lasted 30 minutes, whereas the first 10 minutes was used to stabilization of readings, with subsequent measurement of VO_2 and VCO_2 during 20 minutes. Mean values of these variables were used and inserted in Weir equation for REE measurement (30). Four women (two of each group) did not complete the test and REE was calculated by predictive equation(31) for these individuals.

For calculation of total energy expenditure (TEE), the following variables were used: REE, measured or calculated (as described above); factor activity (FA), considered sedentary for all women (1.3), corresponding to daily life activities (32) and task of metabolic equivalent (MET), calculated based on MET, whereas MET for resistance-type exercise was considered 5.5 (33). The caloric value estimated by METs was multiplied by 3 and divided by 7, once training occurred three times a week. The following formula was used: TEE = (REE x FA) + MET.

Dietary Intake Assessment

Dietary intake was assessed by 24-hour recalls. Three recalls were made at baseline (-4th and -3th weeks), during 5th and 6th weeks and 9th and 10th weeks of intervention, with a total of 9 dietary recalls. Arithmetic mean was held from 6 recalls performed during the intervention for better representation of dietary habits of the PW during the period of intervention. At each time of evaluation, the first 24-hour recall was performed face to face and the others by phone. In addition, 24-hour recalls were performed on non-consecutive days, including two weekdays and one day of the weekend at each moment of the study. Women were asked in detail about all food intake during the assessed day, time of intake and the amount ingested in homely measures over the evaluated days.

The assessment of dietary intake was provided by Dietpro® software (version 5.7i). A database in the program was implemented food usually consumed by the volunteers using food composition tables (USDA - United States Department of Agriculture) (34) in addition to the nutrition labels of manufacturers. Total energy intake (kcal), carbohydrate (g and %), lipid (g and %), protein (g, % and g/kg) and branched chain amino acids (BCAA) were calculated.

Experimental protocol

Diet Intervention

PW were randomized and allocated in two groups: NP or HP. All of them received an individualized and normocaloric dietary plans, containing foods usually consumed and their lists of food replacement. All food plans were made by trained nutritionists.

The NP group received the recommendation of protein intake proposed by RDA, ~$0.8 g \cdot kg^{-1} \cdot d^{-1}$, while HP group, ~$1.2 g \cdot kg^{-1} \cdot d^{-1}$, as advised by new proposals. In order to reach the proposed amount of protein in each group, high quality protein, such as meat, fish, eggs, milk and other dairy products were recommended. Dietary intervention was followed weekly by researchers using phone calls in order to know about the diet adherence and to answer possible doubts.

The amount of carbohydrate was the same in both groups (50% of total caloric value). Lipids were offered in higher amount in the NP group (25-30% of total calories) and lower in the HP group (15% of total calories), for calorie adjustment. PW were not informed in which dietary intervention they were inserted.

Resistance Training Protocol

RT was performed in a public Health and Physical Activity Center at the Federal University of Triangulo Mineiro. The training protocol followed the recommendations of the American College of Sports Medicine Guidelines for hypertrophy (35). RT was performed 3-day-a-week, in non-consecutive days, and at least 48 hours of interval between sessions. All workouts were supervised by a qualified professional. PW were advised not to perform other exercise on other days and times beyond intervention.

Protocol consisted in dynamic exercises. Each training session started with a 10 minutes of warm up (walking). Dynamic exercises were realized for the upper and lower limbs, including: guided squat, leg curl, leg extension, bench press free bar, rowing machine, pull down, triceps pulley and direct threaded bar. Both groups, NP and HP, started RT with one sets of each exercise in the first week and increasing a number per week up to six sets for all exercises. When they reached the six sets (in the six week), they kept this volume until the ten week. At sixth week of RT, the load was adjusted to ensure that PW trained in the proposed intensity. For all the equipment, PW performed 10 to 12 repetitions per sets. The fixed load was 10 to 12-RMs, that is, the load that each volunteer could perform until 10 to 12 repetitions. The interval between sets and exercises was approximately 60 seconds.

The RT was carried out for 10 weeks, sufficient time to denote differences in mass and muscle function (36). Breathing was controlled and PW had expired during the concentric action and inspired by the eccentric action, in order to prevent apnea. During the training sessions, the subjects were instructed to perform the eccentric action in a second and concentric action in a second. Exercises were supervised full time by professionals. A minimum frequency level in the 70% training was standardized, and PW with a lower frequency were excluded from the sample.

Statistical Analysis

The data distribution was determined using Shapiro Wilk test. To compare groups at study baseline Independent *t*-test and Mann-Whitney were used. The values are represented in means ± SD, for parametric variables, and median [interquartile range] for non-parametric data. Generalized Estimating Equation (GEE) with Sequential Sidak post hoc was performed to compare groups and moments; and also the interaction between time *x* treatment. Delta (Δ), which corresponded to final value less initial, was calculated for body composition, strength and functional capacity variables and was compared by independent *t*-test. *P*-value <0.05 was used for statistical significance. SPSS software (version 20.0) was used for statistical analysis.

RESULTS

Baseline characteristics

No differences were found between groups for age, BMI, total lean mass, MMI, strength and functional capacity parameters at baseline (**Table 1**). The mean values of HGS and all functional capacity tests (SPPB, 6MWT, 400MWT, 10MWT and TUG) were adequate, according to each recommendation.

Dietary intake

Calories (kcal), carbohydrate (g) and lipids (g and %) intake did not change during the intervention period for both groups. However, HP reduced carbohydrate intake (%) during intervention, but no differences were found when compared to NP. (**Table 2**). NP group maintained the intake of protein (0.78±0.58 vs 0.87±0.38 $g \cdot kg^{-1} \cdot d^{-1}$, p=0.332), BCAA (8.79±5.62 vs 10.15±4.28 g/day, p=0.117) and leucine (3.46±2.24 vs 4.29±1.76 g/day, p=0.396), whereas HP group increased the intake of these dietary components during the study (protein: 0.82±0.52 vs 1.18±0.56 $g \cdot kg^{-1} \cdot d^{-1}$, p<0.001; BCAA: 9.59±4.84 vs 14.34±4.50 g/day, p<0.001; leucine: 3.82±1.98 vs 6.02±1.92 g/day, p<0.001). Isoleucine intake increased in both groups, but the rise was higher in HP group.

Body composition

Total lean mass (Δ=1.26±0.82 kg vs Δ=1.33±0.68 kg for NP and HP group, respectively; p>0.05) and leg lean mass (Δ= Δ=0.40±0.77 kg vs Δ=0.48±0.47 kg for NP and HP group, respectively; p>0.05) increased similarly in both groups after intervention.

Strength and functional capacity

After intervention, only HP increased bench press RM, leg extension RM and handgrip, however, no differences were found between groups (**Figure 3);** detailed values of each variable is presented in **Supplementary Table 1**. Analyzing the delta (Δ) of these variables, no differences were found for Δ bench press RM (2.75±3.01 vs 1.09±2.25 kg, p=0.153), Δ leg extension RM (6.75±12.46 vs 9.45±7.03 kg, p=0.533), and Δ handgrip: 1.66±3.20 vs 1.90±1.64 kg, p=0.823) in NP and HP, respectively. No differences between groups and/or moments were found for SPPB, 6MWT, 400MWT, 10MWT and TUG (p>0.05) (**Figure 3).**

DISCUSSION

The main finding of present study was that a protein intake according to new proposals (1.18±0.28 $g \cdot kg^{-1} \cdot d^{-1}$) did not result in greater strength and functional capacity gains when compared with an intake proposed by RDA (0.87±0.19 $g \cdot kg^{-1} \cdot d^{-1}$). To the best of our knowledge, this is the first study comparing the effect of these protein recommendations on strength and functional capacity gains in PW after RT protocol.

Besides we found improvements in bench press RM, leg extension RM and HGS (right and left) only in HP group (independently of lean mass gain), there were no differences in relation to NP group, which was confirmed by the absence of differences in delta values between groups. These data show that although a tendency of improvements were found, it is not possible to affirm that ingesting higher amounts of protein lead to higher gains in muscle strength and functional capacity. Interestingly, NP group did not show improvements in muscle function even presenting muscle gain, which show a possible effect of protein intake on muscle function. However, it is possible to speculate that if intervention lasted longer and/or our sample was larger a difference could be found between groups, but new studies are needed to confirm this hypothesis.

Interventional studies comparing RDA recommendations to new proposals of protein intake and the impact on muscle function in PW are lacking. There are only few associative studies (18, 37), which do not permit to conclude a cause and effect relationship. Isanejad *et al.* (2016), demonstrated in a cohort study that higher amounts of protein intake (≥1.2 $g{\cdot}kg^{-1}{\cdot}d^{-1}$) are positively associated with greater handgrip strength, knee extension, 1-RM test, 6 minute walk test and SPPB (18). Gregorio *et al.* (2014), performed a cross-sectional study and reported that older postmenopausal women who consumed more than 0.8 $g{\cdot}kg^{-1}{\cdot}d^{-1}$ of protein compared to less than 0.8 $g{\cdot}kg^{-1}{\cdot}d^{-1}$ (considering the RDA recommendation as cut point) presented better results in SPPB and 1-RM test (37).

Protein intake proposed by RDA ($0.8g{\cdot}kg^{-1}{\cdot}d^{-1}$) is recommended for general population, without distinction between sex or age. This recommendation is based on nitrogen balance method, which has several limitations (12), however, using a new available amino acid oxidation technique, recent studies have shown that the amount of protein recommended by RDA seems to be insufficient to promote maximum protein synthesis in older individuals (16). It has been proposed that older individuals, such as PW, need to ingest an amount of 1.0 to 1.3g of $protein{\cdot}kg^{-1}{\cdot}d^{-1}$ to maintain muscle mass and function (15-17). Nevertheless, we did not observe higher gains in whole-body and leg lean mass when higher amount of protein was ingested. A recent study showed that, acutely, there were also no differences in muscle protein synthesis with similar dietary intervention as in our study (38). Although muscle mass gain does not seem to be directly associated with increases in strength in some cases (39), it is possible to speculate that higher amounts of protein did not promote higher strength gains due to not lead higher lean mass gain. Therefore, the intake of approximately $1.2g{\cdot}kg^{-1}{\cdot}d^{-1}$ of protein seems to be insufficient to promote higher improvements in function and muscle mass gain.

It is possible to speculate that whether PW ingested higher amount of protein during the day a higher increase in muscle strength could be found, however, one of the difficulties in reaching these recommendations by foods is the lack of appetite presenting during aging. Additionally, other factors, such as loneliness, social isolation and depression, besides metabolic changes, can also contributes to low protein intake in this population (40). Moreover, the difficulty of dietary adherence can be explained by the price of protein sources, since these foods are usually more expensive (41).

Our study had some limitations. The assessment of body composition was performed by DEXA, a method that did not control total body water. The use of average protein intake can be another limitation since not all volunteers consumed the exactly amount of protein proposed in each group, whereas some individuals ingested more or less than 0.8 $g{\cdot}kg^{-1}{\cdot}d^{-1}$ or 1.2 $g{\cdot}kg^{-1}{\cdot}d^{-1}$. On the other hand, one of the strengths of the present study was the dietary intervention, which was based on increases in protein intake by various protein sources, which represents a more realistic nutritional intervention in clinical practice. Another point was the use of indirect calorimetry to measure resting energy expenditure, which is an important parameter for dietary prescription. Lately, possible errors of dietary method were minimized performing six 24-hour food recalls during intervention, in order to control the proposed dietary intervention.

In conclusion, a protein intake close to the new proposals (~1.2 $g{\cdot}kg^{-1}{\cdot}d^{-1}$) did not lead to higher increase in strength and functional capacity when compared to RDA (~0.8 $g{\cdot}kg^{-1}{\cdot}d^{-1}$) after 10 weeks of resistance exercise protocol in PW. Further studies lasting more than 10 weeks and/or offering higher protein amount and/or with a larger study sample are needed to evaluate the effect protein intake on muscle function improvement.

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Conflict of Interest

The authors declare no conflict of interests.

Ethics declaration: All experimental procedures were conducted in accordance with the guidelines in the Declaration of Helsinki and approved by the Bioethics Committee of Federal University of Uberlandia in Brazil.

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FIGURES

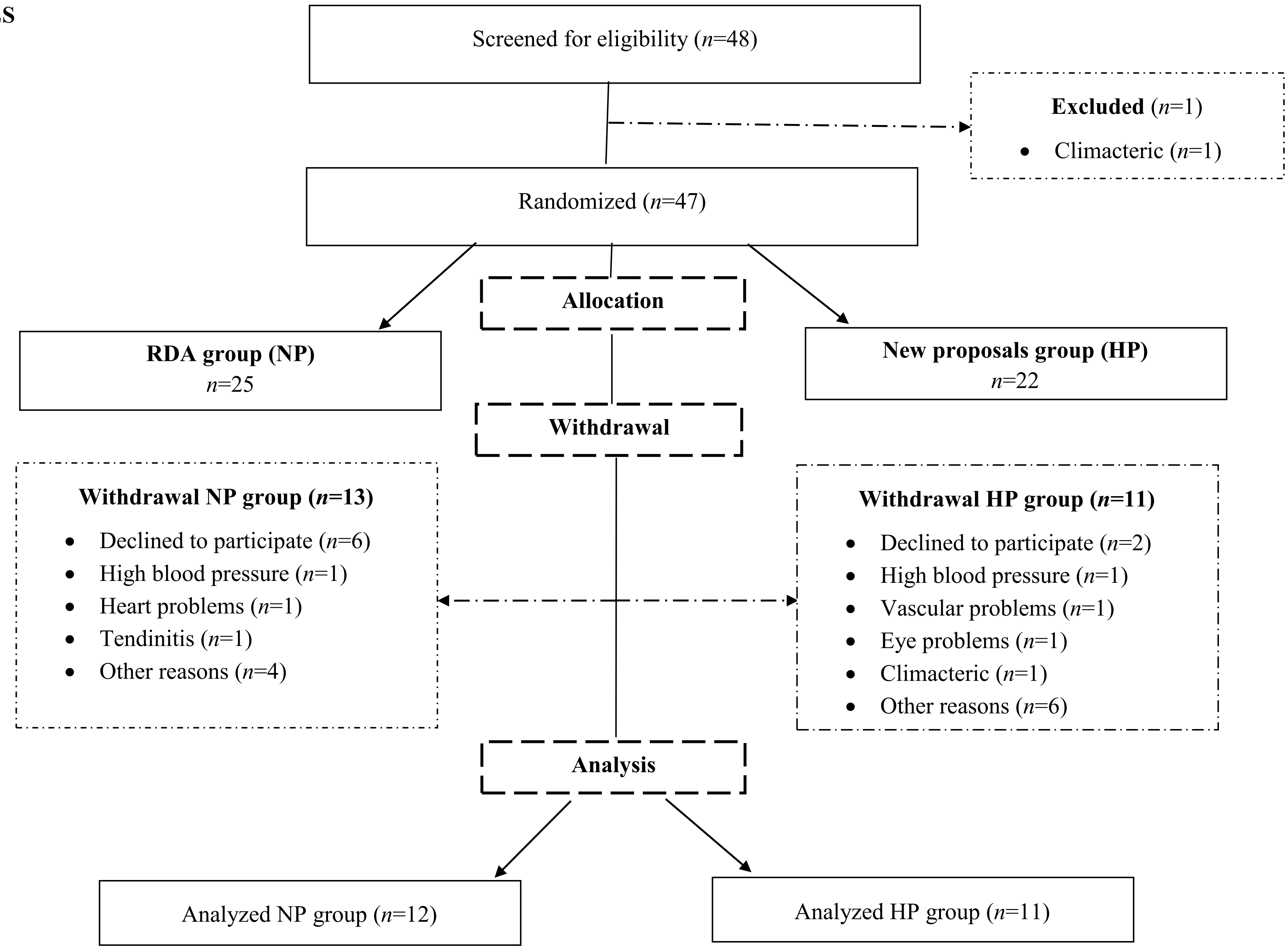

Figure 1. Follow chart of research volunteers.

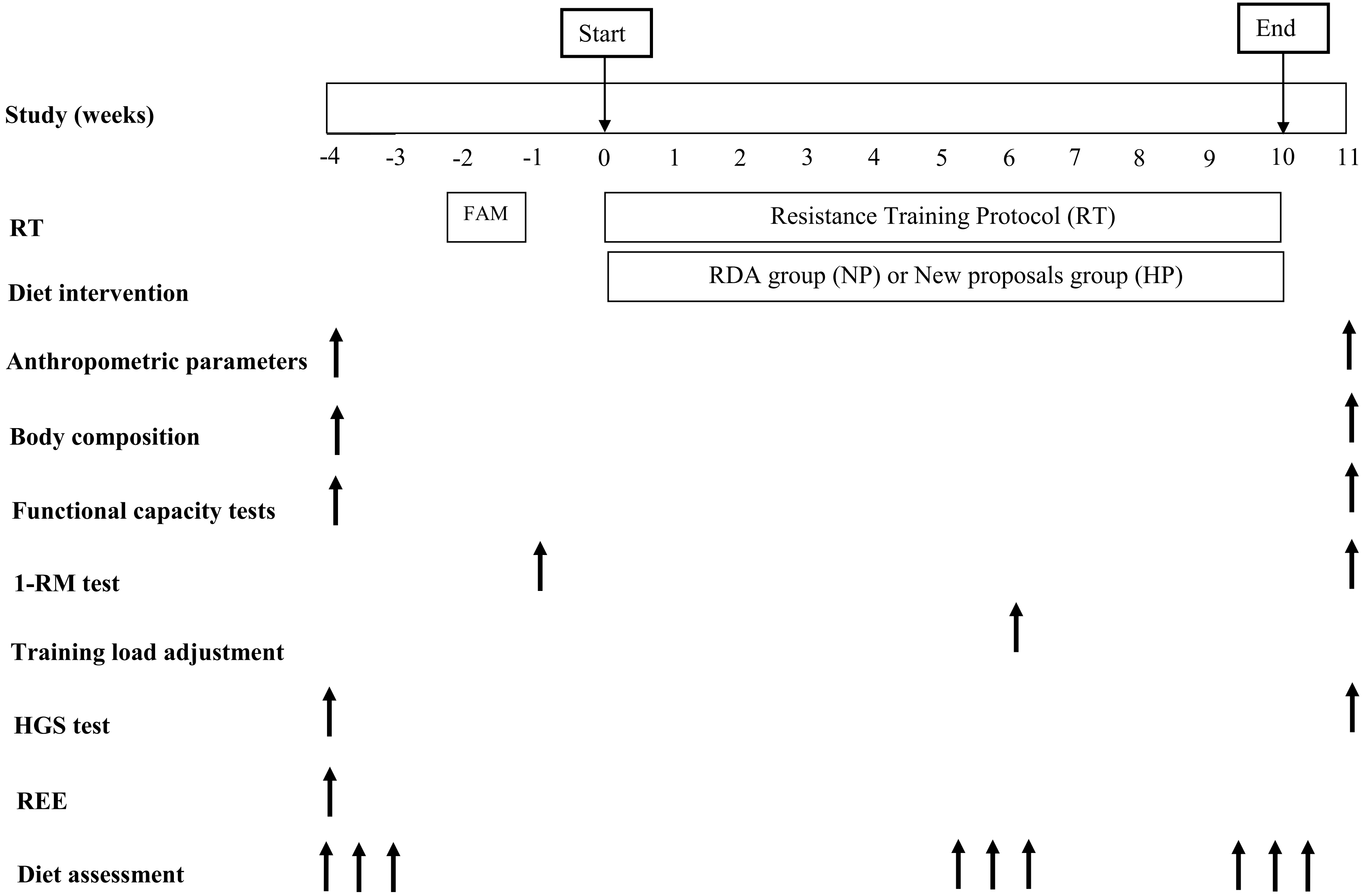

Figure 2. Schematic overview diagram of the study protocol.

FAM, familiarization period; *RT,* Resistance Training Protocol; *NP,* RDA group; *HP,* new proposals group*; 1-RM,* one maximum repetition test; *HGS,* handgrip strength; *REE,* Resting Energy Expenditure.

TABLES

Table 1. Baseline participant's characteristics.

Variables	NP (*n*=12)	HP (*n*=11)	*p-value*
Demographic			
Age (y)	63.00 ± 8.62	63.45 ± 7.67	0.895
Anthropometrics			
Body mass (kg)	69.03 ± 17.06	67.64 ± 15.58	0.840
Height (m)	1.56 ± 0.08	1.55 ± 0.06	0.824
Body Mass Index (kg/m²)	28.38 ± 6.02	28.06 ± 5.46	0.894
Body Composition			
Whole-body lean mass (kg)	37.57 ± 6.18	37.10 ± 6.19	0.857
Leg lean mass (kg)	12.66 ± 2.57	12.71 ± 2.90	0.969
Muscle Mass Index (kg/m²)	6.89 ± 0.95	6.93 ± 1.17	0.934
Strength			
Bench press 1-RM (kg)	33.00 ± 4.95	32.18 ± 6.30	0.900
Leg extension 1-RM (kg)	71.08 ± 15.79	75.27 ± 14.76	0.518
Right Handgrip Strength (kg)	27.08 ± 5.18	27.63 ± 4.74	0.793
Left Handgrip Strength (kg)	24.58 ± 5.43	25.00 ± 5.16	0.853
Functional Capacity			
Balance test - SPPB (score) †	4.00[2.50-4.00]	4.00[4.00-4.00]	0.786
4-meter walk test - SPPB (s)	3.27 ± 0.54	3.14 ± 0.61	0.603
5-time-sit-to-stand test - SPPB (s)	10.52 ± 2.41	9.57 ± 2.22	0.334
Total SPPB †	11.50[10.25-12.00]	12.00[11.00-12.00]	0.740
6-minute walk test (m/s)	1.70 ± 0.30	1.60 ± 0.29	0.407
400-meter walk test (min)	4.03 ± 0.71	3.96 ± 0.45	0.904
10-meter walk test (s)	7.57 ± 1.34	7.49 ± 0.50	0.856
Time Up and Go test (s)	8.00 ± 1.85	7.16 ± 1.25	0.213
Energy expenditure			
Resting Energy Expenditure (kcal)	1582.51 ± 566.35	1394.10 ± 290.98	0.324
Total Energy Expenditure (kcal)	2127.56 ± 750.62	1881.20 ± 388.78	0.332

NP, RDA group; *HP,* new proposals group; *1-RM,* one repetition maximum test; *SPPB,* short physical performance battery. †: non-parametric data. Independent *t* test (mean ± SD) and Mann-Whitney test (median [interquartile range]).

Table 2. Calories, macronutrients and BCAA intake according to moments and groups.

Variables	NP (*n*=12)		HP (*n*=11)		*p-value*		
	Pre	During	Pre	During	*Time*	*Treatment*	*Time x Treatment*
Calories (kcal)	1342.69 ± 423.51 [a]	1439.37 ± 205.85 [a]	1412.12 ± 382.55 [a]	1502.10 ± 214.34[a]	0.267	0.494	0.968
Carbohydrate (g)	161.61 ± 54.37 [a]	168.69 ± 31.06 [a]	182.33 ± 52.63 [a]	179.99 ± 33.33 [a]	0.829	0.237	0.668
Carbohydrate (%)	48.39 ± 8.14 [a, b]	46.90 ± 6.18 [a, b]	51.55 ± 3.77 [a]	47.77 ± 3.62 [b]	0.024	0.296	0.326
Lipids (g)	51.92 ± 16.72 [a]	56.39 ± 12.48 [a]	53.97 ± 16.57 [a]	53.33 ± 10.13 [a]	0.642	0.895	0.535
Lipids (%)	35.03 ± 6.32 [a]	35.46 ± 6.94 [a]	34.16 ± 2.72 [a]	32.04 ± 3.16 [a]	0.400	0.219	0.205
Protein (g)	51.55 ± 15.34 [a]	58.67 ± 10.45 [a]	53.24 ± 13.58 [a]	77.38 ± 10.51 [b]	<0.001	0.012	0.004
Protein (%)	15.53 ± 2.94 [a]	16.37 ± 2.20 [a]	15.34 ± 2.72 [a]	20.64 ± 2.62 [b]	<0.001	0.009	<0.001
Protein (g/kg) †	0.78 ± 0.29 [a]	0.87 ± 0.19 [a]	0.82 ± 0.26 [a]	1.18 ± 0.28 [b]	<0.001	0.111	0.024
Leucine (g)	3.46 ± 1.12 [a]	4.29 ± 0.88 [a]	3.82 ± 0.99 [a]	6.02 ± 0.96 [b]	<0.001	0.002	0.001
Isoleucine (g) †	1.96 ± 0.63 [a]	2.40 ± 0.54 [b]	2.13 ± 0.52 [a,b]	3.42 ± 0.56 [c]	<0.001	0.008	0.011
Valine (g) †	3.37 ± 1.06 [a]	3.45 ± 0.73 [a]	3.64 ± 0.91 [a]	4.90 ± 0.77 [b]	0.002	0.009	0.008
Total BCAA (g)	8.79 ± 2.81 [a]	10.15 ± 2.14 [a]	9.59 ± 2.42 [a]	14.34 ± 2.26 [b]	<0.001	0.002	0.001

NP, RDA group; *HP,* new proposals group*; BCAA* branched-chain amino acids (leucine + isoleucine + valine). Different letters represent statistical difference ($p<0.05$). †: non-parametric data transformed into logarithm for analysis. Generalized Estimating Equations analysis (GEE) was used for to compare groups and moments with Sequential Sidak post hoc. All data described in mean±SD.

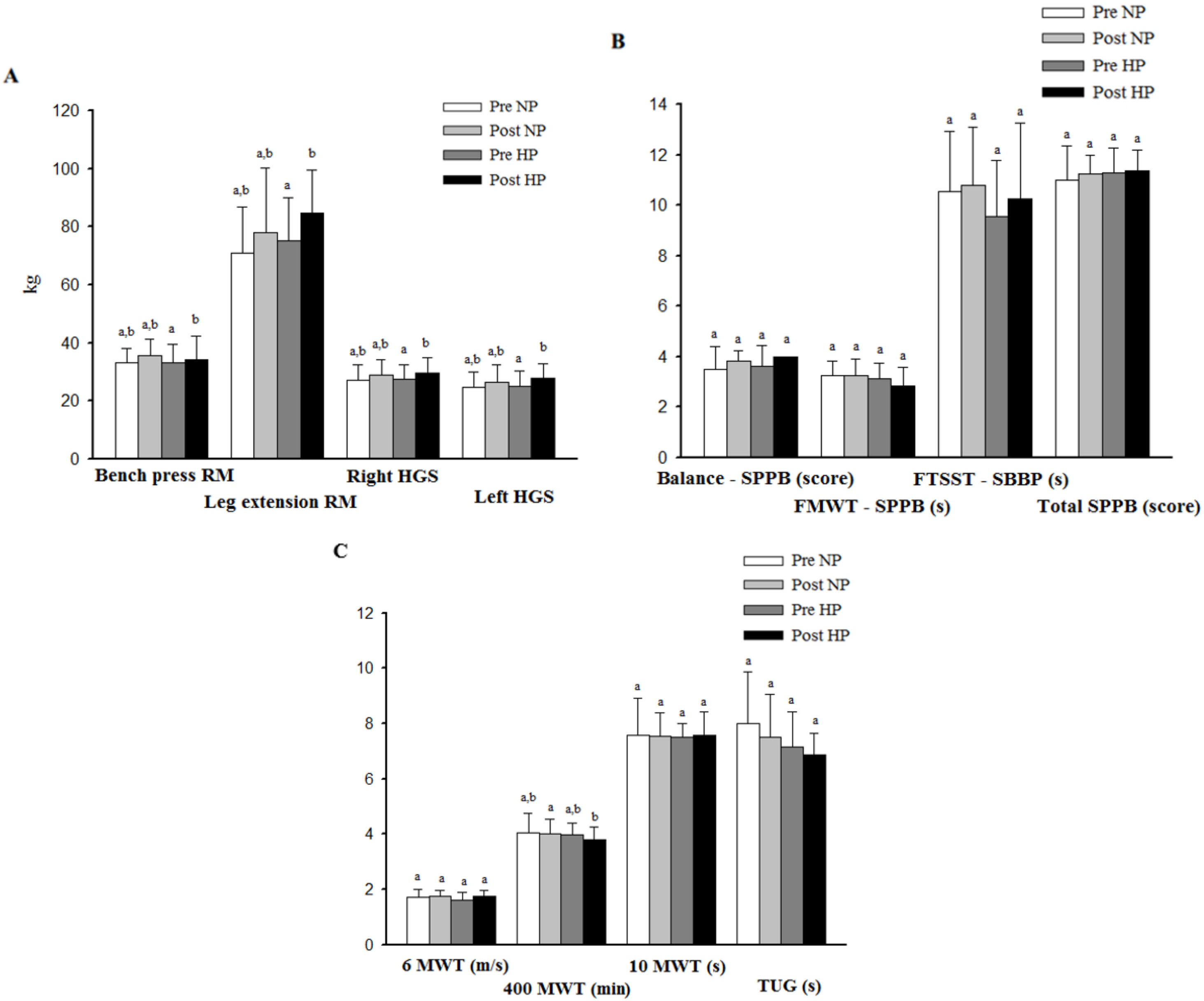

Figure 3. Strength and functional capacity tests according to moments and groups.

NP, RDA group; *HP,* new proposals group*; RM,* maximum repetition test; *HGS,* handgrip strength; *SPPB,* short physical performance battery; *FMWT,* four-meter walk test; *FTSST,* five-time-sit-to-stand test; *6MWT*, six-minute walk test; *400MWT*, 400-meter walk test; *10MWT*, 10-meter walk test; *TUG,* time up and go test. Different letters represent statistical difference ($p<0.05$). For all variables, *time x treatment* >0.05. Generalized Estimating Equations analysis (GEE) was used for to compare groups and moments with Sequential Sidak post hoc. All data is described in mean±SD.

Supplementary Table 1. Strength and functional capacity tests detailed values according to moments and groups.

Variables	NP (*n*=12)		HP (*n*=11)		*p-value*		
	Pre	Post	Pre	Post	*Time*	*Treatment*	*Time x Treatment*
Strength							
Bench press RM (kg)	33.00 ± 4.95 [a,b]	35.50 ±5.56 [a,b]	33.18 ± 6.30 [a]	34.27 ± 7.90 [b]	0.002	0.644	0.215
Leg extension RM (kg)	71.08 ± 15.79 [a,b]	77.83 ± 22.19 [a,b]	75.27 ± 14.76 [a]	84.72 ± 14.61 [b]	<0.001	0.394	0.498
Right HGS (kg)	27.08 ± 5.18 [a,b]	28.75 ± 5.31 [a,b]	27.63 ± 4.74 [a]	29.54 ± 5.34 [b]	<0.001	0.733	0.809
Left HGS (kg)	24.58 ± 5.43 [a,b]	26.41 ± 5.99 [a,b]	25.00 ± 5.16 [a]	27.81 ± 5.10 [b]	<0.001	0.665	0.341
Functional Capacity							
Balance SPPB (score) †	3.50 ± 0.90 [a]	3.83 ± 0.39 [a]	3.63 ± 0.81 [a]	4.00 ± 0.00 [a]	0.049	0.442	0.963
FMWT - SPPB (s)	3.27 ± 0.54 [a]	3.27 ± 0.65 [a]	3.14 ± 0.61 [a]	2.86 ± 0.71 [a]	0.259	0.226	0.245
FTSST - SPPB (s)	10.52 ± 2.41 [a]	10.79 ± 2.29 [a]	9.57 ± 2.22 [a]	10.25 ± 3.02 [a]	0.321	0.396	0.665
Total SPPB (score) †	11.00 ± 1.35 [a]	11.25 ± 0.75 [a]	11.27 ± 1.01 [a]	11.36 ± 0.81 [a]	0.414	0.573	0.699
6MWT (m/s)	1.70 ± 0.30 [a]	1.74 ± 0.24 [a]	1.60 ± 0.29 [a]	1.76 ± 0.19 [a]	0.350	0.665	0.164
400MWT (min)	4.03±0.71 [a,b]	3.99±0.54 [a]	3.96±0.45[a,b]	3.79±0.47 [b]	0.049	0.620	0.310
10MWT (s)	7.57 ± 1.34 [a]	7.53 ± 0.87 [a]	7.49 ± 0.50 [a]	7.58 ± 0.83 [a]	0.849	0.962	0.646
TUG (s)	8.00 ± 1.85 [a]	7.52 ± 1.54 [a]	7.16 ± 1.25 [a]	6.87 ± 0.78 [a]	0.084	0.147	0.658

NP, RDA group; *HP,* new proposals group*; RM, one* maximum repetition; *HGS,* handgrip strength; *SPPB,* short physical performance battery; *FMWT,* four-meter walk test; *FTSST,* five-time-sit-to-stand test; *6MWT*, six-minute walk test; *400MWT*, 400 meter walk test; *10MWT*, 10 meter walk test; *TUG,* time up and go test. Different letters represent statistical difference ($p<0.05$). †: non-parametric data transformed into logarithm for analysis. Generalized Estimating Equations analysis (GEE) was used for to compare groups and moments with Sequential Sidak post hoc. All data described in mean±SD. For all variables, *time x treatment* >0.05.