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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Efeito da suplementação de proteína em diferentes momentos na massa magra, adiposidade e força de mulheres na pós-menopausa praticantes de exercício contra resistência

Uberlândia

2018

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Efeito da suplementação de proteína em diferentes momentos na massa magra, adiposidade e força de mulheres na pós-menopausa praticantes de exercício contra resistência

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.

Orientador: Erick Prado de Oliveira

Co-orientador: Fábio Lera Orsatti

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Ata da defesa de DISSERTAÇÃO DE MESTRADO junto ao Programa de Pós-Graduação em Ciências da Saúde da Faculdade de Medicina da Universidade Federal de Uberlândia.
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Discente: **Flávia Moure Simões de Branco** – Matrícula nº **11612CSD018** - Título do Trabalho: **"Efeito da suplementação de proteína em diferentes momentos na massa magra, adiposidade e força de mulheres na pós-menopausa praticantes de exercício contra resistência."**Às 14:00 horas do dia 07 de fevereiro do ano de 2018, na sala de vídeo conferência do HC/UFU - Campus Umuarama da Universidade Federal de Uberlândia reuniu-se a Banca Examinadora, designada pelo Colegiado do Programa de Pós-Graduação em Ciências da Saúde, assim composta: Professores Doutores: Bruno Gualano (USP) por vídeo conferência, Marina Rodrigues Barbosa (UFU) e Erick Prado de Oliveira (UFU) – orientador da discente presentes no recinto. Iniciando os trabalhos, o presidente da mesa Prof. Dr. Erick Prado de Oliveira apresentou a Comissão Examinadora e a discente, agradeceu a presença do público e concedeu ao discente a palavra para a exposição do seu trabalho. A seguir o senhor presidente concedeu a palavra aos examinadores que passaram a argüir a candidata. Ultimada a argüição, que se desenvolveu dentro dos termos regimentais, em sessão secreta, em face do resultado obtido, a Banca Examinadora considerou a candidata (X) aprovada ( ) reprovada. Esta defesa de Dissertação de Mestrado Acadêmico é parte dos requisitos necessários à obtenção do grau de Mestre. O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, legislação e regulamentação internas da UFU, em especial do artigo 55 da resolução 12/2008 do Conselho de Pós-Graduação e Pesquisa da Universidade Federal de Uberlândia. Nada mais havendo a tratar foram encerrados os trabalhos às 17:00 horas. Foi lavrada a presente ata que após lida e achada conforme foi assinada pela Banca Examinadora.

Prof. Dr. Erick Prado de Oliveira ______________________________
Prof. Dr. Bruno Gualano - Por vídeo conferência
Profa. Dra. Marina Rodrigues Barbosa ______________________________

DEDICATÓRIA

Aos meus pais e principalmente aos meus avós
por sempre investirem e apoiarem minha
formação profissional.

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EPÍGRAFE

"A persistência é o caminho do êxito"

Charles Chaplin

RESUMO

Objetivo: Avaliar o efeito da ingestão de proteína imediatamente após o exercício nos ganhos de massa magra, força e capacidade funcional em mulheres na pós-menopausa. **Metodologia:** Trinta e quatro mulheres na pós-menopausa (60,9 ± 6,7 anos) participaram desse estudo clínico randomizado, duplo-cego de grupos paralelos. As participantes realizaram o mesmo protocolo de exercício de força, no período da manhã, que foi realizado com a carga de 70% de 1-repetição máxima (1-RM), 3 vezes por semana, durante 8 semanas. As voluntárias foram randomizadas em dois grupos: grupo proteína-carboidrato (PC) (n=17), que ingeriu 30 g de *whey protein* imediatamente após o exercício e 30g de maltodextrina a tarde; e grupo carboidrato-proteína (CP) (n=17), que ingeriu 30 g de maltodextrina imediatamente após o exercício e 30 g de *whey protein* a tarde. A massa magra foi avaliada por meio do DEXA, a força de preensão manual por dinamômetro e a força muscular por 1-RM de supino e extensora. A capacidade funcional foi avaliada pelo teste de caminhada de 1-milha. **Resultados:** Os grupos PC (37,3 [35,0-39,7] para 38,1 [35,9-40,5] kg) e CP (38,2 [36,0-40,5] para 38,8 [36,5-41,3] kg) aumentaram a massa magra total após a intervenção ($p < 0,001$). Também foi observado, em ambos os grupos, aumento na força de 1-RM de supino e extensora; e na força de preensão manual ($p < 0,001$). Além disso, o tempo de caminhada de 1-milha reduziu nos dois grupos ($p = 0,019$). Nenhuma diferença foi encontrada para a interação do grupo x tempo para todas as variáveis ($p > 0,05$). **Conclusão:** A ingestão de proteína imediatamente após o exercício de força não promoveu ganhos adicionais de massa magra, força e capacidade funcional em mulheres na pós-menopausa. Esse estudo clínico foi registrado no clinicaltrials.gov NCT03372876.

Palavras-chave: timing, janela de oportunidade anabólica, ganho de massa muscular.

ABSTRACT

Objective: To evaluate the effect of protein intake immediately after resistance exercise on lean mass, strength, and functional capacity gains in postmenopausal women.

Methods: Thirty-four postmenopausal women (60.9 ± 6.7 years) participated in this double-blind, parallel-group, randomized clinical trial. All individuals performed the same resistance training protocol in the morning, 3 times a week, at 70% of 1-maximum repetition (1-RM) during 8 weeks. Participants were randomly assigned to protein-carbohydrate group (PC) (n=17), that ingested 30 g of whey protein immediately after exercise and 30 g of maltodextrin in the afternoon; and to carbohydrate-protein group (CP) (n=17), that ingested 30 g of maltodextrin immediately after exercise and 30 g of whey protein in the afternoon. Lean mass was assessed using dual-energy X-ray absorptiometry, handgrip strength by a dynamometer, and strength was evaluated by 1-RM of bench press and leg extension. One mile walk test was performed to assess the functional capacity.

Results: Both the PC group (37.3 [35.0-39.7] to 38.1 [35.9-40.5] kg) and the CP group (38.2 [36.0-40.5] to 38.8 [36.5-41.3] kg) increased the total lean mass after the intervention ($p < 0.001$). An increase was also found in both groups for 1-RM bench press, 1-RM leg extension and handgrip strength ($p < 0.001$). In addition, the time of 1-mile walk test decreased in both groups ($p = 0.019$). No differences were found for group and time interaction for all variables ($p > 0.05$).

Conclusion: The protein intake immediately after resistance exercise does not promote additional increase in lean mass, strength, and functional capacity in post-menopausal women. This trial was registered at ClinicalTrials.gov as NCT03372876.

Key-words: timing, anabolic window, muscle mass gain.

SUMARIO

1.INTRODUÇÃO

O envelhecimento está associado à mudanças na composição corporal, como o aumento da gordura corporal e a redução da massa muscular, além da redução da força e da capacidade funcional [1, 2]. Neste contexto estão as mulheres na pós-menopausa, que devido à redução de estrógeno, têm maior aumento da perda de massa muscular [3]. Portanto, estratégias que ajudem a reduzir ou reverter os prejuízos do envelhecimento e da pós-menopausa são necessárias, como o consumo de proteína e o exercício de força.

Em relação ao consumo de proteína, estudos agudos mostram que a síntese proteica muscular aumenta com a ingestão de proteína após o exercício de força. Esse período é curto (45 minutos a 1 hora) e é conhecido como janela de oportunidade anabólica. Apesar da existência dessa evidência, o ganho de massa muscular com essa estratégia não está totalmente elucidado em estudos de longa duração. Além disso, há poucos estudos com pessoas mais velhas e não há nenhum estudo com mulheres na pós-menopausa.

2. REVISÃO DE LITERATURA

2.1 *Envelhecimento e massa muscular*

As melhoras das condições de vida e do tratamento de doenças favoreceram o aumento da expectativa de vida e consequentemente o número de idosos na população [4]. Entretanto, o processo de envelhecimento está diretamente associado com a perda progressiva e generalizada de massa muscular esquelética e com redução da função muscular (força e desempenho), o que resulta em um processo conhecido como sarcopenia [1, 2]. Em estágio mais avançado, há perda significativa da capacidade funcional [5]. A função muscular e a sarcopenia estão positivamente correlacionadas com quedas graves [6-8] e dificuldades em realizar as atividades do cotidiano [9].

A perda de massa muscular esquelética relacionada com o progresso da idade é facilitada por uma combinação de diversos fatores [10], dentre eles, o estilo de vida sedentário [11] e consumo proteico inferior àquele considerado ideal [12]. A perda de massa muscular e função acometem de maneira similar homens e mulheres [13]. No entanto, mulheres em período de pós-menopausa merecem atenção especial, pois sofrem com as alterações tanto do envelhecimento quanto do período da menopausa.

2.2 *Pós-menopausa*

É definido pós-menopausa o período após 12 meses da última menstruação, em que há cessação permanente da menstruação, perda da atividade folicular ovariana e, consequentemente, o fim da capacidade de reprodução feminina [14]. Nesta fase, ocorrem várias alterações hormonais em decorrência da perda da atividade folicular ovariana, como a redução da produção de estrógeno e aumento da produção do hormônio folículo estimulante (FSH) e hormônio luteinizante (LH) [14]. O estrógeno é um hormônio sexual feminino e sua redução pode trazer prejuízos para a saúde da mulher, como redução da densidade mineral óssea [15], maior perda de músculo concomitantemente com maior acúmulo de gordura corporal [16, 17] (**Figura 1**). Todas essas alterações advindas da menopausa podem diminuir a qualidade de vida e aumentar o risco do desenvolvimento de doenças cardiovasculares e sarcopenia na mulher na pós-menopausa [15].

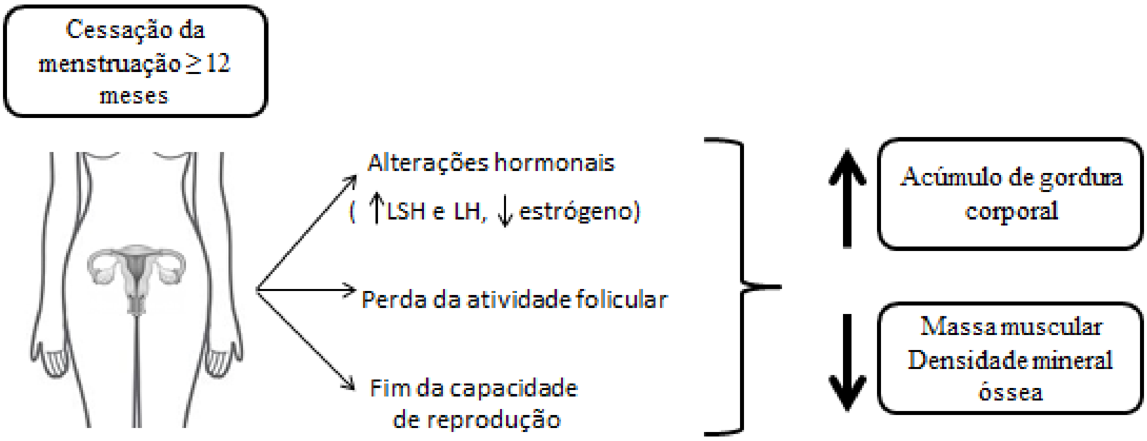

Figura 1. Alterações fisiológicas na mulher na pós-menopausa e suas consequências na composição corporal.

2.3 *Exercício de força*

Intervenções efetivas são necessárias para a prevenção ou tratamento da perda de massa muscular esquelética e força [18]. O treinamento físico de força é conhecido por aumentar a massa muscular em adultos [19, 20] e em idosos [18], sendo, portanto, uma estratégia eficaz no tratamento da perda de músculo esquelético e de força [21-

23], inclusive em mulheres na pós-menopausa [24]. Um estudo recente do nosso grupo de pesquisa, com mulheres na pós-menopausa, mostrou que o exercício de força foi efetivo para aumentar a massa magra total (~1,3 kg) e das pernas após 10 semanas de intervenção. Além disso, as voluntárias aumentaram a força muscular de 1 repetição máxima (1-RM) nos exercícios de supino (~2,5 kg) e cadeira extensora (~7 kg) [25]. Dessa forma, é possível afirmar que o exercício de força é uma estratégia efetiva para atenuar os efeitos do envelhecimento e da menopausa.

2.4 *Proteína*

Segundo Breen e Phillips [26], idosos que procuram tratamento não-farmacológico no auxílio da manutenção ou ganho de massa muscular, devem associar a prática de exercícios de força com a ingestão de proteínas. No músculo esquelético, o incremento ou perda de proteínas é determinado pelo balanço proteico, que é a diferença entre síntese e a degradação de proteína no músculo [27]. Notavelmente, evidências recentes sugerem que indivíduos com idade mais avançada apresentam síntese proteica muscular atenuada no período pós-prandial (período em que ocorre a hiperaminoacidemia), particularmente ou relativamente à baixa ingestão de proteínas [10, 28]. Assim sendo, os idosos apresentam “resistência” ao anabolismo quando comparados com indivíduos jovens [29-31], o que pode explicar, em parte, o declínio na massa muscular relacionado com o avanço na idade [31].

Um estudo conduzido por Moore e colaboradores [31], demonstrou que indivíduos idosos necessitam ingerir uma maior quantidade de proteínas para estimular a síntese proteica de forma satisfatória quando comparados com indivíduos jovens. Além disso, diversos estudos oferecem suporte à premissa de que a recomendação de ingestão proteica para idosos deveria ser superior aos 0,8 gramas/kg/dia propostos pela *Dietary Reference Intakes* em 2005 [26, 32-36]. Os resultados obtidos no estudo de Moore e colaboradores (2014) [31] demonstraram que as taxas máximas de síntese proteica foram similares em jovens e idosos (~0,058% e ~0,056%/h, respectivamente), o que sugere que idosos saudáveis mantém a capacidade de síntese proteica, porém, somente com estímulo nutricional ideal [37-39]. Segundo Moore *et al.* [31], a recomendação de ingestão proteica para jovens é de 0,25-0,3 gramas de proteína/kg de peso/refeição enquanto que para idosos, a recomendação é de 0,4 gramas de proteína/kg de peso/refeição.

Entretanto, apesar de existir esse possível favorecimento para o ganho de massa muscular com doses consideradas ótimas, os dados ainda são inconclusivos quanto a essa conduta. Em um estudo do nosso grupo de pesquisa, com mulheres na pós-menopausa, foi testado o consumo de proteína, durante um protocolo de exercício de força, recomendado pela Recommended Dietary Allowance (RDA) que é de 0,8 e o consumo considerado ideal para o ganho de massa muscular que é de ~1,2 g/kg. Após 10 semanas de intervenção, foi observado aumento da massa magra de ~1,3 kg em ambos os grupos [25].

Para potencializar a síntese muscular promovida pelo exercício, é recomendado que seja ingerida uma quantidade suficiente de proteína rica no em leucina, já que é o principal aminoácido estimulador da mTor na via de síntese proteica muscular [40]. Além disso, é recomendado o consumo de proteína de rápida digestão, como por exemplo, a proteína no soro do leite (*whey protein*), carne de frango ou soja [26]. Desta forma, a dieta prescrita ao indivíduo deve ser equilibrada, com macro e micronutrientes adequadas à necessidade do mesmo. Além disso, diversos pesquisadores recomendam que a proteína seja igualmente distribuída ao longo do dia, de forma que todas as refeições contenham quantidades deste macronutriente suficientes para estimular de maneira satisfatória o anabolismo proteico [41-43].

O estímulo de síntese proteica ocorre por meio da ingestão total de proteínas, além da dose de proteína [44], fonte [20, 45], tempo de ingestão relativo ao exercício (*timing*) [20, 46-48] e a sensibilidade do músculo esquelético à subsequente hiperaminoacidemia [49], os quais têm se mostrado importantes na regularização da resposta de síntese muscular [42].

2.5 *Timing proteico*

Outro fator que parece ser importante para o ganho de massa magra é o momento em que a proteína é consumida. Estudos agudos tem mostrado aumento na síntese proteica muscular quando a proteína é ingerida logo após o treino [50, 51]. Esse fenômeno pode ser explicado pela teoria da "janela de oportunidade anabólica" [52], que é um período curto, com duração de 45 minutos a 1 hora, próximo ao exercício de força, em que a síntese proteica está aumentada [53]. Apesar de haver evidências sobre a existência da janela de oportunidade anabólica, não está totalmente elucidado na literatura sobre sua existência, pois os resultados ainda são muito controversos.

2.6 *Estudos com timing que igualam o consumo de proteína*

Uma das grandes limitações dos estudos com timing é não igualar a quantidade de proteína total entre os grupos. Quando avaliados na literatura somente esses estudos, apenas quatro igualaram o consumo de proteína total e encontram perda[54] ou manutenção[55, 56] da massa muscular. Portanto, seria improvável que o timing teria influência adicional no ganho de massa muscular, já que o exercício de força não foi efetivo. Já está bem estabelecido que o exercício de força é a principal intervenção para estimular os ganhos de massa magra e força [25, 57] e a suplementação sem o exercício não promove esses benefícios[58]. Apenas em um estudo que igualou o consumo de proteína total houve aumento da massa muscular com a intervenção [47]. Foi um estudo randomizado controlado realizado por Cribb e Hayes (2006) com homens *bodybuilders* recreativos, com duração de 10 semanas. Foi suplementado uma mistura de *whey protein,* carboidrato e creatina para um grupo antes e após o treino de força e para outro grupo em horários distantes ao exercício (manhã e noite) e houve maior aumento de massa magra e força nas pessoas que ingeriram proteína próxima ao treino. Porém, foi observado por meio de biópsia intramuscular, que o grupo que consumiu o suplemento próximo ao treino tinha maior quantidade de creatina incorporada no músculo. Desta forma, é difícil atribuir esses resultados positivos do timing de proteína, pois parece que esses efeitos estão mais relacionados com a creatina.

2.7 *Estudos com timing que não igualam o consumo de proteína*

A grande maioria dos estudos com timing não iguala a ingestão de proteína total entre os grupos timing [19, 45, 59-64]. Dessa forma, os grupos que consomem a proteína logo após ou próximo ao exercício de força, ingere quantidades superiores de proteína ao longo do dia. Na literatura, alguns desses estudos encontraram benefícios na massa muscular com o consumo de proteína próximo ao treino [59-63]. Um desses estudos foi realizado com 56 homens jovens destreinados que realizaram exercício de força 5 vezes por semana durante 12 semanas. Foi suplementado 17,5 g de proteína do leite ou soja ou suplemento de carboidrato imediatamente após o exercício e foi encontrado maior aumento da massa livre de gordura no grupo que consumiu a proteína do leite após o exercício. Entretanto, como a intervenção não foi igual entre os grupos, é difícil atribuir esse resultado positivo ao momento em que a proteína foi consumida.

2.8 *Meta-análise timing proteico pós-exercício*

Em uma meta-análise, Schoenfeld e colaboradores [65] avaliaram o efeito do consumo de proteína após o exercício no ganho de massa magra. Os autores concluíram que o consumo proteico após o exercício de força potencializou o ganho de massa magra, entretanto, como a maioria dos estudos incluídos na meta-análise comparou o consumo de proteína após o exercício com um grupo placebo que ingeria carboidrato, o grupo que ingeriu proteína após o exercício também aumentou o consumo total de proteína, o que não permite concluir se o que induziu ao maior ganho de massa magra foi o momento (após exercício) ou o maior consumo proteico total do dia. Sabendo desta limitação, os autores realizaram uma segunda análise, ajustada pelo consumo proteico total, e notou-se que não houve efeito significativo do consumo de proteína após o exercício para potencializar a hipertrofia muscular. Portanto, foi concluído que o consumo de proteína total parece ser mais importante do que o momento para promover a hipertrofia muscular. Entretanto, é importante ressaltar que somente será possível concluir a importância do consumo de proteína após o exercício na hipertrofia muscular quando novos estudos forem realizados comparando grupos que consumam a mesma quantidade de proteína total durante o dia.

2.9 *Populações avaliadas*

Como citado nos tópicos acima, as pessoas mais velhas e principalmente mulheres na pós-menopausa, apresentam mudanças na composição corporal que trazem prejuízos à saúde. Dessa forma, é importante o desenvolvimento de estratégias para atenuar ou reverter essas alterações. Uma dessas estratégias seria o timing proteico. Entretanto, a maioria dos estudos sobre o tema é realizado com adultos jovens [19, 45, 47, 55, 56, 59-64, 66, 67]. Poucos estudos testam o consumo de proteína pós-exercício em idosos [44, 54, 68-70] e mais especificamente em mulheres mais velhas. Um estudo de Wycherley et al. [54] avaliou o efeito da ingestão de proteína antes e após o treinamento de força durante 16 semanas em homens e mulheres mais velhos (57 ± 7 anos), portadores de diabetes tipo 2. Após o período de intervenção, os voluntários reduziram massa livre de gordura, mesmo realizando exercício de força. No nosso conhecimento esse foi o único estudo a avaliar o timing de proteína em mulheres mais velhas. Porém, o estudo era composto por uma amostra mista e não especificamente em mulheres na pós-menopausa.

Apesar de haver muitos dados sobre o timing de proteínas, os estudos ainda apresentam limitações. Dessa forma, a realização do presente estudo justifica-se pela falta de estudos na literatura que sejam mais bem controlados metodologicamente e com pessoas mais velhas, principalmente mulheres na pós-menopausa.

3.OBJETIVOS

3.1 *Objetivo geral*

Avaliar o efeito da suplementação de proteína após o exercício de força na massa magra, adiposidade, força e capacidade funcional em mulheres no período da pós-menopausa praticantes de exercício de força.

3.2 *Objetivos específicos*

- Realizar avaliação antropométrica e da composição corporal;
- Avaliar o fracionamento da dieta e consumo de proteína por refeição;
- Avaliar o estado de saúde geral;
- Avaliar a força e função muscular.

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Protein intake immediately after resistance exercise does not promote additional increase on lean mass, strength and functional capacity in postmenopausal women: a randomized clinical trial

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Abstract

Background and aims: Acute studies have shown that the protein intake immediately post exercise increases the muscle protein synthesis. However, the effect of the protein intake immediately after resistance exercise on lean mass and strength gains in long term studies is still not totally elucidated. In addition, there is no study evaluating it in postmenopausal women. The aim of this study was to evaluate the effect of protein intake immediately after resistance exercise on lean mass, strength, and functional capacity gains in postmenopausal women.

Methods: Thirty-four postmenopausal women (60.9 ± 6.7 years) participated in this double-blind, parallel-group, randomized clinical trial. All individuals performed the same resistance training protocol in the morning, 3 times a week, at 70% of 1-maximum repetition (1-RM) during 8 weeks. Participants were randomly assigned to protein-carbohydrate group (PC) (n=17), that ingested 30 g of whey protein immediately after exercise and 30 g of maltodextrin in the afternoon; and to carbohydrate-protein group (CP) (n=17), that ingested 30 g of maltodextrin immediately after exercise and 30 g of whey protein in the afternoon. Lean mass was assessed using dual-energy X-ray absorptiometry, handgrip strength by a dynamometer, and strength was evaluated by 1-RM of bench press and leg extension. One mile walk test was performed to assess the functional capacity.

Results: Both the PC group (37.3 [35.0-39.7] to 38.1 [35.9-40.5] kg) and the CP group (38.2 [36.0-40.5] to 38.8 [36.5-41.3] kg) increased the total lean mass after the intervention ($p < 0.001$). An increase was also found in both groups for 1-RM bench press, 1-RM leg extension and handgrip strength ($p < 0.001$). In addition, the time of 1-mile walk test decreased in both groups ($p = 0.019$). No differences were found for group and time interaction for all variables ($p > 0.05$).

Conclusion: The protein intake immediately after resistance exercise does not promote additional increase in lean mass, strength, and functional capacity in post-menopausal women. This trial was registered at ClinicalTrials.gov as NCT03372876.

Key-words: timing, anabolic window, muscle mass gain

Introduction

Aging is associated with changes in body composition, such as increased body fat and reduced muscle mass, besides reduction in strength and functional capacity [1, 2]. In this context, postmenopausal women, due to estrogen reduction and aging, have an accelerated increase in muscle mass loss [3]. Considering that the loss of muscle mass and strength are independent factors for mortality in older individuals [4, 5], strategies to prevent it are important. In this way, resistance training and protein intake are non-pharmacological strategies indicate to reverse or attenuate these effects of aging [6, 7]. In relation to protein intake, there are some details that seem to be important for lean mass and strength gains/maintenance, such as the total daily amount [8, 9], quality [10, 11], distribution during the day [12], dose pear meal [13], and timing [14-21].

In respect to protein timing, acute studies have shown an increase in muscle protein synthesis when protein is ingested after resistance exercise [22, 23]. This phenomenon, known as "anabolic window of opportunity" [24], is a short period that lasts from 45 minutes to one hour after the exercise [25]. However, the effect of protein timing on lean mass and strength gains in long term studies is still not totally elucidated [26]. This effect was evaluated in a meta-analysis performed by Schoenfeld et al [27] and it was observed that ingesting protein close to exercise induced to a higher lean mass gain, when compared to the control group. Nevertheless, as the majority of the studies included in the meta-analysis did not match the total protein intake between the groups. The intervention group ingested more protein during the day (~1.7 g/kg) when compared to control group (~1.3 g/kg); therefore, it is not possible to conclude whether the higher lean mass gain occurred due to timing or to higher total protein intake. In the same study [27], the authors performed a sub analysis adjusting for total protein intake and the effect of protein timing on lean mass gain was lost, showing that possibly the higher total protein intake leads to higher lean mass gain, but not the protein timing. However, these results are based on statistical adjustments and randomized controlled clinical trials equalizing the total protein intake during the day are necessary to confirm this data.

In addition, only few studies evaluated the effect of protein timing on lean mass gain in older adults [28-32] and, to the best of our knowledge, there is no study evaluating it in postmenopausal women, which is an important knowledge gap. Thus, the aim of our study was to evaluate the effect of protein timing on lean mass, strength and functional capacity in postmenopausal women following a resistance training protocol. We hypothesized that the timing of protein intake would not induce to additional gains in lean mass, strength and functional capacity when the total protein intake is adequate.

Methods

Research participants

This study was conducted at Federal University of Uberlandia and at Federal University of Triangulo Mineiro, Minas Gerais, Brazil. The postmenopausal women were invited to join the study through television or in local disclosure. Inclusion criteria were: postmenopausal women (at least 1 year of cessation of menstruation, self-reported); at least 6 months without performing resistance exercise and to be disposed to ingest the supplements twice a day. Those volunteers that were diabetics, who were not available to train in the morning and/or were in hormonal replacement were excluded. Dyslipidemia, hypertension and endocrine (hyper or hypothyroidism) were not considered as exclusion criteria. This trial was approved by Federal University of Uberlandia Research Ethics Committees (protocol number 2.023.127) and registered at ClinicalTrials.gov as NCT03372876. All the volunteers were previously informed about the experimental procedures and the possible risks of the study before written consent was provided.

After the disclosure of the study, 141 women showed interest to participate, but only 49 were eligible due to inclusion and exclusion criteria. During the adaptation period, 15 participants dropped out of the study and, after this period, 34 women were randomized (MedCalc® software, version 11.1, Mariakerk, Belgium) into 2 groups. Protein-carbohydrate (PC), who ingested 30 g of whey protein immediately after exercise and 30 g of maltodextrin in the afternoon; and carbohydrate-protein group (CP), who ingested 30 g of maltodextrin immediately after exercise and 30 g of whey protein in the afternoon). The total lean mass values at baseline (after the adaptation period) were used for the randomization of the groups and it was performed by an independent researcher. A flowchart of the progress of this clinical trial is showed in **Figure 1**. An a priori sample size for f test was calculated using G*Power (v. 3.0.10, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany). and the total sample size found was 24 individuals. On the basis of a statistical power (1–*β*- err prob) of 0.80, an effect size F = 0.25, and an overall level of significance of 0.05, 24 individuals were required for this study

Study design

This was a randomized double-blind, parallel-group, clinical trial with 8 weeks of follow-up. Body composition (dual-energy X-ray absorptiometry (DXA)), dietary habits, anthropometric parameters, strength, and functional tests were performed before adaptation period. Due to be previously untrained individuals, 1 week of familiarization and 3 weeks of adaptation were performed to ensure that the exercise protocol during the supplementation would be done correctly and until muscle failure. Before the beginning of the supplementation, body composition, anthropometric parameters, strength and functional tests were repeated to confirm whether changes in these parameters in the adaptation period were observed. After all baseline tests, 8 weeks of protein and carbohydrate supplementation plus resistance exercise were performed. To ensure the correct evaluation of dietary habits, eight 24-hours food recalls (1, 3, 6, 8 weeks) were performed during the supplementation period. At the end of the study, body composition, anthropometric parameters, strength and functional tests were replicated **(Figure 2)**.

Assessments

Anthropometry

Lohman et al. [33] protocol was used to access body mass and height. The body mass was measured in the morning with the volunteers wearing light clothes and without shoes. After these measures, Body Mass Index (BMI) was calculated (body mass/height^2).

Body composition

The volunteers arrived at the laboratory after an overnight fasting and were instructed to wear light clothing, to be free of metal objects and to ingest 2 liters of water in the previous day for standardization of muscle hydration. Total lean mass, leg lean mass, appendicular lean mass and fat mass were accessed 3 times during the study (before adaptation period, at baseline and in the end of intervention) using DXA (Lunar iDXA®, GE Healthcare, Madison, WI, USA) and quantified by software Encore version 14.10 (GE Medical Systems, Madison, WI, USA). The same researcher performed all the exams to avoid measurements error.

Strength measurement

To evaluate upper and lower body muscle strength, 1-RM test was performed in bench press and leg extension exercises. After learning the exercise techniques in 3 familiarization sessions, 1-RM was tested and retested after 48h to avoid the underestimation of the load due to the lack of knowledge of the test by the participant. Initially, volunteers performed a walking warm-up during 5 min and then underwent to a specific warm-up that consisted of 15 repetitions with 30% of 1-RM. The load was adjusted and participants were instructed to perform 10-12 repetitions with 50% of 1-RM after 1 min of rest. After another 1 min of rest, the last warm-up session was performed and consisted in 3-5 repetitions with 80% of 1-RM. When the warm-up was completely, load was increased and the subjects were encouraged to try to perform 1-RM for the first time. If the participant was not able to make a complete movement or made more than one repetition, 1-RM was tested again after 3 min of rest. A maximum of 3 attempts was made for determination of all 1-RM tests.

Isometric handgrip strength was performed on the right hand using a dynamometer (Jamar®). During the assessment, volunteers were standing and were instructed to tighten the device after a voice command. The measurement was performed three times and the highest value of the three measurements was used.

Functional measurement

To access functional capacity, 1-mile walk test was realized on a multi-sport court. The test was performed at baseline (retested after 48h) and in the end of the study. The result of 1-mile walk test was expressed in minutes.

Dietary Assessment

The dietary intake was assessed by 24-h food recall following the 5-step multiple pass [34]. Three dietary recalls were performed (2 weekdays and 1 weekend day) to evaluate the dietary habits before the familiarization (weeks -5 and -4). To correctly assess dietary habits during the supplement intervention, 8 food recalls were performed (weeks 1, 3, 6 and 8) referred to 4 training days, 2 non-training days, and 2 weekend days. The first and the two weekend records were performed in person and the others via phone call after proper familiarization. The evaluated nutrients were carbohydrate, protein, lipid and leucine. Dietpro® software (version 5.7i, Agromídia Softwares®, Minas Gerais, Brazil) was used to perform the analyses of food data and the United States Department of Agriculture (USDA) [35, 36] food composition table was used.

The distribution of protein and leucine intake throughout the day was evaluated on the training days. The meals were divided into: breakfast, morning snack, lunch, afternoon snack, dinner and supper according to the time and which meal the volunteer related.

Experimental Protocol

Resistance training

The resistance exercise was conducted in the Public Health and Physical Activity Centre at the Federal University of Triangulo Mineiro and was performed 3 times a week on non-consecutive days and always in the morning (7 and 8 a.m.) due to timing supplementation protocol. The exercise protocol followed the recommendations of American College of Sports Medicine [37] for muscle hypertrophy. Before the training session, participants realized 5 min of walking warm-up. The resistance exercise consisted in the upper and lower limbs exercises that included bench press (free weight), peck deck (machine), lat pull down (machine), seated low row (machine), leg extension (machine), leg curl (machine), leg press (machine), free weight calf and abdominal. As most women had never performed resistance exercise, before onset the supplement intervention, 3 weeks of progressive adaptation of 1 to 3 sets of 70% of 1-RM (8-12 repetitions) was performed. At the beginning of the intervention (baseline) the resistance exercise remained with 3 sets of 8-12 repetitions (70% 1-RM). As volunteers increased their strength, the loads were readjusted weekly to maintain the training zone of 8-12 repetitions of 70% of 1-RM until the failure. The minimum acceptable training adherence was 70%.

Supplementation

The PC group ingested 30 g of whey protein (Hilmar™ 9010 Instantized Whey Protein Isolate) immediately after exercise (8-9 a.m.) and 30 g of carbohydrate (Maltodextrin Cargill®) at ~3 p.m. The CP group ingested 30 g of carbohydrate immediately after exercise (8-9 a.m.) and 30 g of whey protein at ~3 p.m. The supplements were ingested every day during 8 weeks at the same time of the day (8-9 a.m. and 3 p.m.). The participants received the supplements in an opaque packing from a blind researcher after each training session and the supplements were intake in an opaque bottle drink to guarantee the study blindness. Furthermore, participants received the amount of supplements necessary to 1-wk to ingest in the afternoon period (training days), in the morning and in the afternoon in non-training days. All individuals were instructed to return the empty packing to control the adherence to the supplementation. All the supplements were distributed in separated package containing exactly 30 g of whey or maltodextrin. Both supplements (whey and maltodextrin) were isocaloric and strawberry flavored. The information about the supplements composition is showed in **Supplemental Table 1**.

On training days, the subjects were instructed to refrain the intake of protein food sources during the morning, but they could ingest carbohydrate and fat food sources, keeping their habitual breakfast intake. The lunch was eaten no less than 2 h (~3 h) after cessation of each session (**Figure 3**).

Statistics

All analyzes were performed using SPSS software (version 20.0, IBM Corp, New York, NY, USA). Baseline characteristics and the distribution of protein and leucine during the day were analyzed using Generalized Estimating Equations (GEE) using unstructured correlation and maximum likehood estimation. Medication intake and family history diseases were included as random effects. Moreover, changes in body composition, strength and dietary intake were analyzed by an intention-to-treat analysis using GEE and Sequencial Sidak post-hoc adjusted for the adherence of protein supplementation. Significance was accepted when *P-value* was <0.05. All values were described as means and Wald 95% confidence interval.

Results

Baseline Characteristics

At baseline, no differences were observed between the groups for age, body mass, BMI, lean mass (total, appendicular, leg and trunk), fat mass, strength (bench press, leg extension and handgrip), 1-mile walk test; and calories, protein and carbohydrate intake and supplement adherence (**Table 1**). The mean adherence to the supplementation protocol was ~77% (all individuals). In addition, none of the volunteers were presented sarcopenia.

Dietary Intake during intervention

No differences were found in dietary intake over the time in both groups. When protein and carbohydrate supplementation were included in the dietary intake, an increase in calories, carbohydrate in grams (PC 196 [163-236] to 214 [180-255] g; and CP 200 [175-229] to 249 [226-274] g), g/kg and %, protein in g/kg (PC 0.9 [0.7-1.1] to 1.3 [1.0-1.6] g/kg; and CP 1.0 [0.8-1.2] to 1.3 [1.2-1.4] g/kg), g and % and leucine (g) was found; whereas lipids (g) remained the same and lipids (%) decreased in both groups (**Table 2**).

Distribution of Protein and Leucine on training days

For all meals, no difference was observed in habitual dietary intake between the groups on training days. The protein intake by diet before exercise at breakfast was ~3 g in both groups and at morning snack (post training) was ~2 g in both groups. When the supplement was included after exercise, a protein increase was observed in PC group comparing to CP group at morning snack (29.3 [28.6-30.1] g vs. 1.6 [1.0-2.5] g, $p<0.001$; respectively). At lunch, both groups showed similar protein intake (PC = 26.3 [20.3-34.0] g vs. CP = 29.1 [24.2-35.0] g; $p= 0.531$). At afternoon snack, the protein intake exclusively by food was similar between the groups, but CP group increased the protein intake by supplementation in comparison to PC group (33.4 [31.8-35.0] g vs. 3.7 [2.7-5.3] g; $p <0.001$). The protein intake at dinner and supper was similar between the groups.

For leucine, a small and insignificant amount was ingested by food before the exercise at breakfast (~0.2 g) and after exercise at morning snack (~0.1 g). When the supplement was intake after exercise, an increase in leucine intake at morning snack was observed in PC group (PC = 3.0 [2.9-3.1] g vs. CP = 0.11 [0.07-0.17] g; $p<0.001$). At lunch, both groups showed a similar leucine intake (~2 g). At afternoon snack, both groups ingested the same amount of leucine without the supplement and after the addition of the supplement, CP group had a greater intake of leucine (PC = 0.25 [0.17-0.38] g vs. CP = 3.3 [3.1-3.4] g; $p<0.001$). The leucine intake at dinner (~1.3 g) and supper (~0.15 g) was similar between the groups (**Table 3**).

Muscle strength and Functional capacity

Both groups increased 1-RM in bench press (PC = 29.2 [27.1-31.3] to 33.8 [31.4-36.6] kg and CP = 29.3 [26.2-32.7] to 34.8 [31.3-38.8] kg) and leg extension (PC = 30.0 [26.5-33.8] to 33.8 [31.0-37.0] and CP = 30.7 [26.5-35.7] to 33.3 [29.4-37.8] kg) exercises over time ($p<0.001$). The handgrip strength increased (PC = 27.0 [25.6-28.5] to 28.3 [26.5-30.2] kg and CP = 27.4 [24.8-30.2] to 29.3 [26.6-32.1] kg) in both groups through the time ($p<0.001$). For 1-mile walk, a reduction of the time (in minutes) was found at the end of the study ($p = 0.019$). No difference was observed in group x time interaction effect between the groups for all the variables (**Table 4**).

Anthropometric and Body Composition

No differences were found in body mass in both groups ($p=0.069$). Both the PC group (37.3 [35.0-39.7] to 38.1 [35.9-40.5] kg) and the CP group (38.2 [36.0-40.5] to 38.8 [36.5-41.3] kg) increased the TLM after the intervention ($p<0.001$), but no differences between the groups ($p=0.643$) and group x time interaction ($p=0.408$) were found. Both groups increased LLM ($p<0.001$) with no differences between groups ($p=0.510$) and group x time ($p=0.930$). An increase in MMI was also observed for both groups (PC = 6.9 [6.4-7.5] to 7.1 [6.6-7.6] kg/m^2 vs. CP = 6.8 [6.4-7.3] to 7.0 [6.6-7.5] kg/m^2; $p<0.001$) with no differences between groups ($p=0.839$) and for time x group interaction ($p=0.826$). Additionally, TFM did not change in PC and CP groups over the time. (**Table 4**).

Discussion

The main finding of the present study was that the protein intake immediately after exercise did not promote additional effects on lean mass, strength, and functional capacity gains induced by 8 weeks of resistance exercise in postmenopausal women who ingested ~1.3 g/kg/day of protein. To the best of our knowledge, this is the first study showing these results in previously untrained postmenopausal women. Both PC and CP performed the resistance exercise in the morning and it was not allowed to ingest protein before exercise for both groups. In addition, while the PC ingested ~30 g of protein immediately exercise, the CP group only ingested protein after ~3 hours after exercise (at lunch). Thus, these data refute the hypothesis of the existence of an anabolic window of opportunity for lean mass gain after resistance exercise when both groups (placebo vs. intervention) ingest equal and adequate total protein intake.

The effect of protein timing on lean mass gain is not totally elucidated [26] and it has been studied in young adults [14-21, 38-42] and older adults [28-32], but not specifically in post-menopausal women. These studies evaluated the effect of protein timing comparing pre vs. post exercise [14, 18, 19, 31, 38, 40], only post-exercise [29, 42], close to exercise (pre and post) vs. several hours after exercise [17, 39], and post exercise vs. several hours after exercise [11, 41], as in the present study. A meta-analysis performed by Schoenfeld et al [27] evaluated the effect of protein timing on lean mass gain. Initially, it was noted that the intake of protein close to exercise induced higher lean mass gain; however, most of these studies did not match the total protein intake, and the groups that ingested protein after exercise also increased the total protein intake. Therefore, it does not allow to conclude whether the higher lean mass gain occurred due timing or higher total protein intake. In this same meta-analysis, in a second statistical analysis, after adjustments for total protein intake, the effect of timing on lean mass gains was lost, which is possible to suggest that the total protein intake seems to be more important than the timing. However, these results are based on statistical analysis and controlled randomized clinical trials, as the present study, are necessary to confirm this data. Therefore, our results confirm the conclusions of this meta-analysis [27] because when the total protein intake was equalized, no additional effects were found on lean mass gain in PC group.

Evaluating only the studies that equalized the total protein intake [28, 38, 39], no differences were observed between groups for changes in lean mass, whereas a maintenance [38, 39] or loss [28] of lean mass was observed. However, it would be unlikely that the supplementation timing would induce additional lean mass gain in these studies, since the resistance training was not effective to promote lean mass gain. It is known that resistance exercise is the primary intervention to stimulate lean mass and strength gains [43, 44] and protein supplementation without resistance exercise effects does not lead to lean mass gain [45]. In the present study, the resistance exercise induced to significant total lean mass (~0.7 kg), strength and functional capacity gains. Therefore, this is the first study showing the effect of protein timing, equalizing the total protein intake, in which resistance training was effective to induce lean mass gain; and it was found that the protein timing did not induce to higher lean mass gain, even when resistance exercise is effective. Only a study [17], that evaluated young recreational bodybuilders and equalized the total protein intake between the groups, showed benefits in lean mass gain promoted by the timing. It was concluded that the intake of a mixture of whey, creatine, and carbohydrate immediately before and after resistance exercise induced to higher lean mass and strength gains than the group that consumed the same dose of the same supplement in the morning and late evening. However, it was observed by muscle biopsy that the group that intake the supplement close to exercise had a major incorporation of intramuscular creatine [17]. In this way, it is difficult to attribute these positive results of timing supplementation to protein, since it seems that these effects are more related to creatine alone.

The post-menopausal women of the present study ingested 30 g of isolated whey protein (27.6 g of protein) and 30 g of maltodextrin (placebo). We chose whey as protein source since this is a rich leucine-protein that promote higher muscle protein synthesis than other protein sources [46]. In addition, the absence of carbohydrate in the protein supplement has probably no effect on muscle protein anabolic response, because when adequate doses of protein are ingested there are no further augment in protein synthesis with addition of carbohydrate [47]. Furthermore, we chose this protein dose, because we evaluated older women and it is known that for maximum MPS stimulation in older adults it is recommended to consume ~30 g of high quality protein per meal [12] or ~0.4 g/kg/meal [13]. In the present study, it was offered 27.6 g or ~0.43 g/kg/meal of protein, therefore, we offered the protein dose that stimulate the maximum muscle protein synthesis, which allowed us to evaluate the timing using the ideal dose of protein for this population. This protein dose was not the same comparing to other studies that evaluated protein timing in older adults, since 10 g [48], 20 g [31], and 21 g [28] of protein were offered, whereas these studies offered lower doses than our study. In addition, both supplements were blinded with the same flavor (strawberry flavored) and there were no differences between the groups regarding the correct answers and errors about which supplements were being ingested (data not shown), which confirms the correct blinding in the present study.

The volunteers were instructed to abstain the protein intake in the morning on training days during the intervention. This recommendation was important to exclude the effects of protein intake by food sources on the timing supplementation effects. It is important to remember that this recommendation did not seem to influence the habitual protein intake of these individuals since the low protein intake in the morning is already a habit of Brazilian postmenopausal women found in other [44] and in the present study at baseline (data not shown). Despite this orientation, a consumption of small amount of protein in both groups were observed in breakfast (~3 g) and in morning snack (~1.5 g) due to the protein that is contained in breads and fruits, that were frequently ingested in these two meals. However, these amounts of protein seem to be insignificant for the MPS stimulus and it is not a confounding factor to evaluate the timing. The dietary intake in the present study was assessed by eleven 24 h food recall, being 3 at baseline and 8 during the study (4 on training days, 2 on the weekend, and 2 on non-training days). Therefore, our study provided a reliable evaluation of dietary intake during the intervention. The majority of studies does not assess the dietary intake during the intervention, being evaluated only in the pre and post intervention moments [15, 17, 30, 31, 39, 48]. As whey protein intake can increase satiety [49], it was possible that the protein supplementation may had decreased the protein and calorie intakes of the diet during these interventions. However, in our study, both PC and CP groups maintained the habitual intake of calories and protein during the intervention. When the supplements were added in dietary intake, there was an increase in calories, protein, carbohydrate, and leucine intakes in both groups, with no differences between the interventions. In addition, besides both groups had ingested the same total protein (~1.3 g/kg/day), the distribution of protein during the day (by diet) was the same between groups during the intervention which is an important control to evaluate the effect of the protein timing.

We performed 1-week familiarization and 3-weeks of adaptation of the resistance training due to the evaluated women were previously untrained and they should perform each set until muscle failure during the intervention. We noted that both groups increased LBM (~0.7 kg), strength, and functional capacity similarly after the intervention. This demonstrates that 8-weeks of resistance training was efficient to promote benefits. These gains are in accordance with other studies that evaluated the effects of resistance training in postmenopausal women [3, 44, 50, 51]. In addition, no changes in body fat was observed in both groups, which shows that the individuals did not perform a caloric restriction, that could attenuate the lean mass gain [52]. In addition, even with an increase in caloric intake due to protein and carbohydrate supplementation, there was no fat mass gain, that also seems to be important for optimal lean mass gain in postmenopausal women [53].

The present study has limitations, such as the short follow-up time (8 weeks), the small sample and the absence of a control group without protein supplementation. As strength, the detailed control of the habitual diet allow us to conclude the correct effect of the protein timing on lean mass and strength gains. We controlled the total protein intake, as well as the protein distribution and dose pear meal in both groups.

In conclusion, the protein intake immediately after resistance exercise did not promote additional increase in lean mass, strength, and functional capacity in post-menopausal women. These results refute the existence of an anabolic window of opportunity that can favor the increase in lean mass and muscle function in previously untrained postmenopausal women.

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Authorship

FMSB participated in collection and interpretation of the data, performed statistical analysis, and wrote the manuscript; MASC, LTR, P.C.N and GNO participated in collection, analysis, and interpretation of the data. KRT performed statistical analysis, collaborated in the collection and interpretation of the data; F. L.O. carried out the conception and design of the study and revised 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.

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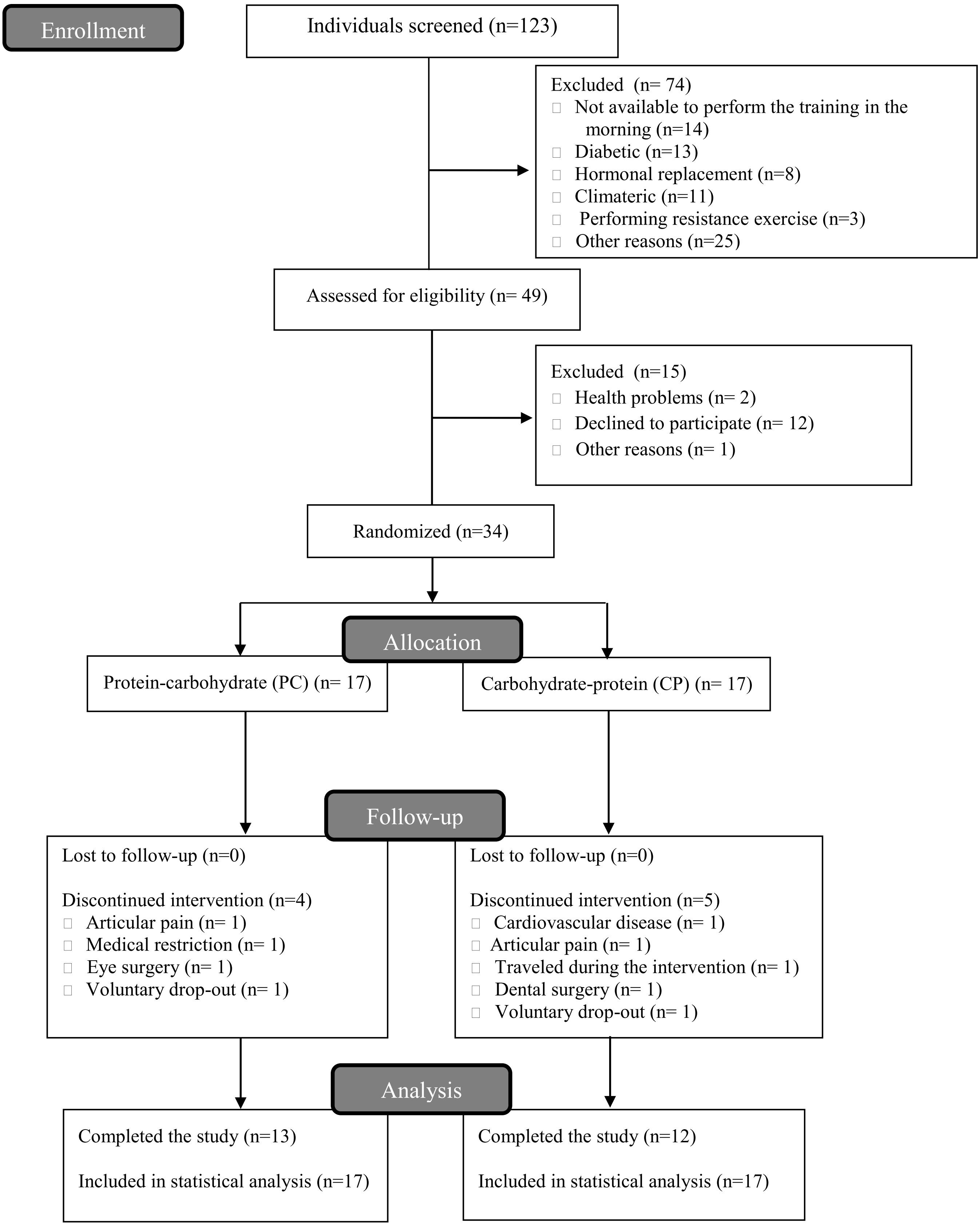

Figure 1. Flowchart of the progress of the trial.

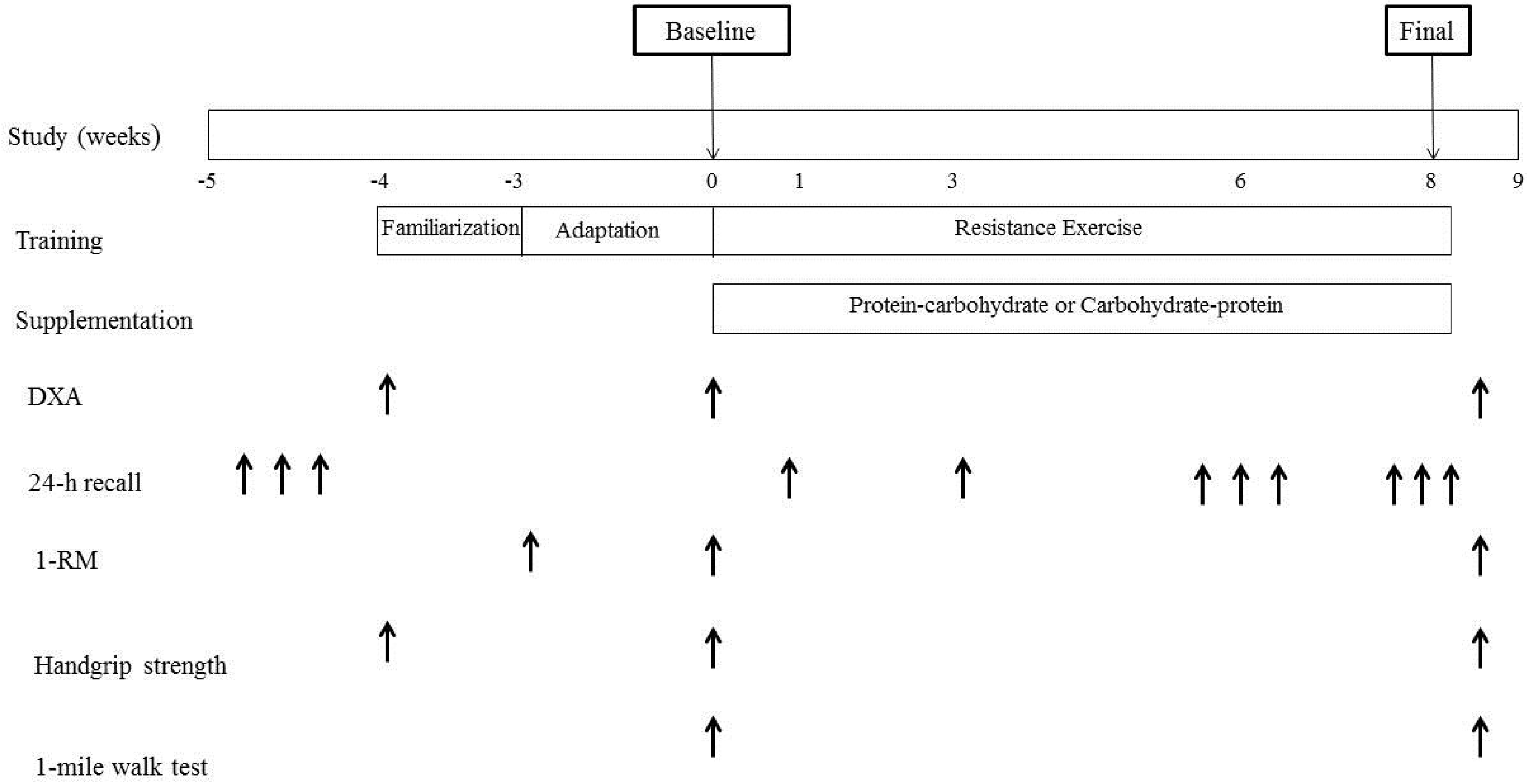

Figure 2. Schematic overview of study design.

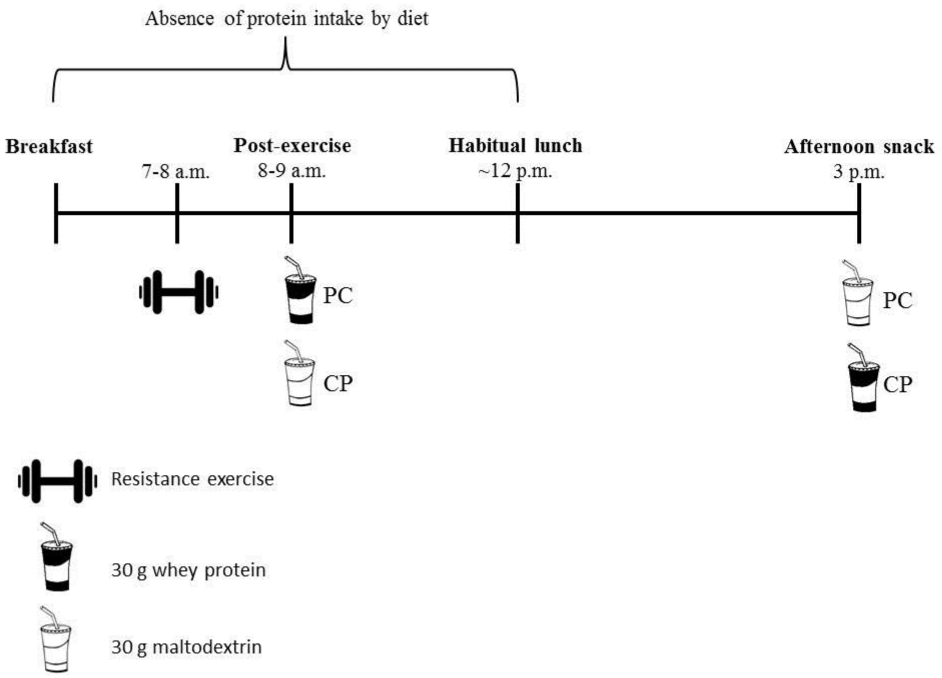

Figure 3. Supplementation protocol on training days. PC: protein-carbohydrate group. CP= carbohydrate-protein group.

TABLE 1 Baseline characteristics of the participants.

Variables	PC (*n*=17)	CP (*n*=17)	*p-value*
Age, *y*	60.5 [57.7-63.3]	61.4 [58.0-65.0]	0.680
Anthropometrics			
Body Mass, *kg*	69.1 [62.2-76.5]	71.3 [65.7-77.3]	0.646
Height, *m*	1.58 [1.56-1.62]	1.59 [1.57-1.62]	0.774
BMI, *kg/m²*	27.9 [25.2-30.8]	27.6 [25.7-29.7]	0.878
Body Composition			
Total lean mass, *kg*	37.3 [35.0-39.7]	38.2 [36.0-40.5]	0.597
Leg lean mass, *kg*	12.9 [11.8-14.2]	13.5 [12.5-14.6]	0.519
Trunk lean mass, *kg*	17.2 [16.3-18.2]	17.5 [16.6-18.4]	0.703
MMI, *kg/m²*	6.9 [6.4-7.5]	6.8 [6.4-7.3]	0.750
Total fat mass, *kg*	29.4 [24.5-35.4]	30.6 [27.2-34.5]	0.727
Total fat mass, *%*	42.5 [38.2-47.2]	43.8 [41.8-46.0]	0.578
Trunk fat mass, *kg*	15.1 [12.3-18.4]	15.3 [13.4-17.4]	0.919
Strength			
Bench press 1-RM (kg)	29.2 [27.1-31.3]	29.3 [26.2-32.7]	0.952
Leg extension 1-RM (kg)	30.0 [26.5-33.8]	30.7 [26.5-35.7]	0.783
Handgrip strength (right hand), *kg*	27.0 [25.6-28.5]	27.4 [24.8-30.2]	0.819
Functional capacity			
1-mile walk test, *minutes*	15.7 [14.7-16.8]	15.3 [14.7-15.9]	0.520
Dietary intake			
Calories, *kcal*	1589 [1371-1842]	1690 [1499-1906]	0.523
Carbohydrate, *g*	196 [163-236]	200 [176-229]	0.844
Carbohydrate, *%*	49.5 [45.4-54.0]	48.0 [44.5-51.8]	0.595
Protein, *g*	62.4 [52.9-73.5]	69.1 [58.7-81.3]	0.386
Protein, *g/kg*	0.9 [0.7-1.1]	1.0 [0.8-1.2]	0.355
Protein, *%*	15.6 [14.2-17.2]	16.5 [14.8-18.3]	0.454
Lipids, *g*	58.4 [49.9-68.4]	60.9 [52.0-71.4]	0.717
Lipids, *%*	33.5 [31.1-36.0]	32.7 [29.5-36.2]	0.718
Supplement adherence, *%*	82.2 [70.4-96.1]	72.9 [58.2-88.8]	0.315

Notes: PC, protein-carbohydrate group; CP, carbohydrate-protein group. BMI: body mass index; MMI: muscle mass index, was calculated by appendicular lean mass (kg) divided by height squared. All values are means and 95% confidence-interval. Significantly different between groups $p < 0.05$.

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

Variables	PC		CP		*p-value*		
	Pre	**During**	**Pre**	**During**	***Group***	***Time***	***Time x Group***
Energy intake, *kcal*	1589 [1371-1842]	1446 [1228-1702]	1690 [1499-1906]	1662 [1478-1869]	0.253	0.239	0.412
Energy intake including supplement, *kcal*	1589 [1371-1842]	1686 [1467-1938]	1690 [1499-1906]	1902[1717-2107]	0.264	0.044	0.506
Carbohydrate intake, *g*	196 [163-236]	184 [150-226]	200 [175-229]	219 [196-244]	0.338	0.819	0.190
Carbohydrate intake including supplement, *g*	196 [163-236]	214 [180-255]	200 [176-229]	249 [226-274]	0.361	0.005	0.234
Carbohydrate intake, *%*	49.5 [45.4-54.0]	50.2 [45.9-54.8]	48.0 [44.5-51.8]	53.4 [49.1-57.9]	0.759	0.057	0.140
Carbohydrate intake including supplement, *%*	49.5 [45.4-54.0]	50.2 [46.7-54.0]	48.0 [44.5-51.8]	52.9 [49.3-56.8]	0.826	0.055	0.157
Protein intake, *g*	62.4 [52.9-73.5]	57.6 [46.8-70.9]	69.1 [58.7-81.3]	64.8 [56.6-74.2]	0.285	0.287	0.905
Protein intake including supplement, *g*	62.4 [52.9-73.5]	85.4 [74.3-98.2]	69.1 [58.7-81.3]	92.7 [84.3-102]	0.286	<0.001	0.859
Protein intake, *g/kg*	0.9 [0.7-1.1]	0.9 [0.6-1.2]	1.0 [0.8-1.2]	0.9 [0.8-1.0]	0.671	0.312	0.673
Protein intake including supplement, *g/kg*	0.9 [0.7-1.1]	1.3 [1.0-1.6]	1.0 [0.8-1.2]	1.3 [1.2-1.4]	0.691	<0.001	0.474
Protein intake, *%*	15.6 [14.2-17.2]	15.8 [14.4-17.4]	16.5 [14.8-18.3]	15.6 [14.5-16.7]	0.798	0.608	0.492
Protein intake including supplement, *%*	15.6 [14.2-17.2]	20.5 [19.2-21.8]	16.5 [14.8-18.3]	19.6 [18.6-20.7]	0.999	<0.001	0.267
Leucine intake, *g*	4.5 [3.7-5.4]	4.2 [3.4-5.3]	4.7 [3.9-5.6]	4.5 [3.9-5.3]	0.591	0.457	0.836
Leucine intake including supplement, *g*	4.5 [3.7-5.4]	7.2 [6.3-8.1]	4.7 [3.9-5.6]	7.5 [6.8-8.2]	0.608	<0.001	0.983
Lipids, *g*	58.4 [49.9-68.4]	52.0 [44.7-59.3]	60.9 [52.0-71.4]	56.5 [46.9-68.0]	0.520	0.103	0.679
Lipids intake, *%*	33.5 [31.1-36.0]	32.6 [30.5-34.8]	32.7 [29.5-36.2]	29.9 [27.6-32.4]	0.200	0.146	0.429
Lipids intake including supplement, *%*	33.5 [31.1-36.0]	27.6 [25.9-29.3]	32.7 [29.5-36.2]	26.0 [23.8-28.5]	0.345	<0.001	0.687

All values are means and 95% confidence-interval. Significantly different between groups $p < 0.05$.

TABLE 3 Distribution of protein and leucine on training days (supplementation timing).

Meal	Groups		*p-value*
	PC (*n*=17)	CP (*n*=17)	
Protein			
Breakfast	2.8 [2.1-3.9]	3.4 [2.5-4.7]	0.445
Morning snack	2.2 [1.5-3.2]	1.6 [1.0-2.5]	0.277
Morning snack including supplement	29.3 [28.6-30.1]	1.6 [1.0-2.5]	<0.001
Lunch	26.3 [20.3-34.0]	29.1 [24.2-35.0]	0.531
Afternoon snack	3.7 [2.7-5.3]	5.3 [4.3-6.7]	0.095
Afternoon snack including supplement	3.7 [2.7-5.3]	33.4 [31.8-35.0]	<0.001
Dinner	18.3 [14.7-22.9]	16.9 [13.4-21.2]	0.610
Supper	2.6 [1.5-4.6]	2.5 [1.3-4.5]	0.885
Leucine			
Breakfast	0.18 [0.13-0.36]	0.22 [0.16-0.31]	0.383
Morning snack	0.13 [0.09-0.20]	0.11 [0.07-0.17]	0.552
Morning snack including supplement	3.0 [2.9-3.1]	0.11 [0.07-0.17]	<0.001
Lunch	2.0 [1.5-2.6]	2.1 [1.7-2.5]	0.914
Afternoon snack	0.25 [0.17-0.38]	0.38 [0.28-0.50]	0.114
Afternoon snack including supplement	0.25 [0.17-0.38]	3.3 [3.1-3.4]	<0.001
Dinner	1.36 [1.09-1.69]	1.20 [0.95-1.51]	0.443
Supper	0.20 [0.10-0.38]	0.23 [0.12-0.44]	0.738

All values are means and 95% confidence-interval. Significantly different between groups $p < 0.05$.

Table 4. Body composition, strength and functional tests values according to moments and groups.

Variables	PC		CP		p-value		
	Pre (n=17)	**Post (n=17)**	**Pre (n=17)**	**Post (n=17)**	***Group***	***Time***	***Time x group***
Body mass (kg)	69.3 [62.6-76.6]	69.6 [62.7-77.3]	70.9 [65.6-76.7]	71.8 [66.2-77.9]	0.678	0.069	0.478
Total lean mass (kg)	37.3 [35.0-39.7]	38.1 [35.9-40.5]	38.2 [36.0-40.5]	38.8 [36.5-41.3]	0.643	<0.001	0.408
Leg lean mass (kg)	12.9 [11.8-14.2]	13.3 [12.2-14.5]	13.5 [12.5-14.6]	13.8 [12.8-14.9]	0.510	<0.001	0.930
Trunk lean mass (kg)	17.2 [16.3-18.2]	17.5 [16.6-18.5]	17.5 [16.6-18.4]	17.5 [16.5-18.6]	0.845	0.036	0.212
Total fat mass (kg)	29.4 [24.5-35.4]	29.3 [24.4-35.3]	30.6 [27.2-34.5]	30.5 [27.0-34.3]	0.727	0.434	0.981
Trunk fat mass (kg)	15.1 [12.3-18.4]	15.2 [12.5-18.5]	15.3 [13.4-17.4]	15.3 [13.3-17.5]	0.946	0.538	0.613
MMI (kg/m^2)	6.9 [6.4-7.5]	7.1 [6.6-7.6]	6.8 [6.4-7.3]	7.0 [6.6-7.5]	0.839	<0.001	0.826
Bench press RM (kg)	29.2 [27.1-31.3]	33.8 [31.4-36.6]	29.3 [26.2-32.7]	34.8 [31.3-38.8]	0.800	<0.001	0.539
Leg extension RM (kg)	30.0 [26.5-33.8]	33.8 [31.0-37.0]	30.7 [26.5-35.7]	33.3 [29.4-37.8]	0.947	<0.001	0.318
Handgrip strength (kg)	27.0 [25.6-28.5]	28.3 [26.5-30.2]	27.4 [24.8-30.2]	29.3 [26.6-32.1]	0.664	<0.001	0.479
1-mile walk test (minutes)	15.7 [14.7-16.8]	15.6 [14.7-16.5]	15.3 [14.7-15.9]	15.0 [14.3-15.7]	0.333	0.019	0.796

Notes: PC, protein-carbohydrate group; CP, carbohydrate-protein group. MMI= Muscle mass index. All values are means and 95% confidence interval. Significantly different between groups $P < 0.05$.

Supplementary Table 1. Nutritional composition of the supplements.

	Whey protein (30 g)	**Maltodextrin (30 g)**
Calories, kcal	120	120
Protein, g	27.6	0
Carbohydrate, g	0.3	30
Fat, g	0.4	0
Leucine, g	2.9	0
Isoleucine, g	1.8	0
Valine, g	1.5	0

6 ANEXOS

6.1

UFU Comitê de Ética em Pesquisa — UNIVERSIDADE FEDERAL DE UBERLÂNDIA/MG — Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito da suplementação de proteína em diferentes momentos na massa magra, adiposidade e força de mulheres pós-menopausadas praticantes de exercício contra resistência

Pesquisador: Erick Prado de Oliveira

Área Temática:

Versão: 2

CAAE: 65638717.9.1001.5152

Instituição Proponente: Faculdade de Medicina

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.023.127

Apresentação do Projeto:

Segundo o protocolo de pesquisa:

"A sarcopenia é um processo de perda muscular involuntária e progressiva que acompanha o avanço da idade, e neste grupo, estão incluídas as mulheres menopausadas. Hábitos alimentares inadequados (dieta com baixa quantidade de proteína) e o sedentarismo estão associados com o aparecimento e agravo desta condição, a qual está relacionada com diminuição da capacidade funcional e maior risco de quedas. Diante do exposto, o desenvolvimento do presente projeto de pesquisa justifica-se pela importância de se observar se o consumo de uma dieta com diferentes quantidades de proteínas influenciam na composição corporal e força muscular de mulheres pós-menopausadas. O objetivo deste estudo é avaliar o efeito do consumo de dietas com diferentes quantidades de proteínas na composição corporal e força muscular de mulheres pós-menopausadas em treinamento com exercícios de contra-resistência. Na avaliação do perfil antropométrico serão aferidos: massa corporal, altura, circunferência da cintura e dobras cutâneas. Também será realizada avaliação da composição corporal por meio de densitometria com emissão de raios-X de dupla energia, bioimpedância e dobras cutâneas. A ingestão alimentar será estimada por meio de recordatório alimentar de 24 horas. A avaliação geral de saúde será avaliada pelo 36-

Endereço: Av. João Naves de Ávila 2121- Bloco "1A", sala 224 - Campus Sta. Mônica
Bairro: Santa Mônica CEP: 38.408-144
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Telefone: (34)3239-4131 Fax: (34)3239-4335 E-mail: cep@propp.ufu.br

6.2

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você está sendo convidada para participar da pesquisa intitulada "**Efeito da suplementação de proteína em diferentes momentos na massa magra, adiposidade e força de mulheres pós-menopausadas praticantes de exercício contra resistência"**, sob a responsabilidade dos pesquisadores Flávia Moure Simões de Branco, Paula Nahas, Luana Thomazetto Rossato, Fábio Lera Orsatti e Erick Prado de Oliveira.

Nesta pesquisa nós estamos buscando entender se a suplementação de proteínas próxima ao treino de força leva a maiores mudanças na quantidade de músculos e gorduras corporais, além de mudanças na força em longo prazo em comparação à suplementação distante do momento do treino. O Termo de Consentimento Livre e Esclarecido será obtido pelas pesquisadoras Flávia Moure Simões de Branco, Paula Nahas e Luana Thomazetto Rossato durante a apresentação da pesquisa.

Na sua participação você irá realizar exercícios físicos e terá orientações e acompanhamento nutricional, além de fornecer informações sobre os seus hábitos alimentares.

Em nenhum momento você será identificada. Os resultados da pesquisa serão publicados e ainda assim a sua identidade será preservada. Você não terá nenhum gasto e ganho financeiro por participar na pesquisa.

Os riscos consistem em: constrangimento ("vergonha") para medição de peso, circunferência da cintura e dobras de gordura, mas serão tomados todos os cuidados para se evitar isso. Além disso, a realização dos exames de bioimpedância e densitometria (esses dois exames são para conhecer a composição corporal, ou seja, quantidade de músculo e de gordura do corpo) não oferecem riscos à saúde. Se por algum motivo você não gostar do sabor do suplemento, poderá parar de toma-lo. Poderá haver o risco de identificação da participante da pesquisa, porém todas as voluntárias serão identificadas por códigos numéricos para minimizar esse risco.

Além disso, serão realizados questionários para avaliar o seu estado de saúde geral, testes de caminhadas e testes para medir sua força. Todos eles são testes simples e rápidos. Você estará sempre acompanhada de um profissional habilitado.

Os benefícios em participar da pesquisa serão a prática de exercícios físicos e o acompanhamento nutricional, importantes para a saúde do organismo. Ao término da intervenção, orientações serão dadas para que você continue uma rotina saudável.

Rúbricas: ________________________ ________________________

Pesquisadores Participante da pesquisa

Você é livre para deixar de participar da pesquisa a qualquer momento sem nenhum prejuízo ou coação. Caso seja esta a sua opção, lhe serão oferecidas orientações para que você tenha uma rotina saudável mesmo não participando da pesquisa.

Uma cópia deste Termo de Consentimento Livre e Esclarecido ficará com você.

Qualquer dúvida a respeito da pesquisa, você poderá entrar em contato com:

- Flávia Moure Simões de Branco. Nutricionista. Universidade Federal de Uberlândia. Endereço: Avenida Pará, 1720- Bloco 2H, Sala 09, *Campus* Umuarama, Uberlândia. Fone: 3218-2389.
- Luana Thomazetto Rossato. . Nutricionista. Universidade Federal de Uberlândia. Endereço: Avenida Pará, 1720- Bloco 2H, Sala 09, *Campus* Umuarama, Uberlândia. Fone: 3218-2389
- Paula Nahas. Nutricionista. Universidade Federal de Uberlândia. Endereço: Avenida Pará, 1720- Bloco 2H, Sala 09, *Campus* Umuarama, Uberlândia. Fone: 3218-2389
- Fábio Lera Orsatti. Docente nos Programas de Pós-Graduação Stricto Sensu em Educação Física e Ciências da Saúde. Líder do Grupo de Estudo e Pesquisas em Biologia do Exercício e Coordenador do Laboratório de Pesquisa em Biologia do Exercício Físico. Vinculado ao Instituto de Ciências da Saúde (ICS), na Universidade Federal do Triângulo Mineiro (UFTM). Avenida Tutunas, 490, Tutunas, Uberaba. Telefone: Fone: 3318-5504.
- Erick Prado de Oliveira. Professor Adjunto I, Curso de Nutrição, Faculdade de Medicina, Universidade Federal de Uberlândia. Endereço: Avenida Pará, 1720- Bloco 2U, Sala 20, *Campus* Umuarama, Uberlândia. Fone: 3218-2084.

Poderá também entrar em contato com o Comitê de Ética na Pesquisa com Seres-Humanos:

- Universidade Federal de Uberlândia: Av. João Naves de Ávila, nº 2121, bloco A, sala 224, Campus Santa Mônica – Uberlândia –MG, CEP: 38408-100; fone: (34)32394131.
- Universidade Federal do Triângulo Mineiro: (34) 3700-6776.

O CEP é um colegiado independente criado para defender os interesses dos participantes das pesquisas em sua integridade e dignidade e para contribuir para o desenvolvimento da pesquisa dentro de padrões éticos conforme resoluções do Conselho Nacional de Saúde.

Uberlândia, ____de____de 201____

Uberaba, ____de____de 201____

Assinatura dos pesquisadores

Eu aceito participar do projeto citado acima, voluntariamente, após ter sido devidamente esclarecido.

Participante da pesquisa