UNIVERSIDADE FEDERAL DE UBERLÂNDIA

INGESTÃO DE PROTEÍNA DE ACORDO COM AS NOVAS PROPOSTAS NÃO AUMENTA O GANHO DE MASSA MAGRA QUANDO COMPARADO COM AS RECOMENDAÇÕES PROPOSTAS PELA RDA EM MULHERES PÓS-MENOPAUSADAS APÓS PROTOCOLO DE EXERCÍCIO DE FORÇA: UM ENSAIO CLÍNICO RANDOMIZADO

LUANA THOMAZETTO ROSSATO

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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.

Área de concentração: Ciências da Saúde.

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"O que sabemos é uma gota; o que ignoramos é um oceano."

Isaac Newton

RESUMO

Introdução: Estudos recentes têm sugerido que a ingestão de proteína de acordo com a *Recommended Dietary Allowance* (RDA) (0,8 g/kg/dia) parece ser insuficiente para indivíduos mais velhos. **Objetivo:** Avaliar o efeito de uma nova proposta de ingestão de proteína em relação à recomendação da RDA sobre a massa magra (MM) de mulheres pós-menopausadas (PM) durante protocolo de exercício de força. **Material e métodos:** Vinte e três PM (63,2 ± 7,8 anos) foram randomizadas em dois grupos. O grupo com ingestão semelhante a nova proposta (n=11) (hiperproteico - HP) recebeu um plano dietético com ~1,2 g/kg/dia de proteína, enquanto que o grupo com recomendação da RDA (n=12) (normo proteico - NP) foi instruído a ingerir ~0,8 g/kg/dia de proteína. Ambos os grupos realizaram o mesmo protocolo de treinamento de força, que consistiu de três vezes por semana, com progressão do número de séries (de 1 para 6 séries) e 8-12 repetições. A intervenção ocorreu durante 10 semanas. A avaliação da composição corporal foi realizada por densitometria com emissão de raios-X de dupla energia. A dieta foi avaliada por nove recordatório alimentar de 24 horas. **Resultados:** Durante o protocolo, o grupo HP apresentou maior ingestão de proteína (1,18±0,3 vs 0,87±0,2 g/kg/dia, p=0,008) e leucina (6,0±1,4 vs 4,3±0,9 g/dia, p<0,001) do que o grupo NP, respectivamente. No final da intervenção, houve aumento de MM tanto no HP (37,1±6,2 vs 38,4±6,5 kg, p=0,004) como no grupo NP (37,6±6,2 vs 38,8±6,4 kg, p<0,001), sem diferenças entre os grupos (p=0,572). **Conclusão:** O consumo de proteína próximo à nova proposta não resultou em maior ganho de MM quando comparado à recomendação da RDA em mulheres PM realizando exercícios de força durante 10 semanas. Este ensaio clínico foi registado no clinicaltrials.gov, com o protocolo NCT03024125.

Palavras-chave: Massa Magra. Proteína. Recomendações da RDA. Ingestão de Leucina. Mulheres Pós-menopausadas.

ABSTRACT

Introduction: Recent studies have been suggested that protein intake according to Recommended Dietary Allowance (RDA) (0.8 $g \cdot kg^{-1} \cdot d^{-1}$) seems to be insufficient for older individuals. **Objective:** The aim of this study was to evaluate the effect of a new proposal of protein intake compared to RDA recommendation on lean body mass (LBM) gain in postmenopausal women (PMW) practicing resistance exercise. **Material and methods:** Twenty-three PMW (63.2±7.8y) were randomised into two groups. The group with new proposal (n=11) (high protein - HP) received a dietary plan with ~1.2 $g \cdot kg^{-1} \cdot d^{-1}$ of protein, while the group with RDA recommendation (n=12) (normal protein - NP) was instructed to ingest ~0.8 $g \cdot kg^{-1} \cdot d^{-1}$ of protein. Both groups performed the same resistance training protocol, which consisted of three times a week, with progression of the number of series (from 1 to 6 sets) and 8-12 repetitions. The intervention occurred during 10-weeks. Body composition evaluation was performed by dual-energy X-ray absorptiometry. The diet was evaluated by nine 24-hour food recall. **Results:** During the protocol, HP group presented higher intake of protein (1.18±0.3 vs. 0.87±0.2 $g \cdot kg^{-1} \cdot d^{-1}$, $p=0.008$) and leucine (6.0±1.4 vs 4.3±0.9 g/day, $p<0.001$) than the NP group, respectively. At the end, there were an increase in LBM both in HP (37.1±6.2 vs 38.4±6.5 kg, $p=0.004$) and in NP (37.6±6.2 vs 38.8±6.4 kg, $p<0.001$), with no differences between groups ($p=0.572$). **Conclusion:** Therefore, we concluded that a protein intake close to new proposals did not lead to higher LBM gain, when compared to RDA recommendation in PMW performing resistance exercise during 10-weeks. This trial was registered at clinicaltrials.gov as NCT03024125.

Key words: Lean Body Mass. Protein. RDA Recommendations. Leucine Intake. Postmenopausal Women.

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LISTA DE ABREVIATURAS E SIGLAS

1-RM - 1 repetição máxima

ACSM - American College of Sports Medicine

AI - Adequate intake

AS - Afternoon snack

BCAA - Branched-chain amino acids

BMI - Body mass index

BR - Breakfast

CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico

CO2 - Carbon dioxide

DI - Dinner

DRI - Dietary reference intake

DXA - Dual-energy X-ray absorptiometry

EAR - Estimated Average Requirement

EDTA - Ethylenediamine tetraacetic acid

FA - Factor activity

FAO - Food and Agriculture Organization of the United Nations

FAPEMIG - Fundação de Amparo à Pesquisa de Minas Gerais

FBSGO - Federação Brasileira das Sociedades de Ginecologia e Obstetrícia

FSH - Hormônio folículo estimulante

GEE - Generalized Estimating Equation

HP - High protein

IBGE - Instituto Brasileiro de Geografia e Estatística

IL-6 - Interleucina-6

IOM - Institute of Medicine

LBM - Lean body mass

LH - Hormônio luteinizante

LLM - Leg lean mass

LU - Lunch

MET - Metabolic equivalent

MM - Massa magra

MMI - Muscle mass index

MPS - Muscle protein synthesis

MS - Morning snack

mTOR - Proteína alvo da rapamicina em mamíferos (*Mammalian Target of Rapamycin*)

NP - Normal protein

O_2 - Oxygen

PM - Pós-Menopausadas

PMW - Postmenopausal women

RDA - Recommended dietary allowance

REE - Resting energy expenditure

SD - Standard deviation

SE - Standard error

SPM - Síntese proteica muscular

SU - Supper

TEE - Total energy expenditure

TFM - Total fat mass

TF - Treinamento de força

TLM - Total lean mass

TNF-α - Fator de necrose tumoral α

TrFM - Trunk fat mass

UL - Tolerable Upper Intake Level

USDA - United States Department of Agriculture

VCO2 -Volume of carbon dioxide

VO2 - Volume of oxygen

WHO - World Health Organization

Δ - Delta, diferença entre os momentos

SUMÁRIO

1 INTRODUÇÃO

A expectativa de vida tem aumentado na maioria dos países, inclusive no Brasil, resultando em crescimento do número de pessoas mais velhas (IBGE, 2013). O envelhecimento tem impacto sobre a saúde e está diretamente associado com a perda de massa muscular esquelética, além de redução da função muscular (força e desempenho), o que resulta em um processo conhecido como sarcopenia (FIELDING et al., 2011; KIM; CHOI, 2013). Mulheres pós-menopausadas (PM) merecem atenção especial, pois nesta fase ocorre cessação permanente da menstruação, resultante da perda da atividade folicular ovariana e consequente redução da produção de estrogênios (BURGER et al., 2007), situação que pode levar à maior perda de músculo concomitantemente com maior acúmulo de gordura corporal (MALTAIS et al., 2009).

De acordo com Leenders e colaboradores (2013), são necessárias intervenções efetivas na prevenção e/ou tratamento das consequências prejudiciais à saúde que podem ocorrer devido à perda de massa muscular esquelética e força. O tratamento não-farmacológico para indivíduos mais velhos nessa situação deve associar a prática de exercícios de força com a ingestão adequada de proteínas (BREEN; PHILLIPS, 2012).

Diante do exposto, são necessárias estratégias que visem a manutenção e melhora da saúde nessa população, com consequente impacto na qualidade de vida.

2 REVISÃO DA LITERATURA

2.1 Pós-menopausa

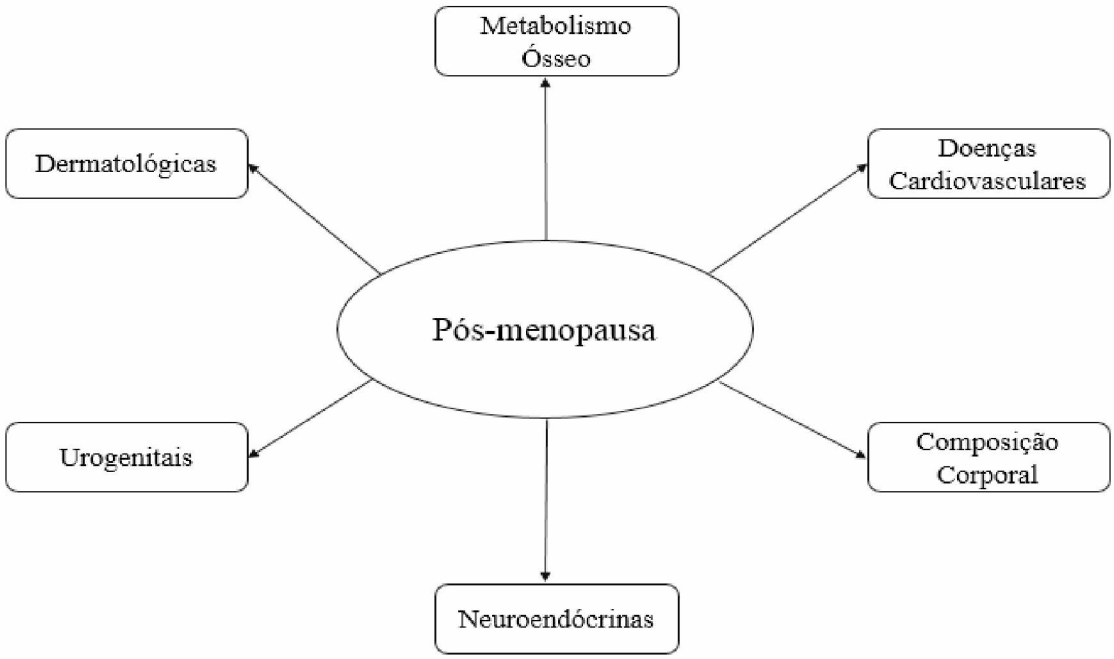
A transição do período reprodutivo para o não reprodutivo no sexo feminino é caracterizada por oscilações no ciclo menstrual e conhecida como climatério ou perimenopausa. A menopausa é a cessação total da menstruação e compreende o período entre a última menstruação e os doze meses subsequentes. A pós-menopausa é o período ao qual se refere o tempo de vida após a menopausa (WHO, 1981).

Quando recrutados, os folículos ovarianos sofrem alterações, produzindo o hormônio luteinizante (LH) e hormônio folículo estimulante (FSH). Após ação do LH, o colesterol é convertido em androgênios (androstenediona e testosterona), os quais são posteriormente convertidos em estrogênios (principalmente o estradiol) por ação do FSH. Com o avançar da idade, ocorre diminuição do número de folículos, e consequentemente, menor recrutamento dos mesmos. Essa situação leva à diminuição da fertilidade, declínio

nos níveis de estrogênios e elevação dos níveis plasmáticos de FSH. Como não há mais síntese de estradiol por este processo, a maior parte dos estrogênios é produzida pela conversão periférica da androstenediona em estrona, com ação bem mais fraca que o estradiol (FBSGO, 1995).

Segundo o Manual de Orientação sobre o Climatério, proposto pela Federação Brasileira das Sociedades de Ginecologia e Obstetrícia (FBSGO, 1995), essas mudanças hormonais acabam levando à diversas alterações, apresentadas na figura abaixo (Figura 1).

Figura 1 – Alterações corporais que afetam as mulheres no período da pós-menopausa.



2.2 Alterações na composição corporal durante a pós-menopausa

Condições físicas e de saúde que são relacionadas à idade, como a sarcopenia (perda de massa muscular associada à perda de força e/ou função) por exemplo, não se desenvolvem de maneira aguda (PADDON-JONES; LEIDY, 2014). Após os 50 anos de idade, há maior perda de massa muscular, o que coincide com os anos da menopausa (ALOIA et al., 1991). Segundo um estudo conduzido por Janssen e colaboradores (JANSSEN et al., 2002), ocorre acréscimo de 20% na prevalência de baixa quantidade de massa muscular em mulheres na faixa etária de 50-59 anos quando comparadas com

mulheres de 40-49 anos, sugerindo que o aumento da prevalência de pouca massa muscular ocorre ao mesmo tempo em que há mudanças hormonais em decorrência da menopausa. Além disso, segundo Rolland e colaboradores (ROLLAND et al., 2007), após a menopausa, a perda de massa muscular anual é na ordem de 0,6%.

Possivelmente, as mudanças hormonais ocorridas durante este período levariam à diminuição da massa muscular esquelética (MALTAIS et al., 2009), além de aumentar o percentual de gordura e redistribuir a gordura subcutânea para gordura visceral (CARR, 2003). Entretanto, os mecanismos pelos quais o decréscimo dos níveis de estrogênios afetaria a composição corporal de mulheres PM ainda não estão bem elucidados.

Tem sido sugerido que a diminuição da concentração plasmática de estrogênios pode estar associada com o aumento dos níveis circulantes de citocinas pró-inflamatórias, como o fator de necrose tumoral α (TNF-α) e interleucina-6 (IL-6), as quais podem contribuir com o aparecimento da sarcopenia (ROUBENOFF, 2003). Além disso, os estrogênios parecem exercer efeito direto sobre a massa muscular, uma vez que o músculo esquelético apresenta receptores na membrana celular, no citoplasma e na membrana nuclear (BROWN, 2008). Em adição, alterações no próprio tecido muscular também têm sido reportadas (MESSIER et al., 2011). Mulheres PM apresentam duas vezes mais tecido muscular não contrátil e gordura intramuscular, quando comparadas com mulheres jovens (JUBRIAS et al., 1997).

As vias de sinalização de como o estrogênio regula o acúmulo de gordura ainda não estão totalmente elucidadas (VAN PELT et al., 2015). Entretanto, estudos com ratos têm mostrado que a interrupção da sinalização de estrogênios (por meio de manipulação genética ou intervenção cirúrgica) induz mudanças na composição corporal (HEINE et al., 2000; ROGERS et al., 2009; WITTE et al., 2010). A desregulação de receptores de estrogênios ERα resulta em aumento de gordura corporal, sugerindo que esses receptores apresentam efeito protetor contra essa situação (HEINE et al., 2000). Estudos que reduzem os estrogênios circulantes têm mostrado maior adiposidade corporal em ratos quando comparados ao controle (ROGERS et al., 2009; WITTE et al., 2010). Segundo Van Pelt, Gavin e Kohrt (2015), devido ao potencial impacto adverso na saúde após a menopausa, é necessário compreender melhor os mecanismos de ações metabólicas dos estrogênios nas mulheres.

Estudos transversais, de coorte e randomizados de terapia hormonal são utilizados para investigar os efeitos da menopausa sobre a composição corporal, sendo este último, considerado o padrão-ouro, apesar de apresentar limitações (tipo de hormônio, dose, via

de administração e mudança abrupta nos níveis hormonais quando fisiologicamente essas mudanças ocorrem lentamente) (VAN PELT; GAVIN; KOHRT, 2015). Mulheres em terapia hormonal parecem apresentar menor circunferência da cintura (ESPELAND et al., 1997), peso, massa gorda total e do tronco (JENSEN et al., 2003).

Apesar dos mecanismos não estarem totalmente claros, a pós-menopausa tem sido associada com alterações negativas importantes na composição corporal, merecendo, portanto, maior atenção quanto as consequências e cuidados para melhor tratamento ou manutenção da saúde dessa população.

2.3 Exercício de força

O protocolo de treinamento de força (TF) recomendado pelo *American College of Sports Medicine* preconiza que sejam realizados treinos com frequência de 2-3 vezes por semana, contendo de 1-3 séries de 8-12 repetições, com carga entre 70-85% de uma repetição máxima (1-RM) e que contenha exercícios uni e multiarticulares (ACSM, 2009). Diversos estudos têm mostrado benefícios do TF à saúde geral, afetando positivamente parâmetros cardiovasculares (SHAW et al., 2016) e ósseos (GOMBOS et al., 2016), além de estar associado com a redução de inflamação (PHILLIPS et al., 2012). Além disso, o TF é conhecido por aumentar a massa muscular em adultos (HARTMAN et al., 2007) e idosos (LEENDERS et al., 2013), sendo portanto, uma estratégia eficaz no tratamento da perda de músculo esquelético e de força (FRONTERA et al., 1988; CHARETTE et al., 1991; KOSEK et al., 2006), inclusive em mulheres PM (ORSATTI et al., 2008; NUNES et al., 2016).

Shaw e colaboradores (2016) realizaram um estudo de intervenção com 37 mulheres PM. As voluntárias foram submetidas a um protocolo de exercício de força, o qual continha 10 exercícios e eram realizados 3 séries com 12 repetições entre 67-85% de 1-RM, com frequência de duas vezes por semana. Após 6 semanas de intervenção, foram observadas melhoras em medidas antropométricas e força. Nunes e colaboradores (2016) recentemente demonstraram que o TF com alto volume (6 séries por exercício) parece impactar em melhoras nos indicadores de adiposidade abdominal (circunferência da cintura e razão cintura quadril), metabolismo lipídico (colesterol total e LDL-colesterol) e ainda, prevenir o aumento de IL-6 em mulheres PM.

Em outro estudo conduzido por Conceição e colaboradores (2013), 20 mulheres PM foram randomizadas em dois grupos: grupo intervenção (realizava 10 exercícios de força, 3 séries cada, entre 8-10 RM, 3 vezes/semana) e grupo controle (não realizou

nenhum tipo de exercício). Após 16 semana de intervenção e avaliação da composição corporal por antropometria, os pesquisadores observaram aumento da massa magra (Δ=2,46%/ $p<0,05$) no grupo intervenção, bem como diminuição da massa gorda total (Δ=-7,0,6%, $p<0,05$) e do percentual de gordura (Δ=-6,75%, $p<0,05$).

Assim sendo, o TF é uma estratégia para estimular e promover a hipertrofia muscular, além de promover diversos outros benefícios à saúde geral, sendo indicado inclusive para mulheres PM.

2.4 Proteína

A ingestão de proteínas leva à estimulação da síntese proteica muscular (SPM) e diversos fatores podem modular essa estimulação, como a dose de aminoácidos/proteína (VERDIJK et al., 2009), fonte (CANDOW et al., 2006; HARTMAN et al., 2007), tempo de ingestão relativo ao exercício (CRIBB; HAYES, 2006; HARTMAN et al., 2007) e a sensibilidade do músculo esquelético à subsequente hiperaminoacidemia (CHURCHWARD-VENNE et al., 2012).

Estudos recentes têm sugerido que indivíduos com idade mais avançada apresentam SPM atenuada no período pós-prandial (período em que ocorre a hiperaminoacidemia), particularmente ou relativamente à baixa ingestão de proteínas (PADDON-JONES et al., 2008; BAUER et al., 2013). Um estudo conduzido por Moore e colaboradores (2015) demonstrou que indivíduos idosos necessitam ingerir maior quantidade de proteínas para estimular a SPM de forma satisfatória quando comparados com indivíduos jovens. Desta forma, esses indivíduos apresentam "resistência" ao anabolismo quando comparados aos jovens, situação que pode explicar, em parte, o declínio na massa muscular relacionado com o avanço na idade (GUILLET et al., 2004; CUTHBERTSON et al., 2005; MOORE et al., 2015). Além disso, diversos estudos oferecem suporte à premissa de que a recomendação de ingestão proteica para idosos deveria ser superior aos 0,8 g/kg de peso/dia, propostos pela Dietary Reference Intake (DRI) (BARTALI et al., 2012; BREEN; PHILLIPS, 2012; KIM et al., 2012; TIELAND, DIRKS, et al., 2012; GRAY-DONALD et al., 2014; PHILLIPS et al., 2016).

2.5 Recomendação de ingestão proteica pela DRI *vs.* novas propostas

As DRIs formam um conjunto de valores de referência de ingestão para nutrientes específicos, os quais incluem: *Estimated Average Requirement* (EAR), *Recommended Dietary Allowance* (RDA), *Adequate Intake* (AI) e *Tolerable Upper Intake Level* (UL).

A escolha do valor de referência a ser utilizado depende de alguns pontos importantes, tais como o critério de adequação nutricional e a população-alvo. A RDA é a ingestão média diária de determinado nutriente que satisfaça a necessidade de quase toda a população (97-98%) de um determinado grupo e gênero.

A atual RDA de ingestão proteica é de 0,8 g/kg de peso/dia para indivíduos com idade igual ou superior a 19 anos (IOM, 2002), inclusive para mulheres PM. Entretanto, essa recomendação tem sido questionada por ser insuficiente na máxima estimulação da SPM em indivíduos mais velhos (PHILLIPS et al., 2016). Concomitantemente, pesquisas têm proposto que a ingestão de aproximadamente 1,2 g de proteína/kg de peso/dia seria mais eficiente na manutenção da massa magra (BAUER et al., 2013; MCDONALD et al., 2016; PHILLIPS et al., 2016).

Recentemente, dois estudos demonstraram que as necessidades diárias de ingestão proteica seriam superiores as recomendações da DRI. Esses estudos utilizaram a técnica de oxidação de aminoácido, a qual é superior ao balanço nitrogenado, metodologia utilizada para chegar no atual valor da RDA (IOM, 2002; PHILLIPS et al., 2016). Um estudo avaliou o efeito da ingestão de sete diferentes quantidades de proteína (entre 0,1 e 1,8 g/kg) na SPM em seis mulheres idosas. Foi observado que a EAR de proteína na máxima estimulação da SPM foi de 0,85g/kg de peso e a RDA de 1,15g/kg de peso, sendo estes valores 29% e 44% superiores a atual recomendação para adultos, respectivamente (TANG et al., 2014). Um outro estudo também avaliou a ingestão de várias doses de proteína (entre 0,2 e 2,0 g/kg) na SPM de doze mulheres idosas. Os pesquisadores observaram que a EAR foi de 0,96g/kg de peso e a RDA de 1,29g/kg de peso (RAFII et al., 2015).

Houston e colaboradores (2008) avaliaram a associação entre a ingestão de proteína e mudanças na massa magra em 2066 voluntários durante 3 anos. Após ajustes para características demográficas, hábito de fumar, consumo de álcool, nível de atividade física, uso de esteroides orais, doenças (diabetes, isquemia cardíaca, insuficiência cardíaca congestiva, doença pulmonar e câncer), e gordura corporal, foi observada associação entre ingestão proteica total (β(SE): 6.38 (2.74), p=0,020) e proteína animal (β(SE): 6.58 (2.75), p=0,020) e massa magra. Além disso, aqueles indivíduos que estavam no quinto quintil de ingestão (1,2 g/kg de peso/dia) perderam cerca de 40% menos massa magra do que aqueles indivíduos que estavam no primeiro quintil (0,8 g/kg de peso/dia). McDonald e colaboradores (2016) avaliaram a ingestão alimentar de 368 indivíduos por 6 anos, os quais foram categorizados em idade superior ou inferior a 65

anos. Foi observado que aqueles indivíduos com maior ingestão de proteína (~1,26g/kg de peso/dia), apesar de não terem ganhado, foram aqueles que apresentaram maior manutenção de massa magra ao longo tempo

Entretanto, essa nova proposta de ingestão ainda não é um consenso. Um estudo recentemente avaliou a SPM aguda (após três dias de ingestão) comparando o consumo de 0,8 e 1,2 g de proteína/kg/dia em homens idosos, na presença e ausência de exercício. Não foi observada diferença na estimulação da SPM entre ambas ingestões de proteína (MURPHY et al., 2016).

Atualmente, há apenas estudos que avaliaram a SPM de maneira aguda e parece que essa síntese aguda não está correlacionada com a hipertrofia muscular a longo prazo (MITCHELL et al., 2014). Além disso, não foram encontrados estudos controlados e randomizados que tenham avaliado cronicamente a ingestão de 1,2 g de proteína/kg de peso/dia, tanto para a população em geral como para mulheres PM. Portanto, são necessárias mais pesquisas nesta área, que avaliem diversas populações, inclusive mulheres PM.

2.6 Distribuição de proteína ao longo do dia

Atualmente, tem sido dada grande importância à avaliação da ingestão de proteína em cada refeição ao invés de se avaliar apenas a ingestão de proteína total diária (BAUER et al., 2013; MURPHY et al., 2016). Uma distribuição mais uniforme de proteína ao longo do dia parece estimular a SPM mais efetivamente (MAMEROW et al., 2014). Segundo a teoria proposta por diversos pesquisadores, conforme a quantidade de proteína ingerida aumenta, também ocorre elevação na SPM, porém até que uma dose "ótima" seja alcançada (CUTHBERTSON et al., 2005; YANG et al., 2012; MOORE et al., 2015). Após essa dose ótima ser atingida, o músculo se tornaria refratário a um contínuo aumento na ingestão de proteína e consequente aminoacidemia, sem aumento na SPM, levando então, a teoria de dose-resposta saturável (MURPHY et al., 2016).

Moore e colaboradores (2015) compararam a SPM pós-prandial em indivíduos jovens e idosos com o objetivo de observar se o envelhecimento poderia afetar a SPM. Segundo a pesquisa, jovens precisam de 0,24 g de proteína/kg de peso/refeição (IC95% 0,18-0,30) enquanto idosos precisam de 0,4 g de proteína/kg de peso/refeição (IC95% 0,21-0,59) para estimular a máxima SPM. Adicionalmente, foi observado que ambos os grupos (jovens e idosos) apresentam taxas de síntese parecidas, sugerindo que indivíduos mais velhos mantém a capacidade anabólica, porém apenas com estímulo nutricional

suficiente. Segundo Paddon-Jones e Leidy (2014), o consumo de refeições contendo cerca de 30-40 g de proteína de alta qualidade seria mais interessante para o estímulo da SPM.

Recentemente Macnaughton e colaboradores (2016) publicaram um estudo onde foi avaliada a SPM após a ingestão de 20 ou 40g de proteína (*whey protein*) em homens jovens que realizaram um treino com exercícios de força para membros inferiores e superiores. Os pesquisadores encontram que a ingestão de 40g de proteína foi superior em estimular a SPM após um treino para o corpo todo, quando comparada a ingestão de 20g (MACNAUGHTON et al., 2016). No entanto, este foi o primeiro estudo a encontrar que seria necessária uma dose ainda superior para jovens. Para indivíduos mais velhos, ainda faltam pesquisas que avaliem doses superiores de proteína na SPM e também na hipertrofia muscular a longo prazo, após a realização de diferentes protocolos de exercícios.

Não foram encontrados estudos que tenham avaliado a ingestão proteica entre as refeições de mulheres PM, entretanto, várias pesquisas têm mostrado um padrão desigual de ingestão de proteínas ao longo do dia em diversas populações (TIELAND et al., 2012; VALENZUELA et al., 2013; USDA, 2016), onde geralmente, apenas uma única refeição teria a quantidade adequada de proteína (comumente o almoço ou o jantar). Além disso, a maioria dos estudos observou apenas o teor proteico das refeições (TIELAND et al., 2012; VALENZUELA et al., 2013; MAMEROW et al., 2014; USDA, 2016), não sendo levado em consideração a qualidade da proteína.

Grande parte dos estudos utilizam o *whey protein*, uma proteína com rápida digestibilidade, absorção e rica em leucina (VAN VLIET et al., 2015). Ainda não se sabe se uma refeição mista precisaria de uma dose proteica diferente de uma proteína isolada (MURPHY et al., 2016). Segundo uma revisão recentemente publicada (MURPHY et al., 2016), tendo como assunto central a distribuição de proteína ao longo do dia, o ideal para pessoas mais velhas seria o consumo de pelo menos 3 refeições diárias com 0,4 g de proteína/kg de peso com fonte proteica de alto valor biológico acrescida a proteína de menor qualidade (ex. grãos e vegetais), podendo o valor total de proteína diária ser superior a 1,2 g/kg de peso.

2.7 Leucina

Uma fonte proteica com boa qualidade depende de sua digestibilidade e composição de aminoácidos essenciais (FAO, 2011), e as proteínas de origem vegetal apresentam qualidade inferior quando comparadas as proteínas de origem animal, como

carnes, ovos e produtos lácteos (VAN VLIET et al., 2015). Em adição, as proteínas de origem animal têm um teor mais elevado do aminoácido essencial leucina (VAN VLIET et al., 2015), o qual parece atuar na estimulação pós-prandial da SPM (MURPHY et al., 2016).

Valina, isoleucina e leucina constituem os aminoácidos de cadeia ramificada (*branch chain amino acid* – BCAA), e particularmente este último, a leucina, apresenta potencial anabólico, com capacidade de estimular a SPM via sinalização da proteína alvo da rapamicina em mamíferos (*Mammalian Target of Rapamycin* - mTOR) (DE BANDT, 2016). Apesar da recomendação de ingestão de leucina ainda não estar bem clara, a necessidade do indivíduo parece aumentar com o avanço da idade (KATSANOS et al., 2006), tendo sido demonstrado que é necessário ingerir entre 2-3 g de leucina/refeição para a estimulação máxima da SPM (CASPERSON et al., 2012).

McDonald e colaboradores (2016) recentemente publicaram um estudo longitudinal conduzido com 368 indivíduos que participaram da coorte de *Danish WHO-MONICA (Multinational MONItoring of TRends and determinants in CArdiovascular disease*). A ingestão de leucina foi avaliada no momento basal e após seis anos, em homens e mulheres (entre 35-65 anos). A avaliação da composição corporal foi realizada através de bioimpedância elétrica. Foi observado que os indivíduos mais velhos que estavam no quarto quartil de ingestão de leucina (7,1 g/dia) apresentaram manutenção de massa magra ao longo do tempo, enquanto que aqueles com ingestão inferior apresentaram perdas na massa magra durante os seis anos (β=0,434, p=0,030). Entretanto, os indivíduos que ingeriram maior quantidade de leucina também consumiram mais proteína (quarto quartil: 1,26 g proteína/kg/dia). Assim, não está totalmente claro se é a maior ingestão de leucina ou o maior consumo proteico que proporcionou maior manutenção de massa magra ao longo do tempo.

Uma meta-análise avaliou o efeito da suplementação de leucina em parâmetros antropométricos e de força e encontrou resultados positivos para o ganho de massa corporal, massa magra e índice de massa corporal (KOMAR et al., 2015). Entretanto, a maioria dos estudos não avaliou a quantidade proteica da dieta ou sua distribuição ao longo do dia. Desta forma, mais estudos que avaliem a influência da leucina sobre a massa magra, inclusive em mulheres PM são necessários para melhor avaliar se há um efeito desse BCAA na hipertrofia muscular e qual seria esse efeito.

3 OBJETIVOS

3.1 Objetivo geral

Avaliar o efeito de dietas com diferentes quantidades de proteínas (nova proposta ou recomendação das DRIs), na composição corporal de mulheres pós-menopausadas realizando treinamento físico com exercícios de força.

3.1 Objetivos específicos

- Realizar avaliação antropométrica e de composição corporal;
- Avaliar os hábitos alimentares das mulheres;
- Avaliar a ingestão de leucina.

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5. ARTIGO

Research Article

Protein intake according to new proposal does not improve lean mass gain when compared with RDA recommendations in postmenopausal women after resistance exercise protocol: a randomised clinical trial

Short title: Protein intake and lean mass gain

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ABSTRACT

Recent studies have been suggested that protein intake according to Recommended Dietary Allowance (RDA) (0.8 $g \cdot kg^{-1} \cdot d^{-1}$) seems to be insufficient for older individuals. The aim of this study was to evaluate the effect of a new proposal of protein intake compared to RDA recommendation on lean body mass (LBM) gain in postmenopausal women (PMW) practicing resistance exercise. Twenty-three PMW (63.2±7.8y) were randomised into two groups. The group with new proposal (n=11) (high protein - HP) received a dietary plan with ~1.2 $g \cdot kg^{-1} \cdot d^{-1}$ of protein, while the group with RDA recommendation (n=12) (normal protein - NP) was instructed to ingest ~0.8 $g \cdot kg^{-1} \cdot d^{-1}$ of protein. Both groups performed the same resistance training protocol, which consisted of three times a week, with progression of the number of series (from 1 to 6 sets) and 8-12 repetitions. The intervention occurred during 10-weeks. Body composition evaluation was performed by dual-energy X-ray absorptiometry. The diet was evaluated by nine 24-hour food recall. During the protocol, HP group presented higher intake of protein (1.18±0.3 vs. 0.87±0.2 $g \cdot kg^{-1} \cdot d^{-1}$, p=0.008) and leucine (6.0±1.4 vs 4.3±0.9 g/day, p=0.000) than the NP group, respectively. At the end, there were an increase in LBM both in HP (37.1±6.2 vs 38.4±6.5 kg, p=0.004) and in NP (37.6±6.2 vs 38.8±6.4 kg, p<0.001), with no differences between groups (p=0.572). Therefore, we concluded that a protein intake close to new proposals did not lead to higher LBM gain, when compared to RDA recommendation in PMW performing resistance exercise during 10-weeks. This trial was registered at clinicaltrials.gov as NCT03024125.

Key words: lean body mass, protein, RDA recommendations, leucine intake, postmenopausal women.

INTRODUCTION

Aging is associated with a gradual and progressive loss of muscle mass and function[1,2]. In addition, older individuals present attenuated muscle protein synthesis (MPS) in postprandial period, characterizing a process called anabolic resistance[3,4]. Middle-aged adults have similar negative metabolic consequences of aging[5], and in this context postmenopausal women (PMW) are included. This population deserves special attention because during this period a decrease in serum estrogen levels is observed[6,7], which seems to have direct and indirect effects on skeletal muscle[8]. These effects may lead to greater muscle and strength loss, concomitantly with greater accumulation of body fat[9].

Resistance training and adequate protein intake are known strategies to increase lean body mass (LBM)[10,11]. A greater intake of high-quality proteins (leucine-rich proteins) seems to promote additional muscle gain in resistance exercise protocol[12]. Thus, the association of both interventions is an effective strategy for preventing loss and also promote LBM gain[13].

In this context, protein intake proposed by Recommended Dietary Allowance (RDA) for older adults, including PMW, is 0.8 $g \cdot kg^{-1} \cdot d^{-1}$[14]. However, current studies have shown that higher doses of protein, approximately 1.2 $g \cdot kg^{-1} \cdot d^{-1}$, seem to be more effective in stimulating MPS or muscle maintenance[15,16,17,18,19,20], suggesting that RDA recommendation seems to be insufficient for older individuals, although this is not a consensus[21]. A recent study compared the intake of 1.2 $g \cdot kg^{-1} \cdot d^{-1}$ with 0.8 $g \cdot kg^{-1} \cdot d^{-1}$ in older men, during 3 days, and showed no difference in acute MPS stimulation after exercise[21]. However, considering acute measurements of MPS does not seem to be directly associated with longer-term changes in muscular hypertrophy[22], long-term studies are needed to evaluate the effect of these intake of protein on LBM gain to confirm these results, also in PMW.

Therefore, the aim of our study was to evaluate the effect of the protein intake according to RDA recommendations (0.8 $g \cdot kg^{-1} \cdot d^{-1}$) compared to new protein proposals (1.2 $g \cdot kg^{-1} \cdot d^{-1}$) on LBM gain in PMW after resistance exercise protocol. We hypothesized that a protein intake close to new proposals would enhance LBM gain associated with resistance exercise in PMW.

METHODS

Research participants

This trial was a single-blind, randomised, parallel prospective clinical trial, conducted at the Federal University of Uberlandia and in partnership with the Federal University of Triângulo Mineiro, Minas Gerais, Brazil. Inclusion criteria for participants were women in the postmenopausal period (cessation of menstruation for at least one year; self-reported) and sedentary. Those who presented orthopaedic limitation, hormone replacement, alcoholic habits and any stage of kidney disease were excluded. The study was approved by Federal University of Uberlandia Research Ethics (protocol number 1.733.512) and Federal University of Triângulo Mineiro Research Ethics Committee (protocol number 1.090.676). This research was registered at ClinicalTrials.gov as NCT03024125.

Initially, 48 participants were recruited and one volunteer was excluded from the study. Once consent was obtained, all the other participants were randomly assigned by MedCalc® software (version 11.1) into two groups: RDA protein recommendations (~0.8 $g \cdot kg^{-1} \cdot d^{-1}$) (normal protein, NP) and new protein proposals (~1.2 $g \cdot kg^{-1} \cdot d^{-1}$) (high protein, HP). After losses occurred during the intervention, 11 volunteers were part of HP group and 12 subjects in NP group (Figure 1). An independent researcher performed randomisation.

Study design

Before the beginning of the study, body composition (dual-energy X-ray absorptiometry - DXA), dietary habits, anthropometric parameters, resting energy expenditure (REE) and blood samples were assessed. The evaluation of these analyses lasted two weeks before the intervention. Additionally, a two-week familiarization period was done to ensure that PMW were able to perform all resistance exercises safely and correctly. Resistance training and diet intervention were performed during ten weeks. Dietary intake was also assessed at 5-6 and 9-10 weeks. At the end of the study, DXA was performed again. The study protocol is described in Figure 2.

Assessments

Anthropometry

Body mass and height was measured according to protocol proposed by Lohman et al.[23] (balance and stadiometer Líder®, Araçatuba, Brazil). After these measurements, body mass index (BMI) was calculated, whereas body weight in kilograms was divided by height in meters squared.

Body composition

Body composition (lean and fat mass) was assessed using DXA (Lunar iDXA®, GE Healthcare, USA) and quantified by software Encore version 14.10. Twenty-four hours before the evaluation, the volunteers were instructed to ingest two litters of water to standardize the level of muscle hydration and were oriented to 8 to 10 hours of fasting, as previously reported [24]. The volunteers wore light and comfortable clothes without the presence of metal objects. The equipment was used manually and all analyses were performed by the same researcher. The body scan was divided into arms, legs, trunk and head.

Resting energy expenditure (REE) and total energy expenditure (TEE)

Resting energy expenditure was measured by indirect calorimetry (open circuit the mixing-chamber system), using VO2000 (MedGraphics, Ann Arbor, USA). The device was turned on 30 minutes before the examination for heating, proper stabilization and calibration of the analysers of O_2 and CO_2 with an ambient air[25]. The volunteers started the test after 12 hours of overnight fast, six to eight hours of sleep, without intense physical activity in the previous 48 hours of the examination and 24 hours without caffeine consumption before the test. The test was performed in a quiet room with controlled temperature[26]. There was a 10-minutes acclimatization period for reading stabilization, and then VO_2 and VCO_2 were measured for a period of 20 minutes. Mean values of these variables were used and inserted in Weir equation[27] for REE measurement. Four women (two of each group) did not complete the test and REE was estimated by the FAO/WHO/UNU equation[28].

For the calculation of total energy expenditure, the following variables were used: REE was measured or calculated (as described above); 1.3 was used for all as factor activity (FA) (concerning daily activities). For calculation of metabolic equivalent (MET), we used the value 5.5 MET for resistance exercise[29]. After being estimated effective training time, MET training was calculated and then multiplied by 3 (number of

155 times that physical training occurred a week) and the value divided by 7 (corresponding
156 to the days of the week). The following formula was used: TEE = (REE * FA) + MET[30].
157
158 ***Dietary assessment***
159 Dietary intake was assessed by 24-hour food recall. Three recalls were performed
160 at baseline (weeks -4 and -3), during 5-6 weeks and 9-10 weeks of intervention, with a
161 total of 9 dietary recalls. Arithmetic mean was held from 6 recalls performed during the
162 intervention for better representation of the dietary habits of the volunteers during the
163 period of intervention. The recalls were performed on non-consecutive days, and referring
164 to two weekdays and one day of the weekend at each moment of the study. Two recalls
165 were conducted in person and others via phone call after adequate familiarisation.
166 Analyses of food data were performed using Dietpro® software (version 5.7i) and United
167 States Department of Agriculture (USDA)[31,32] food composition table was used. In
168 addition, nutrition labels of manufacturers were also utilised.
169 The distribution of protein and leucine throughout the day was evaluated
170 according to the meals described by volunteers, divided into: breakfast, morning snack,
171 lunch, afternoon snack, dinner and supper. No fixed meal times were set. The 24-hour
172 food recall was evaluated according to the pre-established meals, taking into account the
173 time and type of food consumed.
174
175 ***Blood Samples***
176 Blood samples (16 ml) were collected between 7:30 AM and 9:00 AM after an
177 overnight fast (8–10 h), and placed in a dry tube with gel separator or EDTA (vacuum-
178 sealed system; Vacutainer®, England). Urea and creatinine were analysed by automated
179 colorimetric. These variables were measured to evaluate renal function. was used to
180 evaluated the samples.
181
182 ***Experimental Protocol***
183 ***Dietary Intervention***
184 After randomisation of the sample into two groups, both received individualized
185 dietary plans, with their lists of food substitutions. All diets were normocaloric. The NP
186 group contained ~0.8g protein·kg^{-1}·d^{-1}in their dietary plans while the group HP contained
187 ~1.2g protein·kg^{-1}·d^{-1}. The amount of carbohydrate was the same in both groups (50% of
188 total caloric value). Lipids were offered a lesser amount in the HP group (15% of total

calories) and higher in NP group (25-30% of total calories), for calorie adjustment. The participants did not know which intervention group were part (single blind).

Resistance Training

The resistance training was conducted in a public Health and Physical Activity Centre at the Federal University of Triângulo Mineiro and the protocol followed the recommendations of the American College of Sports Medicine, with the aim of muscle hypertrophy[33]. The minimum acceptable frequency for training was 70% and resistance training was realized three times a week and at least 48 hours of rest between sessions. Initially, the women started with 10 minutes of warm-up (walking). Dynamic resistance exercises were realized for the upper and lower limbs, including guided squat (free weight), leg curl (machine), leg extension (machine), bench press (free weight), rowing (machine), pull down (machine), triceps pulley and arm curl (free weight). All volunteers attended a 1-week familiarization period with low loads in order to learn the techniques. The load of 12-RM was applied for all resistance exercise and 8-12 repetitions were done in each set. The interval between sets and exercises was 60 seconds. The resistance training was carried out for 10 weeks[34]. The groups started with one series of each exercise in the first week and increased a set per week up to six series for all exercises. When they reached to six series (in the sixth week), they kept this volume until the tenth week. In the sixth week, there was a readjustment of the load for volunteers who were able to perform more than 12 RM. The load was increased until the volunteer remained in the range of 8-12 repetitions. During the training sessions, the subjects were instructed to perform the eccentric action in a second and the concentric action in a second. The exercises were supervised all the time by trained professionals, who were blind to nutritional intervention.

Statistics

The sample size calculation was performed using the following equation: $n=[(Z\alpha/_2*\sigma)/E]^2$, where for $Z\alpha/_2$ was used 1.96, for σ was used 6.7 (standard deviation found in the study of Longland et al.[35], and for E was used 3 (referring to 3 kg of lean mass). The data distribution was determined using the Shapiro-Wilk test. Independent *t*-Test or Mann-Whitney was used to compare groups at baseline. Generalized Estimating Equation (GEE) with sequential Sidak post hoc was performed to compare groups and moments, and evaluate the interaction between time x treatment. Delta (Δ), final minus

initial value, was calculated for protein ($g \cdot kg^{-1} \cdot d^{-1}$) and leucine (g/day) intake, body composition measurements, such as total lean mass (TLM) (kg), leg lean mass (LLM) (kg), total fat mass (TFM) (kg) and trunk fat mass (TrFM) (kg). The values were represented in means ± SD for the *t*-test and GEE, and in median [interquartile range] for Mann-Whitney. *P-value* <0.05 was used for statistical significance and SPSS software (version 20.0) for statistical analysis.

RESULTS

Baseline characteristics

Table 1 presents the baseline characteristics of PMW. No differences were found for age, anthropometrics, body composition, biochemical parameters, and resting energy expenditure between groups.

Dietary intake

At baseline, both group presented the same intake for all diet components evaluated. No changes were observed for calories, carbohydrate (g) and lipid (g and %) intake between groups or moments. Carbohydrate intake (%) remained the same in NP group, but decreased in HP group during the intervention, however, no differences were found between groups. HP increased isoleucine, and valine intake regarding time, intervention and interaction (Table 2).

NP group maintained the intake of protein (0.78±0.3 vs 0.87±0.29 $g \cdot kg^{-1} \cdot d^{-1}$, p=0.332), BCAA (8.8±2.81 vs 10.15±2.14 g/day, p=0.117) and leucine (3.46±1.12 vs 4.3±0.88 g/day, p=0.396), whereas HP group increased the intake of these dietary components during the study (protein: 0.82±0.29 vs 1.18±0.3 $g \cdot kg^{-1} \cdot d^{-1}$, p<0.001; BCAA: 9.59±2.42 vs 14.34±2.25 g/day, p<0.001; leucine: 3.82±0.99 vs 6.02±0.93 g/day, p<0.001). The values of protein (p=0.013), BCAA (p=0.005) and leucine (p=0.006) were higher in HP when compared to NP group during the study. All deltas of HP group were significantly higher than NP (protein: 0.35±0.23 vs 0.09±0.22 $g \cdot kg^{-1} \cdot d^{-1}$, p=0.013; BCAA: 4.76±2.48 vs 1.35±2.64 g/day, p=0.005; leucine: 2.20±1.07 vs 0.83±1.06 g/day, p=0.006, respectively) (Figure 3).

Distribution of protein and leucine during the day

An increase in protein intake was observed at breakfast by HP group in comparison to baseline (6.56±1.08 vs 12.72±1.61 g, $p<0.001$), and this value was also higher than in NP group during the study (12.72±1.61 vs 5.52±0.50 g, $p=0.001$). At lunch, HP group showed higher protein intake comparing to baseline (20.59±5.01 vs 32.41±7.08 g, $p<0.001$), being the only group and moment to reach the recommendation of protein intake per meal (~30 g per meal). However, the amount consumed by HP group was not significantly different from NP group (32.41±7.08 vs 26.89±7.12 g, $p=0.122$, respectively). At supper, NP group presented a reduction in intake in relation to time (2.85±1.62 vs 1.09±0.84 g, $p=0.001$), but no difference was found between groups ($p=0.085$). For the other meals, no significant differences were observed during the study. In relation to leucine intake, we noted a reduction by NP (0.29±0.16 vs 0.18±0.11 g, $p=0.024$) and an increase in HP (0.40±0.30 vs 0.95±0.50 g, $p=0.001$), whereas the HP value was higher than the same moment of NP ($p<0.001$). At lunch and dinner, HP group increased the consumption (1.58±0.12 vs 2.57±0.61 g, $p<0.001$; 1.19±0.13 vs 1.69±0.16 g, $p=0.17$; respectively), but there was no difference between groups ($p>0.05$). For the other meals, no significant differences were observed (Figure 4).

Body composition

Both NP group (37.57±6.17 vs 38.83±6.46 kg, $p<0.001$; $\Delta=1.26\pm0.82$ kg) and HP group (37.10±6.19 vs 38.43±6.49 kg, $p<0.001$, $\Delta=1.33\pm0.68$ kg) presented TLM gains after exercise protocol, however, no difference between groups was found. Additionally, both groups had LLM gain (NP: 12.66±2.57 vs 13.07±2.67 kg, $p=0.003$, $\Delta=0.40\pm0.77$; HP: 12.70±2.89 vs 13.19±3.2 kg, $p<0.001$, $\Delta=0.48\pm0.47$), but there was no difference between groups. Regarding TFM and TrFM, no differences were found at the end of the study in both groups (Figure 5).

DISCUSSION

The main finding of the present study was that, contrary to our initial hypothesis, a protein intake according to new proposals (1.18±0.3 $g \cdot kg^{-1} \cdot d^{-1}$) did not result in higher LBM gain when compared with an intake proposed by RDA (0.87±0.29 $g \cdot kg^{-1} \cdot d^{-1}$) after 10-week resistance training protocol in PMW.

Recent studies have shown that protein intake recommended by RDA seems to be insufficient to enhance the maximum MPS in elderly women and to maintain muscle mass during aging, therefore higher doses have been proposed[15,16,17,18,19,20]. This difference in protein dose recommendation is possibly due to the methodologies applied. Protein intake proposed by RDA used nitrogen balance method, which has several limitations[36]. Nowadays, new proposal of protein intake is based on amino acid oxidation technique, which is an independent tracer-based method that circumvents many nitrogen balance limitations[17], being a more advanced methodology. In our study, we did not find a greater LBM gain in HP group, suggesting that the new protein proposal seems to have no additional benefits in long-term changes of LBM in older women.

However, protein intake values compared in our study were obtained through means of each group, and some individuals ingested higher or lower doses than the average of the group. However, it does not seem to be a limitation in our study because when the LBM gain of the participants that only ingested an amount equal to or less than 0.8 $g \cdot kg^{-1} \cdot d^{-1}$ was compared to those who ingested equal to or greater than 1.2 $g \cdot kg^{-1} \cdot d^{-1}$ the results remained the same (data not shown). Furthermore, the recommendations of protein intake for maximum muscle hypertrophy proposed by American College of Sports Medicine position is 1.2 - 2.0 $g \cdot kg^{-1} \cdot d^{-1}$[37], therefore, we can speculate that active PMW may need an even higher quantity of protein to promote higher LBM gains than the offered at present study. A study published by Tieland et al.[12] evaluated the LBM gain in elderly men and women performing 2 times/week resistance training during 24 weeks. The researchers observed a gain of ~1.3 kg of LBM in the group with consumption of ~1.3 $g \cdot kg^{-1} \cdot d^{-1}$ of protein (with whey protein supplementation), whereas in placebo (with consumption of 0.9 $g \cdot kg^{-1} \cdot d^{-1}$ of protein), no LBM change was observed. At present study, the PMW gained ~1.30 kg of LBM regardless of protein consumption. These differences in both studies probably occurred due to our sample was younger, and the PMW performed resistance exercise 3 times/week (six sets of 8-12 repetitions), which containing exercises that recruited large muscle groups of upper and lower limbs. However, it is possible to speculate that if our volunteers were older, the consumption of ~0.8 $g \cdot kg^{-1} \cdot d^{-1}$ of protein could promote lower LBM gain, but new studies are needed to confirm it.

Besides total protein intake, the amount of protein intake per meal seems to be important for MPS and/or LBM gain, although this is not a consensus[38]. A balanced distribution of total protein intake, with meals contained at least 30-40 g of high quality

protein seems to be more effective in stimulating rates of MPS throughout the day in older individuals[39]. However, there are no chronic studies assessing the protein consumption by meals and their implications in LBM gain in PMW, which is an important knowledge gap[40]. In our study, only the HP group reached this threshold at lunch (32.41 ± 7.08 g protein), whereas the NP group did not reach the recommendation at any meal. A research conducted by Farsijani et al.[41] showed that over 2 years, people who consumed larger amounts of protein and more distributed throughout the day presented a greater amount of LBM. Another study found that more frequent consumption of meals containing between 30 and 45 g of protein presents higher association with LLM and strength[42]. Therefore, only one meal reaching the recommended protein intake per meal, like as found in our study, may be not able to produce higher changes in LBM, and possibly, more meals reaching that threshold are needed, thereby increasing the amount of protein intake per day.

Moreover, controlled experiments have elucidated that amounts of protein containing about 2-3 grams of leucine can stimulate maximum MPS[43,44,45]. In our research, both NP and HP group reached the recommendation of leucine per meal at lunch (2.05±0.44 and 2.57±0.61 g, respectively). In addition, HP group increased the intake of leucine in ~2 grams per day, but this increase was distributed throughout the day and not in only one meal, which did not seem to be sufficient to induce maximum MPS. McDonald et al.[15], in a longitudinal study, showed that older individuals (>65 years) ingesting 1.26 g protein·kg^{-1}·d^{-1} and 7.10 g/day of leucine presented higher LBM maintenance during 6 years, whereas lower intakes were associated with LBM loss. In our study, the HP group presented similar intake of both protein and leucine, but no higher increase in LBM was found when compared to lower protein intake group. Although an interventional study (present study) can be not compared to a longitudinal study, it is possible to speculate that the length of time that ~1.2 g·kg^{-1}·d^{-1} vs ~0.8 g·kg^{-1}·d^{-1} of protein is ingested may be important to promote greater gains in LBM. Therefore, new interventional studies lasting more than 10 weeks evaluating the effect RDA versus new proposal recommendations of protein plus resistance exercise on LBM gain in PMW are needed.

At present study, a higher protein intake did not result in changes of body fat. As we offered a normocaloric eating plan, the absence of fat loss was already expected. A normocaloric diet was prescribed because our aim was to observe the hypertrophy, and it is known that the caloric restriction can attenuate this process[46]. In addition, there were

no fat mass gain at present study, which also seems important to promote maximum hypertrophy because the increase of trunk adiposity is correlated with lower LBM gain in PMW performing resistance training protocol[24].

In our study, both groups gained LBM (~1.3 kg) in similarly way after the intervention protocol, showing that the resistance training was able to promote muscle hypertrophy in PMW after 10-weeks of training. This gain is in agreement with the literature, which shows that resistance training was effective in leading to muscular hypertrophy[47], including PMW[8].

As the main limitation of our study, we can mention the high withdrawal found in both groups, which reduced our sample and the statistical power of the comparisons. As strengths, indirect calorimetry was used to measure the resting energy expenditure of individuals for diet prescription. Additionally, our dietary intervention was based in increasing protein intake by a variety of protein sources, and not only one type of protein, and it represents a more realistic nutritional management in clinical practice.

In conclusion, the intake of new protein proposals (~1.2 $g \cdot kg^{-1} \cdot d^{-1}$) did not promote higher LBM gain when compared to RDA (~0.8 $g \cdot kg^{-1} \cdot d^{-1}$) in PMW after resistance exercise protocol exercises. Further studies, with greater time, are needed to evaluate the effect of these two protein recommendations on LBM gain.

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Authorship

L.T.R participated in collection and interpretation of the data, performed statistical analysis, and wrote the manuscript; P.C.N and F.M.S.B participated in collection, analysis, and interpretation of the data. F.M.M., A.P.S., and M.A.S.C.collaborated in the collection and interpretation of the data; F. L.O. carried out the conception and design of the study and wrote the manuscript. E.P.O. carried out the conception and design of the study, participated in the interpretation of the data, wrote, and contributed with the revision of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

None.

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TABLE 1 Baseline participant characteristics.

Variables	NP	HP	*p-value*
	***n*=12**	***n*=11**	
Age, *y*	63.00±8.62	63.45±7.67	0.895
Anthropometrics			
Weight, *kg*	69.03±17.06	67.64±15.58	0.840
Height, *m*	1.56±0.08	1.55±0.06	0.824
BMI, kg/m^2	28.38±6.02	28.06±5.46	0.894
Body Composition			
Total lean mass, *kg*	37.57±6.18	37.10±6.19	0.857
Leg lean mass, *kg*	12.66±2.57	12.70±2.90	0.969
MMI, kg/m^2	6.89±0.95	6.93±1.17	0.934
Total fat mass, *kg**	24.48 [20.53-33.54]	27.72 [20.62-34.21]	0.786
Total fat mass, *%*	40.75±7.01	40.95±4.73	0.938
Trunk fat mass, *kg**	13.20 [11.25-19.61]	13.58 [11.65-18.22]	0.740
Biochemical parameters			
Creatinine, *mg/dL*	0.78±0.16	0.80±0.18	0.768
Urea, *mg/dL**	27.70 [24.40-35.77]	34.10 [30.30-36.00]	0.104
Energy expenditure			
REE, *kcal*	1582.51±566.35	1394.10±290.98	0.324
TEE, *kcal*	2127.56±750.62	1881.20±388.77	0.332

Notes: BMI: body mass index; MMI: muscle mass index, was calculated by lean mass (kg) divided by height squared; REE: rest energy expenditure; TEE: total energy expenditure; *: data with non-normal distribution.

Independent *t*-test or Mann-Whitney; mean ± SD or median [interquartile range].

TABLE 2 Intake of calories, macronutrients, and BCAAs according to moments and groups**.**

Variables	NP (*n*=12)		HP (*n*=11)		*p-value*		
	Pre	**During**	**Pre**	**During**	***Time***	***Intervention***	***Interaction***
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	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	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
Isoleucine, *g**	1.96±0.63[a]	2.40±0.54[b,c]	2.13±0.52[a,c]	3.42±0.56[d]	<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

Notes: BCAA: branched-chain amino acid.

Generalized Estimating Equation (GEE) with sequential Sidak post hoc; all values are mean ± SD; *: data with non-normal distribution.

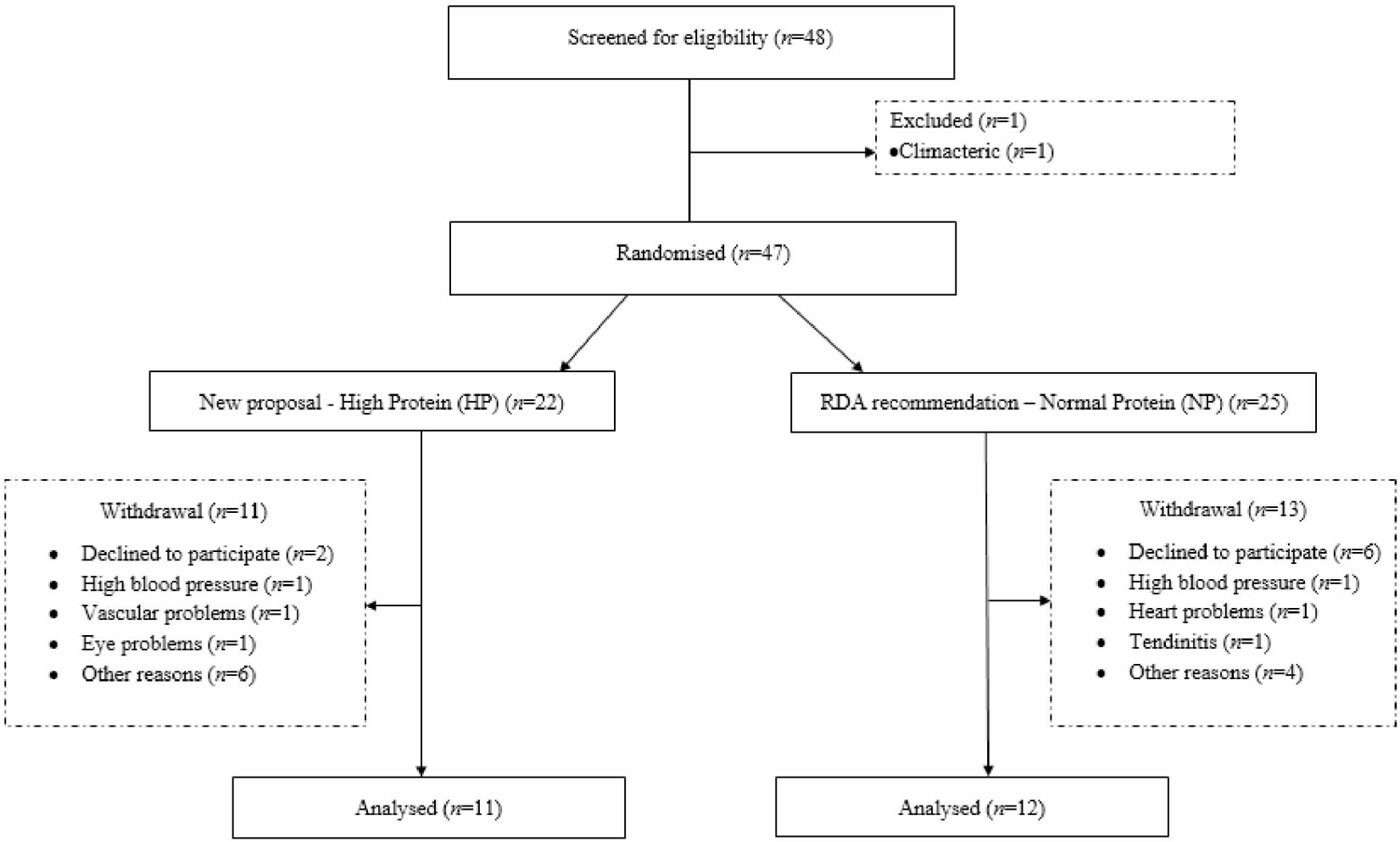

FIGURE 1 Randomisation of research volunteers.

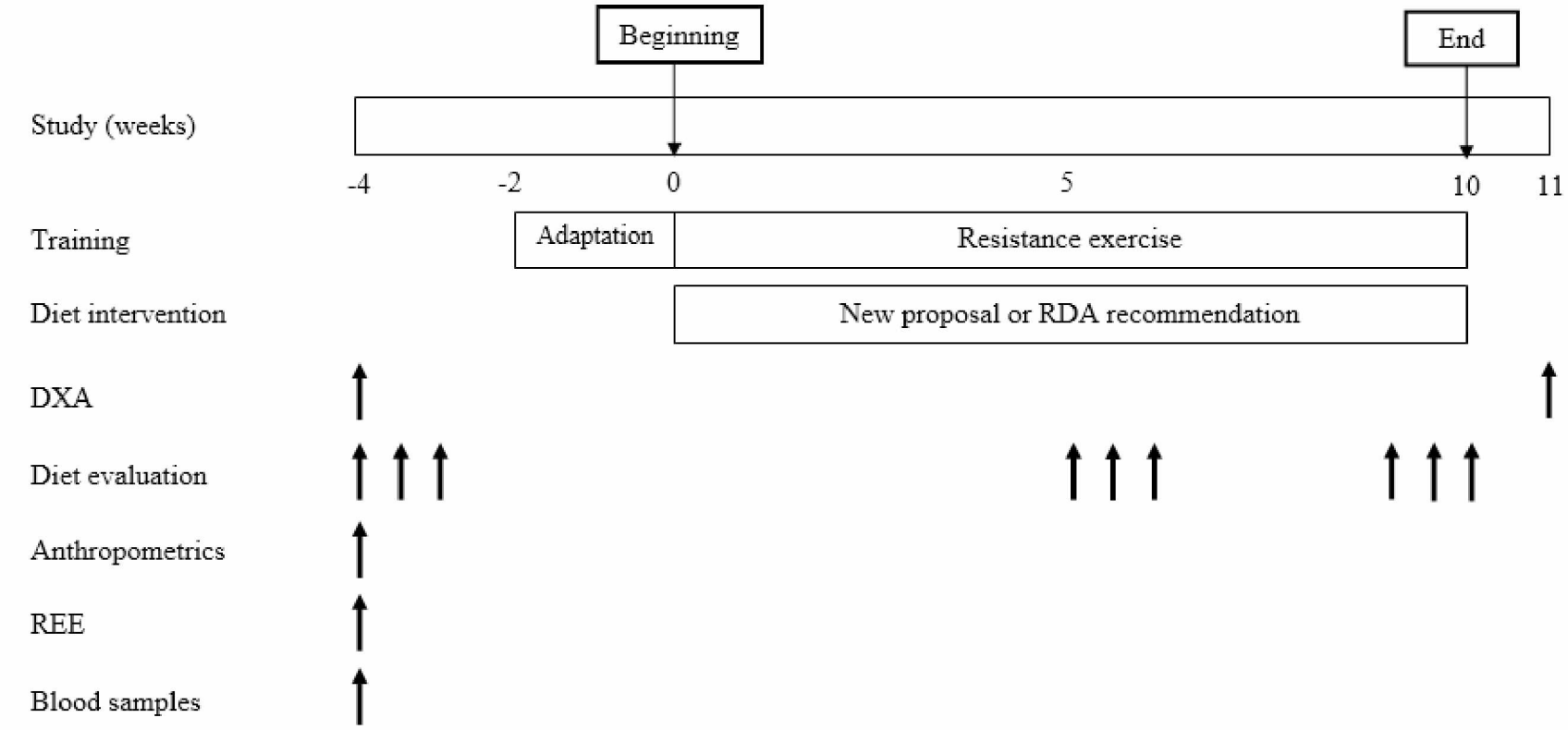

FIGURE 2 Schematic overview diagram of the study protocol.

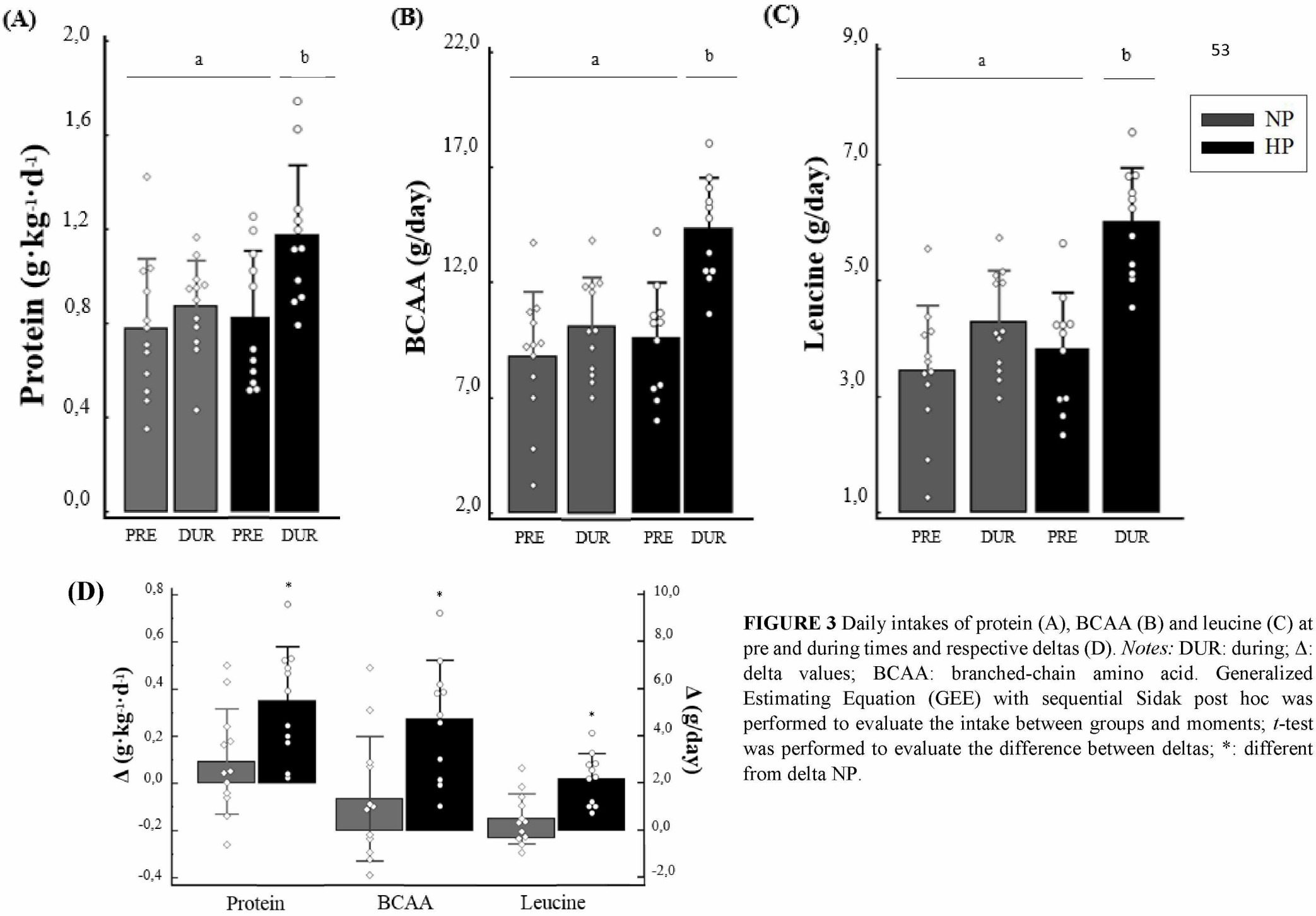

FIGURE 3 Daily intakes of protein (A), BCAA (B) and leucine (C) at pre and during times and respective deltas (D). *Notes:* DUR: during; Δ: delta values; BCAA: branched-chain amino acid. Generalized Estimating Equation (GEE) with sequential Sidak post hoc was performed to evaluate the intake between groups and moments; *t*-test was performed to evaluate the difference between deltas; *: different from delta NP.

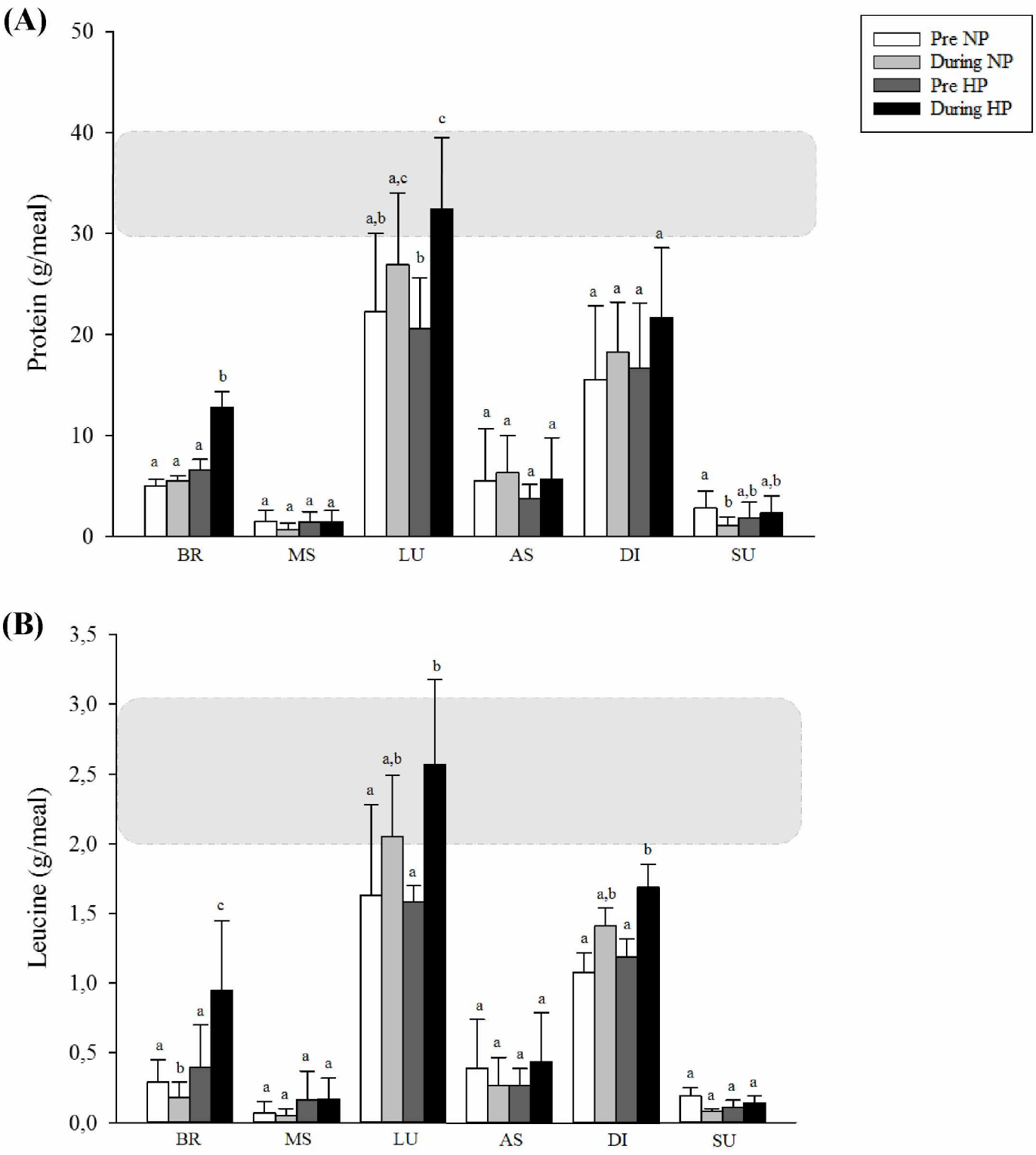

FIGURE 4 Distribution of protein (A) and leucine (B) according to meals.

Notes: BR: breakfast; MS: morning snack; LU: lunch; AS: afternoon snack; DI: dinner; SU: supper. The darker rectangle in the graph represents the ideal amount that volunteers should achieve in consumption. Generalized Estimating Equation (GEE) with sequential Sidak post hoc.

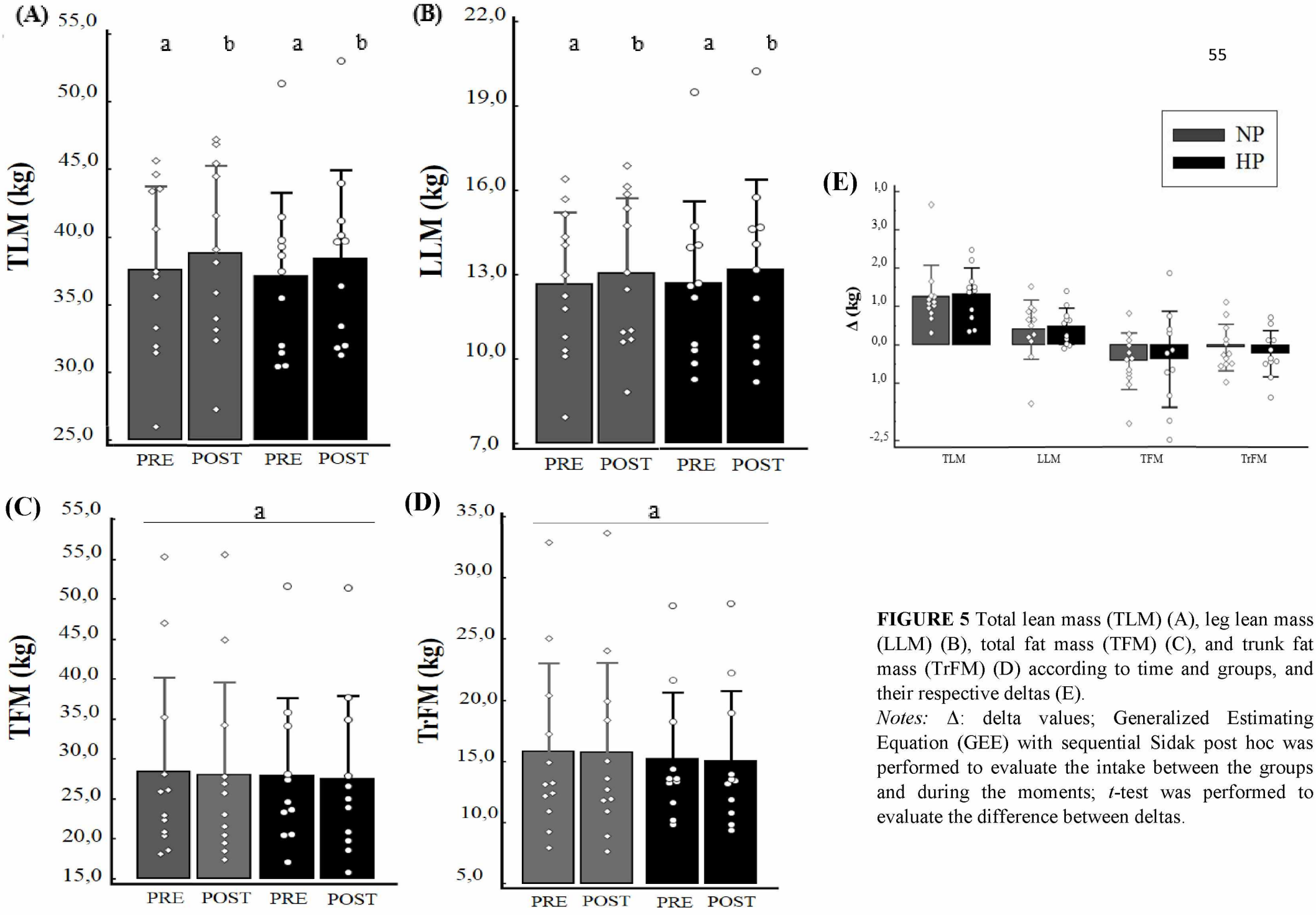

FIGURE 5 Total lean mass (TLM) (A), leg lean mass (LLM) (B), total fat mass (TFM) (C), and trunk fat mass (TrFM) (D) according to time and groups, and their respective deltas (E).

Notes: Δ: delta values; Generalized Estimating Equation (GEE) with sequential Sidak post hoc was performed to evaluate the intake between the groups and during the moments; *t*-test was performed to evaluate the difference between deltas.