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Ácido graxo poli-insaturado ômega-3 e sua associação com a massa muscular de indivíduos participantes do *National Health and Nutrition Examination Survey*

Uberlândia

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FLÁVIA MOURE SIMÕES DE BRANCO

Ácido graxo poli-insaturado ômega-3 e sua associação com a massa muscular de indivíduos participantes do *National Health and Nutrition Examination Survey*

Tese 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 Doutor em Ciências da Saúde

Área de concentração: Ciências da Saúde.

Orientador: Erick Prado de Oliveira

Co-orientador: Catarina Machado Azeredo

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UNIVERSIDADE FEDERAL DE UBERLÂNDIA
Coordenação do Programa de Pós-Graduação em Ciências da Saúde
Av. Pará, 1720, Bloco 2H, Sala 11 - Bairro Umuarama, Uberlândia-MG, CEP 38400-902
Telefone: (34) 3225-8628 - www.ppcsa.famed.ufu.br - ppcsa@famed.ufu.br

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Reuniu-se em web conferência pela plataforma Zoom, em conformidade com a PORTARIA Nº 36, DE 19 DE MARÇO DE 2020 da COORDENAÇÃO DE APERFEIÇOAMENTO DE PESSOAL DE NÍVEL SUPERIOR - CAPES, pela Universidade Federal de Uberlândia, a Banca Examinadora, designada pelo Colegiado do Programa

pela Universidade Federal de Uberlândia, a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Ciências da Saúde, assim composta: Profs. Drs. Hamilton Roschel (USP), Paula Candido Nahas (UNA), Cibele Aparecida Crispim (UFU), Geórgia das Graças Pena (UFU) e Erick Prado de Oliveira (UFU), orientador da candidata.

Iniciando os trabalhos, a presidente da mesa, Prof. Dr. Erick Prado de Oliveira, apresentou a Comissão Examinadora e a candidata, agradeceu a presença do público, e concedeu a Discente a palavra para a exposição do seu trabalho. A duração da apresentação da Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

A seguir o senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos(às) examinadores(as), que passaram a arguir o(a) candidato(a). Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o(a) candidato(a):

Aprovada.

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Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata que após lida e achada conforme foi assinada pela Banca Examinadora.

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EPÍGRAFE

"O sábio não é o homem que fornece as verdadeiras respostas; é quem faz as verdadeiras perguntas"

Claude Lévi-Strauss

RESUMO

Introdução: Os ácidos graxos ômega-3 (ω-3) têm efeitos anti-inflamatórios e podem aumentar indiretamente a síntese de proteína muscular, tornando o músculo mais sensível à absorção de aminoácidos; entretanto, a associação entre plasma ω-3 e massa muscular em indivíduos mais jovens não é bem conhecida. Além disso, têm-se discutido a influência da ingestão de proteína na síntese proteica muscular estimulada pelo ω-3. **Objetivos:** Avaliar a associação entre os ácidos graxos ω-3 plasmáticos e o índice de massa muscular apendicular (IMMA) em indivíduos jovens e de meia-idade e de acordo com o sexo; e avaliar a associação entre os ácidos graxos ω-3 plasmáticos e o IMMA de acordo com a ingestão de proteína diária (<0,8 g/kg/dia ou ≥0,8 g/kg/dia). **Metodologia:** Trata-se de um estudo transversal que avaliou 1.037 indivíduos de 20 a 59 anos do *National Health and Nutrition Examination Survey* (NHANES) 2011-2012. O método de espectrometria de massa por cromatografia gasosa foi usado para avaliar os ácidos graxos ω-3 do plasma. A massa magra foi avaliada por absorciometria de raios-X de dupla energia (DXA) e o IMMA (kg/m^2) foi calculado pela massa magra apendicular (kg) dividida pela altura ao quadrado. Análises de regressão linear foram realizadas para avaliar a associação entre IMMA e ω-3 plasmático total, ácido docosahexaenóico (DHA), ácido eicosapentaenóico (EPA) e ácido alfa-linolênico (ALA) para a amostra total, de acordo com a idade (20 a 44 anos e 45 a 59 anos) e sexo e separadamente pela ingestão de proteína (baixa ingestão de proteína: <0,8 g / kg; ou adequado: ≥0,8 g / kg). **Resultados:** O ω-3 total, DHA, EPA e ALA plasmáticos não foram associados com o IMMA na amostra total, de acordo com sexo e idade após ajustes para fatores de confusão. Entretanto, o ω-3 total, ALA e DHA plasmáticos associaram-se positivamente ao IMMA em indivíduos com baixo consumo de proteínas, mas nenhuma associação foi observada em indivíduos com ingestão adequada de proteínas. **Conclusão:** Em conclusão, os ácidos graxos ω-3 plasmáticos não estão associados ao IMMA em indivíduos jovens e de meia-idade, independentemente do sexo. Entretanto, os ácidos graxos ω-3 plasmáticos foram associados ao IMMA em indivíduos com baixa ingestão proteica.

Palavras-chave: Ômega-3; Massa muscular; Ingestão proteica.

ABSTRACT

Background: Omega-3 (ω-3) fatty acids have anti-inflammatory effects and can indirectly increase muscle protein synthesis, making muscle more sensitive to amino acid uptake; however, the association between plasma ω-3 and muscle mass in younger individuals is not well known. Furthermore, the influence of protein intake on muscle protein synthesis stimulated by ω-3 has been discussed. **Aim:** To evaluate the association between plasma ω-3 fatty acids and appendicular muscle mass index (IMMA) in young and middle-aged individuals and according to sex; and to evaluate the association between plasma ω-3 fatty acids and IMMA according to daily protein intake (<0.8 g/kg/day or ≥0.8 g/kg/day). **Methods:** This is a cross-sectional study that evaluated 1,037 individuals aged 20 to 59 years from the National Health and Nutrition Examination Survey (NHANES) 2011-2012. The gas chromatography mass spectrometry method was used to evaluate the plasma ω-3 fatty acids. Lean mass was assessed by dual energy X-ray absorptiometry (DXA) and IMMA (kg/m2) was calculated as appendicular lean mass (kg) divided by height squared. Linear regression analyzes were performed to assess the association between IMMA and total plasma ω-3, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and alpha-linolenic acid (ALA) for the total sample, according to age (20 to 44 years and 45 to 59 years) and sex and separately by protein intake (low protein intake: <0.8 g / kg; or adequate: ≥0.8 g / kg). **Results:** Total ω-3 plasma, DHA, EPA, and ALA were not associated with IMMA in the total sample according to sex and age after adjustments for confounding factors. However, total ω-3, plasma ALA and DHA were positively associated with IMMA in individuals with low protein intake, but no association was observed in individuals with adequate protein intake. **Conclusion:** In conclusion, plasma ω-3 fatty acids are not associated with IMMA in young and middle-aged individuals, regardless of sex. However, plasma ω-3 fatty acids have been associated with IMMA in individuals with low protein intake.

Keywords: Omega-3; Muscle mass; Protein intake.

SUMÁRIO

1. INTRODUÇÃO

Os ácidos graxos ômega-3 (ω-3) são gorduras poli-insaturadas de cadeia longa que apresentam insaturação entre o terceiro e o quarto átomos de carbono. No organismo humano, os ácidos graxos ω-3 são considerados essenciais já que não podem ser sintetizados e, portanto, devem ser ingeridos via alimentação ou suplementação (ARTERBURN; HALL; OKEN, 2006). Os principais ácidos graxos ω-3 são o ácido alfa linolênico (ALA; 18:3n-3), que é encontrado especialmente em fontes vegetais como nozes, sementes e óleos de linhaça, canola e soja; já o ácido eicosapentaenóico (EPA; 20: 5n-3) e o ácido docosahexaenóico (DHA; 22: 6n-3) são encontrados principalmente em peixes gordurosos (MCGLORY, C.; CALDER, P. C.; NUNES, E. A., 2019).

O ω-3 tem sido cada vez mais estudado devido seus efeitos benéficos em vários sistemas do organismo humano como na saúde cardiovascular e perfil lipídico (CABO; ALONSO; MATA, 2012), na cognição (CARDOSO; AFONSO; BANDARRA, 2016), perfil inflamatório (CALDER, 2017) e mais recentemente tem surgido interesse dos benefícios do ω-3 na massa muscular (CARDOSO; AFONSO; BANDARRA, 2016), já que esse tecido tem importante papel no estado de saúde, pois parece ser associado à força, capacidade funcional e qualidade de vida (THIEBAUD; LOENNEKE; ABE; FAHS *et al.*, 2017; TROMBETTI; REID; HARS; HERRMANN *et al.*, 2016).

Especula-se que o ω-3 possa beneficiar a massa muscular de duas principais formas: reduzindo a inflamação e facilitando a capitação de aminoácidos pelas células musculares. Em relação ao primeiro mecanismo, uma vez que a inflamação é uma das causas da perda muscular (BREEN; PHILLIPS, 2011; ROUBENOFF, 2007) e o ω-3 tem efeitos anti-inflamatórios (MCGLORY, CHRIS; CALDER, PHILIP C.; NUNES, EVERSON A., 2019), indivíduos que tem maiores quantidades desse ácido graxo no organismo podem ter redução das citocinas pró-inflamatórias preservando a massa muscular. Além disso, tem sido proposto que o ω-3 pode ter efeitos na massa muscular devido ao seu aumento indireto na síntese proteica muscular. Os ácidos graxos ômega-3 podem alterar a fluidez da membrana das células musculares, melhorando a captação de

aminoácidos e, consequentemente, tornando a célula mais sensível a estímulos para a síntese proteica muscular (SMITH; ATHERTON; REEDS; MOHAMMED *et al.*, 2011a).

Adicionalmente, tem-se discutido se a ingestão de proteína pode ter influência no efeito do ômega-3 sobre a massa muscular. Especula-se que quando a ingestão de proteína está inadequada, o ω-3 poderia auxiliar na síntese proteica muscular, portanto, tendo benefícios nesse tecido (MCGLORY, C.; CALDER, P. C.; NUNES, E. A., 2019). Dessa forma, é pertinente avaliar a relação entre a concentração de ácidos graxos ω-3 no organismo e a massa muscular e adicionalmente verificar se essa relação é dependente da ingestão proteica diária.

2. FUNDAMENTAÇÃO TEÓRICA

2.1 Omega-3

Os ácidos graxos ω-3 são gorduras poli-insaturadas de cadeia longa que apresentam insaturação (dupla ligação) a partir do terceiro carbono da estrutura química. Esses ácidos graxos são considerados essenciais, ou seja, o organismo humano não tem a capacidade de produzi-lo, e, portanto, devem ser consumidos via alimentação ou suplementação (ARTERBURN; HALL; OKEN, 2006). O ω-3 é um componente estrutural essencial das membranas fosfolipídicas dos tecidos de todo o corpo e pode alterar a fluidez das membranas celulares (CONNOR, 2000). Os ácidos graxos ω-3 são abundantes especialmente na retina e no cérebro e tem uma grande importância durante o desenvolvimento fetal (NEURINGER; CONNOR; LIN; BARSTAD *et al.*, 1986). Em adição, existem benefícios do consumo dessas gorduras para a saúde cardiovascular, no perfil lipídico (CABO; ALONSO; MATA, 2012), especialmente na redução de triglicerídeos sanguíneos (DURRINGTON; BHATNAGAR; MACKNESS; MORGAN *et al.*, 2001; SKULAS-RAY; KRIS-ETHERTON; HARRIS; VANDEN HEUVEL *et al.*, 2010), na cognição (CARDOSO; AFONSO; BANDARRA, 2016) e na inflamação (CALDER, 2017).

Apesar das evidências do consumo ω-3 na saúde cardiovascular ainda serem conflitantes (AUNG; HALSEY; KROMHOUT; GERSTEIN *et al.*, 2018; BALK; ADAMS; LANGBERG; HALLADAY *et al.*, 2016), algumas sociedades trazem recomendações sobre seu consumo do ω-3 para a saúde cardiovascular. Segundo a Sociedade Brasileira de Cardiologia, a suplementação de 1 g/dia de ômega-3 de origem animal (EPA e DHA) pode ser recomendada para diminuir o risco cardiovascular e a

suplementação de 2 a 4 g/dia de ômega-3 de origem animal (EPA e DHA) pode ser recomendada para indivíduos com hipertrigliceridemia grave (>500 mg/dL). Além disso, a *American Heart Association* recomenda a ingestão de pelo menos 2 porções de peixes por semana, principalmente de peixes gordurosos como salmão, sardinha e atum, para a redução do risco de doenças cardíacas (KRIS-ETHERTON; HARRIS; APPEL, 2003).

2.2 Tipos de ácidos graxos ômega-3

Os principais ácidos graxos ω-3 encontrados nos alimentos e suplementos são o ALA, EPA e o DHA, entretanto, através de interconversões metabólicas que adicionam carbonos ou saturações, esses ácidos graxos podem ser convertidos e se transformarem em tipos diferentes do que foi consumido. A cadeia de interconversões se inicia com o ALA e ocorre principalmente no fígado no retículo endoplasmático. Durante o processo de conversão, a enzima desaturase adiciona insaturações (duplas ligações) e a enzima elongase adiciona 2 unidades de carbono ao esqueleto do ácido graxo. Para a conversão final da cadeia em ALA para DHA, é necessário que aconteça uma reação de β-oxidação, que retira 2 carbonos da estrutura molecular (ARTERBURN; HALL; OKEN, 2006). A cadeia de conversões é mostrada de forma detalhada na **Figura 1**.

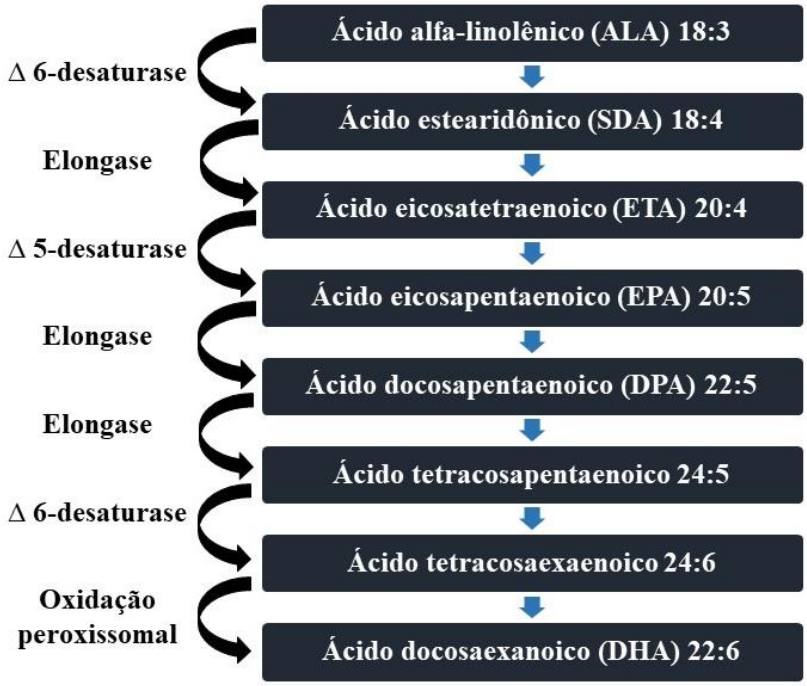


Figura 1. Sequência de reações que leva a conversão de ácido alfa-linolênico (ALA) em ácido eicosapentaenoico (EPA) e ácido docosahexaenoico (DHA).

A conversão de ALA para EPA e DHA pode variar de acordo com o sexo e também devido à ingestão de ácidos graxos ômega-6. Em relação ao sexo, as mulheres podem converter maiores quantidades de ALA, por volta de 21% em EPA e 9% em DHA; já para os homens, é estimado que a conversão seja de 8% e 4%, respectivamente (ARTERBURN; HALL; OKEN, 2006). Adicionalmente, a quantidade de ácido linoleico (ω-6) da dieta também pode influenciar na taxa de conversão ALA. Isso pode ser explicado porque o ALA e os ácidos graxos ω-6 competem pelo substrato na membrana fosfolipídica e também pela enzima Δ 6-desaturase, que é a mesma enzima utilizada pelo ALA no processo de conversão (ARTERBURN; HALL; OKEN, 2006). Estima-se que dietas ricas em ω-6 podem reduzir a conversão de ALA em até 40% (EMKEN; ADLOF; GULLEY, 1994).

2.3 Fontes

Os ácidos graxos ω-3 podem ser encontrados tanto em fontes vegetais como fontes animais. O ALA pode ser ingerido através do consumo de fontes vegetais como sementes, castanhas, linhaça, óleos vegetais como canola, linhaça e soja. Já as principais fontes de EPA e DHA são peixes gordurosos de água fria como o salmão, sardinha e atum. Além das fontes alimentares de ω-3, muitos suplementos contendo esses ácidos graxos são comercializados como uma forma de adequar o baixo consumo via dieta. Os óleos de peixes, algas e krill são os suplementos fontes de EPA e DHA mais comuns; já os óleos de linhaça e nozes são suplementos fontes de ALA.

Em relação às fontes mais consumidas pela população americana, um estudo com participantes do *National Health and Nutrition Examination Survey* (NHANES) dos anos de 2003-2008, avaliou o consumo de ω-3 pela população americana e mostrou que os alimentos que mais contribuíam para a ingestão desses ácidos graxos eram os peixes e os grãos (RICHTER; BOWEN; MOZAFFARIAN; KRIS-ETHERTON *et al.*, 2017). Entretanto, apesar de haver o consumo de alimentos fonte desses ácidos graxos, grande parte da população, por volta de 90%, tinha o consumo de ω-3 abaixo do recomendado, e, apesar dessa baixa ingestão, apenas 6,2% faziam uso de suplementos (RICHTER; BOWEN; MOZAFFARIAN; KRIS-ETHERTON *et al.*, 2017).

2.4 Ômega-3 e inflamação

A inflamação é fundamental para a defesa do organismo contra patógenos (CALDER, 2015). Essa reação inflamatória cria um ambiente hostil e inicia a morte dos patógenos através de mudanças no sistema imune do hospedeiro. A resposta inflamatória

envolve interações entre muitos tipos de células e a produção de um grande número de mediadores químicos. Um suprimento aumentado de sangue ao local da inflamação e um aumento na permeabilidade da parede vascular são os passos iniciais da resposta inflamatória. Assim, é possível que o plasma e moléculas grandes cruzem o endotélio, para entregar mediadores que tem ação anti-inflamatória no local da inflamação. Esses mediadores podem ser derivados de lipídios, como, por exemplo, as prostaglandinas e os leucotrienos (CALDER, 2015). Nesse contexto, estão os ácidos graxos ω-3 que podem produzir mediadores que podem ter ação anti-inflamatória.

Entretanto, para entender a ação do ω-3 a inflamação, é preciso entender também a ação dos ácidos graxos ω-6 nesse contexto. Tanto o ω-3 como o ω-6 são substratos na síntese de mediadores lipídicos, que são conhecidos como eicosanoides. Esses eicosanoides exercem funções de regulação e mediação das respostas inflamatórias, podendo ter respostas diferentes se for advindo de ácidos graxos ω-6 ou ω-3 (SERHAN; BRAIN; BUCKLEY; GILROY *et al.*, 2007). Os ácidos graxos ω-6 e ω-3, mais especificamente o ácido aracdônico e o EPA competem entre si pelas vias enzimáticas ciclooxigenase (COX) e lipooxigenase (5-LOX) para a produção de mediadores lipídicos. Os ácidos graxos ω-6 produzem prostaglandinas da série 2, leucotrienos da série 4 e tromboxano A2 que são eicosanoides pró-inflamatórios; já os ácidos graxos ω-3 produzem prostaglandinas da série 3, leucotrienos de série 5 e tromboxano A3 que são eicosanoides menos inflamatórios e podem até ter ação anti-inflamatória (TILLEY; COFFMAN; KOLLER, 2001). Dessa forma, a composição da membrana fosfolipídica de ácidos graxos pode mediar se serão produzidos eicosanoides mais ou menos inflamatórios de acordo com a quantidade de ácidos graxos ω-6 ou ω-3.

2.5 Ômega-3 e massa muscular

Além dos benefícios do ω-3 na saúde cardiovascular e perfil lipídico (CABO; ALONSO; MATA, 2012), na cognição (CARDOSO; AFONSO; BANDARRA, 2016), perfil inflamatório (CALDER, 2017), mais recentemente tem-se investigado a relação dessas gorduras com a massa muscular (ROSSATO; SCHOENFELD; DE OLIVEIRA, 2020). Especula-se que os ácidos graxos ω-3 possam beneficiar o músculo por duas formas distintas: reduzindo a inflamação e facilitando a capitação de aminoácidos pelo músculo. Em relação à influência da inflamação na massa muscular, a expressão aumentada de citocinas pró-inflamatórias como IL-1, IL-6 e TNF e proteínas de fase aguda como a proteína C-reativa são conhecidos por desencadear reguladores de

proteólise que, por sua vez, promovem a perda muscular (CROSSLAND; SKIRROW; PUTHUCHEARY; CONSTANTIN-TEODOSIU *et al.*, 2019). Dessa forma, como os ácidos graxos ω-3 podem modificar a produção dessas substâncias que favorecem a inflamação, essas gorduras poderiam trazer benefícios para a massa muscular, preservando esse tecido (MCGLORY, C.; CALDER, P. C.; NUNES, E. A., 2019). Já em relação à captação de aminoácidos, especula-se que o ω-3, através da incorporação nas membranas celulares do músculo, aumenta a fluidez da membrana e facilita a captação de aminoácidos, aumentando assim, a síntese proteica muscular (SPM) (DA BOIT; SIBSON; SIVASUBRAMANIAM; MEAKIN *et al.*, 2017; ROSSATO; SCHOENFELD; DE OLIVEIRA, 2020).

Estudos realizados tanto em indivíduos jovens e de meia idade (SMITH; ATHERTON; REEDS; MOHAMMED *et al.*, 2011b) como em idosos (SMITH; ATHERTON; REEDS; MOHAMMED *et al.*, 2011a) avaliaram a SPM após a suplementação de ω-3 durante 8 semanas e encontraram aumento da resposta anabólica com a ingestão desses ácidos graxos. Em contrapartida, McGlory e colaboradores (MCGLORY; WARDLE; MACNAUGHTON; WITARD *et al.*, 2016) avaliaram o efeito da suplementação de 5 g/dia de óleo de peixe (3,5g de EPA e 0,9g de DHA) ou de óleo de coco por 8 semanas na SPM de homens jovens que realizaram uma sessão aguda de exercício de força unilateral, seguido pela ingestão de 30 g de proteína de soro de leite. Os autores encontraram aumento da atividade de p70S6K1 somente no grupo óleo de coco, sugerindo uma atenuação da SPM relacionada com à suplementação ω-3. Uma das possíveis explicações para esses achados discrepantes na SPM pode ser devido à diferença da ingestão de proteína/aminoácidos de acordo com os estudos. Entretanto, a discussão sobre a influência da ingestão proteica na suplementação de ω-3 será abordada com mais detalhes no tópico seguinte.

Sobre a associação do ω-3 com a massa muscular, poucos estudos avaliaram essa relação. Reinders e colaboradores (REINDERS; SONG; VISSER; EIRIKSDOTTIR *et al.*, 2015) conduziram um estudo com 836 idosos (66-96 anos) e avaliaram a associação entre os ácidos graxos plasmáticos ω-3 e a área muscular transversal da coxa. Além disso, Belury et al. (BELURY; COLE; BAILEY; KE *et al.*, 2016) avaliaram a associação entre a composição de ω-3 em eritrócitos com massa magra em 139 indivíduos de meia-idade (~ 44,3 anos). Ambos os estudos não encontraram associação entre ω-3 e massa muscular.

Entretanto, nesses dois estudos não foi avaliada a associação de acordo com a ingestão proteica.

Em adição, uma revisão de literatura avaliou a evidência atual sobre a ingestão de ω-3 na composição corporal e função física, mostrou que os dados ainda são conflitantes (ROSSATO; SCHOENFELD; DE OLIVEIRA, 2020). Além de vários estudos não mostrarem benefícios com a suplementação de ω-3, os estudos que mostram resultados positivos têm importantes falhas metodológicas, como a falta de controle da realização de atividade física pelos participantes e também a falta de avaliação da ingestão dietética, especialmente da ingestão proteica. Portanto, ainda não é possível determinar com clareza se a ingestão de ω-3 pode favorecer o ganho de massa muscular.

2.6 Ômega-3 e ingestão proteica

A influência da ingestão de proteínas ou infusão de aminoácidos na SPM estimulada pelo ω-3 tem sido discutida, uma vez que a proteína é o principal nutriente para estimular o anabolismo muscular. Smith et al. (SMITH; ATHERTON; REEDS; MOHAMMED *et al.*, 2011b) mostraram que a suplementação de ω-3 durante 8 semanas aumentou o estímulo anabólico em 50% após a infusão de aminoácidos em indivíduos jovens e de meia-idade. No entanto, McGlory e colaboradores (MCGLORY; WARDLE; MACNAUGHTON; WITARD *et al.*, 2016) avaliaram o efeito da suplementação de 5 g/dia de óleo de peixe ou óleo de coco por 8 semanas em homens jovens que realizaram uma sessão aguda de exercício de força unilateral seguido pela ingestão de 30 g de proteína de soro de leite. Foi encontrado que a suplementação de ω-3 não aumentou a SPM.

Esses achados discrepantes podem ser parcialmente explicados devido às diferenças na administração de proteínas; enquanto McGlory et al. (MCGLORY; WARDLE; MACNAUGHTON; WITARD *et al.*, 2016) usaram uma dose ideal para estimular a MPS por meio da ingestão de proteína de soro de leite; Smith et al. (SMITH; ATHERTON; REEDS; MOHAMMED *et al.*, 2011b) realizaram infusão contínua de aminoácidos que simulou uma dose subótima de proteína. Um estudo realizado por Moore e colaboradores (MOORE; CHURCHWARD-VENNE; WITARD; BREEN *et al.*, 2015) objetivou avaliar qual seria a dose ótima de proteína para estimular a SPM máxima em indivíduos jovens e idosos. Os autores encontraram que para jovens, a SPM máxima acontecia com a ingestão de 0,24 g de proteína/ kg de peso (~20 g por refeição) e para

indivíduos idosos a SPM máxima ocorria com a dose de 0,4 de proteína/ kg de peso (~30g por refeição). Portanto, no estudo de McGlory, os indivíduos consumiam uma dose suficiente para estimular a SPM de indivíduos jovens e de meia idade. Adicionalmente, as *Dietary Reference Intakes* (DRIs) através das *Recommended Dietary Allowances* (RDA) sugerem que para indivíduos acima de 19 anos, a ingestão de proteína diária adequada seria de 0,8 g/ kg de peso/ dia (PADOVANI; AMAYA-FARFÁN; COLUGNATI; DOMENE, 2006), e, portanto, doses inferiores a essa são consideradas inadequadas..

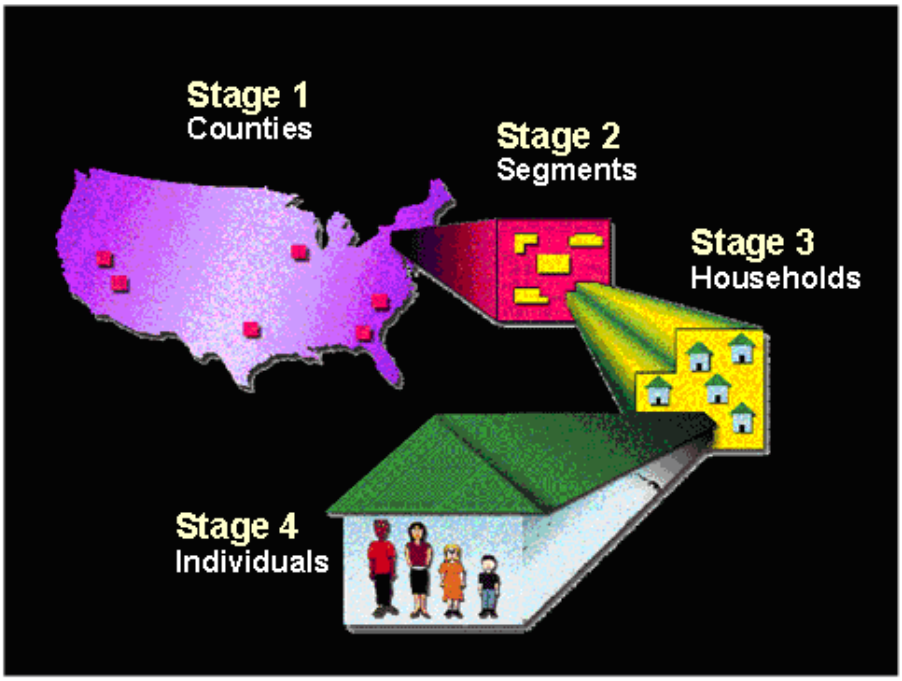
Assim, é possível especular que quando a ingestão de proteína está inadequada (abaixo de 0,8 g/ kg de peso/ dia ou de 20-30 g/refeição), é provável que uma quantidade maior de ω-3 na célula possa aumentar a SPM e apresentar benefícios para a massa muscular. Porém, até o momento, nenhum estudo avaliou a relação entre ω-3 e massa muscular de acordo com a ingestão de proteína.

2.7 National Health and Nutrition Examination Survey

O *National Health and Nutrition Examination Survey* (NHANES) é um programa de estudos que visa investigar os hábitos alimentares e de saúde de crianças, adultos e idosos não institucionalizados dos Estados Unidos. O NHANES é desenvolvido pelo Centro Nacional de Estatísticas de Saúde (NCHS), que faz parte do Centro de Controle e Prevenção de Doenças (CDC). A pesquisa tem o objetivo produzir estatísticas em relação aos hábitos da população e investigar os riscos à saúde a fim de traçar estratégias populacionais de prevenção e tratamento dos problemas identificados. Além dos questionários aplicados, o NHANES também consiste em medidas médicas, odontológicas e fisiológicas, bem como exames bioquímicos, composição corporal, avaliação da força muscular, dentre outras medidas. O programa do NHANES teve início nos anos 60 e em 1999 se tornou uma pesquisa contínua que é realizada por biênios e tem foco em mudança em uma variedade de medidas de saúde e nutrição para atender às necessidades populacionais. A pesquisa examina uma amostra nacionalmente representativa de cerca de 10.000 pessoas a cada ano.

As amostras NHANES não são aleatórias simples e sim amostras probabilísticas complexas em multi-estágio. Portanto, esse tipo de amostragem seleciona os participantes para que os dados coletados sejam representativos da população civil não

institucionalizada dos Estados Unidos. Um resumo de como são selecionados os participantes do NHANES está descrito abaixo:



Four Stages of NHANES Sampling Procedure

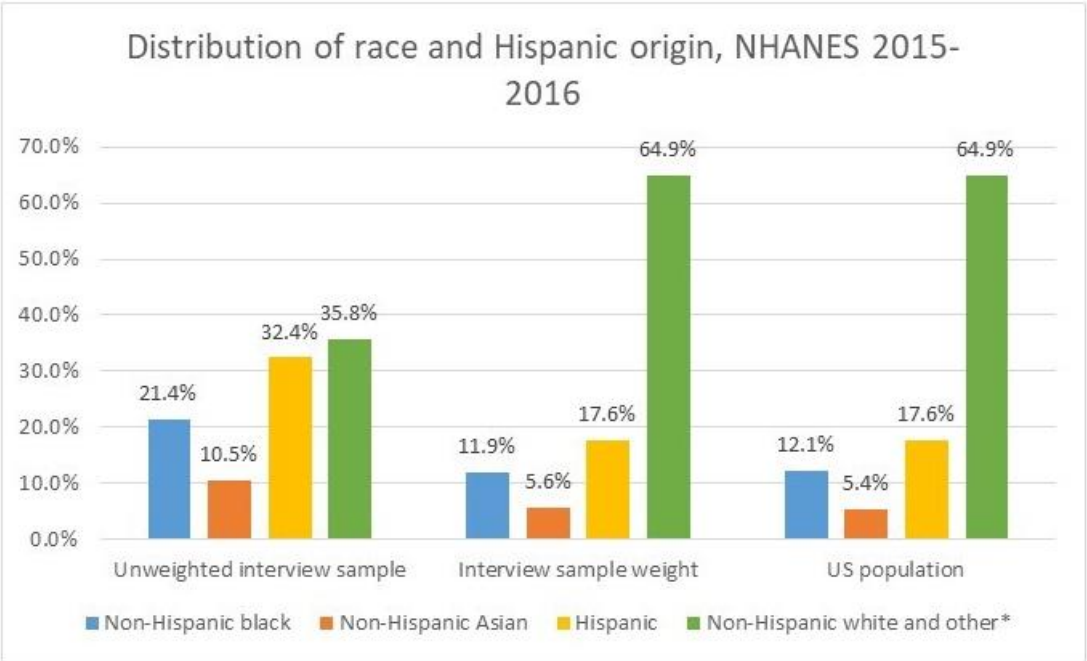
Estágio 1: São selecionadas as unidades primárias de amostragem (PSUs). As PSUs geralmente são condados isolados ou, em alguns casos, grupos de condados. Essas unidades primárias são selecionadas com probabilidade proporcional a uma medida de tamanho (PPS). Amostragem PPS significa que unidades de amostragem com populações maiores são mais prováveis de serem selecionadas do que aquelas com populações menores, ou seja, é uma média ponderada de contagens da população, onde os pesos são calculados para dar probabilidade relativamente maior de seleção para PSUs com proporções mais altas de indivíduos dentro dos subgrupos demográficos.

Estágio 2: As PSUs da amostra são divididas em segmentos (geralmente quarteirões da cidade ou seu equivalente). Assim como acontece na PSU, os segmentos de amostra são selecionados com PPS.

Estágio 3: Os domicílios dentro de cada segmento são listados e uma amostra é sorteada aleatoriamente. Em áreas geográficas onde a população tem uma proporção maior de um determinado grupo de idade, etnia ou renda selecionado para sobreamostragem, a probabilidade de seleção para esses grupos é maior do que em outras áreas.

Etapa 4: Os indivíduos são escolhidos para participar do NHANES a partir de uma lista de todas as pessoas que residem em domicílios selecionados. Em média, cerca de 2 pessoas da amostra são selecionadas por domicílio elegível.

A partir dessa seleção em multi-estágio, os pesos amostrais no banco de dados são criados para cada indivíduo participante do NHANES. O peso amostral é uma medida do número de pessoas na população representada por uma única pessoa da amostra, refletindo a probabilidade desigual de seleção. Quando a probabilidade de seleção desigual é aplicada, os pesos da amostra são usados para produzir uma estimativa nacional imparcial. O uso do peso amostral para as análises dos dados do NHANES é imprescindível para aumentar a confiabilidade e a precisão das estimativas de indicadores de estado de saúde. Os pesos da amostra permitem obter estimativas nacionais que refletem as verdadeiras proporções relativas desses grupos na população dos Estado Unidos como um todo. A figura abaixo mostra a diferença na estimativa da proporção das raças em uma análise com e sem o uso dos pesos amostrais e como é a distribuição verdadeiramente da população americana. É possível observar que as análises utilizando os pesos se aproxima muito mais dos dados reais da população.



Graph Comparing NHANES Unweighted Interview Sample, Weighted Interview Sample, and U.S. Population

3. OBJETIVOS

3.1 Objetivo geral

Avaliar a associação entre os ácidos graxos poli-insaturados ω-3 plasmáticos e a massa muscular.

3.2 Objetivos específicos

-Avaliar a associação entre os ácidos graxos poli-insaturados ω-3 plasmáticos e a massa muscular de acordo com o sexo e idade (20 a 44 anos e 45 a 59 anos);

- Avaliar a associação entre os ácidos graxos poli-insaturados ω-3 plasmáticos e a massa muscular de acordo com a ingestão de proteína diária (<0,8 g/kg/dia e ≥0,8 g/kg/dia).

Original article

Plasma omega-3 is not associated with appendicular muscle mass index in young and middle-aged individuals: Results from NHANES 2011-2012

Short title

Association between plasma omega-3 and AMMI

Abstract

The aim of this study was to evaluate the association between plasma ω-3 and appendicular muscle mass index (AMMI) in young and middle-aged individuals and also to evaluate whether these associations are sex-specific. A cross-sectional study was performed evaluating 1037 individuals aged 20 to 59 years from the National Health and Nutrition Examination Survey (NHANES) 2011-2012. Plasma ω-3 was evaluated by gas chromatography mass spectrometry and lean mass was assessed by dual-energy x-ray absorptiometry (DXA). Total plasma ω-3, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and alpha-linolenic acid (ALA) were not associated with AMMI in total sample, men and women after adjustments for confounders. Plasma ω-3 and its subtypes were not associated with AMMI in a sub analysis evaluating young (20 to 44 y) and middle-aged (45 to 59 y) individuals separately. In conclusion, plasma ω-3 fatty acids are not associated with AMMI in young and middle-aged individuals regardless of sex.

Key-words: Lean mass; Omega-3; Polyunsaturated fatty acids; body composition.

Introduction

Muscle mass has an important role on health status, since it is associated with strength, functional capacity and quality of life (THIEBAUD; LOENNEKE; ABE; FAHS *et al.*, 2017; TROMBETTI; REID; HARS; HERRMANN *et al.*, 2016). Several factors can predict the muscle mass amount, such as age (WILKINSON; PIASECKI; ATHERTON, 2018), genetic heritability (PRIOR; ROTH; WANG; KAMMERER *et al.*, 2007), hormones (FIELDING; VELLAS; EVANS; BHASIN *et al.*, 2011), inflammation (PEAKE; DELLA GATTA; CAMERON-SMITH, 2010), oxidative stress (MENG; YU, 2010), exercise (FIELDING; VELLAS; EVANS; BHASIN *et al.*, 2011) and dietary intake (CERMAK; RES; DE GROOT; SARIS *et al.*, 2012; FIELDING; VELLAS; EVANS; BHASIN *et al.*, 2011). Regarding dietary intake, although protein seems to be the most important nutrient for muscle mass (CERMAK; RES; DE GROOT; SARIS *et al.*, 2012), other nutrients have also been studied for this purpose, such as omega-3 (ω-3) fatty acids (BELURY; COLE; BAILEY; KE *et al.*, 2016; REINDERS; SONG; VISSER; EIRIKSDOTTIR *et al.*, 2015; ROSSATO; SCHOENFELD; DE OLIVEIRA, 2020).

Omega-3 are polyunsaturated fatty acids that can be found specially in oily fish foods, but it can also be found in seeds and nuts mainly in three different forms: alpha-linolenic acid (ALA; 18:3n-3), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (MOZAFFARIAN; WU, 2011). Since ω-3 has anti-inflammatory effects and can indirectly increase the muscle protein synthesis making the muscle more sensitive to amino acids uptake regardless of age (MCGLORY; CALDER; NUNES, 2019; ROSSATO; SCHOENFELD; DE OLIVEIRA, 2020; SMITH; ATHERTON; REEDS; MOHAMMED *et al.*, 2011a; b), it is possible to suggest that individuals who have higher levels of plasma ω-3 fatty acids (blood marker of ω-3 intake (SUN; MA; CAMPOS; HANKINSON *et al.*, 2007)) would have more muscle mass; however, the current evidence is still limited (ROSSATO; SCHOENFELD; DE OLIVEIRA, 2020).

Few cross-sectional studies have evaluated the association between ω-3 intake and muscle/lean mass in free-living individuals (BELURY; COLE; BAILEY; KE *et al.*, 2016; REINDERS; SONG; VISSER; EIRIKSDOTTIR *et al.*, 2015). Belury et al. (BELURY; COLE; BAILEY; KE *et al.*, 2016) found no association between ω-3 composition in erythrocyte and lean mass in 139 healthy middle-aged individuals. Reinders et al. (REINDERS; SONG; VISSER; EIRIKSDOTTIR *et al.*, 2015) observed no association

between plasma ω-3 polyunsaturated fatty acids and muscle size in older adults (REINDERS; SONG; VISSER; EIRIKSDOTTIR *et al.*, 2015). The null results may reflect limited power to detect an association of small magnitude, and a mixture of effects because they have evaluated men and women together (BELURY; COLE; BAILEY; KE *et al.*, 2016; REINDERS; SONG; VISSER; EIRIKSDOTTIR *et al.*, 2015). Recently, our research group showed a sex-specific association between ω-3 intake and muscle strength in older adults, in which older men (but not women) who ingested more ω-3 presented higher strength (ROSSATO; DE BRANCO; AZEREDO; RINALDI *et al.*, 2020). However, to date, it is unknown whether there is also a sex-dependent association between ω-3 and muscle mass amount. This is an important knowledge gap in the literature since there are sex-differences in ω-3 metabolism (LENG; WINTER; AUKEMA, 2018; MENDONCA; CAYER; PAULS; WINTER *et al.*, 2018).

The limited evidence does not support an association between ω-3 and muscle mass (BELURY; COLE; BAILEY; KE *et al.*, 2016; REINDERS; SONG; VISSER; EIRIKSDOTTIR *et al.*, 2015; ROSSATO; SCHOENFELD; DE OLIVEIRA, 2020); however, more studies are needed evaluating men and women separately, as well as other age-range since the causes of muscle mass loss can be different according to age-range due to different levels of inflammation and anabolic resistance (BREEN; PHILLIPS, 2011; ROUBENOFF, 2007). The aim of the present study was to examine the association between plasma ω-3 fatty acids and appendicular muscle mass index (AMMI) in young and middle-aged individuals. We also aimed to evaluate whether these associations are sex-specific.

Methods

Design and participants

This is a cross-sectional study conducted with data from the National Health and Nutrition Examination Survey (NHANES) from 2011-2012. NHANES is a survey developed by the National Center for Health Statistics (NCHS) to evaluate health and nutritional data for adults and children from a multi-stage representative sample of non-institutionalized people from the United States. NHANES cross-sectional data are public and can be found on the Centers for Disease Control and Prevention (CDC) website. In NHANES 2011-2012, 9756 individuals were evaluated. However, in the present study, individuals aged under 20 years, and with missing information on anthropometric, body

composition, and plasma omega-3 fatty acids data; individuals who did not have dietary data assessment and women who were breastfeeding were excluded. Thus, the present study evaluated 1037 individuals (556 men and 481 women) aged 20-59 years (**Figure 1)**. NHANES is a public data set and all participants provided a written informed consent, consistent with approval from the National Center for Health Statistics Research Ethics Review Board (NCHS ERB) (Protocol #2011-17 for NHANES cycle 2011-2012).

Dietary intake

Dietary intake was evaluated through two 24-hour food recalls for each volunteer. The first 24-hour food recall interview was collected in-person and the second was collected by telephone 3 to 10 days later. For individuals who had two 24-hour food recalls, the mean of nutrients was calculated, while for individuals who had only the first 24-hour food recall (but not the second day) the data for this single day was used (n=97). Dietary data were collected using the Automated Multiple Pass Method (MOSHFEGH; RHODES; BAER; MURAYI *et al.*, 2008), which conducts an interview in 5 steps (quick list, forgotten foods, time and occasion, detail cycle and final probe). To process dietary intake, USDA's Food and Nutrient Database for Dietary Studies (FNDDS) 2011-2012 was used. The underlying nutrient values for FNDDS 2011-2012 were based on values in the USDA National Nutrient Database for Standard Reference, release 26, produced by USDA's Nutrient Data Lab (http://www.ars.usda.gov/nutrientdata). Total ω-3 intake was calculated from the sum of ALA (18:3n-3), DHA (22:6n-3), EPA (20:5n-3), and docosapentaenoic acid (DPA, 22:5n-*3)*. Omega-6 intake was evaluated only by linoleic acid (18:2n-6), since this was the only omega-6 available in 2011-2012 NHANES data.

Body composition

Body composition was assessed using a whole-body dual-energy x-ray absorptiometry (DXA) Hologic Discovery model A densitometers (Hologic, Inc., Bedford, Massachusetts), using software version Apex 3.2. For DXA evaluation, only individuals up to 59 years old were eligible. For this reason, older adults were not included in the present study. In addition, pregnant women, people who used radiographic contrast material in the 7 days prior to the exam and individuals with body weight over 450 pounds (~200 kg) and height over 6'5" (1.96 m) were excluded from the DXA exam.

Appendicular muscle mass index (AMMI) was calculated by dividing appendicular lean mass (legs and arms lean mass excluding bone mass; kg) to height squared (BAUMGARTNER; KOEHLER; GALLAGHER; ROMERO *et al.*, 1998).

Plasma fatty acids

A 0.5-mL sample of plasma or serum was required to allow for repeated analyses and a volume of 100uL was required per analysis. The appropriate amount of plasma or serum was dispensed into a Nalge 2.0-mL cryovial or another plastic screw-capped vial labeled with the specimen identification and stored at -70ºC. Profile of 30 fatty acids was evaluated by gas chromatography – mass spectrometry. Total plasma ω-3 was calculated from the sum of ALA (18:3n-3), EPA (20:5n-3), DHA (22:6n-3), SDA (SDA 18:4n-3) and DPA (22:5n-3). Regarding ω-6 fatty acids, linoleic acid (18:2n-6), arachidonic acid (20:4n-6), eicosadienoic acid (20:2n-6), docosapentaenoic acid (22:5n-6), homo-gamma-linolenic acid (20:3n-6), docosatetraenoic acid (22:4n-6) and gamma-linolenic acid (18:3n-6) were evaluated and the sum of these fatty acids was considered as total plasma ω-6. Total saturated fatty acids were capric acid (C10:0), lauric acid (C12:0), myristic acid (14:0), pentadecanoic acid (C15:0), palmitic acid (16:0), margaric acid (C17:0), stearic acid (18:0), arachidic acid (20:0), docosanoic acid (22:0), tricosanoic acid (C23:0) and lignoceric acid (24:0). All fatty acids were expressed in μmol/L and the blood samples were collected after an overnight fast. More details about plasma fatty acids evaluation are provided by the NHANES manual (NHANES, 2011).

Potential confounders

Several variables were considered as potential confounders and were included as adjustments in the analyses. Demographic data evaluated in the present study were age (years), annual family income (0 to $19,999, $20,000 to 54,999, 55,000 to 74,999 or over $75,000), race (non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, other races), marital status (married, widowed, divorced, separated, never married and living with partner), and educational level (less than 9^{th} grade, 9 to 11^{th} grade, high school graduation, some college and college graduation or above). Smoking status (yes or no), diabetes (pre-diabetes, yes or no), arthritis (yes or no) and hypertension (presence or absence) were evaluated as health conditions and lifestyle habits. Physical

activity level included moderate and vigorous exercises (yes or no), and dietary intake variables included protein (g/day), alcohol (g/day) and total energy (kcal/day). Body fat (kg) was also inserted in the adjustments. Menopause (yes or no) was additionally adjusted for women.

Statistical analyses

Sociodemographic, dietary intake, anthropometric, body composition, physical activity, medical conditions and lifestyle characteristics were described for total sample, and for men and women separately. The comparison between men and women was performed through regression analysis. The continuous variables were described as mean and standard deviation, and the categorical variables were described as percentage and confidence interval. Additionally, a missing category was created for annual family income, diabetes, hypertension, arthritis and smoking status to categorize individuals who had missing data in these variables. Linear regression analysis (coefficients and 95% confidence intervals) was performed to evaluate whether AMMI is associated with total plasma ω-3, DHA, EPA, and ALA. The analyses were performed for total sample and according to sex. A sub analysis was performed evaluating young adults (20 to 44 y) and middle-aged individuals (45 to 59 y) separately. The significance of the associations was considered when p-value <0.05. Analyses were performed without adjustment (model 1) and adjusted for protein, energy and alcohol intakes; smoking status, age, physical activity, annual family income, marital status, race, educational level, menopause (only for women), diabetes, hypertension, arthritis, body fat and sex (only for total sample) (model 2). Since plasma fatty acids were part of a NHANES subsample, all analyses were performed using the "svy" command to incorporate information on the ‘fatty acid subsample 2 year’ sample weight, primary sampling units and strata for correct variance estimation. The analyses were performed using Stata software version 14.0 (StataCorp, College Station, TX, USA).

Results

Individual's characteristics

The characteristics of the total sample and separated by sex are shown in **Table 1**. Evaluating the total sample, the individuals had an average age of 39.6 ± 11.8 years, were

predominantly men, non-Hispanic white, married/living as married, non-smokers, had annual family income over $ 75,000, and a higher education level. The majority did not have diabetes, hypertension and arthritis; and were not physically activity. Most individuals presented adequate AMMI. Men had a lower educational level and a higher proportion of hypertensive individuals, smokers and were more physically active compared to women. Additionally, men had higher weight, height, lean mass (total and appendicular), fat mass (kg and %), higher di-etary intake of energy, carbohydrate, protein, lipids, mono and polyunsaturated fats, total omega-3, ALA, linoleic acid, fiber and alcohol. In relation to plasma fatty acids, men had higher plasma levels of ALA and women had higher levels of plasma DHA.

AMMI and plasma ω-3

Table 2 shows the linear regression between plasma ω-3 levels and AMMI in total sample and according to sex. Plasma ALA was positively associated with AMMI for total sample (but not for men and women separately) in the unadjusted analysis (model 1); however, this association was no longer significant after adjustments for confounders (model 2). In the unadjusted analyses (model 1), total plasma ω-3, DHA, and EPA levels were not associated with AMMI in total sample, as well as for men and women. This lack of association was also observed after adjustments for confounders (model 2).

AMMI and plasma ω-3 according to age-range

Supplemental Table 1 shows the linear regression between plasma ω-3 and AMMI according to specific age-ranges (young and middle-aged individuals). For total sample, plasma ALA was positively associated with AMMI in young adults in the unadjusted analyses. However, this association was no longer significant after adjustments for confounders. No association was observed in young and middle-aged men in models 1 and 2. For women, in the unadjusted analyses, total plasma ω-3, EPA, and DHA were negatively associated with AMMI in middle-aged women, while no association was observed in young individuals. However, plasma ω-3 and its subtypes were not associated with AMMI after adjustments for confounders (model 2) for both young and middle-aged women.

Discussion

We found that total plasma ω-3 and its subtypes (ALA, DHA and EPA) were not associated with AMMI in total sample, neither in subgroups of sex and age. These results suggest that there is no association between plasma ω-3 fatty acids and muscle mass. To the best of our knowledge, this is the first study evaluating this association in a representative sample that included young and middle-aged individuals.

To date, only a limited number of cross-sectional studies have evaluated the association between ω-3 fatty acids and muscle/lean mass (BELURY; COLE; BAILEY; KE *et al.*, 2016; REINDERS; SONG; VISSER; EIRIKSDOTTIR *et al.*, 2015), and these associations were only performed evaluating men and women together and none was conducted with an age-range that included young individuals. Reinders et al. (REINDERS; SONG; VISSER; EIRIKSDOTTIR *et al.*, 2015) evaluated the association between ω-3 plasma fatty acids and thigh cross-sectional muscle area in 836 older adults (66-96 y), whereas Belury et al. (BELURY; COLE; BAILEY; KE *et al.*, 2016) associated the ω-3 composition in erythrocyte with lean mass in 139 middle-aged individuals (~44.3 y). Both studies (BELURY; COLE; BAILEY; KE *et al.*, 2016; REINDERS; SONG; VISSER; EIRIKSDOTTIR *et al.*, 2015) showed that ω-3 was not associated with muscle mass. These results are in agreement with the present study, since the ω-3 plasma fatty acids and its subtypes were not associated with AMMI. Therefore, this lack of association seems to occur independent of the differences between the studies, such as age-range, body composition methods (computed tomography vs. DXA) and forms to assess ω-3 (erythrocyte content vs. plasma). In addition, in the present study, the absence of association remained even after the analyses were performed separated by sex, which shows that a sex-specific association seems unlikely.

It has been proposed that ω-3 may have effects on muscle mass due to its indirect increase on muscle protein synthesis (MCGLORY; CALDER; NUNES, 2019; ROSSATO; SCHOENFELD; DE OLIVEIRA, 2020). Omega-3 fatty acids may alter membrane fluidity of the muscle cells, improving the uptake of amino acids and, consequently, making the cell more sensitive to stimuli for muscle protein synthesis (SMITH; ATHERTON; REEDS; MOHAMMED *et al.*, 2011a). However, we did not find significant associations between ω-3 and muscle mass when young and middle-aged individuals were evaluated. The lack of association in young individuals can possibly be explained because these individuals likely present normal muscle protein synthesis

(BREEN; PHILLIPS, 2011); therefore, the effect of ω-3 in enhancing the protein synthesis may not be relevant. In addition, since middle-aged individuals are at the beginning of the muscle mass loss and anabolic resistance (KELLER; ENGELHARDT, 2013), it is possible to suggest that plasma ω-3 may not have an effect to improve the protein synthesis. Furthermore, it has been speculated that ω-3 may enhance the muscle anabolism mainly when the intake of protein is insufficient to stimulate a maximum muscle protein synthesis (ROSSATO; SCHOENFELD; DE OLIVEIRA, 2020). The individuals evaluated in the present study ingested adequate amounts of protein (~1.0 g/kg/day), which seems to be an optimal dose for muscle maintenance in young and middle-aged individuals (CAMPBELL; JOHNSON; MCCABE; CARNELL, 2008). Therefore, we showed that plasma ω-3 was not associated with muscle mass in individuals who ingested adequate amounts of protein, but this relationship is still unknown in individuals ingesting low protein intake.

Since inflammation is one of the causes of muscle wasting (BREEN; PHILLIPS, 2011; ROUBENOFF, 2007), individuals ingesting higher amounts of ω-3 can have reduced pro-inflammatory cytokines (MCGLORY; CALDER; NUNES, 2019) preserving muscle mass (SHARMA; DABUR, 2020). However, no association was observed in the present study, even when men and women were evaluated separately. Studies conducted with rats show that there are sex-differences in ω-3 metabolism (LENG; WINTER; AUKEMA, 2018; MENDONCA; CAYER; PAULS; WINTER *et al.*, 2018), which justifies the evaluation according to the sex. Oxylipins (the main mediators of PUFAs anti-inflammatory effects in the body (GABBS; LENG; DEVASSY; MONIRUJJAMAN *et al.*, 2015)) are predominantly higher in rat males (LENG; WINTER; AUKEMA, 2018; MENDONCA; CAYER; PAULS; WINTER *et al.*, 2018); however, these oxylipins were measured in adipose tissue (MENDONCA; CAYER; PAULS; WINTER *et al.*, 2018), kidney, liver and serum (LENG; WINTER; AUKEMA, 2018); and the formation of oxylipins in muscle cell is unknown. Nevertheless, it would be possible that men may have greater formation of oxylipins in muscle cells than women, which could contribute for greater anti-inflammatory effects on muscle mass, resulting in higher muscle maintenance. However, despite the possible sex-differences in the ω-3 metabolism, the present study showed no association between plasma ω-3 and AMMI both in men and women. This lack of association can be possible explained because the muscle mass loss induced by inflammation occurs mainly in older adults or in specific

clinical conditions (LONDHE; GUTTRIDGE, 2015; PEAKE; DELLA GATTA; CAMERON-SMITH, 2010), and may have few effects on muscle mass loss in young and middle-aged individuals. Future studies should be performed evaluating the association or effect of ω-3 in other populations according to the sex.

The present study has limitations. The cross-sectional design does not allow to determine a cause-effect relationship. Our data cannot be extrapolated for older adults, people with sarcopenia and/or muscle wasting diseases, and for individuals ingesting low protein intake. Since the analyzes are representative of the USA population, these data are not valid for individuals from other countries. As strengths, we evaluated plasma ω-3, which are blood markers that estimate ω-3 consumption for 2-4 weeks; however, as red blood cells reflect a longer ω-3 intake (4-6 months), future studies should also evaluate the association of red blood cells fatty acid content with muscle mass (ARTERBURN; HALL; OKEN, 2006). Appendicular lean mass was evaluated through DXA, which is a reliable body composition method. Additionally, the large sample size increased the power to detect associations of small magnitude, and the sample design allowed to evaluate a representative U.S. population, ensuring generalizability of our results. Finally, we adjusted our analyses for several important covariates, reducing the risk of bias due to confounding.

Conclusion

Plasma ω-3 fatty acids are not associated with AMMI in young and middle-aged individuals regardless of sex. Longitudinal studies should be performed to confirm the absence of association between plasma ω-3 and muscle mass.

Conflicts of interest

The authors declare no conflicts of interest.

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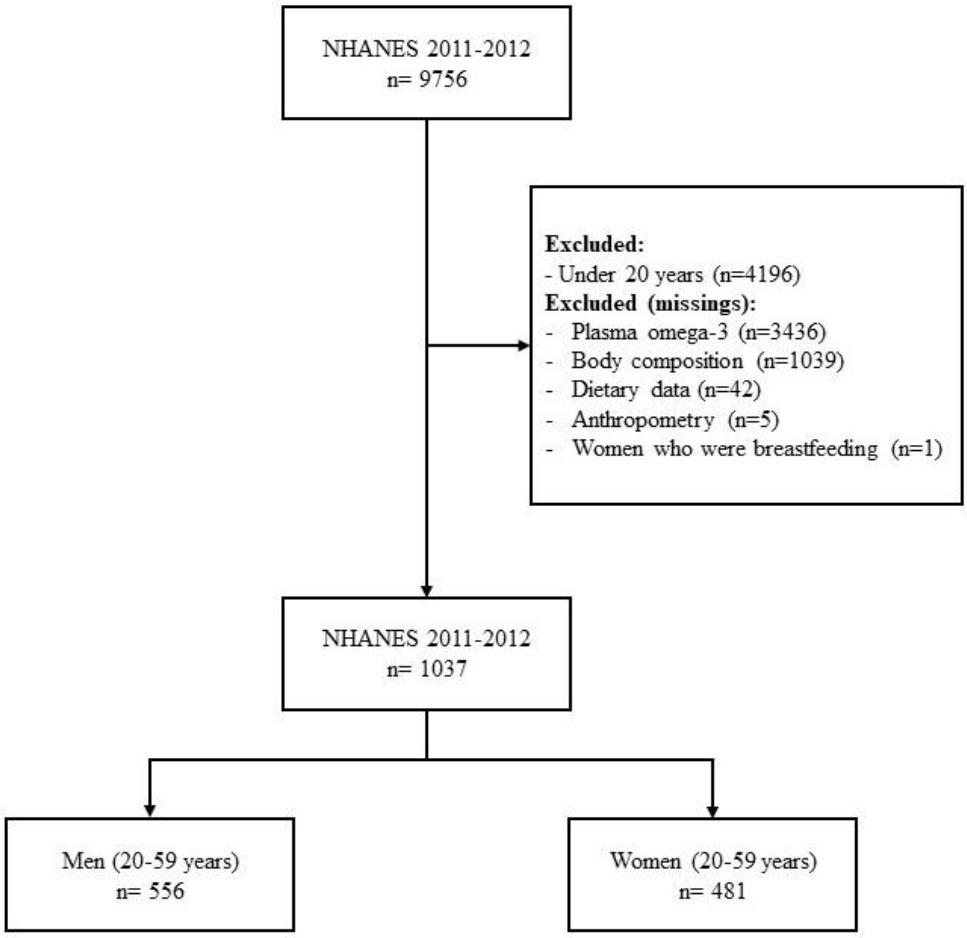

Figure 1. Flowchart of the sample selection from NHANES 2011-2012.

Table 1. Sociodemographic, anthropometric and body composition characteristics in total sample and according to sex. NHANES, 2011-2012.

Variables	Total	Men	Women	p-value
Age, years	39.6 (11.8)	39.3 (11.5)	39.9 (12.1)	0.578
20-44y, %	61.7 (54.7-68.2)	65.3 (57.6-72.3)	57.7 (48.9-66.1)	0.084
45-59y, %	38.8 (31.7-45.3)	34.7 (27.7-42.4)	42.3 (33.9-51.1)	
Non-Hispanic white, %	65.4 (56.7-73.2)	66.7 (57.3-74.9)	64.1 (54.8-72.4)	0.375
Sex, %				
Men	52.4 (48.3-56.5)	-	-	-
Women	47.6 (43.5-51.7)	-	-	-
Marital status, %				0.846
Single/divorced/widowed/never married	39.5 (34.3-44.9)	39.8 (34.1-45.8)	39.1 (32.4-46.3)	
Married/ living as married	60.5 (55.1-65.7)	60.2 (54.2-65.9)	60.9 (53.7-67.6)	
Annual family income, %				
$0-19.999	18.3 (13.6-24.2)	16.6 (11.5-23.3)	20.2 (13.6-28.8)	0.361
$20.000-54.999	32.8 (27.9-38.2)	33.2 (27.1-39.8)	32.5 (26.8-38.7)	
$55.000-74.999	11.7 (8.0-16.6)	11.0 (6.8-17.2)	12.4 (8.5-17.4)	
Over $75.000	34.3 (28.0-41.3)	36.3 (29.0-44.2)	32.2 (25.1-40.2)	
Missing	2.9 (1.9-4.2)	2.9 (1.5-5.7)	2.7 (1.9-4.0)	
Educational level, %				0.013
High school graduate or under	35.2 (29.0-41.9)	40.0 (32.3-48.2)	29.9 (23.6-37.0)	
Some college or above	64.8 (58.0-71.0)	60.0 (51.7-67.7)	70.1 (62.9-76.3)	
Health conditions and habits, %				
Hypertension	23.1 (18.6-28.3)	25.9 (20.3-32.4)	20.0 (15.8-25.2)	0.031
Missing	0.12 (0.02-0.65)	0.10 (0.02-0.46)	0.14 (0.02-1.11)	
Diabetes				0.501
Pre-diabetes	1.2 (0.5-3.2)	1.2 (0.3-3.9)	1.3 (0.5-3.7)	
Yes	5.0 (3.3-7.4)	4.8 (3.0-7.9)	5.2 (3.3-8.1)	
No	93.7 (91.3-95.4)	94.0 (91.8-95.6)	93.4 (90.1-95.5)	
Missing	0.1 (0.05-0.4)	-	0.1 (0.01-0.8)	
Smoking				0.016
Yes	22.1 (17.6-27.5)	26.4 (19.6-34.4)	17.5 (13.7-22.0)	
No	77.8 (72.5-82.3)	73.5 (65.4-80.3)	82.5 (78.0-86.3)	
Missing	0.1 (0.07-0.5)	0.1 (0.01-0.9)	-	
Arthritis				0.106
Yes	14.2 (11.4-17.4)	12.2 (9.0-16.2)	16.4 (12.2-21.6)	
No	85.7 (82.4-88.5)	87.8 (83.8-90.9)	83.5 (78.2-87.6)	
Missing	0.1 (0.01-0.7)	-	0.1 (0.02-1.4)	
Physical activity %				
Moderate PA				0.004
Yes	40.4 (36.0-44.8)	46.0 (39.7-52.5)	34.1 (29.3-39.3)	
No	59.6 (55.1-63.9)	54.0 (47.5-60.3)	65.8 (60.7-70.7)	
Vigorous PA				<0.001
Yes	23.8 (19.4-28.8)	33.7 (26.9-41.2)	12.9 (8.6-18.8)	
No	76.2 (71.2-80.5)	66.3 (58.7-73.0)	87.1 (81.1-91.4)	
Anthropometrics				

Weight, kg	82.6 (19.9)	88.7 (18.1)	75.8 (19.6)	<0.001
Height, m	1.69 (0.09)	1.76 (0.06)	1.62 (0.07)	<0.001
Body mass index, kg/m^2	28.5 (6.1)	28.5 (5.4)	28.5 (6.8)	0.890
Body composition				
Total lean mass, kg	53.1 (12.9)	61.6 (9.7)	43.7 (8.7)	<0.001
Appendicular lean mass, kg	23.2 (6.3)	27.5 (4.6)	18.4 (4.1)	<0.001
AMMI, kg/m^2	7.92 (1.63)	8.85 (1.27)	6.90 (1.35)	<0.001
Total fat mass, kg	27.4 (10.9)	24.9 (9.6)	30.1 (11.5)	<0.001
Total fat mass, %	32.6 (8.2)	27.2 (5.8)	38.7 (6.0)	<0.001
Plasma Fatty Acids				
Total plasma ω-3, µmol/L	361 (153)	363 (161)	359 (143)	0.618
ALA, µmol/L	92.1 (50.5)	95.8 (56.5)	88.1 (42.5)	0.019
EPA, µmol/L	62.4 (46.7)	63.1 (44.4)	61.7 (49.1)	0.615
DHA, µmol/L	150.6 (69.9)	145.2 (68.1)	156.5 (71.3)	0.016
Linoleic acid (ω-6), µmol/L	3809 (846)	3844 (905)	4772 (775)	0.207
Dietary intake				
Energy, kcal	2210 (841)	2531 (860)	1855 (657)	<0.001
Carbohydrate, g	271 (108)	305 (114)	233 (87.0)	<0.001
Protein, g	85.0 (36.2)	98.1 (39.0)	70.5 (26.1)	<0.001
Protein, g/kg	1.06 (0.47)	1.14 (0.51)	0.97 (0.41)	<0.001
Lipids, g	82.4 (38.8)	94.1 (40.3)	69.6 (32.8)	<0.001
Saturated fat, g	26.5 (13.4)	30.2 (13.8)	22.4 (11.7)	<0.001
Monounsaturated fat, g	29.5 (14.8)	33.9 (15.5)	24.6 (12.3)	<0.001
Polyunsaturated fat, g	19.5 (10.4)	22.1 (11.1)	16.7 (8.9)	<0.001
Total ω-3, g	1.88 (1.08)	2.08 (1.14)	1.67 (0.96)	0.001
ALA, g	1.78 (1.03)	1.96 (1.09)	1.57 (0.92)	0.001
EPA, g	0.02 (0.06)	0.03 (0.07)	0.02 (0.05)	0.167
DHA, g	0.06 (0.12)	0.06 (0.12)	0.05 (0.11)	0.150
Linoleic acid, g	17.3 (9.4)	19.6 (10.0)	14.8 (8.0)	<0.001
Fiber, g	18.2 (9.2)	19.7 (9.8)	16.5 (8.3)	<0.001
Alcohol, g	10.4 (23.7)	14.4 (29.4)	6.0 (13.9)	<0.001

Notes: DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; ALA: alpha linolenic acid.
Data described as mean (standard deviation) or percentage (confidence interval).

Table 2. Linear regression between plasma omega-3 and appendicular muscle mass index in individuals aged 20-59 y. NHANES, 2011-2012.

	Model 1			**Model 2**		
	β	95%CI	p-value	β	95%CI	p-value
ω-3						
Total	-0.00005	-0.0009; 0.0008	0.912	-0.0002	-0.0005; 0.0001	0.255
Men	0.00008	-0.0007; 0.0009	0.820	-0.00009	-0.0006; 0.0004	0.695
Women	-0.0004	-0.0014; 0.0006	0.375	-0.0003	-0.0007; 0.0002	0.243
ALA						
Total	0.0020	0.0006; 0.0035	0.009	-0.0007	-0.0019; 0.0004	0.200
Men	0.0002	-0.0014; 0.0018	0.793	-0.0009	-0.0022; 0.0004	0.172
Women	0.0014	-0.0024; 0.0052	0.454	-0.0004	-0.0023; 0.0014	0.650
EPA						
Total	-0.0003	-0.0036; 0.0029	0.837	-0.0004	-0.0017; 0.0010	0.582
Men	0.0001	-0.0029; 0.0032	0.918	-0.00006	-0.0023; 0.0023	0.998
Women	-0.0013	-0.0039; 0.0012	0.151	-0.0007	-0.0019; 0.0004	0.186
DHA						
Total	-0.0017	-0.0039; 0.0004	0.101	0.00009	-0.0010; 0.0012	0.868
Men	0.0002	-0.0020; 0.0024	0.849	0.0006	-0.0008; 0.0020	0.377
Women	-0.0014	-0.0035; 0.0005	0.144	-0.0001	-0.0012; 0.0010	0.768

Notes: DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; ALA: alpha linolenic acid. Model 1: Without adjustment. Model 2: Total sample: adjusted for protein, energy and alcohol intakes, smoking, age, sex, physical activity, family income, marital status, race, educational level, diabetes, hypertension, arthritis and body fat. Analyses performed by sex did not include "sex" in adjustment. For women, menopause was included as adjustment. Values shown as coefficients and 95% confidence intervals (95%CI).

Supplementary Table 1. Linear regression between plasma omega-3 and appendicular muscle mass index according to sex and age-range. NHANES, 2011-2012.

	Total sample						Men						Women					
	Model 1			Model 2			Model 1			Model 2			Model 1			Model 2		
	β	95%CI	p-value	β	95%CI	p-value	β	95%CI	p-value	β	95%CI	p-value	β	95%CI	p-value	β	95%CI	p-value
ω-3																		
20-44 y	0.0006	-0.0010; 0.0022	0.428	0.00008	-0.0006; 0.0008	0.815	0.0010	-0.0009; 0.0029	0.297	0.0001	-0.0010; 0.0013	0.814	0.0002	-0.0013; 0.0017	0.777	-0.00008	-0.0009; 0.0007	0.817
45-59 y	-0.0030	-0.0014; 0.0008	0.585	-0.0003	-0.0009; 0.0003	0.284	-0.0003	-0.0014; 0.0007	0.490	-0.0002	-0.0010; 0.0006	0.597	-0.0011	-0.0021; -0.0001	0.028	-0.0003	-0.0009; 0.0003	0.320
ALA																		
20-44 y	0.0043	0.0006; 0.0081	0.026	-0.0003	-0.0028; 0.0021	0.765	0.0019	-0.0020; 0.0058	0.323	-0.0003	-0.0032; 0.0027	0.850	0.0027	-0.0030; 0.0084	0.337	-0.0003	-0.0030; 0.0023	0.785
45-59 y	0.0005	-0.0021; 0.0031	0.704	-0.0007	-0.0024; 0.0010	0.378	-0.0010	-0.0041; 0.0021	0.517	-0.0011	-0.0032; 0.0009	0.248	0.0002	-0.0048; 0.0052	0.930	-0.0005	-0.0022; 0.0012	0.558
EPA																		
20-44 y	0.0020	-0.0045; 0.0085	0.519	-0.0002	-0.0019; 0.0015	0.782	0.0031	-0.0048; 0.0112	0.414	0.0001	-0.0045; 0.0047	0.957	-0.00001	-0.0042; 0.0041	0.993	-0.0006	-0.0026; 0.0014	0.542
45-59 y	-0.0010	-0.0042; 0.0020	0.489	-0.0004	-0.0024; 0.0014	0.604	-0.0007	-0.0041; 0.0026	0.627	-0.0003	-0.0042; 0.0036	0.887	-0.0029	-0.0050; -0.0008	0.039	-0.0004	-0.0021; 0.0012	0.583
DHA																		
20-44 y	-0.0021	-0.0052; 0.0009	0.154	0.0009	-0.0007; 0.0026	0.238	0.0015	-0.0020; 0.0052	0.375	0.0011	-0.0009; 0.0032	0.275	-0.0003	-0.0031; 0.0025	0.825	0.0005	-0.0011; 0.0021	0.527
45-59 y	-0.0010	-0.0037; 0.0015	0.398	-0.0004	-0.0017; 0.0009	0.502	-0.0002	-0.0031; 0.0026	0.865	0.0003	-0.0016; 0.0022	0.721	-0.0027	-0.0048; -0.0007	0.012	-0.0005	-0.0021; 0.0011	0.522

Notes: DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; ALA: alpha linolenic acid. Model 1: Without adjustment. Model 2: adjusted for protein, energy and alcohol intake, smoking, age, sex, physical activity, family income, marital status, race, educational level diabetes, hypertension, arthritis, body fat. Analyses performed by sex did not include "sex" in adjustment. For women, menopause was included as adjustment. Values shown as coefficients and 95% confidence intervals (95%CI).

Original Article

Plasma omega-3 fatty acids are positively associated with appendicular muscle mass index only in adults ingesting low protein intake: Results from NHANES 2011-2012

Abstract

Background: Omega-3 (ω-3) fatty acids can indirectly increase the muscle protein synthesis making the muscle more sensitive to amino acids uptake; therefore, omega-3 may promote benefits on muscle mass mainly when protein intake is low. However, no study has evaluated the association between ω-3 and muscle mass according to protein intake. **Aim:** To evaluate the association of plasma ω-3 with the appendicular muscle mass index (AMMI) in adults according to the protein intake. **Methods:** Cross-sectional study that evaluated 1037 individuals aged from 20 to 59 years from the National Health and Nutrition Examination Survey (NHANES) 2011-2012. Gas chromatography mass spectrometry method was used to assess plasma ω-3 fatty acids. The lean mass was evaluated by dual-energy x-ray absorptiometry (DXA) and AMMI (kg/m^2) was calculated by appendicular lean mass (kg) divided by height squared. Linear regression analysis was performed to assess the association between total plasma ω-3, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), alpha-linolenic acid (ALA), and EPA+DHA with AMMI, according to protein intake (<0.8 g/kg and ≥0.8 g/kg; <0.8 g/kg, 0.8 - 1.2 g/kg, 1.2 - 1.6 g/kg and >1.6 g/kg). **Results:** Plasma ω-3, ALA, EPA, DHA and EPA+DHA were positively associated with AMMI in individuals who had low protein intake (<0.8 g/kg). No association was observed in individuals with protein intake more than 0.8 g/kg. **Conclusion:** Plasma ω-3 fatty acids were positively associated with AMMI only in individuals with low protein intake.

Key-words: Lean mass; Omega-3; Polyunsaturated fatty acids; Protein intake.

Introduction

Omega-3 (ω-3) fatty acids are long chain polyunsaturated fatty acids and their main forms are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are mainly found in fatty fish (MCGLORY; CALDER; NUNES, 2019). Additionally, alpha-linolenic acid (ALA), another ω-3 type, can be found in plant sources and can be endogenously converted to EPA and DHA, making them conditionally essential (MCGLORY; CALDER; NUNES, 2019). Omega-3 has been studied for improvements in in lipid profile, blood pressure (CABO; ALONSO; MATA, 2012), inflammation (CALDER, 2017), and cognition (CARDOSO; AFONSO; BANDARRA, 2016); but more recently the effects of ω-3 intake on muscle mass have been also investigated (ROSSATO; SCHOENFELD; DE OLIVEIRA, 2020).

It has been speculated that ω-3 indirectly increases muscle protein synthesis (MPS) through incorporation into cell membranes, favoring cellular amino acid uptake (DA BOIT; SIBSON; SIVASUBRAMANIAM; MEAKIN *et al.*, 2017; ROSSATO; SCHOENFELD; DE OLIVEIRA, 2020). The influences of protein intake or infusion on MPS stimulated by ω-3 has been discussed (MCGLORY; WARDLE; MACNAUGHTON; WITARD *et al.*, 2016; SMITH; ATHERTON; REEDS; MOHAMMED *et al.*, 2011). Smith et al. (SMITH; ATHERTON; REEDS; MOHAMMED *et al.*, 2011) showed that ω-3 supplementation for 8-weeks increased the anabolic stimulus by 50% after the infusion of amino acids in middle-aged individuals. However, McGlory et al. (MCGLORY; WARDLE; MACNAUGHTON; WITARD *et al.*, 2016) evaluated the effect of ω-3 supplementation for 8 weeks in young men who performed an acute bout of unilateral resistance exercise followed by the intake of 30 g of whey protein, and ω-3 supplementation did not increase the MPS. These discrepant findings can be partially explained due to differences in protein administration; whereas McGlory et al. (MCGLORY; WARDLE; MACNAUGHTON; WITARD *et al.*, 2016) used an optimal dose to stimulate MPS through whey protein intake; Smith et al. (SMITH; ATHERTON; REEDS; MOHAMMED *et al.*, 2011) performed continuous amino acid infusion which simulated a suboptimal protein dose. Thus, it is likely that a greater amount of ω-3 in the cell may increase the MPS when the dose of protein is insufficient to stimulate a maximum MPS response. Therefore, it is possible to speculate that ω-3 would also have benefits on muscle mass mainly in individuals who ingest protein below

the recommendation. However, no study has evaluated the relationship between ω-3 and muscle mass according to the protein intake.

The Recommended Dietary Allowance (RDA) (PADOVANI; AMAYA-FARFÁN; COLUGNATI; DOMENE, 2006) of protein for adults is 0.8 g/kg/day, and a protein intake lower than this amount is considered inadequate. Thus, the aim of the present study was to evaluate the association of plasma ω-3 with the AMMI in young and middle-aged individuals, according to the protein intake recommendation established by the RDA. We hypothesized that plasma omega-3 would be associated with lean mass only in individuals with low protein intake.

Methods

Design and participants

This is a cross-sectional study using data from the National Health and Nutrition Examination Survey (NHANES) from 2011-2012. NHANES is a survey conducted by the National Center for Health Statistics (NCHS) to evaluate health and nutritional data from a multi-stage representative sample of non-institutionalized people from the United States. In NHANES 2011-2012, 9756 individuals were evaluated. Individuals who had missing information in the main variables such as anthropometric, body composition, and plasma omega-3 fatty acids data, individuals aged under 20 years, who did not have dietary data assessment and women who were breastfeeding were excluded. Therefore, our study included 1037 individuals, who were further divided according to protein intake: <0.8 g/kg/day or ≥0.8 g/kg/day (**Figure 1**). All participants provided a written informed consent, consistent with approval from the National Center for Health Statistics Research Ethics Review Board (NCHS ERB) (Protocol #2011-17 for NHANES cycle 2011-2012) and the dataset is publicly available (https://www.cdc.gov/nchs/nhanes/irba98.htm).

Dietary intake

The evaluation of dietary intake was performed through two 24-hour food recalls for each individual and the first 24-hour food recall was collected in-person and the second was performed by telephone 3 to 10 days later. The Automated Multiple Pass Method (MOSHFEGH; RHODES; BAER; MURAYI *et al.*, 2008) was used to collected dietary data, which consists in an interview in 5 steps (quick list, forgotten foods, time

and occasion, detail cycle and final probe) and USDA's Food and Nutrient Database for Dietary Studies (FNDDS) 2011-2012 was used to process dietary data. The usual dietary intake was estimated with National Cancer Institute (NCI) method (TOOZE; KIPNIS; BUCKMAN; CARROLL *et al.*, 2010). The sum of ALA (18:3n-3), DHA (22:6n-3), EPA (20:5n-3), and docosapentaenoic acid (DPA, 22:5n-*3*) was considered as total ω-3 intake. Omega-6 intake was evaluated only by linoleic acid (18:2n-6), since this was the only omega-6 available in 2011-2012 NHANES data.

Body composition

A whole-body dual-energy x-ray absorptiometry (DXA) Hologic Discovery model A densitometers (Hologic, Inc., Bedford, Massachusetts) and software version Apex 3.2 were used to asses body composition. Due to the eligibility of DXA examination, people over 59 years were excluded from the study, as well as pregnant women, individuals who used radiographic contrast material in the 7 days prior to the exam and individuals with body weight over 450 pounds (~200 kg) and height over 6'5" (1.96 m). For the calculation of the AMMI appendicular lean mass (legs and arms lean mass excluding bone mass in kilograms) was divided to height squared (BAUMGARTNER; KOEHLER; GALLAGHER; ROMERO *et al.*, 1998).

Plasma fatty acids

For the analysis of blood fatty acids, the sample was collected after an overnight fast. A 0.5-mL sample of plasma or serum was required to allow for repeated analyses and a volume of 100uL was required per analysis. The appropriate amount of plasma or serum was dispensed into a Nalge 2.0-mL cryovial or another plastic screw-capped vial labeled with the specimen identification and stored at -70ºC. Fatty acids were evaluated by gas chromatography – mass spectrometry. Total plasma ω-3 was calculated from the sum of ALA (18:3n-3), EPA (20:5n-3), DHA (22:6n-3), SDA (SDA 18:4n-3) and DPA (22:5n-*3)*. Regarding ω-6 fatty acids, linoleic acid (18:2n-6) was considered as plasma ω-6. All fatty acids were expressed in μmol/L. More details about plasma fatty acids analysis are available on the NHANES manual (NHANES, 2011).

Potential confounders

We assessed some demographic, health conditions and lifestyle habits, body composition and dietary variables as potential confounders to the association between

plasma ω-3 and AMMI. Demographic data were: age (years), annual family income (0 to $19,999, $20,000 to 54,999, 55,000 to 74,999 or over $75,000) and race (non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, other races). As health conditions and lifestyle habits we assessed smoking status (yes or no), arthritis (yes or no) and physical activity level (moderate and vigorous exercises; yes or no) were included. Finally, we assessed alcohol consumption (g/day), total energy intake (kcal/day), and body fat (kg).

Statistical analyses

Sociodemographic, anthropometric, dietary intake, body composition, physical activity, medical conditions, and lifestyle data were described for total sample and according to protein intake (<0.8 g/kg/day or ≥0.8 g/kg/day). Due to missing data in some categorical variables, a missing category was created for annual family income, diabetes, hypertension, arthritis and smoking status. Associations between plasma ω-3, DHA, EPA, and ALA and AMMI were evaluated through linear regression models according to protein intake. Analyses were performed without adjustment (model 1) and energy and alcohol intakes, smoking, age, sex, physical activity, family income, race, arthritis and body fat (model 2). The significance of the associations was considered when p-value <0.05. Since plasma fatty acids were part of a NHANES subsample, all analyses were performed using the "svy" command to incorporate information on the 'fatty acid subsample 2 year' sample weight, primary sampling units and strata for correct variance estimation. The analyses were performed using Stata software version 14.0 (StataCorp, College Station, TX, USA).

Results

Individual's characteristics

Characteristics of the total sample and according to protein intake are shown in **Table 1.** Individuals with adequate protein intake had lower body weight, height, BMI, total and appendicular lean mass, AMMI and fat mass (kg and %), and a lower proportion of non-Hispanic whites, diabetic and hypertensive individuals compared with those with inadequate protein intake. In addition, individuals with adequate protein intake ingested more energy, carbohydrate, protein (g and g/kg), lipids, saturated, monounsaturated and

polyunsaturated fats, total ω-3, linoleic acid and fiber. They also had higher levels of plasma EPA compared to individuals with low protein intake.

AMMI and plasma ω-3 according to protein intake <0.8 g/kg and ≥0.8 g/kg

Table 2 shows the linear regression between plasma ω-3 levels and AMMI according to protein intake. No association was observed between plasma ω-3 and AMMI in the unadjusted model (Model 1). However, after adjustments for confounders, plasma ω-3, ALA, EPA, DHA and EPA+DHA were positively associated with AMMI in individuals with low protein intake. No association was found in individuals who had adequate protein intake (Model 2).

AMMI and plasma ω-3 according to protein intake <0.8 g/kg, 0.8 - 1.2 g/kg, 1.2 - 1.6 g/kg and >1.6 g/kg

Plasma ALA and DHA were positively associated with AMMI only in individuals with a protein intake between 1.2 and 1.6 g/kg in the unadjusted model (Model 1). However, after adjustments for confounders, plasma ω-3, ALA, EPA, DHA and EPA+DHA were positively associated with AMMI in individuals with low protein intake (<0.8 g/kg) (**Table 3**).

Discussion

The main finding of the present study was that plasma ω-3, ALA and DHA were positively associated with AMMI only in individuals ingesting low protein (< 0.8 g/kg/day). These results suggest that the association between ω-3 and muscle mass seems to be influenced by daily protein intake. To the best of our knowledge, this is the first study evaluating this association in a representative sample of adults.

To date, the association between ω-3 and muscle mass is not fully elucidated, since only a few cross-sectional studies have evaluated this association (BELURY; COLE; BAILEY; KE *et al.*, 2016; REINDERS; SONG; VISSER; EIRIKSDOTTIR *et al.*, 2015). Reinders et al. (REINDERS; SONG; VISSER; EIRIKSDOTTIR *et al.*, 2015) conducted a study with 836 older adults (66-96 y) and evaluated the association between ω-3 plasma fatty acids and thigh cross-sectional muscle area. Additionally, Belury et al. (BELURY; COLE; BAILEY; KE *et al.*, 2016) evaluated the association between the ω-3 composition in erythrocyte with lean mass in 139 middle-aged individuals (~44.3 y). Both studies

(BELURY; COLE; BAILEY; KE *et al.*, 2016; REINDERS; SONG; VISSER; EIRIKSDOTTIR *et al.*, 2015) found no association between ω-3 and muscle mass. However, daily protein intake was not evaluated and maybe the lack of association could be partially explained due to adequate protein intake in both studies.

A study by McGlory and colleagues (MCGLORY; WARDLE; MACNAUGHTON; WITARD *et al.*, 2016) evaluated the muscle protein synthesis after 8 weeks of ω-3 or coconut oil supplementation followed by the ingestion of 30 g of whey protein. The authors found that ω-3 supplementation suppressed the anabolic signaling, without affecting MPS. This result can be explained because the intake of 30 g of protein is known to maximally stimulate MPS (MOORE; CHURCHWARD-VENNE; WITARD; BREEN *et al.*, 2015), and; therefore, ω-3 supplementation in this scenario would not lead to greater benefits in MPS. In contrast, Smith et al. (SMITH; ATHERTON; REEDS; MOHAMMED *et al.*, 2011) found an increase in MPS after supplementation of ω-3 for 8 weeks with the infusion of amino acids and insulin. The infusion of amino acids stimulates MPS submaximally, so in this case, ω-3 could influence the increase in MPS. The mechanism by which ω-3 increases MPS is through incorporation into cell membranes, favors cellular amino acid uptake, thus increasing MPS (DA BOIT; SIBSON; SIVASUBRAMANIAM; MEAKIN *et al.*, 2017; ROSSATO; SCHOENFELD; DE OLIVEIRA, 2020). Therefore, for individuals who have an inadequate daily protein intake, ω-3 could optimize the uptake of amino acids, which may benefit muscle mass.

Our study has some limitations. A cause-effect relationship cannot be determined due to the cross-sectional design of the study. In addition, our results cannot be extrapolated to older adults, since this population was not evaluated in the present study. As strengths we can highlight the representative sample of US individuals, which increases the power to detect associations of small magnitude and the generalizability of the results. In addition, plasma ω-3 were evaluated, these are blood markers of long-term (2-4 weeks) ω-3 consumption. A reliable body composition method (DXA) was used to assess AMMI. Finally, linear regression analyses were adjusted for several important cofounder covariates, which reduces the risk of bias.

Conclusion

Plasma ω-3 fatty acids were positively associated with AMMI only in individuals with low protein intake. This is the first study to show this association according to daily

protein intake. These findings suggest that ω-3 could have benefits in muscle mass when protein intake is inadequate.

Conflicts of interest

The authors declare no conflicts of interest.

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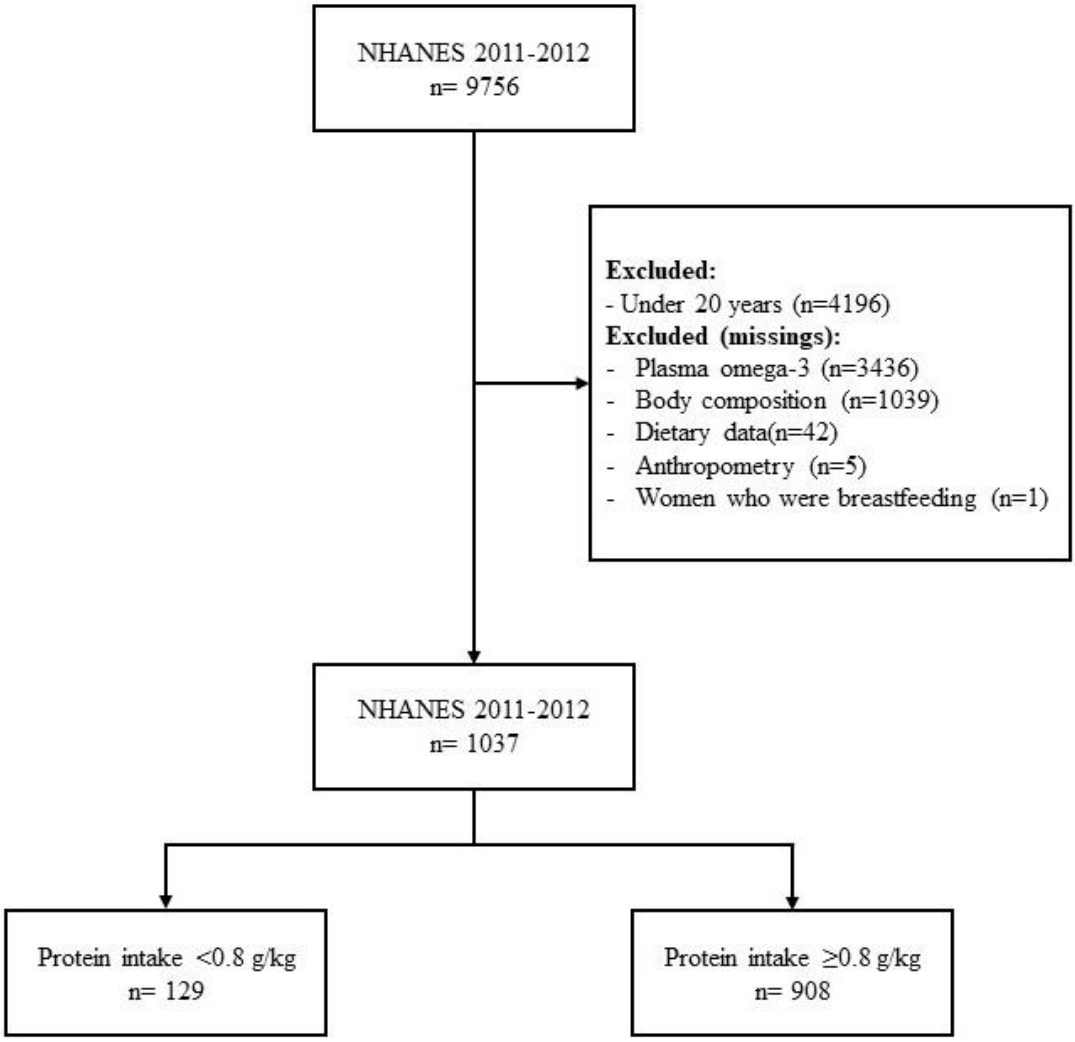

Figure 1. Flowchart of the sample selection from NHANES 2011-2012.

Table 1. Sociodemographic, anthropometric and body composition characteristics according to protein intake. NHANES, 2011-2012.

Variables	Total sample	Protein intake <0.8 g/kg	Protein intake ≥0.8 g/kg	p-value
Age, years	39.6 (11.8)	40.1 (11.6)	39.5 (11.8)	0.733
Non-Hispanic white, %	65.4 (56.7-73.2)	70.6 (61.0-78.6)	64.8 (55.9-72.7)	0.031
Sex, %				0.164
Men	52.4 (48.3-56.4)	58.2 (48.7-67.0)	51.6 (47.3-55.9)	
Women	47.6 (43.5-51.7)	41.8 (32.9-51.2)	48.4 (44.1-52.6)	
Marital status, %				0.242
Single/divorced/widowed/never married	39.5 (34.3-44.9)	45.5 (31.8-59.9)	38.7 (33.9-43.6)	
Married/ living as married	60.5 (55.1-65.7)	54.5 (40.1-68.1)	61.3 (56.3-66.1)	
Annual family income, %				0.145
$0-19.999	18.3 (13.6-24.2)	27.8 (13.3-49.2)	17.0 (12.6-22.5)	
$20.000-54.999	32.8 (27.9-38.2)	37.8 (23.6-54.4)	32.2 (27.7-37.0)	
$55.000-74.999	11.7 (8.0-16.7)	11.5 (5.3-23.3)	11.7 (8.1-16.6)	
Over $75.000	34.3 (28.0-41.3)	18.6 (10.0-31.9)	36.4 (29.7-43.7)	
Missing	2.9 (1.9-4.2)	4.3 (0.9-17.7)	2.7 (2.0-3.6)	
Educational level, %				0.472
High school graduate or under	35.2 (29.0-41.9)	30.1 (17.5-46.6)	35.9 (29.0-43.4)	
Some college or above	64.8 (58.0-71.0)	69.9 (53.4-82.5)	64.1 (56.6-71.0)	
Health conditions and habits				
Hypertension, %	23.1 (18.6-28.3)	42.0 (32.2-52.4)	20.6 (16.0-26.1)	0.001
Missing	0.1 (0.02-0.6)	0.2 (0.03-1.9)	0.1 (0.02-0.5)	
Diabetes, %				0.010
Pre-diabetes	1.2 (0.5-3.2)	5.0 (1.4-16.5)	0.7 (0.006-2.0)	
Yes	5.0 (3.3-7.4)	9.5 (6.6-13.5)	4.4 (2.6-7.3)	
No	93.7 (91.3-95.4)	85.5 (76.6-91.4)	94.8 (92.0-96.6)	
Missing	0.1 (0.005-0.4)	-	0.1 (0.006-0.5)	
Smoking %				0.100
Yes	22.1 (17.6-27.5)	30.9 (20.5-43.7)	20.9 (16.2-26.7)	
No	77.8 (72.5-82.3)	69.1 (56.3-79.5)	79.0 (73.2-83.8)	
Missing	0.1 (0.007-0.5)	-	0.1 (0.007-0.5)	
Arthritis				0.295
Yes	14.2 (11.4-17.4)	18.7 (10.4-31.2)	13.6 (10.7-17.0)	
No	85.7 (82.5-88.5)	81.3 (68.7-89.6)	86.3 (82.7-89.2)	
Missing	0.1 (0.009-0.7)	-	0.1 (0.01-0.8)	
Physical activity %				
Moderate PA				0.412
Yes	40.4 (36.0-44.8)	43.8 (34.0-54.1)	39.9 (35.5-44.5)	
No	59.6 (55.1-63.9)	56.2 (45.9-66.0)	60.1 (55.5-64.5)	
Vigorous PA				
Yes	23.8 (19.4-28.8)	23.4 (9.9-46.1)	23.9 (19.2-29.2)	0.962
No	76.2 (71.2-80.5)	76.6 (53.9-90.1)	76.1 (70.8-80.7)	
Anthropometrics				
Weight, kg	82.6 (19.9)	115.1 (17.8)	78.2 (15.7)	<0.001
Height, m	1.69 (0.09)	1.74 (0.09)	1.69 (0.09)	<0.001
Body mass index, kg/m^2	28.5 (6.11)	37.8 (6.4)	27.3 (4.9)	<0.001
Body composition				
Total lean mass, kg	53.1 (12.9)	68.7 (11.5)	51.0 (11.5)	<0.001
Appendicular lean mass, kg	23.2 (6.3)	30.1 (5.8)	22.3 (5.8)	<0.001
AMMI, kg/m^2	7.92 (1.63)	9.80 (1.50)	7.67 (1.48)	<0.001
Total fat mass, kg	27.4 (10.9)	43.7 (12.7)	25.3 (8.6)	<0.001
Total fat mass, %	32.6 (8.2)	37.5 (7.8)	32.0 (8.1)	<0.001
Plasma Fatty Acids				
Total plasma ω-3, µmol/L	361 (153)	345 (124)	363 (156)	0.193
ALA, µmol/L	92.1 (50.5)	94.2 (46.8)	91.8 (51.0)	0.566

EPA, µmol/L	62.4 (46.7)	54.6 (30.0)	63.5 (48.4)	0.026
DHA, µmol/L	150 (69.9)	140 (60.5)	151 (70.9)	0.204
Linoleic acid, µmol/L	3809 (846)	3855 (862)	3803 (844)	0.470
Total plasma saturated, µmol/L*	4028 (1392)	4136 (983)	4014 (1436)	0.524
Dietary intake				
Energy, kcal	2257 (310)	2121 (296)	2276 (307)	<0.001
Carbohydrate, g	279 (34.8)	266 (32.7)	281 (34.8)	0.001
Protein, g	87.1 (11.9)	80.2 (10.2)	88.0 (11.8)	<0.001
Protein, g/kg	1.10 (0.27)	0.70 (0.07)	1.16 (0.24)	<0.001
Lipids, g	84.5 (13.8)	80.4 (14.0)	85.1 (13.6)	0.009
Saturated fat, g	27.3 (4.2)	26.0 (4.3)	27.5 (4.2)	0.009
Monounsaturated fat, g	30.2 (5.2)	28.8 (5.6)	30.4 (5.1)	0.010
Polyunsaturated fat, g	20.3 (3.4)	19.4 (3.3)	20.4 (3.4)	0.015
Total ω-3, g	2.15 (0.34)	2.01 (0.29)	2.17 (0.34)	0.001
ALA, g	1.86 (0.32)	1.72 (0.27)	1.87 (0.32)	0.002
EPA, g	0.022 (0.006)	0.021 (0.05)	0.022 (0.006)	0.132
DHA, g	0.062 (0.030)	0.057 (0.02)	0.063 (0.03)	0.109
Linoleic acid, g	17.9 (3.0)	17.2 (3.0)	18.0 (3.0)	0.026
Fiber, g	18.2 (4.3)	16.7 (3.6)	18.4 (4.4)	0.003
Alcohol, g	10.6 (12.4)	8.4 (11.9)	10.9 (12.5)	0.131

Notes: DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; ALA: alpha linolenic acid. Data described as mean (standard deviation) or percentage (confidence interval).

Table 2. Linear regression between plasma omega-3 and appendicular muscle mass index according to protein intake (<0.8 g/kg and ≥0.8 g/kg). NHANES, 2011-2012.

	Model 1			Model 2		
	β	95%CI	p-value	β	95%CI	p-value
Protein intake <0.8 g/kg						
Omega-3	0.0020	-0.0014; 0.0053	0.231	0.0030	0.0013; 0.0046	**0.002**
ALA	0.0023	-0.0078; 0.0125	0.628	0.0063	0.0020; 0.0107	**0.008**
EPA	0.0079	-0.0014; 0.0172	0.091	0.0073	0.0005; 0.0142	**0.037**
DHA	0.0044	-0.0006; 0.0009	0.078	0.0057	0.0022; 0.0093	**0.004**
EPA + DHA	0.0033	-0.0001; 0.0067	0.057	0.0040	0.0010; 0.0071	**0.013**
Protein intake ≥0.8 g/kg						
Omega-3	-0.00003	-0.0010; 0.0010	0.950	-0.0002	-0.0007; 0.0003	0.394
ALA	0.0018	-0.00009; 0.0037	0.061	-0.0010	-0.0022; 0.0001	0.076
EPA	0.0002	-0.0031; 0.0035	0.905	-0.0001	-0.0018; 0.0015	0.857
DHA	-0.0018	-0.0039; 0.0003	0.087	0.0001	-0.0012; 0.0015	0.828
EPA + DHA	-0.0007	-0.0021; 0.0007	0.312	-0.00002	-0.0009; 0.0008	0.947

Notes: DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; ALA: alpha linolenic acid. Model 1: Without adjustment. Model 2: adjusted for energy and alcohol intakes, smoking, age, sex, physical activity, family income, race, arthritis and body fat. Values shown as coefficients and 95% confident intervals (95%CI).

Table 3. Linear regression between plasma omega-3 and appendicular muscle mass index according to protein intake. NHANES, 2011-2012.

	Model 1			Model 2		
	β	95%CI	p-value	β	95%CI	p-value
Protein intake <0.8 g/kg						
Omega-3	0.0020	-0.0014; 0.0053	0.231	0.0030	0.0013; 0.0046	**0.002**
ALA	0.0023	-0.0078; 0.0125	0.628	0.0063	0.0020; 0.0107	**0.008**
EPA	0.0079	-0.0014; 0.0172	0.091	0.0073	0.0005; 0.0142	**0.037**
DHA	0.0044	-0.0006; 0.0009	0.078	0.0057	0.0022; 0.0093	**0.004**
EPA + DHA	0.0033	-0.0001; 0.0067	0.057	0.0040	0.0010; 0.0071	**0.013**
Protein intake 0.8 - 1.2 g/kg						
Omega-3	-0.0001	-0.0012; 0.0009	0.717	-0.0003	-0.0010; 0.0004	0.400
ALA	-0.0005	-0.0016; 0.0028	0.585	-0.0005	-0.0021; 0.0010	0.509
EPA	-0.0004	-0.0042; 0.0034	0.834	-0.0009	-0.0035; 0.0018	0.499
DHA	-0.0014	-0.0040; 0.0012	0.273	-0.0004	-0.0027; 0.0018	0.697
EPA + DHA	-0.0006	-0.0023; 0.0010	0.440	-0.0003	-0.0017; 0.0010	0.612
Protein intake 1.2 - 1.6 g/kg						
Omega-3	-0.00005	-0.0007; 0.0006	0.858	0.0003	-0.0003; 0.0008	0.321
ALA	0.0033	0.0011; 0.0055	**0.005**	-0.0009	-0.0026; 0.0008	0.286
EPA	-0.0002	-0.0023; 0.0018	0.817	0.0009	-0.0006; 0.0023	0.236
DHA	-0.0015	-0.0031; -0.0003	**0.046**	0.0011	-0.0001; 0.0024	0.078
EPA + DHA	-0.0007	-0.0016; 0.0002	0.121	0.0006	-0.00007; 0.0013	0.077
Protein intake >1.6 g/kg						
Omega-3	-0.0001	-0.0023; 0.0021	0.905	0.0005	-0.0007; 0.0017	0.397
ALA	-0.0020	-0.0113; 0.0072	0.639	-0.0019	-0.0056; 0.0017	0.271
EPA	-0.0006	-0.0041; 0.0028	0.698	0.0017	-0.0011; 0.0046	0.208
DHA	0.0006	-0.0037; 0.0049	0.766	0.0022	-0.0037; 0.0049	0.087
EPA + DHA	0.00007	-0.0020; 0.0022	0.944	0.0012	-0.0004; 0.0028	0.130

Notes: DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; ALA: alpha linolenic acid. Model 1: Without adjustment. Model 2: adjusted for energy and alcohol intakes, smoking, age, sex, physical activity, family income, race, arthritis and body fat. Values shown as coefficients and 95% confident intervals (95%CI).

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8. CONCLUSÃO DA TESE

Os ácidos graxos ω-3 plasmáticos não estão associados ao IMMA em indivíduos jovens e de meia-idade, independentemente do sexo. Entretanto, os ácidos graxos ω-3 plasmáticos foram positivamente associados com o IMMA em indivíduos com baixa ingestão proteica (<0,8 g/kg), indicando que possivelmente o ω-3 pode ter influência na massa muscular quando a ingestão de proteína está inadequada.