ALESSANDRA DA SILVA

EFEITOS DO CONSUMO REGULAR DE CASTANHA-DO-BRASIL (*Bertholletia excelsa* H.B.K.) ASSOCIADA À RESTRIÇÃO CALÓRICA SOBRE MARCADORES DE RISCO CARDIOMETABÓLICO, ESTRESSE OXIDATIVO E DE LONGEVIDADE CELULAR EM MULHERES EM RISCO CARDIOMETABÓLICO: ESTUDO CASTANHAS BRASILEIRAS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência da Nutrição, para obtenção do título de *Doctor Scientiae*.

Orientador: Josefina Bressan

Coorientadores: Helen Hermana M. Hermsdorff
Walmir da Silva

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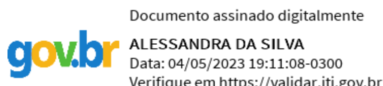

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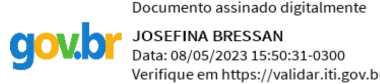

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Orientadora

À educação e saúde gratuitas e de qualidade, pois são a base para o desenvolvimento de uma sociedade.

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"To an extent that has surprised us and the rest of the scientific community, telomeres do not simply carry out the commands issued by your genetic code. Your telomeres, it turns out, are listening to you. They absorb the instructions you give them. The way you live can, in effect, tell your telomeres to speed up the process of cellular aging. But it can also do the opposite."

— Elizabeth Blackburn (Nobel Prize in Physiology or Medicine, 2009),
The Telomere Effect: The New Science of Living Younger

RESUMO

DA SILVA, Alessandra, D.Sc., Universidade Federal de Viçosa, fevereiro de 2023. **Efeitos do consumo regular de castanha-do-brasil (*Bertholletia excelsa* H.B.K.) associada à restrição calórica sobre marcadores de risco cardiometabólico, estresse oxidativo e de longevidade celular em mulheres em risco cardiometabólico: estudo castanhas brasileiras.** Orientadora: Josefina Bressan. Coorientadores: Helen Hermana Miranda Hermsdorff e Walmir da Silva.

O consumo de catanha-do-brasil (CB) no contexto da alimentação habitual foi associado a benefícios à saúde, por meio de mecanimos como melhora do perfil lipídico, status antioxidante, estresse oxidativo, inflamação, entre outros. Objetivou-se avaliar o efeito do consumo de CB aliada a dieta restrita em calorias por oito semanas sobre indicadores antropométricos e de composição corporal, marcadores de risco cardiometabólico, de saúde endotelial, estresse oxidativo e de longevidade celular em mulheres em risco cardiometabólico. Para isso, 25 mulheres receberam dieta restrita em 500 calorias + 2 unidades/dia de CB (~340 µg de selênio) (GCB) e em paralelo 24 mullheres receberam apenas dieta restrita em 500 kcal (GC). Indicadores antropométricos (peso, altura, perímetros da cintura, do pescoço e do quadril), de composição corporal (massa de gordura, massa magra e massa livre de gordura do corpo total e das regiões do tronco, adroide e ginóide), marcadores bioquímicos (marcadores do metabolismos lipídico, glicídico e hepático), de estresse oxidativo e status antioxidante (selênio, óxido nítrico, malondialdeído, superóxido dismutase, glutationa peroxidase, LDL oxidada, dano ao DNA), função endotelial (moléculas de adesão) e de longevidade celular (telomerase e comprimento absoluto dos telômeros) foram avaliados antes e após o período de intervenção. Testes de hipóteses bilaterais foram usados para avaliar os efeitos intra e entre grupos. O software SPSS versão 20.0 foi usado para as análises, e adotou-se um nível de significância estatística de 5% em todas as análises. A perda de peso, o dano ao DNA e o comprimento telomérico foram similares entre os grupos avaliados após o período de intervenção. No entanto, as participantes do GCB apresentaram aumento dos níveis séricos de selênio, bem como da atividade antioxidante total do plasma pelo método de redução do ferro, aumento de apolipoproteína A e também dos níveis plasmáticos da enzima telomerase comparado ao GC. Além disso, o GCB teve redução mais acentuada da circunferência da cintura, da relação cintura-estatura, de enzimas hepáticas, de óxido nítrico e de malondialdeído em paralelo a percentuais de massa magra e livre de gordura mais preservados em relação ao GC. Os níveis plasmáticos e a expressão mRNA de superóxido dismutase 1 diminuíram após o consumo de CB comparado a linha de base. Adicionalmente, observamos correlações significantes entre

alterações nos níveis séricos de selênio e as mudanças em parte desses marcadores. Conclui-se que a incorporação da CB em um regime de restrição calórica de 8 semanas levou a melhora em marcadores de risco cardiometabólico, status antioxidante, estresse oxidativo e de longevidade celular em mulheres em risco cardiometabólico. Os achados aqui resportados podem embasar novos mecanismos pelos quais o consumo de CB exerce efeitos benefícos a saúde humana, bem como a inter-relação entre selênio, adiposidade, aterogênese, estresse oxidativo e longevidade.

Palavras-chave: Estresse oxidativo. Longevidade. Nutrigenômica. Oleaginosas. Perda de peso. Selênio. Telomerase. Telômeros.

ABSTRACT

DA SILVA, Alessandra, D.Sc., Universidade Federal de Viçosa, February, 2023. **Effects of regular Brazil nut (*Bertholletia excelsa* H.B.K.) consumption associated to an energy-restricted diet on cardiometabolic risk markers, endothelial health, oxidative stress, and cellular longevity in women at cardiometabolic risk.** Adviser: Josefina Bressan. Co-advisers: Helen Hermana Miranda Hermsdorff and Walmir da Silva.

Brazil nut (BN) consumption in the context of the usual diet was associated with health benefits, through mechanisms such as improved in the lipid profile, antioxidant status, oxidative stress, inflammation, among others. The objective was to evaluate the effect of BN consumption combined with an energy-restricted diet for eight weeks on anthropometric and body composition indicators, cardiometabolic risk markers, endothelial health, oxidative stress, and cellular longevity in women at cardiometabolic risk. For this, 25 women received an energy-restricted diet of 500 calories + 2 units/day of BN (~340 µg of selenium) (BN-group) and in parallel, 24 women received only an energy-restricted diet of 500 kcal (CG). Anthropometric indicators (weight, height, waist, neck, and hip circumferences), body composition (fat mass, lean mass, and fat-free mass of the total body and trunk, adroid, and gynoid regions), biochemical markers (markers of lipid, glucose, and liver metabolism), oxidative stress and antioxidant status (nitric oxide, malondialdehyde, superoxide dismutase, glutathione peroxidase, oxidized LDL, DNA damage), endothelial function (adhesion molecules) and cell longevity (telomerase and absolute telomere length) were evaluated before and after the intervention period. Two-way hypothesis tests were used to assess intra- and between-group effects. SPSS software version 20.0 was used for the analyses, and a statistical significance level of 5% was adopted for all analyses. Weight loss, DNA damage, and telomere length were similar between groups after the intervention period. However, the BN-group participants showed increased serum selenium levels, increased plasma total antioxidant activity by the iron reduction method, increased apolipoprotein A, and increased plasma levels of the enzyme telomerase compared to the CG. In addition, the BN-group had a more pronounced reduction in waist circumference, waist-hip ratio, liver enzymes, nitric oxide, and malondialdehyde in parallel with more preserved percentages of lean and fat-free mass compared to the CG. Plasma levels and mRNA expression of superoxide dismutase 1 decreased after BN consumption compared to baseline. Additionally, we observed significant correlations between changes in serum selenium levels and changes in some of these markers. It is concluded that an 8-week energy-restricted regimen improved markers of cardiometabolic risk, antioxidant status,

oxidative stress, and cellular longevity in women at cardiometabolic risk. The findings reported here may support new mechanisms by which BN consumption exerts beneficial effects on human health, as well as the interrelationship between selenium, adiposity, atherogenesis, oxidative stress, and longevity.

Keywords: Oxidative stress. Longevity. Nutrigenomic. Oilseeds. Weight loss. Selenium. Telomerase. Telomere.

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LISTA DE SIGLAS E ABREVIATURAS

8-OHdG, 8-hydroxyguanosine

ALT, alanine transaminase

ANCOVA, Analysis of covariance

Apo, apolipoprotein

AST, aspartate transaminase

BMI, body mass index

BN, Brazil nut

BN-group, brasil nut group

CAAE, certificado de apresentação de apreciação ética

CAD, coronary artery disease

CB, castanha-do-brasil

cDNA, complementary DNA

CG, control group

CSN, conselho nacional de saúde

DAAT, Deep-Abdominal-Adipose-Tissue Index

DEX, Dual-energy X-ray absorptiometry

DHA, docosahexaenoic acid

DNA, Deoxyribonucleic acid

DNS, Departamento de Nutrição e Saúde

EER, Estimated Energy Requirement

EPA, eicosapentaenoic acid

ESF, Estratégia de Saúde da Família

Fe, ferro

FFM, fat-free mass

FLI, fatty liver index

FM, fat mass

FRAP, ferric reducing antioxidant power

GC, grupo controle

GCB, grupo castanha-do-brasil

GGT, gamma-glutamyl transferase

GPX1, Glutathione peroxidase 1

H_2O_2, peróxido de hidrogênio

HC, hip circumference

HDL-c, high-density lipoprotein cholesterol

HOMA-IR, homeostatic model assessment for insulin resistance

ICAM-1, intercellular adhesion molecule 1

IMC, índice de massa corporal

JBI, Joanna Briggs Institute

LAMECC, Laboratory of Energy Metabolism and Body Composition

LDL-c, low-density lipoprotein cholesterol

LM, lean mass

MCI, mild cognitive impairment

MDA, malondialdehyde

MUFA, monounsaturated fatty-acid

n-3 PUFA, omega-3 polyunsaturated fatty acid

NASF, Núcleo de Apoio a Saúde da Família

NC, neck circumference

NO, nitric oxide

Nrf2, nuclear-eritrode-factor-2

O_2, oxigênio

O_2^-, radical superóxido

PC, perímetro da cintura

POF, Pesquisa de Orçamentos Familiares

PUFA, polyunsaturated fatty-acid

qPCR, Real Time Quantitative polymerase chain reaction

qRT-PCR, Real-Time Quantitative Reverse Transcription PCR

RCE, relação cintura-estatura

RDA, dietary reference intakes

REBEC, Brazilian Registers of Clinical Trials

RNA, Ribonucleic acid

SD, standard deviation

SFA, saturated fatty-acid

SOD1, superoxide dismutase 1

SPSS, Statistical Package for Social Sciences

T2DM, type 2 diabetes mellitus

TCLE, Termo de Consentimento Livre e Esclarecido

TyG index, triglyceride-glucose index

UFV, Universidade Federal de Viçosa

UL, Tolerable Upper Intake Level

VAI, Visceral adiposity index

VCAM-1, vascular cell molecule 1

VLDL-c, very-low density lipoprotein cholesterol

WC, waist circumference

WHtR, Waist-to-height ratio

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1. INTRODUÇÃO GERAL

O excesso de peso, caracterizado pelo sobrepeso e a obesidade, cresce de forma exponencial a cada ano, sendo fator de risco para as doenças cardiovasculares, independente de outras morbidades associadas ao excesso de peso como a hipertensão arterial, a dislipidemia aterogênica e o diabetes mellitus tipo 2 (COSTA *et al.*, 2018). O ganho de peso, e em especial, o acúmulo de gordura na região abdominal parece acelerar a progressão da aterosclerose, por meio do intenso processo inflamatório e de estresse oxidativo, influenciando diretamente a homeostase de órgãos vitais como o coração, o fígado, os rins, e outros (KOLIAKI; LIATIS; KOKKINOS, 2019; WU; BALLANTYNE, 2020). Assim, consequências locais e sistêmicas decorrentes da expansão do tecido adiposo conectam o excesso de peso e as condições crônicas não-transmissíveis.

Diante desse cenário, estratégias que visam a conter o crescente avanço da obesidade se fazem necessárias. Dentre os fatores passíveis de mudanças, destaca-se o excesso de consumo alimentar, o qual é está associados ao desenvolvimento da obesidade. Afshin et al (2019) observaram que a melhora na qualidade da alimentação pode prevenir uma em cada cinco mortes no mundo, exercendo papel chave na redução de marcadores de risco para condições crônicas não transmissíveis (AFSHIN *et al.*, 2019).

A perda de peso por meio da restrição calórica diária é uma das mais bem consolidadas estratégias (JENSEN *et al.*, 2014); no entanto, associa-se a muitos desafios como a manutenção a longo prazo (MARTIN *et al.*, 2022). Por sua vez, o consumo de alimentos funcionais, apesar de não levarem a redução do peso por si só, tendem a ser grandes aliados no processo de perda de peso (KONSTANTINIDI; KOUTELIDAKIS, 2019; SUNKARA; VERGHESE, 2014). As castanhas, por exemplo, são oleaginosas palatáveis, ricas em nutrientes e não nutrientes, os quais estão associados ao controle da fome e da saciedade, ao controle glicêmico, termogênese, entre outros, que em conjunto podem ser favoráveis ao processo de emagrecimento (FRANCO ESTRADA *et al.*, 2022; TAN; DHILLON; MATTES, 2014; TINDALL *et al.*, 2019). No entanto, são escassos os estudos que avaliam os possíveis efeitos a saúde do consumo de castanhas no contexto da restrição calórica. Em um ensaio clínico controlado randomizado de 16 semanas, o consumo de 42 g/dia de pistache associado a déficit calórico de 500 a 1000 kcal/dia não teve efeitos superiores na redução de peso, índice de massa corporal (IMC), perímetro da cintura (PC) e outros marcadores de risco cardiometabólico em adultos com sobrepeso/obesidade (ROCK *et al.*, 2020). Em outro estudo, o consumo de um mix de castanhas

brasileiras (30g de castanha de caju + 15g de castanha-do-brasil (CB)) associado à dieta restrita em calorias não proporcionou maior perda de peso comparado a dieta restrita em calorias livre de castanhas, mas diminuiu o percentual de massa gordurosa ao mesmo tempo em que aumentou a massa magra e a massa livre de gordura em mulheres com risco cardiometabólico. Além disso, recente trabalho de nosso grupo mostrou que a inserção do mix de castanhas brasileiras no plano alimentar restrito em calorias diminuiu a molécula de adesão vascular 1 (VCAM-1) comparado ao grupo controle (CALDAS *et al.*, 2022).

Até a data, nenhum estudo avaliou os efeitos do consumo de CB no contexto de déficit calórico na saúde, sobretudo em indicadores antropométricos e de composição corporal, perfil bioquímico, na saúde endotelial, estresse oxidativo e em marcadores de longevidade celular. A castanha-do-brasil é uma matriz alimentar originária da região Amazônica, que possui alto conteúdo lipídico, com detaque para as gorduras insaturadas como o ácido graxo ômega 3 e é uma das mais ricas fontes do mineral selênio, podendo alcançar valores acima de 400 µg em apenas uma unidade (ALCÂNTARA *et al.*, 2022; CARDOSO *et al.*, 2017). Recente revisão sistemática mostrou os efeitos benéficos como melhora do status antioxidante, perfil lipídico, estresse oxidadito e inflamação quando a CB é consumida no contexto alimentar habitual (DA SILVA *et al.*, 2022). Além de ser constituinte de enzimas antioxidantes, o selênio parece exercer papel reguladar na adipogênse (ABO EL-MAGD *et al.*, 2022).

Por sua vez, a longevidade associada a uma expectativa de vida saudável tem sido o foco de várias investigações nos últimos tempos (EKMEKCIOGLU, 2020). O atrito prematuro dos telômeros é um dos mecanismos envolvidos no desenvolvimento de doenças e, consequentemente no comprometimento da expectativa de vida. Os telômeros são partes de DNA não codificantes ricos em guaninas com função de proteger o DNA genômico e tendem a diminuir ao longo da vida consistindo em uma das teorias do envelhecimento. No entanto, esse encurtamento parece poder ser acelerado por fatores ambientais e assim orginar de maneira prematura as doenças relacionadas ao envelhecimento. O contrário também parece acontecer. O estresse oxidativo e a inflamação são os conhecidos mecanismos, até o momento, por tráz do encurtamento prematuro dos telômeros (BLACKBURN; EPEL; LIN, 2015; COLUZZI; LEONE; SGURA, 2019; JURK *et al.*, 2014). Nesse sentido, a nutrigenômica se destaca como campo de estudo de notável importância nas investigações sobre a alimentação e a modução da expressão gênica. Estudos sugerem que a perda de peso se associa a manutenção ou ao elongamento do comprimento telomérico (CARULLI *et al.*, 2016; HEILBRONN *et al.*, 2006). Além disso, evidencias sugerem haver uma relação entre a alta adesão à dieta Mediterrânea bem como o consumo de alguns antioxidantes, frutas e vegetais, com telômeros mais longos

(CANUDAS *et al.*, 2020; GALIÈ *et al.*, 2020). No entanto, existe uma lacuna em ensaios clínicos que comprovem esses achados.

O consumo de castanhas sobre o comprimento dos telômeros também tem sido avaliado; contudo, os estudos ainda são limitados e heterogênos quanto ao tipo de castanha, tempo de intervenção, método de avaliação do comprimento telomérico e amostra estudada (CANUDAS *et al.*, 2019; FREITAS-SIMOES *et al.*, 2018; GARCÍA-CALZÓN *et al.*, 2016; WARD *et al.*, 2021). Ademais, nenhum estudo avaliou o efeito do cosumo de castanhas, em especial a CB, no contexto de déficit calórico sobre o coprimento telômerico, enzima telomerase e genes relacionados a esses marcadores.

2. HIPÓTESES

As hipóteses do presente estudo foram que o consumo de CB associado a dieta restrita em 500 kcal por 8 semanas irá:

1) Potencializar a perda de peso, bem como a redução das demais medidas antropométricas concomitante a uma mudança favorável da composição corporal com preservação da massa magra;
2) Melhorar marcadores bioquímicos de risco cardiometabólico, como perfil lipídico, glicídico e hepático;
3) Aumentar o consumo e níveis séricos de selênio aliado ao aumento do potencial antioxidante do plasma;
4) Aumentar a expressão e os níveis das enzimas antioxidantes e do fator de transcrição das enzimas antioxidantes em paralelo a diminuição de marcadores de estresse oxidativo e de disfunção endotelial;
5) Manter ou elongar o comprimento dos telômeros, bem como aumentar as concentrações plasmáticas da telomerase e modular a expressão de genes relacionado ao complexo shelterina e telomerase.

3. OBJETIVOS

3.1 Objetivo geral

Avaliar os efeitos do consumo regular de duas unidades de CB associado à dieta restrita em 500 kcal ao dia por oito semanas sobre indicadores antropométricos e de composição corporal, marcadores de risco cardiometabólico, saúde endotelial, estresse oxidativo e longevidade celular.

3.2 Objetivos específicos

Artigo de Revisão 1

- Revisar sistematicamente o efeito da suplementação com n-3 PUFA no comprimento dos telômeros e na atividade da enzima telomerase em humanos.

Artigo de Revisão 2

- Revisar sistematicamente os efeitos do consumo regular de CB na saúde humana, discutir os mecanismos envolvidos nesses efeitos, avaliar criticamente a literatura disponível e propor perspectivas futuras no campo da ciência da nutrição.

Artigos Originais

- Caracterizar as voluntárias de acordo com variáveis antropométricas, de composição corporal, bioquímicas e de consumo alimentar;
- Avaliar o efeito do consumo de 2 unidades de CB associada à dieta restrita em 500 kcal/dia por oito semanas sobre:

Artigo Original 1

- Medidas antropométricas (peso, perímetros da cintura, do quadril e do pescoço) e de composição corporal (quantidade total e das regiões do tronco, androide e ginóide de massa de gordura, massa magra e massa livre de gordura);
- IMC e relação cintura-estatura (RCE);
- Índice de adiposidade visceral (VAI) e índice de acúmulo abodominal de tecido adiposo (DAAT);
- Relação entre as variações séricas de selênio e mudanças nos indicadores antropométricos e de composição corporal.

Artigo Original 2

- Marcadores bioquímicos [colesterol total, lipoproteína de baixa densidade (LDL-c), lipoproteína de alta densidade (HDL-c), lipoproteína de muito baixa densidade (VLDL-c), triglicerídeos, Apolipoproteína (Apo) A, B, E, glicemia, insulina, fosfatase alcalina, aspartato transaminase - AST, alanina transaminase - ALT, e gama-glutamil transferase – GGT);
- Modelo homeostático para avaliação da resistência à insulina (HOMA-IR), índice triglicerídeos-glicose (TyG), índice de fígado gorduroso (FLI), índices de Castelli I e II;
- Níveis séricos de selênio e a relação entre as variações séricas de selênio com as mudanças plasmáticas nos marcadores bioquímicos.

Artigo Original 3

- Marcadores de status antioxidante [níveis da enzima antioxidantes glutationa peroxidade (GPX1), soperóxido dismutase (SOD) e potencial antioxidante do plasma pelo método de redução de ferro (FRAP)];
- Marcadores de estresse oxidativo e de saúde endotelial [malondialdeído (MDA), proteína de adesão celular vascular 1 (VCAM-1), molécula de adesão intercelular 1 (ICAM-1), Lipoproteína de Baixa Densidade Oxidada (LDL-ox) e óxido nítrico (ON)]
- Expressão de genes das enzimas antioxidantes GPX1, SOD1e catalase e do fator nuclear eritroide 2 relacionado ao fator 2 (Nrf2).

- Relação entre as variações séricas de selênio com as mudanças nos marcadores de status antioxidante, saúde endotelial, extresse oxidativo e expressão gênica.

Artigo Original 4

- Comprimento absoluto dos telômeros do DNA;
- Concentrações plasmáticas de telomerase e do marcador de dano ao DNA 8-hydroxydeoxyguanosine (8-OHdG);
- Relação entre as variações séricas e de consumo de selênio com as mudanças plasmáticas de comprimento telomérico, telomerase e 8-OHdG.

4. METODOLOGIA

4.1 Aspectos Éticos e Registro clínico

Os procedimentos descritos no presente projeto de pesquisa estão de acordo com a Resolução CNS/466 de 2012 (CNS, 2012) que trata dos princípios éticos na pesquisa clínica no Brasil. A proposta do presente projeto faz parte dos estudos intitulados "Efeito do consumo diário de castanhas brasileiras sobre redução do peso e composição corporal, metabolismo energético, apetite, ingestão alimentar, reguladores metabólicos e marcadores genéticos" e "Efeito do consumo diário de castanha-do-brasil (*Bertholetia excelsa* h.b.k.) sobre redução do peso e composição corporal, metabolismo energético, apetite, ingestão alimentar, reguladores metabólicos e marcadores genéticos", os quais já foram aprovados pelo Comitê de Ética em Pesquisa com Seres Humanos da Universidade Federal de Viçosa (UFV) (CAAE: 92004818.0.0000.5153 e 21448719.0.0000.5153, respectivamente) (**ANEXO A**). Ademais, os dois projetos estão registrados no Registro Brasileiro de Ensaios Clínicos (ReBEC) (números dos registros: RBR-3ntxrm e RBR-8zfn5c, respectivamente).

As mulheres selecionadas receberam informações sobre os objetivos e procedimentos a serem realizados no estudo. Aquelas que aceitaram as condições, termos e objetivos, bem como concordaram com a coleta de materiais biológicos (sangue, fezes e urina), assinaram o Termo de Consentimento Livre e Esclarecido em duas vias (**APÊNDICE A**).

Ao final da intervenção, cada participante recebeu um relatório individual com os resultados das avaliações antropométricas, de composição corporal e bioquímicas. Além disso, foram orientadas a respeito da importância da alimentação saudável.

4.2 Desenho do estudo

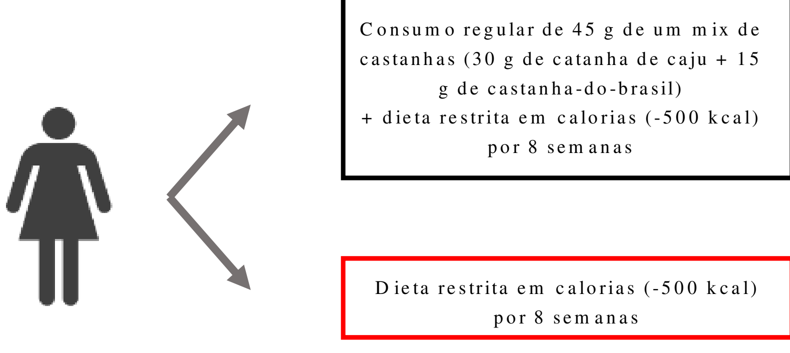
O estudo Castanhas Brasileiras foi desenhado com o intuito de avaliar os efeitos do consumo regular (diário) de castanhas nativas do Brasil associada à dieta restrita em calorias sobre marcadores de risco cardiometabólico, metabolismo energético, apetite, inflamação, estresse oxidativo, marcadores genéticos, de função endotelial em amostras de sangue. A presente pesquisa avaliou dados coletados de 2 estudos pertencentes ao estudo Castanhas Brasileiras, sendo eles:

Estudo I: Tratou-se de um ensaio clínico, randomizado, controlado, com o objetivo de avaliar o efeito do consumo regular de um mix de castanhas brasileiras (30 g de castanha de caju + 15 g de castanha-do-brasil) associado à dieta restrita em calorias comparado ao consumo de dieta restrita em calorias sem o consumo de castanhas em mulheres adultas com excesso de peso por 8 semanas. A coleta de dados do Estudo I ocorreram entre outubro de 2018 e outubro de 2019 em Viçosa, Minas Gerais, Brasil.

Estudo II: Tratou-se de um ensaio clínico, não randomizado com o objetivo de avaliar o efeito do consumo regular de 2 unidades de castanha-do-brasil associado à dieta restrita em calorias em mulheres adultas com excesso de peso por 8 semanas. A coleta de dados do Estudo II ocorreram entre junho de 2021 e dezembro de 2021 em Viçosa, Minas Gerais, Brasil.

A presente pesquisa avaliou os dados do grupo controle coletados no **Estudo I** e, também os dados provenientes da intervenção com a castanha-do-brasil (**Estudo II**) (**Figura 1**). Dessa forma, trata-se de ensaio clínico não-randomizado, controlado, paralelo, realizado com mulheres adultas em risco cardiometabólico.

a) Estudo I - Ensaio clínico randomizado, controlado



b) Estudo II - Ensaio clínico não-randomizado

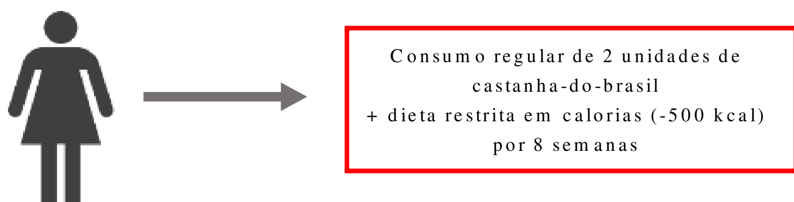


Figura 1: Esquematização dos estudos que foram utilizados na presente pesquisa. Os itens destacadas em vermelho fizeram parte da presente pesquisa.

4.3 Critérios de inclusão e não inclusão no estudo - Elegibilidade

As participantes elegíveis para o estudo deveriam atender a todos aos seguintes critérios:

-Ser mulher com idade entre 20 e 55 anos (a idade de 55 anos foi escolhida considerando que a menopausa pode enconrrer entre 51 e 55 anos (MUSTAFA; SOUZA; SENA, 2021)). O estudo foi conduzido com mulheres visto que as prevalências de excesso de peso e de obesidade são maiores nesse sexo e também pelo fato de ser o sexo que mais procura participar de projetos.

- adultas não na menopausa e no climatério;

- não fazendo uso de reposição hormonal;

-Ter excesso de gordura corporal (≥ 32%) e perímetro da cintura elevado (≥ 80 cm);

-Ter sobrepeso (IMC ≥ 27 kg/m^2) associado a pelo menos mais um outro componente da síndrome metabólica (triglicerídeos ≥150 mg/dL, pressão arterial sistólica >130 e/ou diastólica >85 mmHg ou glicemia de jejum >100 mg/dL) ou obesidade (IMC ≥ 30 kg/m^2) independente da presença dos demais componentes da síndrome metabólica.

Po outro lado, não foram incluídas no estudo:

-Mulheres com idade < 20 anos ou > 55 anos;

-Mulheres que apresentavam IMC < 30 kg/m^2 sem a presença de pelo menos mais um componente da síndrome metabólica, perímetro da cintura < 80 cm, percentual de gordura corporal $< 32\%$;

-Gestantes, lactantes, ou mulheres na menopausa;

-Atletas;

-Fumantes;

-Com histórico de HIV, doença ou alterações digestiva, hepática, renal, cardiovascular, tireoide, câncer, doenças inflamatórias e desordens alimentares;

-Histórico de abuso de drogas e/ou álcool;

-Aversão ou alergia a castanhas;

-Episódio infeccioso no último mês;

-Uso de medicamentos anti-inflamatórios, corticoides, antibióticos e outros que possam afetar o apetite e metabolismo energético;

-Instabilidade ponderal no peso (5% do peso usual) nos últimos 3 meses;

-Consumo usual de ≥ 1 unidade/dia castanha-do-brasil e também de outras castanhas;

-Consumo de álcool > 21 unidades ($\approx$168g) por semana;

-Problemas dentários que interfiram na mastigação;

-Consumo de suplementos vitamínicos, minerais e ômega 3.

4.4 Recrutamento

As participantes do estudo foram recrutadas na comunidade local (Viçosa, Minas Gerais, Brasil) ou arredores por meio da afixação de cartazes e distribuição de panfletos no comércio local, nas Estratégias de Saúde da Família (ESF), nos Núcleos de Apoio à Saúde da Família (NASF) e divulgação do estudo em emissoras de rádio e redes sociais, deixando a disposição um número de telefone e endereço de e-mail para contato.

Após contato telefônico ou por e-mail, foi realizada uma triagem telefônica para agendar uma visita presencial para aquelas participantes pré-selecionadas e interessadas em participar do estudo.

Na primeira visita, as participantes foram informadas sobre todos os procedimentos do estudo, e se estivessem em acordo, assinaram o Termo de Consentimento Livre Esclarecido (TCLE) (**APÊNDICE A**) para participar do mesmo. Em seguida, foi verificada a elegibilidade

das participantes por meio de um questionário de seleção presencial semiestruturado com questões referentes a história clínica e alimentar, dados sociodemográficos, antropométricos e de composição corporal, pressão arterial e exames bioquímicos. A respeito dos exames bioquímicos, as participantes apresentaram exames atuais (dos últimos 3 meses) de glicose de jejum, triglicerídeos e HDL-c. Ainda, na ocasião dessa primeira visita, as voluntárias selecionadas foram orientadas quanto ao preenchimento de três registros alimentares de 24 horas, em dois dias da semana e um dia de final de semana, para avaliação do seu consumo habitual.

4.5 Intervenção

A intervenção consistiu no consumo diário de duas unidades de castanha-do-brasil ao dia associada à restrição calórica (-500 kcal) (grupo teste) ou somente restrição calórica (-500 kcal) (grupo controle) (dados coletados no Estudo I), por um período de oito semanas. A quantidade de castanha-do-brasil foi definida com base em seu teor de selênio, objetivando não ultrapassar a UL (RDA para Se: 45 µg/dia e UL para Se: 400 µg/dia). Além disso, evidências mostram efeitos benéficos do consumo de castanha-do-brasil com teor de selênio de 290 µg por mulheres obesas (COMINETTI *et al.*, 2012). Cada unidade de castanha-do-brasil utilizada em nosso estudo possui 173,6 µg de selênio. Então, dobramos o número de castanhas para aproximar a quantidade de selênio contida nas castanhas de estudos anteriores e que não ultrapasse o UL.

Todas as participantes receberam um plano alimentar com cinco cardápios nutricionalmente balanceados, cada um com cinco refeições (café da manhã, colação, almoço, café da tarde e jantar). A ingestão total de energia foi estimada usando o *Estimated Energy Requeriment* (EER) para mulheres adultas com sobrepeso ou obesidade (INSTITUTE OF MEDICINE, 2005) e em seguida foram descontadas 500 kcal / dia para a prescrição alimentar conforme recomendações do guia de gerenciamento do excesso de peso e obesidade em adultos (JENSEN *et al.*, 2014). A distribuição média de macronutrientes foi de 20%, 30% e 50% da energia diária de proteínas, lipídios e carboidratos, respectivamente. Para o grupo teste, as dietas foram calculadas incluindo a energia fornecida pela porção diária de 2 unidades de castanha-do-brasil. As castanhas foram entregues às participantes quinzenalmente.

Todos os aconselhamentos dietéticos foram individualizados e fornecidos por nutricionistas. Ao longo do período do estudo, as participantes receberam instruções para usar

apenas óleo de soja no preparo das refeições consumidas ao longo do dia. O óleo de soja é comumente usado no Brasil no preparo dos alimentos e por essa razão padronizamos o uso deste para todas as participantes.

O tempo de 8 semanas de intervenção foi escolhido porque foi o tempo mínimo em que mulheres com obesidade severa tiveram benefícios do consumo de CB no contexto alimentar habitual (COMINETTI *et al.*, 2012).

4.6 Alimento teste

As castanhas-do-brasil (*Bertholletia excelsa* h.b.k.) usadas no presente estudo são da marca ECONUT® (https://econut.com.br/) cultivadas pela Agropecuária Aruanã Ltda. As castanhas foram recebidas em embalagens com atmosfera modificada, obtidas de uma mesma safra, e após recebimento das mesmas no LAMECC do Departamento de Nutrição e Saúde (DNS) da UFV foram porcionadas em embalagens laminadas, seladas a vácuo (Seladora Selovac modelo 200 B) e armazenadas em freezer a -20°C até o momento de sua distribuição às participantes.

Para análise de composição nutricional, as castanhas foram trituradas em liquidificador até a obtenção de uma farinha. Da farinha foram preparados extratos para determinar as concentrações dos minerais (magnésio, selênio e zinco) por meio de espectrofotometria de absorção atômica; perfil lipídico por meio de cromatografia a gás.

Os teores de umidade, proteína, lipídios, cinzas e fibras foram determinados, pelos métodos descritos pela AOAC (2016). A quantidade total de carboidratos foi calculada por diferença: 100 – (% água + % proteína + % lipídios + % cinzas).

4.7 Monitoramento de adesão ao protocolo do estudo e efeitos adversos

O dia experimental inicial (coleta de sangue e demais dados na linha de base) coincidiu com o primeiro dia de intervenção. Assim, nesse dia, as voluntárias receberam orientações referentes aos hábitos de vida e de consumo alimentar, com entrega de um plano alimentar semanal restrito em calorias (-500 kcal/dia) e equilibrado em macronutrientes (normoglicídica, normoproteica e normolipídica), o qual foi seguido durante todo período do estudo (8 semanas). As participantes também receberam 2 unidades de castanha-do-brasil ECONUT® em pacotes laminados individuais embalados à vácuo. A quantidade foi suficiente para consumo diário até

a data da primeira visita de monitoramento. Quando necessário, foi fornecido um adicional de 20% de castanha-do-brasil, considerando o eventual compartilhamento com familiares.

O grupo controle (dados coletados no estudo I do estudo Castanhas Brasileiras), também compareceu às visitas de monitoramento sendo submetidos à mesmas avaliações realizadas nos grupos teste, com exceção da distribuição das castanhas. Esse grupo foi orientado a não consumir castanhas durante o período de intervenção.

Para estimular a adesão ao protocolo da pesquisa, as participantes receberam uma cartilha, elaborada pelos pesquisadores, contendo informações sobre todos os procedimentos do estudo e com orientações a serem seguidas durante o período de intervenção. As participantes também foram orientadas a registrar na cartilha qualquer alteração no tipo e/ou dosagem de medicamentos de uso contínuo, bem como mudanças no estado de saúde geral. Monitoramentos telefônicos, mensagens por WhatsApp® e por e-mail também foram recursos usados para aumento da adesão ao estudo.

A adesão ao protocolo do estudo foi avaliada por meio da avaliação do consumo alimentar, por meio da concentração de selênio sérica e também por meio da perda de peso (estimativa de perda de peso em 1 mês: aproximadamente 2 kg).

4.8 Critérios de descontinuidade do estudo

Durante o período de 8 semanas de intervenção, as participantes que não seguiram o plano alimentar, que não fizeram o consumo de CB ou que manifestaram qualquer reação adversa ao consumo das castanhas foram descontinuadas da pesquisa. Mulheres que engravidaram ou manifestarem sintomas da menopausa (diagnosticada pelo médico) também foram descontinuadas do estudo.

4.9 Coleta de dados

Ao início e ao final do período de intervenção, as participantes compareceram a Divisão de Saúde da UFV pela manhã após jejum noturno (10-12h) para coleta de sangue e realização do exame de Dual-energy X-ray Absortiometry (DEXA). Ainda de jejum, elas foram encaminhadas ao laboratório de metabolismo energético e de composição corporal (LAMECC) para aferição de medidas antropométricas, como representado na Figura 2.

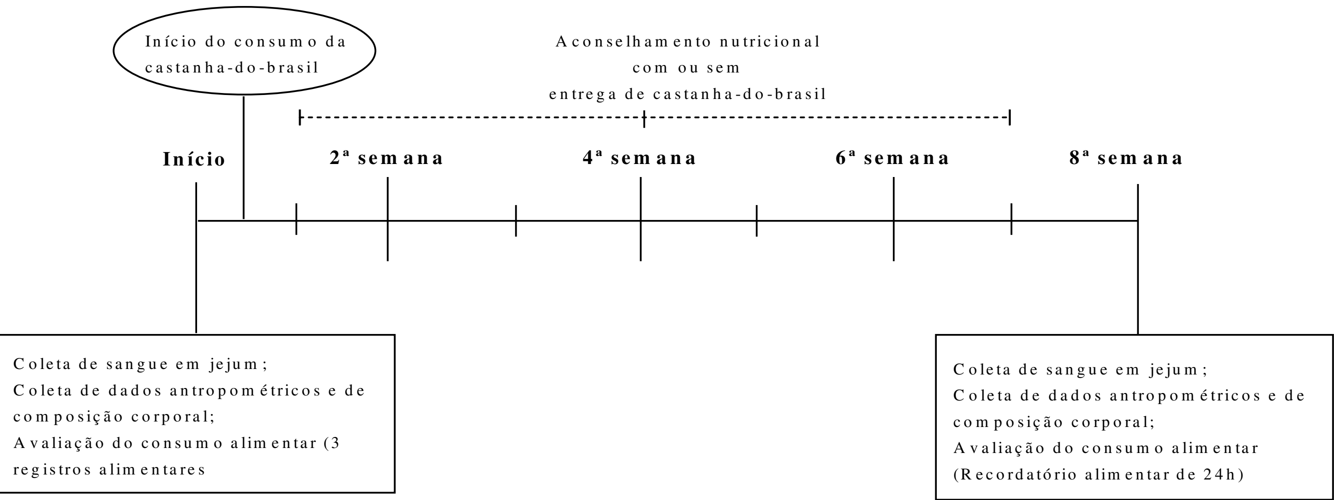


Figura 2: Desenho esquemático da coleta de dados no período de intervenção.

4.9.1 Indicadores antropométricos e de composição corporal

As medidas antropométricas (peso, altura, e perímetros da cintura, do quadril e do pescoço) foram obtidas com as participantes trajando roupas leves e sem sapatos, seguindo recomendações padronizadas.

O peso corporal foi avaliado por Inbody 230 (Biospace Corp., Seul, Coreia do Sul). A estatura foi medida com estadiômetro (modelo Seca 206, Hamburgo, Alemanha). Em ambas as situações as participantes estavam em pé, em posição firme, com os braços relaxados e cabeça no plano horizontal. O perímetro da cintura foi medido com fita inelástica (precisão 0,1 cm), mantida em plano horizontal com a participante em pé confortavelmente e com peso distribuído uniformemente em ambos os pés. O IMC foi obtido pela razão entre o peso em kg e a altura em metros ao quadrado. Foram consideradas com sobrepeso quando o IMC for ≥ 25 kg/m² e com obesidade quando o IMC ≥ 30 kg/m² (WHO, 2020).

O perímetro da cintura foi aferido na posição umbilical, ao final da expiração normal, com fita inelástica (precisão 0,1 cm). Os perímetros do quadril e do pescoço foram aferidos com fita inelástica na extensão posterior máxima do glúteo e no meio do pescoço, respectivamente (BEN-NOUN; LAOR, 2003). A relação cintura-estatura (RCE) foi obtida pela razão entre o perímetro da cintura e a altura em cm. O Índice de Tecido Adiposo Abdominal Profundo (DAAT, cm^2) foi calculado por meio da equação para mulheres: - 278 + [- 0,86 x peso (kg)] + [5,19 x perímetro da cintura (cm)].

A composição corporal das participantes foi aferida por meio do exame do DEXA com tecnologia fan-beam (Lunar Prodigy Advance DXA System, versão 13,31, GE Lunar), seguindo o protocolo recomendado pelo fabricante, na Divisão de Saúde da UFV. Os valores em quilogramas de massa gorda, massa magra, massa livre de gordura e massa total foram obtidos do corpo total e de regiões como tronco, andróide e ginoide. Posteriormente, foram calculados os percentuais em relação às medidas corporais totais A área andróide está entre as costelas e a pelve, enquanto a região ginóide inclui os quadris e a parte superior das coxas e se sobrepõe às regiões da perna e do tronco.

Para garantir a fidedignidade do exame DEXA, as participantes foram orientadas nos dias de exame: trajar roupas leves, retirar quaisquer acessórios de metal (brincos, piercings, anéis, etc), não estar em período menstrual, não fazer atividades que exigem esforço e consumir alimentos com cafeína 1 dia anterior ao exame, estar de bexiga vazia e jejum total por no mínimo 10h.

4.9.2 Coleta e separação das amostras biológicas

Ao início e ao final da intervenção, todas as participantes foram submetidas à coleta de sangue em jejum. Foram coletados aproximadamente 50 ml de sangue em tubos adequados para sorologia, tubos EDTA e tubos para análise de elementos traços. Os tubos foram centrifugados à 3500 rpm por 10 minutos à 4 °C para separação de soro e plasma. Foi utilizado Ficoll Paque® (GE Healthcare Life Sciences, Chalfont St Giles, UK) para suspensão de células periféricas mononucleares (PMBC). As amostras de PBMC foram imersas no Tri Reagent® e o RNA foi isolado seguindo o guia do usuário do TRIzol® Reagent (Invitrogen, ThermoFisher Scientific®). Resumidamente, o clorofórmio foi adicionado à amostra homogeneizada com TRIzol®, e o sobrenadante foi misturado com isopropanol. Em seguida, foram realizadas sucessivas lavagens com etanol e, por fim, o RNA foi reconstituído em água ultrapura. A razão de absorbância (A260/A280) indicou uma pureza ideal para os RNAs extraídos com valores variando de 1,8 a 2,0. A qualidade e integridade dos RNAs extraídos também foram verificadas usando um gel de agarose 1,2% corado com brometo de etídio.

O material coletado foi aliquotado em microtubos e armazenado em ultrafreezer (Thermo Scientific/Forma 900 Series®) a -80º C para posteriores análises.

a) Avaliação de marcadores bioquímicos

Marcadores do metabolismo lipídico e glicídico, bem como enzimas hepáticas foram analisados. Colesterol total sérico, colesterol da lipoproteína de baixa densidade (LDL-c), colesterol da lipoproteína de alta densidade (HDL-c), triglicerídeos e glicose foram analisados pelo método colorimétrico. O colesterol da lipoproteína de muito baixa densidade (VLDL-c) foi calculado usando a equação de Friedewald [triglicerídeos (mg/dl) / 5] (FRIEDEWALD; LEVY; FREDRICKSON, 1972). Glicose sérica, aspartato aminotransferase (AST), alanina aminotransferase (ALT), gama-glutamil transferase (GGT) e fosfatase alcalina foram analisadas pelo método enzimático. A insulina sérica foi determinada pelo método de quimioluminescência. A apolipoproteína E sérica foi determinada pelo método de imunonefelometria. As apolipoproteínas A1 e B foram medidas pelo método de Turbidimetria.

O HOMA-IR foi calculado por meio da fórmula: glicemia de jejum (mg / dl) x insulina sérica de jejum (µU / ml) / 405 (MATTHEWS *et al.*, 1985).

O índice triglicerídeos-glicose foi calculado por meio da fórmula: Ln [triglicerídeos (mg / dl) x glicemia de jejum (mg / dl) / 2] (SIMENTAL-MENDÍA; RODRÍGUEZ-MORÁN; GUERRERO-ROMERO, 2008).

O índice de adiposidade visceral foi calculado por meio da fórmula para mulheres: perímetro da cintura (cm) / (36,58 + 1,89 x IMC (kg/m²)) x (triglicerídeos (mmol/l)) / 0,81 x (1,52 / HDL (mmol / l)) (AMATO *et al.*, 2010).

Os índices de Castelli I e II foram obtidos por meio das fórmulas: colesterol total / HDL-c e LDL-c / HDL-c, respectivamente. O colesterol total menos o HDL-c foi calculado para obter o não-HDL-c. A razão Apo B/Apo A1 também foi calculada.

O índice de fígado gorduroso (FLI) foi calculado por meio da fórmula: $(e^{0.953*\log(\text{triglicerídeos}) + 0.139*\text{IMC} + 0.718*\log(\text{GGT}) + 0.053*\text{perímetro da cintura} - 15.745}) / (1 + e^{0.953*\log(\text{triglicerídeos}) + 0.139*\text{IMC} + 0.718*\log(\text{GGT}) + 0.053*\text{perímetro da cintura} - 15.745}) * 100$ (BEDOGNI *et al.*, 2006).

b) Avaliação plasmática de SOD, FRAP, MDA e óxido nítrico

A atividade da superóxido dismutase (SOD) foi avaliada por sua capacidade de catalisar a redução do radical superóxido (O_{2-}) a peróxido de hidrogênio (H_2O_2) e O_2, usando pirogalol. A SOD foi lida em um espectrofotômetro a 570 nm e expressa em unidade por mililitro de plasma (DIETERICH *et al.*, 2000).

A capacidade antioxidante do plasma foi avaliada pela capacidade de redução férrica do plasma (FRAP) com base na redução de Fe3+ para Fe2+ no plasma por antioxidantes não enzimáticos e subsequente complexação de Fe2+ com 2,4,6-tri (2-piridil) s-triazina para formar um cromóforo Fe2+ 22,4,6-tri (2-piridil)-s-triazina (2-piridil)-s-triazina (BENZIE; STRAIN, 1996). A absorbância do cromóforo a 595 nm foi lida em um espectrofotômetro e o FRAP foi expresso em micromoles por mililitro.

O malondialdeído (MDA) foi determinado de acordo com o método descrito por Buege et al. usando um ácido tiobarbitúrico reativo (BUEGE; AUST, 1975). A absorbância do cromóforo a 535 nm foi lida em um espectrofotômetro e o MDA foi expresso em micromoles por mililitro.

A concentração de óxido nítrico (NO) foi determinada usando o reagente de Griess de acordo com o protocolo proposto por Grisham et al. (1996) (GUEVARA *et al.*, 1998). As placas foram lidas em espectrofotômetro a 570 nm, e o NO foi expresso em micromoles por mililitro. Todos os marcadores foram avaliados em triplicata e os valores médios foram usados.

c) Avaliação dos níveis plasmáticos de GPX1, OxLDL, ICAM-1, VCAM-1, 8-OHdG e telomerase

Os níveis plasmáticos de GPX1, OxLDL, ICAM-1 e VCAM-1 foram estimados usando kits de ensaio ELISA disponível comercialmente para humanos: GPX1, LDL-ox, ICAM-1/CD54, VCAM-1/C, 8-OHdG e telomerase. Todas as análises foram realizadas de acordo com as instruções do fabricante (Elabscience®, EUA).

100 µl de plasma, 100 µl de plasma diluído 5 vezes, 100 µl de plasma diluído 100 vezes, 100 µl de amostras de plasma diluídas 2 vezes, 50 µl de amostras de plasma e 100 µl de amostras de plasma diluídas 100 vezes foram necessários para análise de GPX1, LDL-ox, ICAM-1, VCAM-1, 8-OHdG e telomerase, respectivamente.

As amostras foram pipetadas em plascas de 96 poços e incubadas por 90 minutos a 37 ºC. Em seguida, o líquido foi descartatado e adiciou-se imediatamente em cada poço 100 µl de "Biotynilated Detection Ab working solution" e a placa foi incubada por 60 minutos a 37 ºC. O líquido da plasca foi aspirado e lavado por três vezes com "Wash Buffer solution". Depois, adicionou-se em cada poço 100 µl de "HRP conjugated working solution". A placa foi incubada por 30 minutos a 37 ºC e logo após, o líquido foi aspirado e a placa lavada por cinco vezes com "Wash Buffer solution". Em seguida, adicionou-se 90 µl de "Substrate Reagent" e a placa

incubada por 15 minutos a 37 ºC. Por fim, foi acrescentado 50 µl de “Stop Solution” e as placas foram lidas a 450 nm.

GPX1 e LDL-ox foram expressos em pg/ml enquanto ICAM-1, VCAM-1, 8-OHdG e telomerase foram expressos em ng/ml. Os valores de LDL-ox, ICAM-1, VCAM-1 e telomerase foram corrigidos pelo fator de diluição.

d) Expressão gênica

A expressão mRNA de SOD1, GPX1, catalase e fator nuclear eritroide 2 relacionado ao fator 2 (Nrf2) foi avaliada a partir de PBMC usando a reação em cadeia da DNA-polimerase quantitativa em tempo real com transcriptase reversa (qRT-PCR). O RNA foi extraído com Tri Reagent®. Em seguida, o cDNA foi sintetizado com o kit de transcrição reversa de cDNA de alta capacidade (Applied Biosystems®). O fluoróforo PowerUpTM SYBRTM Green Master Mix (Applied Biosystems®) foi usado. A amplificação por PCR foi realizada usando o Sistema de Detecção de Sequência ABI Prism 7500 (Applied Biosystems®) sob condições padrão de ciclo de expressão mRNA.

As condições de amplificação para todas as reações foram: 95°C por 5 minutos, 40 ciclos de desnaturação a 95°C por 15 segundos cada, e anelamento e extensão a 60°C por 60 segundos. Após 40 ciclos de amplificação, a curva de dissociação foi obtida pela etapa adicional com elevação gradual da temperatura de 60 °C a 94 °C (0,01 °C/s). Os dados quantitativos de PCR em tempo real foram obtidos como valores de Ct que foram usados posteriormente na análise estatística. As réplicas técnicas foram consideradas adequadas com valores de Coeficiente de Variação (CV) atingindo no máximo 10%.

A expressão dos genes foi normalizada contra o gene endógeno b-globina, e o nível de expressão foi calculado usando o método ΔΔCT (delta delta threshold cycle) (LIVAK; SCHMITTGEN, 2001). Foram utilizadas as seguintes sequências de primers: SOD1 (F: 5’GGTGTGGCCCGATGTGTCTATT3’; R: 5’CTGCTTTTTCATCGACCACCA3’); GPX1 (F: 5'GCGGCGGCCCAG TCGGTGTA3'; R: 5'GAGCTTGGGGTCGGTCATAA3'); catalase (F: 5'TCCGGGATCTTTTTAACGCCATTG3'; R: 5'TCGAGCACGGTAGGGACAGTTCAC3'); Nrf2 (F: 5'-TTCAGCCAGCCCAGCACATC-3; R: 5'-CGTAGCCGAAGAAACCTCATTGTC-3) e b-globina F: GCTTCTGACACAACTGTGTTCACTAGC; R: CACCAACTTCATCCACGTTCACC).

e) Avaliação do comprimento dos telômeros

O comprimento absoluto dos telômeros de leucócitos de cada amostra de DNA foram estimados usando a reação em cadeia da DNA-polimerase quantitativa em tempo real (qPCR). Este é um método otimizado e validado proposto por O'Callaghan e Fenech (2011) (O’CALLAGHAN; FENECH, 2011) baseado no método de Cawthon para medir o comprimento relativo dos telômeros (CAWTHON, 2002), mas modificado pela introdução de um padrão de oligômero para medir o comprimento absoluto dos telômeros.

Resumidamente, este método realiza reações separadas para os genes TEL, 36B4 e b-globina. As sequências de iniciadores para quantificação do comprimento absoluto dos telômeros foram teloF (CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGG TTTGGGTT) e teloR (GGCTTGCCTTACCCTTACCCTTACCC TTACCCTTACCCT). Os iniciadores de gene de cópia simples foram 36B4F (CAGCAAGTGGGAAGGTGTAATCC) e 36B4R (CCCATTCTATCATCAACGGGTACAA). Os iniciadores usados como padrão endógeno foram a b-globina F (GCTTCTGACACAACTGTGTTCACTAGC) e a b-globina R (CACCAACTTCATCCACGTTCACC). Os inciaidores usados para a curva padrão foram padrão Telomero (TTAGGG-14) e padrão 36B4 (CAGCAAGTGGGAAGGTGTAACCCGTCTCCACAGACAAGGCCAGGACTCGTTTGT ACCCGTTGATGATAGAATGGG). Todos os iniciadores são da Sigma Aldrich® e foram purificados pelo fabricante através do uso de HPLC. Cada amostra foi processada em triplicata na placa de PCR de 96 poços (ABI 7300 Sequence Detection System, Applied Biosystems®). Foi utilizada a média dos valores de thresold.

A mistura principal usada na ténica de qPCR continha o fluorófo PowerUpTM SYBRTM Green (Applied Biosystems®), pares de primers, a amostra de DNA e água ultrapura para completar o volume final. O comprimento absoluto dos telômeros foi calculado plotando o sinal de fluorescência da amostra realizado para o gene TEL em uma curva padrão. O comprimento relativo dos telômeros foi calculado como a razão entre o comprimento do DNA do telômero da curva padrão e o comprimento do DNA 36B4.

As condições de ciclagem de PCR (tanto para telômeros quanto para amplicons 36B4) foram: 10 min a 95°C, seguido de 40 ciclos de 95°C por 15 seg, 60°C por 1 min, seguido por uma curva de dissociação (ou fusão) (O’CALLAGHAN; FENECH, 2011).

4.10 Atividade física e consumo alimentar

O nível de atividade física habitual foi estimado por meio do Questionário Internacional de Atividade Física (IPAQ) versão curta (**ANEXO B**), no início e ao final da intervenção.

O consumo de alimentos foi avaliado por meio de registros alimentares de três dias não consecutivos (um final de semana e dois dias da semana) (**APÊNDICE B**) aplicados no início do estudo e por 1 recordatório alimentar de 24 horas aplicado ao final do estudo. As quantidades consumidas dos alimentos foram convertidas em gramas ou mililitros. Os dados de consumo foram inicialmente digitados no Excel e em seguida inseridos no *software* REC24h-ERICA (BARUFALDI et al., 2016), que possui um banco de dados composto por uma lista de itens incluídos no banco de dados de compras de alimentos e bebidas da Pesquisa de Orçamentos Familiares (POF). Os alimentos faltantes no ERICA foram inseridos pelos pesquisadores

4.11 Análises estatísticas

As análises estatísticas foram realizadas utilizando-se o *software* SPSS (versão 22.0, USA) e o nível de significância adotado foi de 5% bilateral. Os seguintes testes estatísticos foram realizados: Teste de Shapiro-Wilk e análises gráficas complementares para avaliação da distribuição das variáveis. Teste t de *Student* ou Mann-Whitney para comparações de variáveis quantitativas paramétricas e não paramétricas, respectivamente, entre grupos. Teste t pareado ou de Wilcoxon para comparações de variáveis quantitativas paramétricas e não paramétricas, respectivamente, dentro dos grupos. Os dados foram apresentados como média (desvio padrão) ou mediana (IC 95%). Além disso, foram realizadas análises de correlação e de Qui-quadrado de Pearson.

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5. RESULTADOS

5.1 ARTIGO 1 – Artigo de revisão publicado - Prostaglandins, Leukotrienes and Essential Fatty Acids (FI: 3.015) - doi.org/10.1016/j.plefa.2022.102451

Effect of omega-3 fatty acid supplementation on telomere length and telomerase activity: A systematic review of clinical trials

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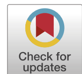

Effect of omega-3 fatty acid supplementation on telomere length and telomerase activity: A systematic review of clinical trials

Alessandra da Silva [a,*], Brenda Kelly Souza Silveira [a], Helen Hermana Miranda Hermsdorff [a], Walmir da Silva [b], Josefina Bressan [a]

[a] Laboratory of Energy Metabolism and Body Composition. Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil
[b] Laboratory of Animal Biotechnology. Animal Science Department, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil

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ABSTRACT

Evidence suggests antioxidant and anti-inflammatory properties of omega-3 polyunsaturated fatty acids (n-3 PUFA). However, the effect of supplementation of this fatty acid profile on the telomere length and the telomerase enzyme activity was not revised yet. The PubMed and Embase® databases were used to search for clinical trials. A total of six clinical trials were revised. Omega-3 PUFA supplementation did not statistically affect telomere length in three out of three studies but affected telomerase activity in two out of four studies. The supplementation increased telomerase enzyme activity in subjects with first-episode schizophrenia. Besides, it decreased telomerase enzyme activity without modulating the effects of Pro12Ala polymorphism on the PPARγ gene in type 2 diabetes subjects. The methodological differences between the studies and the limited number of studies on the theme suggest that further studies are needed to elucidate the effects of n-3 PUFA supplementation on telomere length and telomerase enzyme activity in humans.

Abbreviations

DHA	docosahexaenoic acids
EPA	eicosapentaenoic acids
n-3 PUFA	omega-3 polyunsaturated fatty acid
MUFA	monounsaturated fatty acid
Nrf2	factor-2 related erythroid nuclear transcription factor 2
NF-kB	nuclear factor kappa B
PBMC	peripheral blood mononuclear cells
PPARγ	peroxisome proliferator activated receptor gamma
SFA	saturated fatty acid
SPMs	specialized pro-family mediators
TRF1	telomeric repeat-binding factor 1

1. Introduction

aging-related diseases have been responsible for a crescent number of premature deaths worldwide [1,2]. Type 2 diabetes, cardiovascular diseases, chronic kidney disease, neurodegenerative diseases, mental disorders [3,4], and even central fat accumulation [5] are some examples of common morbidities in aging. Telomere shortening is directly related to the chronological age of individuals and is proposed as a programmed theory of the cellular aging process [6,7]. Furthermore, telomere attrition has been associated with all these diseases [8–10].

Telomeres are complex structures formed by proteins and repeated sequences of DNA (5′-TAGGG-3′) located at the end of chromosomes, responsible for avoiding chromosomes fusion, for protecting the DNA from oxidative damage suffered throughout life, thus keeping genomic stability [11,12]. In its turn, the telomerase enzyme is responsible for maintaining telomere length [12,13]. In addition to genetic factors and the own cell division, nongenetic factors such as environmental, lifestyle events, and behavior are associated with telomeres shortening, leading to senescence and consequent cell dysfunction [11]. Although telomere shortening is a natural process in the organism [14], studies have shown that it can be accelerated by oxidative stress and inflammation [15,16].

Considering the etiology of age-related diseases linked to oxidative stress and inflammation process, nutrition is one of the behavioral and modifiable risk factors that can interfere with these processes [17,18]. Thus, it becomes necessary to investigate the role of foods and nutrients

* Corresponding author at: Laboratory of Energy Metabolism and Body Composition. Department of Nutrition and Health, Universidade Federal de Viçosa. Avenida pH Rolfs, s/n. Viçosa, MG 36570-900, Brasil.
E-mail address: alessandra.silva2@ufv.br (A. da Silva).

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Table 1
Characterization of six clinical trials that investigated the n-3 polyunsaturated fatty acid supplementation on telomere length and/or in telomerase enzyme activity.

Authors, year, and country	Sample characteristics	Intervention characteristics	Method	Study design and follow-up	Markers	Main results
Holub et al., 2020 USA	**n:** 30 adults with T2DM **Age:** 56.6 (SD 8.9) y **BMI:** 34.6 (SD 7.5) kg/m^2	−81 mg/d of aspirin alone in capsules for 7 days −4 g/day of n-3 PUFA fish oil (EPA: 1600 mg + DHA: 800 mg) alone in capsules for 28 days **-**Combination of n-3 PUFA fish oil and aspirin in capsules for another 7 days.	TRAPeze telomerase detection kit; RT-PCR (sample: PBMC)	8-week sequential therapy unblinded clinical trial	Relative telomerase activity (copies)	No statistically significant change in telomerase enzyme copy number compared to baseline and between interventions
Pawełczyk et al., 2017 Poland	**n:** 71 subjects with a first episode of schizophrenia **Age:** G1: 23.2 (SD 4.8) y G2: 23.3 (SD 4.8) y	**G1:** 2.2 g/day of n-3 PUFA fish oil (EPA: 1320 mg + DHA: 880 mg) + 0.2% of vitamin E in capsules **G2:** olive oil (MUFA: 73.9% + PUFA: 9.8%) + 0.2% of vitamin E in capsules	TE ELISA kit (sample: PBMC)	Randomized placebo-controlled clinical trial −26 weeks	Telomerase concentration	**G1:** ↑ levels of telomerase *vs.* **G2**
Toupchian et al., 2016 Iran	**n:** 72 PPARy Pro12Ala polymorphism genotyped subjects with T2DM **Age:** **G1:** 55.9 (SD 7.8) y **G2:** 56 (SD 7) y	**G1:** 2.4 g/day of n-3 PUFA fish oil (EPA: 400 mg + DHA: 1450 mg) in capsules **G2:** 600 mg of paraffin in capsules	Telomeric repeat amplificaton protocol (TRAP); PCR-Elisa RT-PCR (sample: PBMC)	Double-blind randomized controlled clinical trial - 8 weeks	Telomerase activity	**G1:** ↓ telomerase activity *vs.* baseline and **G2** No statistically significant change in telomerase activity between genotypes
Barden et al., 2016 Australia	**n:** 74 subjects with chronic kidney disease **Age:** 56.5 (SEM 1.4) y **BMI:** 27.3 (SEM 0.5) kg/m^2	**G1:** 4 g/day of n-3 PUFA (EPA: 460 mg + DPA: 38 mg + DHA: 380 mg) in capsules **G2:** 200 mg/day of coenzyme Q10 in capsules **G3:** Combination of 4 g/day de n-3 PUFA + 200 mg/day of coenzyme Q10 in capsules **G4:** 4 g/day of olive oil in capsules	qPCR (sample: PBMC and neutrophils)	Double-blind randomized placebo-controlled clinical trial −8 weeks	Absolute telomere length (kb/diploide genome)	No statistically significant change in telomere length compared to baseline and between interventions n-3 PUFA supplementation: ↑ telomere length in neutrophils when results were adjusted by total neutrophil count *vs.* subjects who did not receive n-3
O' Callaghan et al., 2014 Australia	**n:** 33 older adults with mild cognitive impairment **G1:** **Age:** 74.8 (SD 5) y **BMI:** 28.1 (SD 4.1) kg/m^2 **G2:** Age: 74.2 (SD 7) y BMI: 26.8 (SD 2.6) kg/m^2 **G3:** Age: 73 (SD 3.9) y BMI: 28.1 (SD 5.3) kg/m^2	**G1:** fish oil (EPA: 1670 mg + DHA: 160 mg) in capsules **G2:** fish oil (EPA: 400 mg + DHA: 1550 mg) in capsules **G3:** 2.2 g/day of linoleic acid from safflower oil in capsules (total de 4 cápsulas diárias)	qPCR (sample: whole blood)	Double-blind, randomized placebo-controlled clinical trial −6 months (24 weeks)	Absolute telomere length (kb/diploide genome)	No statistically significant change in telomere length compared to baseline and between interventions
Kiecolt-glaser et al., 2013 EUA	**n:** 106 adults and older adults with overweight **G1:** **Age:** 50.6 (SD 6.5) y **BMI:** 30.7 (SD 3.8) kg/m^2 **G2:** **Age:** 50.3 (SD 7.8) y **BMI:** 31.7 (SD 4.5) kg/m^2 **G3:** **Age:** 51.2 (SD 8.9) y **BMI:** 31.1 (SD4.8) kg/m^2	**G1:** 2.5 g/day of n-3 PUFA fish oil (EPA: 2080 mg + DHA: 340 mg) + 1 UI vit. E in capsules **G2:** 1.25 g/day n-3 PUFA fish oil (EPA: 1040 mg + DHA: 170 mg) + 1 UI vit. E in capsules **G3:** SFA:MUFA:PUFA ratio= 37:42:21 + 1 UI vit. E in capsules	TRAPeze telomerase detection kit qPCR (sample: peripheral blood lymphocytes)	Double-blind, randomized placebo-controlled clinical trial −4 months (16 weeks)	Telomere length (base pairs) and telomerase activity	No statistically significant change in telomere length and telomerase activity between interventions ↓ n-6 PUFA:n-3 PUFA ratio = ↑ telomere length

Legend: G, group; BMI, body mass index; SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; RT-PCR, reverse transcription polymerase chain reaction; qPCR, quantitative real-time PCR; EPA, eicosapentaenoic fatty acid; DHA, docosahexaenoic fatty acid; PBMC, peripheral blood mononuclear cell.

Identification

Records identified through PubMed/Medline database searching (n=32)

Records identified through Embase® database searching (n=275)

Screening

Records after duplicates removed (n=290)

Records screened (n=290)

Records excluded (n=275)

Eligibility

Full-text articles assessed for elegibility (n=15)

Records excluded, with reasons (n=9)

Other study design (n=6)
animal study (n=1)
intervention with a combination of nutraceutical supplements (n=1)
Maternal supplementation of omega-3 PUFA (n=1)

Included

Studies included in systematic review (n=6)

Fig. 1. Flowchart of studies included in the systematic review.

in developing, progressing, and protecting against diseases and the mechanism behind longevity and a healthy lifespan.

Well-described reviews of epidemiological studies [19,20] and also randomized clinical trials [20] showed that high adherence to the Mediterranean diet [19], as well as the consumption of some antioxidants, fruits, and vegetables, are associated with longer telomeres length [20]. On the other hand, several studies have investigated the role of n-3 polyunsaturated fatty acid (n-3 PUFA) supplementation as a potential modulators of telomere length and telomerase activity [21–25]. Two review studies also briefly cited the results of some original studies on the effect of n-3 PUFA supplementation on telomere length and/or telomerase activity [20,26]. Despite this, no review has focused on critically evaluating these studies, particularly reviewing omega-3 supplementation alone rather than in combination with other compounds.

N-3 PUFA is a long-chain fatty acid present in significant amounts in plant and animal foods. Health beneficial effects of n-3 PUFA supplementation primarily attributed to its antioxidant and anti-inflammatory properties have been suggested [17,27,28]. Eicosapentaenoic acids (EPA) and docosahexaenoic acids (DHA) present mainly in fish oil have been associated with cardiovascular risk reduction [29]. However, it has not been answered if the supplementation of n-3 PUFA affects the modulation of the senescence process through telomere length and telomerase enzyme activity. Thus, this systematic review aims to review the effect of n-3 PUFA supplementation on telomere length and telomerase enzyme activity in humans.

2. Materials and methods

2.1. Protocol and registration

This systematic review was carried out according to the guideline "Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)" [30] and was registered in PROSPERO (https://www.crd.york.ac.uk/prospero/), registration number CRD42020186349.

2.2. Literature search

Studies were identified by searching the following electronic databases: MEDLINE / PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Embase® (https://www-embase.ez35.periodicos.capes.gov.br/a/#/picoSearch). The keywords chosen for the search were based on MeSH terms and a list of synonyms suggested by Embase®. The intervention and outcome fields of the PICOS (population/intervention/comparator/outcome/study design) search strategy were used for the research. Then, filters were used to select studies in humans and clinical trials. Table 1 lists the key terms used in the search. The last search was carried out in June 2021. The search was performed independently by two authors (AS and BKSS). First, a selection was made by titles and abstracts. Then, the

articles were read in full and eligible studies were selected. Any disagreement between the authors was resolved by consensus. Finally, a backward search was performed to identify possible relevant articles to be included in the review. Duplicate articles were removed manually.

2.3. Eligibility criteria

The following criteria were applied for the inclusion of studies: (1) original clinical trials, randomized or not, controlled or not; (2) studies that evaluated the effect of consuming any dose of n-3 PUFA at any time; (3) studies that evaluated telomere length or the expression of telomerase enzyme activity. If data were duplicated in more than 1 study, the most complete and detailed study was included. The following exclusion criteria were applied: (1) studies with observational design, reviews, congress abstracts, letters, protocol articles, notes; (2) no investigation of telomere length or telomere-related markers; (3) interventions in which fatty acids were consumed along with mineral/vitamin supplements, other nutritional supplements; and (4) interventions that included behavioral modifications, such as physical activity.

2.4. Selection of studies and data extraction

The studies were selected by analyzing titles, abstracts, and full texts by two independent authors (AS and BKSS), and differences were resolved by consensus. In the absence of the whole article or when additional information was needed to compile the results, an email was sent to the corresponding author requesting the article or information.

Of the studies eligible for the review, two independent authors (AS and BKSS) extracted the following data from the studies: i) name of the first author, year of publication, and country of study, ii) sample characteristics (number of participants, presence of diseases, age, and body mass index), iii) characteristics of the intervention (description of each intervention group, as well as the doses of n-3 PUFA used), iv) study design and duration, v) analysis technique used to measure the telomeres length and telomerase and cell type used for the analyses, vi) markers evaluated in the study (telomere length and/or telomerase, and vii) main results.

2.5. Risk of bias assessment

The risk of bias was assessed independently by two authors (AS and BKSS) following the Joanna Briggs Institute Reviewer's Manual (JBI). The purpose of this appraisal is to assess the methodological quality of a study and to determine the extent to which a study has addressed the possibility of bias in its design, conduct, and analysis. Thirteen questions for randomized clinical trials and nine questions for non-randomized clinical trials were answered for each study included in the systematic review. Finally, the answers to these questions were classified as Yes, No, Unclear, or not applicable [31].

3. Results

3.1. Studies selection

We identified three hundred and seven articles in our search and removed nineteen duplicate articles. During the screening of titles and abstracts, two hundred and seventy-five articles were excluded for not meeting the eligibility criteria. Fifteen articles remained for full-text evaluation, and then nine articles were excluded. Other study designs (observational studies), animal studies, and interventions with a combination of nutraceutical supplements were the reasons for excluding the studies. As a result, six articles met the eligibility criteria and were included in this systematic review [21–25,32] (**Fig. 1**). The characteristics of these studies are presented in Table 1.

3.2. Studies characteristics

The six eligible studies were published between 2013 and 2020. Five studies were randomized [21–23,25,33], only one was a sequential intervention [32], and four were double-blinded [21–23,25]. The studies were conducted in Australia [22,23], US [21,32], Iran [25], and Poland [24]. A total of 386 subjects were evaluated, being adolescents and adults with first episodes of schizophrenia [24], adults with chronic kidney disease [23], older adults with mild cognitive impairment [22], and adults with overweight [21]. In addition, three studies assessed telomerase activity [24,25,32], another two studies assessed telomere length [22,23] and one study evaluated both markers [21]. Of the studies that evaluated telomerase activity, the intervention period ranged from 28 days to 6 months, with doses of EPA from 1040 mg to 2080 mg/day and DHA between 170 mg to 880 mg/day. On the other hand, of the studies that evaluated telomere length, the intervention period ranged from 8 weeks to 6 months, with doses of EPA from 400 mg to 2080 mg/day and DHA between 160 mg to 1550 mg/day.

Controls used in the studies were olive oil [23,24], aspirin [32], n-6 PUFA [22], paraffin [25], and a mixture of oils - SFA:MUFA:PUFA ratio of 37:42:21 [21]. Furthermore, in all studies, n-3 PUFA were taken in capsules and taken in the context of a habitual diet. Peripheral blood mononuclear cells (PBMC) were used in most studies.

3.3. Results of individual studies

In summary, omega-3 PUFA supplementation did not statistically affect telomere length in three out of three studies but affected telomerase activity in two out of four studies. In patients with chronic kidney disease, the supplementation of 4 g of n-3 PUFA (EPA: 460 mg + DHA: 380 mg) or combined with the supplementation of coenzyme Q10 did not change telomere length in eight weeks compared to the supplementation of coenzyme Q10 alone and, also with olive oil. However, when analyzed together, those who received n-3 PUFA had increased neutrophil telomere length adjusted by total neutrophil count compared to patients who did not receive n-3 PUFA. This result was independent of age, sex, and body mass index [23]. In another study, the supplementation of different doses of EPA and DHA (EPA: 1.670 mg + DHA: 160 0.16 mg and EPA: 400 mg + DHA: 1550 mg) did not affect telomere length in older adults with mild cognitive impairment compared to baseline and the supplementation of 2.2 g/day of safflower oil in six months [22].

In line with these findings, one study found that the supplementation of different doses of n-3 PUFA (EPA: 1040 mg + DHA: 170 mg and EPA: 2080 mg + DHA: 340 mg, respectively) did not impact telomere length and telomerase enzyme activity of overweight older adults over four months. Such results are of comparison between doses and with the placebo group. To further explore the data obtained, the authors unified the groups that received both doses of n-3 PUFA and observed that the decrease in the n-6 PUFA:n-3 PUFA ratio was associated with an increase in telomere length [21]. In subjects with type 2 diabetes mellitus, the supplementation of 4 g of n-3 PUFA (EPA: 1600 mg + DHA: 800 mg) for twenty-eight days also did not affect the copy number of the telomerase enzyme activity compared to baseline and aspirin consumption [32].

On the other hand, supplementation of 2.4 g of n-3 PUFA (EPA: 400 mg + DHA: 1450 mg) for eight weeks decreased the telomerase enzyme activity of subjects with type 2 diabetes without modulating effects of PPARγ Pro12Ala polymorphism compared to the placebo group [25]. In another study, supplementation of 4 g of n-3 PUFA (EPA: 1320 mg + DHA: 880 mg) for twenty-six weeks more pronouncedly increased telomerase enzyme levels in subjects diagnosed with first-episode schizophrenia compared to the placebo group who received olive oil. However, after eight weeks of intervention, this effect was not observed [24].

3.4. Risk of bias

Supplementary Figure 1 summarizes the results of the risk of bias assessment. Most of the studies (76%) did not address the possibility of bias in its design, conduct, and analysis. For example, the intention-to-treat analysis was mentioned in just one article, which justifies the prevalence of increased risk of bias on the item "Patients analyzed in the groups to which they were randomized". In addition, most studies did not mention the number and training of raters and their intra and inter reliability, which confer some unclear answers.

4. Discussion and conclusions

This is the first systematic review of clinical trials focusing on supplementation of n-3 PUFA as a potential modulator of telomere length and telomerase enzyme activity in humans. Despite being a natural phenomenon of cell fate [12], telomere shortening has been associated with behavioral characteristics such as food consumption [19,20]. In this sense, behavioral changes focused on improving dietary constituents have shown promising results in maintaining telomere length or decelerating telomere attrite [34] and in greater telomerase enzyme activity [24].

To date, six clinical trials investigated the effects of n-3 PUFA supplementation on telomere length (EPA: 400 mg to 2080 mg + DHA: 160 mg to 1550 mg) and/or telomerase enzyme activity (EPA: 1040 mg to 2080 mg + DHA: 170 mg to 880 mg), with unanimity regarding the absence of effect on telomere length. Omega-3 PUFA supplementation did not statistically affect telomere length in three out of three studies but affected telomerase activity in two out of four studies. Similar results were observed when n-3 PUFA was supplemented with other compounds. The supplementation of NucleVital®Q10 complex, which contains n-3 PUFA (1350 mg), ubiquinone (300 mg), astaxanthin (15 mg), lycopene (45 mg), lutein palmitate (30 mg), zeaxanthine palmitate (6 mg), L-selenomethionine (330 mg), cholecalciferol (30 μg), and α-tocopherol (45 mg) for twelve weeks unchanged telomere length, but increased telomerase levels by more than 25% in healthy subjects [33]. Interestingly, linseed-oil-supplemented pigs showed lower levels of the shelterin telomeric repeat-binding factor 1 (TRF1) protein compared to the control group [35]. TRF1 increased is associated with oxidative stress and is involved in the negative regulation of the telomere length by inhibiting the telomerase activity [36–38] suggesting a possible protective role of n-3 PUFA from linseed oil on telomere attrition.

Despite the lack of statistical significance, a trend towards lesser shortening of telomeres after n-3 PUFA supplementation could be observed [21–23]. Thus, this result may be relevant from a clinical point of view. Considering that telomere attrition is associated with the triggering of diseases [38], slowing down this process, can be advantageous, even if it is minimal.

The anti-inflammatory and antioxidant bioactivity of n-3 PUFA is recognized in the literature either from vegetables [39] or from animal sources such as fish oil [40]. The increase in reactive oxygen species in the organism parallel to the state of imbalance redox and inflammation seem to contribute to telomeric attrition and, consequently, exposure of genetic material to oxidation. In most studies included in this review, the subjects evaluated had some morbidity, such as type 2 diabetes, chronic kidney disease, overweight, and schizophrenia. Both inflammation and oxidative stress are a consequence and are also involved in the progression of these morbidities [41,42]. Thus, it would be precisely through these two mechanisms of action that we expected to observe the beneficial effects of n-3 PUFA supplementation on telomere length and telomerase activity [7].

Studies suggest that peroxidation of n-3 PUFA increases the concentration of 4-hydroxyhexenal in the cytosol, which activates factor-2 related erythroid nuclear transcription factor 2 (Nrf2). Thus, antioxidant enzymes are produced, giving antioxidant characteristics to this fatty acid profile [43,44]. In this sense, EPA and DHA could attenuate oxidative stress in vascular endothelial cells through upregulation of Nrf2 [18].

In addition to antioxidant effects, n-3 PUFA has anti-inflammatory properties. N-3 PUFA's are involved in the synthesis of specialized pro-family mediators (SPMs), such as resolvins, maresins, and protectins, which are involved in the resolution of inflammation [45]. Besides, dietary fats have a key role in modulating cell membranes, and thus inflammatory mediators such as leukotrienes, prostaglandins, among others, can be produced depending on the lipid composition of the membranes. In this sense, a balanced diet between the proportions of n-6 PUFA: n-3 PUFA can favor the balance between arachidonic fatty acid and EPA in cell membranes. When removed by the phospholipase A2 enzyme into cells, the EPA fatty acids will be bioconverted by the lipoxygenase and cyclooxygenase 1 enzymes into unique leukotrienes, prostaglandins, and thromboxanes. These substances have anti-inflammatory characteristics, unlike the leukotrienes produced by arachidonic acid. The anti-inflammatory capacity of n-3 PUFA is also related to the disarticulation of the complete TAK-1-TAB-1/2-ubiquitin. EPA and DHA fatty acids bind to the GPR120 receptor on cell membranes, recruiting the β-arrestin protein. When activated by GPR120, β-arrestin rescues the binding protein 1/2 (TAB-1/2) to TAK-1 from the inflammatory protein complex initiated by inflammatory mediators such as TNF or by activation of toll-like receptors, preventing dephosphorylation of the kappa B inhibitor kinase (IKK). Thus, nuclear factor kappa B (NF-kB) does not target the cell nucleus to stimulate the production of cytokines and other inflammatory mediators. Furthermore, n-3 PUFA increases the expression of the peroxisome proliferator-activated receptor γ (PPARγ) and consequently negatively impacts the activity of the transcription factor NF-kB through the mechanism of transrepression [28,46]. In addition to these effects, n-3 PUFA also modulates the expression of sterol regulatory element-binding protein (SREBP), decreasing the liver's synthesis of fatty acids and cholesterol [47].

In one of the revised studies, the decrease in the n-6:n-3 PUFA ratio was associated with longer telomere length [21]. This association may indicate that the imbalance between these two types of fat consumption is related to inflammatory processes and oxidative stress, which are the main links in the cellular senescence process. The imbalance between the consumption of n-6:n-3 fatty acids, typical of the Western dietary pattern, may favor the production of serial pair pro-inflammatory mediators such as leukotrienes, prostaglandins, and thromboxanes [28]. In patients with stable coronary artery disease, there was an inverse relationship between baseline blood levels of marine n-3 PUFA and the rate of telomere shortening over five years [48]. In another study, higher n-6: n-3 PUFAs ratio and lower EPA and DHA were associated with shorter telomere length in the Chinese population [49]. Furthermore, in the Nurse's Health Study, the n-6 PUFA linoleic acid intake was inversely associated with telomere length [50].

In subjects with type 2 diabetes, supplementation of n-3 PUFA for 28 days numerically increased telomerase activity, but without statistical significance in relation to aspirin consumption and the same amount of EPA and DHA combined with the consumption of aspirin [32]. In contrast, supplementation of a lower dose of EPA and higher dose of DHA compared to the study above [32] decreased telomerase levels in type 2 diabetes subjects with PPARy Pro12ALA polymorphism within eight weeks [25]. In the first study [32], the short intervention time may be insufficient to detect a statistically significant increase in telomerase activity. Studies have shown that subjects with the rs1801282 variant may have decreased type 2 diabetes susceptibility and may have positive, beneficial effects on insulin sensitivity and body mass index [51, 52]. Despite this, DHA-enriched fish oil upregulated cyclin-dependent kinase inhibitor 2A expression, a marker of cell senescence, which is related to inhibition of the telomerase activity [25].

Subjects with first-episode schizophrenia seem to benefit from n-3 PUFA supplementation, as they had increased telomerase enzyme activity after six months of supplementation compared to supplementation

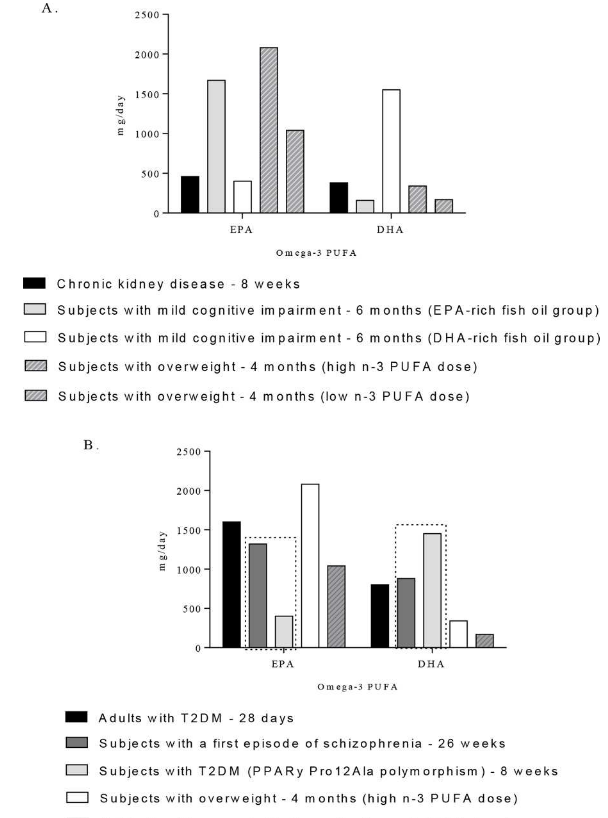

Fig. 2. Comparison of EPA and DHA doses used in studies that evaluated the effects of n-3 supplementation on: **A.** telomere length and **B.** telomerase enzyme activity. Data dashed are from studies that observed a statistically significant effect of n-3 PUFA supplementation on telomerase enzyme activity.

with olive oil [24]. In contrast, the supplementation with a higher dose of EPA but a lower dose of DHA compared to the prior study did not impact telomerase activity in adults and older adults with overweight compared to the placebo group after sixteen weeks. Evidence suggests that oxidative stress and inflammation are involved in the pathology of schizophrenia [53]. Due to the bond between schizophrenia pathophysiology and the anti-inflammatory and antioxidant properties of n-3 mentioned earlier, individuals with the disease may benefit from supplementation with EPA + DHA. A detail of all three studies that observed a trend toward less telomere shortening after supplementation with n-3 PUFA [21–23] is that the dose of EPA was higher than those of DHA. Furthermore, studies have shown improvement in lipid profile markers after EPA supplementation, whereas DHA supplementation appears to increase low-density lipoprotein cholesterol concentrations [54–56]. In another study, EPA, but not DHA, markedly activated the sirtuin 1 gene expression in THP1 cells [57]. In fact, molecular mechanisms link telomeres to sirtuin expression, which are positively related to telomere stabilization [58]. Despite this, the mechanisms of EPA and DHA in cell senescence markers still need to be further studied. It is noteworthy that the studies differed regarding the doses of EPA and DHA supplemented, intervention time, profile and number of evaluated subjects, and comparison group (Fig. 2). These observations may justify the different results regarding telomerase activity between studies and allow us to infer that more studies are necessary for the field.

In this systematic review, we critically analyzed the studies that assessed the effects of the n-3 PUFA supplementation on telomere length and telomerase enzyme activity. Heterogeneous studies were compared in this review. Differences between studies regarding the doses of EPA and DHA supplemented, intervention time, profile and number of evaluated subjects, and the comparison group were detected and critically assessed. It is necessary to conduct more studies with n-3 PUFA supplementation and other promising fatty acid sources. In addition, it would be interesting to assess markers related to telomere constituents such as proteins from the shelterin complex and others. Finally, we emphasize the lack of human studies on mechanisms behind the effects of n-3 PUFA on the senescence process through modulation of telomere length and telomerase enzyme activity.

Supplementation of n-3 PUFA had no statistically significant effect on telomere length, but showed a tendency to reduce telomere attrition in three of three studies that assessed telomere length. The supplementation increased telomerase enzyme activity in subjects with first-episode schizophrenia. Besides, it decreased telomerase enzyme activity without modulating the effects of Pro12Ala polymorphism on the PPARγ gene in type 2 diabetes subjects. The methodological differences between the studies and the limited number of studies on the theme allow us to infer that further studies are needed to elucidate the effects of n-3 PUFA supplementation on telomere length and telomerase enzyme activity in humans.

CRediT authorship contribution statement

Alessandra da Silva: Writing – original draft, Writing – review & editing, Visualization. **Brenda Kelly Souza Silveira:** Writing – original draft, Writing – review & editing, Visualization. **Helen Hermana Miranda Hermsdorff:** Writing – review & editing, Supervision. **Walmir da Silva:** Writing – review & editing, Supervision. **Josefina Bressan:** Writing – review & editing, Supervision.

Declarations of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.plefa.2022.102451.

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Effects of Regular Brazil Nut (Bertholletia excelsa H.B.K.) Consumption on Health: A Systematic Review of Clinical Trials

Review

Effects of Regular Brazil Nut (*Bertholletia excelsa* H.B.K.) Consumption on Health: A Systematic Review of Clinical Trials

Alessandra da Silva [1], Brenda Kelly Souza Silveira [1], Brenda Vieira Machado de Freitas [1], Helen Hermana M. Hermsdorff [1,2] and Josefina Bressan [1,*]

[1] Laboratory of Energy Metabolism and Body Composition, Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa 36570-900, MG, Brazil
[2] Institute of Public Policies and Sustainable Development, Universidade Federal de Viçosa, Viçosa 36570-900, MG, Brazil
* Correspondence: jbrm@ufv.br; Tel.: +55-31-3612-5220

Abstract: The Brazil nut (BN) is a promising food due to its numerous health benefits, but it is still necessary to systematically review the scientific evidence on these benefits. Thus, we examined the effects of regular BN consumption on health markers in humans according to the health state (with specific diseases or not) of the subjects. PubMed, Embase®, and Scielo databases were used to search for clinical trials. The PRISMA guideline was used to report the review, and the risk of bias for all studies was assessed. Twenty-four studies were included in the present review, of which fifteen were non-randomized. BNs were consumed in the context of a habitual free-living diet in all studies. Improvement in antioxidant status through increased levels of selenium and/or glutathione peroxidase activity in plasma, serum, whole blood, and/or erythrocytes was observed in all studies that evaluated antioxidant status, regardless of the health state of the sample. In addition, healthy subjects improved lipid markers and fasting glucose. Subjects with obesity had improvement in markers of lipid metabolism. Subjects with type 2 diabetes mellitus or dyslipidemia improved oxidative stress or DNA damage. Subjects undergoing hemodialysis benefited greatly from BN consumption, as they improved lipid profile markers, oxidative stress, inflammation, and thyroid function. Older adults with mild cognitive impairment improved verbal fluency and constructional praxis, and controversial results regarding the change in a marker of lipid peroxidation were observed in subjects with coronary artery disease. In conclusion, the benefits of BN consumption were found in different pathways of action and study populations.

Keywords: functional foods; metabolic risk factors; nuts; nutrition; oilseeds; selenium

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1. Introduction

The Brazil nut (BN) (*Bertholletia excelsa* H.B.K.) is an edible seed of the Brazil nut tree native to the Amazonia biome. According to data provided by International Nut & Dried Fruit (INC), Bolivia is the main producer of BNs, followed by Peru and Brazil. The world consumption of BNs, considering a total supply of BNs minus ending stock, was 26,250 tons in 2020/2021 [1]. Like other nuts such as walnuts, hazelnuts, macadamia, pistachios, and almonds, several bioactive compounds are also present in BNs [2]. Unsaturated fats, minerals, vitamins, fibers, and phytochemicals give nuts potential and recognized beneficial health effects [3]. The BN contains smaller amounts of protein compared to plant-based protein sources such as tropical vegetables; however, in 100 g of BNs, 14.3 g is from protein. Although small doses of BNs are recommended daily due to the selenium (Se) content (depending on the growing area, one unit is sufficient to reach the recommended daily intake of Se), BNs can be a potential alternative source of protein in diets [4]. However, unlike other nuts, the BN is the richest source of selenium (Se), a key element in forming antioxidant defense systems, modulation of the immune system, and helping to prevent ageing-related diseases [5].

In 2017, Cardoso et al. revised the health benefits of BNs and addressed their composition, safety, planting, processing, and use [3]. One study regarding BN safety and toxicity showed the absence of health risks regarding the presence of aflatoxins and radioactivity in 30 samples of BN originating from the Amazon [6]. In addition to the heavy metal barium, other elements may be present in BNs, such as strontium. However, the forms and levels of these elements in BNs do not seem harmful to human health [3,7,8]. Other review articles on BNs are also available. However, they have specific objectives and do not provide the most current findings of all studies that explore the effects of regular BN consumption on human health [3,7–11]. Hence, we aim to carry out a systematic review regarding the effects of regular BN consumption on human health. We also discussed the mechanisms involved in these effects and critically assessed available literature and future perspectives on nutrition science.

2. Materials and Methods

2.1. Protocol and Registration

This systematic review was carried out per the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)" guidelines [12] and was registered in PROSPERO (https://www.crd.york.ac.uk/prospero/, accessed on 11 September 2022), registration number CRD42021198965.

2.2. Literature Search

We identified studies by searching the following electronic databases: MEDLINE/PubMed (https://pubmed.ncbi.nlm.nih.gov/, accessed on 11 September 2022), Embase®, and Scielo. The descriptors used were based on Medical Subject Headings (MeSH) (Appendix A Table A1). We conducted an exhaustive literature review using "intervention" from PICOS (population/intervention/comparator/outcome/study design) search criteria to identify all studies with the BN in any health outcome.

Filters were used to select studies on humans and clinical trials when available. We also searched for articles on this theme in the reference list of previously published review articles [3,7–11]. Table A1 lists the search terms used. The last search was conducted in March 2022. The search was performed independently by two authors (AS and BKSS). First, titles and abstracts were selected. The articles were then read in full, and eligible studies were selected. Any disagreement between authors was resolved by consensus. Duplicate articles were removed using Mendeley® software.

2.3. Eligibility Criteria

The following eligibility criteria were considered to answer our research question, "What are the effects of regular BN consumption on human health?"

(i). Original clinical trials, randomized or not, controlled or not.
(ii). Studies that evaluated the effects of regular BN consumption on any human health marker.
(iii). Studies with any dose and time of intervention.
(iv). Studies in which BNs were consumed as whole seed.
(v). Studies evaluating any health marker, anthropometrics, or body composition and those examining lipid, glucose, kidney, liver, and other markers.
(vi). Studies with any subject profile (healthy subjects or subjects with comorbidities), and with any sex.

The following exclusion criteria were applied:

(i). Postprandial studies.
(ii). Studies with children or animals, observational designs, reviews, congress abstracts, letters, protocol articles, notes, and in vitro analyses.
(iii). Studies that did not investigate the effects of the BN on human health markers
(iv). Interventions that supplemented BNs with minerals/vitamins or other nutritional enhancements.

(v). Interventions with BN oil or flour.
(vi). Interventions that included behavioral modifications, such as physical activity.

Studies with children were not considered due to different Se requirements compared to other ages.

2.4. Selecting Studies and Data Extraction

Two independent authors (AS and BKSS) selected the studies by analyzing the titles, abstracts, and full texts. Disagreements between the authors were resolved by consensus. In the absence of a full article or when additional information was needed to compile the results, an email was sent to the corresponding author requesting the article or information. Two independent authors (AS and BKSS) extracted the following data from the eligible studies:

(i). Name of the first author, year of publication, and country.
(ii). Sample characteristics (number of participants, presence of diseases, age, and body mass index (BMI).
(iii). Characteristics of the intervention (description of each intervention group and the doses of BNs used with their Se contents).
(iv). Study design and duration.
(v). Markers evaluated in the study.
(vi). Observed results.

We grouped the markers evaluated by the studies into major categories: antioxidant status (referred to Se, glutathione peroxidase (GPx), and/or SELENOP levels analyzed in plasma, serum, erythrocytes), lipid metabolism markers (high-density lipoprotein (HDL)-c, low-density lipoprotein (LDL)-c, total cholesterol (TC), triglycerides (TGs), TC/HDL-c, LDL-c/HDL-c, very-low-density lipoprotein (VLDL)-c, paraoxonase 1, Apo-A1, and Apo B), inflammatory markers (C-reactive protein (CRP), tumor necrosis factor (TNF), interleukins (IL), tool-like receptors, nuclear factor kappa B (NF-κB)), anthropometry and body composition markers (weight, BMI, waist circumference, and fat mass), glucose metabolism markers (glucose, hemoglobin A1c (HbA1c), and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)), thyroid function markers (thyroid-stimulating hormone (TSH) and triiodothyronine (T3)), cognitive function markers (a consortium to establish a registry for Alzheimer's disease, verbal fluency, Boston naming test, and constructional praxis), and oxidative stress or DNA damage markers (oxidized (ox)LDL-c, 8-Epi-Prostaglandin F2 alpha (8-epi-PGF2α), malondialdehyde, nuclear factor erythroid 2-related factor 2 (Nrf2), nicotinamide adenine dinucleotide phosphate (NAD(P)H): quinone oxidoreductase 1 (NQO1), thiobarbituric acid reactive substances (TBARS), and 8-hydroxy-2′-deoxyguanosine(8-OHdG)).

2.5. Bias Risk Assessment

Two authors (AS and BVMF) independently assessed the risk of bias, following the Joanna Briggs Institute (JBI) Reviewer's Manual. This appraisal assesses methodological quality and determines the extent to which a study has addressed the possibility of bias in its design, conduct, and analysis. Thirteen questions for randomized clinical trials and nine questions for non-randomized clinical trials were answered for each study included in the systematic review. The answers to these questions were classified as yes, no, unclear, or not applicable [13]. A third author (BKSS) resolved disagreements.

3. Results and Discussion

3.1. Study Selection

We identified 463 records and removed 27 duplicates. During the titles and abstracts screening, 404 articles were excluded because they did not meet the eligibility criteria. Thirty-two articles remained for full-text evaluation. However, eight were excluded. Postprandial studies (n = 2), abstract congress (n = 1), unavailable articles (n = 1), intervention with granulated BN flour (n = 3), and child studies (n = 1) were excluded. Eleven articles

were revised [3]. As result, 24 articles were incorporated per the eligibility criteria (Figure 1). Tables 1 and 2 present the characteristics of these studies.

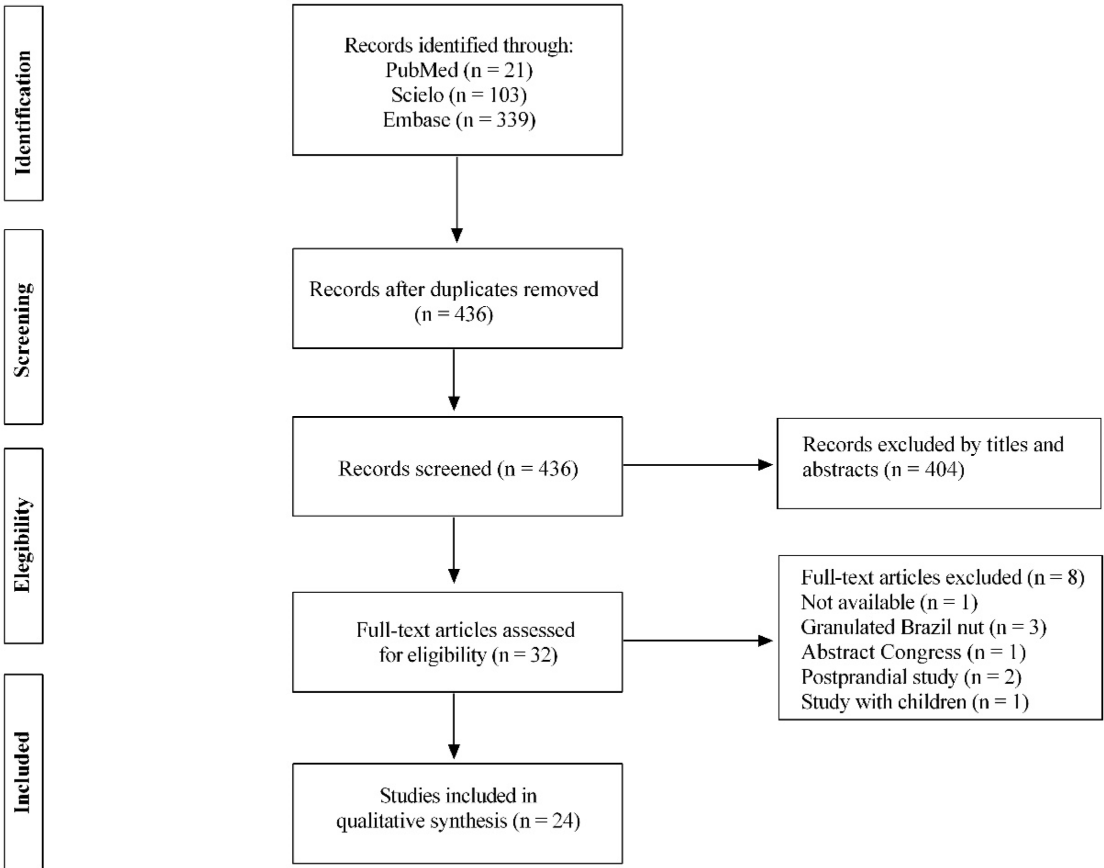


Figure 1. Flowchart of studies included in the systematic review.

Table 1. Randomized clinical trials evaluating the effects of Brazil nut consumption on human health (n = 9).

Reference Author, Year, Country	Sample Characteristics	Intervention Characteristics	Study Design and Duration	Evaluated Markers	Results
		Placebo-controlled clinical trials			
[14] New Zealand	n: 59 healthy subjects G1: Age: 49.5 (SD 8.6) y BMI: 28.2 (SD 5.3) kg/m^2 G2: Age: 45.6 (SD 11) y BMI: 26 (SD 3.6) kg/m^2 G3: Age: 42.5 (SD 9.9) y BMI: 25.9 (SD 4.2) kg/m^2	G1: two units/day of BN (~53 µg of Se) G2: selenomethionine supplement (97.5 ± 11.1 µg of Se) G3: placebo (0.038 ± 0.036 µg of Se)	Randomized, controlled clinical trial 12 weeks	Antioxidant status	G1, G2: ↑ Se and GPx activity in plasma vs. G3 G1: ↑ GPx activity in whole blood vs. G2, G3
[15] Brazil	n: 17 obese female adolescents Age: 15.4 (SD 2) y BMI: 35.6 (SD 3.3) kg/m^2	G1: 15–25 g/day (three to five units) of BN (108.5 ± 27 µg of Se) G2: placebo (one capsule/day containing lactose)	Randomized, non-blinded pilot trial 16 weeks	Antioxidant status Lipid and glucose metabolism markers Inflammation Anthropometry Oxidative stress	G1: ↓ total cholesterol, triglycerides, and ox-LDL vs. G2 G1: ↑ RBCV vs. G2 G1: ↑ Se and RBCV max vs. baseline ↔ BMI, waist circumference, insulin, glycemia, HOMA-IR, CRP, HDL-c, GPx-3, and 8-epi-PGF2α

Table 1. *Cont.*

Reference Author, Year, Country	Sample Characteristics	Intervention Characteristics	Study Design and Duration	Evaluated Markers	Results
		Controlled clinical trials			
[16] Brazil	n: 20 older adults with mild cognitive impairment Age: 77.7 (SD 5.3) y	G1: one unit/day of BN (288.75 µg of Se) G2: control	Randomized, controlled clinical trial 24 weeks	Antioxidant status Oxidative stress Cognition	G1: ↑ Se in plasma and erythrocytes vs. G2 G1: ↑ GPx activity in erythrocytes, verbal fluency, construction praxis vs. G2 ↔ ORAC, MDA, CERAD total score, Boston naming test, word list learning test, word list recall
[17] Brazil	n: 20 older adults with mild cognitive impairment Age: 77.7 (SD 5.3) y	One unit/day of BN (288.75 µg of Se)	Secondary analysis of a randomized controlled clinical trial, which evaluated only the group that received BN 24 weeks	Antioxidant status Oxidative stress	↔ Se in plasma and erythrocytes, GPx, ORAC, and MDA activity among genotypes ↑ expression of GPx1 mRNA and selenoprotein P in CT + TT allele carriers for rs1050450 over time ↑ selenoprotein mRNA expression and ↓ GPX1 mRNA expression in A-carriers for rs7579 and GG-carriers for rs3877899
[18] Australia	n: 32 healthy subjects Age: 60 (52–76) y	G1: six units/day of BN (~48 µg of Se) G2: four capsules containing 800 mg of (-) epigallocatechin-3-gallate/day G3: combination of G1 and G2 interventions	Randomized, controlled clinical trial 6 weeks	Antioxidant status Kidney function marker Glucose Inflammation Thyroid function markers Genes/proteins related to the colorectal cancer oncogenesis	G1: ↑ Se and plasma urea vs. G2 G3: ↑ Se and ↓ plasma creatinine vs. G2 ↔ glycemia, CRP, TSH, T3 and T4, mRNA expression of Ac-H3 histones, Ki-67, SELENOP, NF-κB, β-catenin, c-Myc, cyclin D1, and DNNT1 between groups G1 and G2: ↑ SELENOP mRNA expression vs. baseline G1: ↔ DNMT1, NF-κB, c-Myc, and cyclin D1 mRNA expression vs. baseline G1 and G2: ↓ β-catenin vs. baseline
[19] Brazil	n: 55 women with obesity G1: Age: 40.4 (SD 9) y BMI: 34.6 (30.8–37.4) kg/m^2 G2: Age: 39.4 (SD 9.5) y BMI: 34.8 (33.1–40.2) kg/m^2	G1: one unit/day of BN (~1261 µg of Se) G2: control	Randomized, controlled clinical trial 8 weeks	Antioxidant status Inflammation Endothelial function markers	G1: ↑ Se in plasma and erythrocytes, GPx1 activity, selenoprotein P, gene expression for selenoproteins, TNF-α, IL-6, IL-10, TLR2, TLR4 and ↓ GPx1 gene expression vs. G2 ↔ CRP, MCP-1, IL-6, IL-10, IL-1 β, TNF-α, IFN-γ, fibrinogen
[20] Brazil	n: 54 women with obesity G1: Age: 40.4 (SD 9) y BMI: 34.9 (SD 4.7) kg/m^2 G2: Age: 39.4 (SD 9.5) y BMI: 36.6 (SD 6.5) kg/m^2	G1: one unit/day of BN (~1261 µg of Se) G2: control	Randomized, controlled clinical trial 8 weeks	Antioxidant status	G1: ↑ Se in plasma and erythrocytes, expression of miR-454-3p and miR-584-5p vs. G2

Table 1. *Cont.*

Reference Author, Year, Country	Sample Characteristics	Intervention Characteristics	Study Design and Duration	Evaluated Markers	Results
[21] Brazil	n: 42 subjects with coronary artery disease G1: Age: 63.3 (SD 6.7) y BMI: 29.3 (SD 5.6) kg/m^2 G2: Age: 63.3 (SD 8) y BMI: 28.6 (SD 4.8) kg/m^2	G1: one unit/day of BN (~290.5 µg of Se) G2: control	Randomized, controlled clinical trial 12 weeks	Lipid metabolism markers Inflammation Oxidative stress	↔ Nrf2, NF-κB, and NQO1 mRNA expression, TC, HDL-c, LDL-c, TG, TC/HDL-c, LDL-c/HDL-c, and TBARS G1: ↓ TBARS (?) vs. G2
[22] Brazil	n: 36 subjects with coronary artery disease G1: Age: 63 (SD 6.7) y BMI: 28.5 (SD 4.5) kg/m^2 G2: Age: 64.6 (SD 7.2) y BMI: 29.4 (SD 5.3) kg/m^2	G1: one unit/day of BN (~290.5 µg of Se) G2: control	Randomized, controlled clinical trial 12 weeks	Lipid metabolism markers Inflammation Oxidative stress	↔ PPARβ/δ and NF-κB mRNA expression, CRP, TC, HDL-c, LDL-c, TG, and TNF G2: ↓ TBARS vs. G1

Legend: ↑, increased; ↓, decreased; ↔ unchanged; G, group; SD, standard deviation; BN, Brazil nut; Se, selenium; BMI, body mass index; GPx, glutathione peroxidase; RBCV, red blood cell velocity; oxLDL-c, oxidized low-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; CRP, C-reactive protein; 8-epi-PGF2α, 8-Epi-Prostaglandin F2 Alpha; HDL-c, high-density lipoprotein cholesterol; DNA, deoxyribonucleic acid; ORAC, oxygen radical absorbance capacity; MDA, malondialdehyde; TSH, thyroid-stimulating hormone; T3, triiodothyronine; T4, thyroxine; SELENOP, selenoprotein P; TNF, tumor necrosis factor; interleukin 6; IL-10, interleukin 10; TLR -2, tool-like receptor 2; TLR-4, tool-like receptor 4; MCP-1, monocyte chemoattractant protein 1; IL-1β, interleukin 1β; IFN- γ, interferon gamma; NF-κB, nuclear factor kappa B; Nrf2, nuclear factor erythroid 2-related factor 2; NQO1, NAD(P)H: quinone oxidoreductase 1; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglycerides; TBARS, thiobarbituric acid reactive substances; PPARβ/δ, peroxisome proliferator-activated receptors β/δ; AST, aspartate transaminase; ALT, alanine transaminase; GGT, gamma-glutamyl transferase; hs-CRP, high-sensitivity C-reactive protein; CERAD, consortium to establish a registry for Alzheimer's disease.

3.2. Studies' Characteristics

Approximately 93% of the publications were from research conducted in Brazil between 2008 and 2022. Thirteen [17,19–22,26,31–37] of the twenty-four studies included in this systematic review are new compared to the review carried out by Cardoso et al. (2017).

Table 2. Non-randomized clinical trials evaluating the effects of Brazil nut consumption on human health (n = 15).

Reference	Sample Characteristics	Intervention Characteristics	Study Design and Duration	Evaluated Markers	Results
		Controlled clinical trials			
[23] Brazil	n: 25 subjects on hemodialysis G1 (n = 13): Age: 57.1 (SD 12) y BMI: 24.4 (SD 3.2) kg/m^2 G2 (n = 12): Age: 52 (SD 15.5) y BMI: 26.1 (SD 5.8) kg/m^2	G1: one unit/day of BN (~5 g with 290.5 µg of Se) G2: control	Controlled clinical trial 12 weeks	Antioxidant status Inflammation Oxidative stress	G1: ↑ mRNA expression of Nrf2, NAD(P)H: quinone oxidoreductase 1 (NQO1) and ↓ mRNA expression of NF-κB vs. G2 G1: ↓ MDA, IL-6 vs. baseline
		Uncontrolled clinical trial			
[24] Brazil	n: 15 normolipidemic subjects Age: 27.3 (SD 3.9) y BMI: 23.8 (SD 2.8) kg/m^2	45 g/day of BN (11 units with 862.65 µg of Se)	Clinical trial 15 days	Antioxidant status Anthropometry Lipid metabolism markers	↑ Se in plasma and reception of cholesteryl esters by HDL-c ↔ weight, total cholesterol, LDL-c, HDL-c, TG, Apo A-I, Apo B, HDL-c diameter, PON 1 activity, % cholesterol, TG, and phospholipid transfer

Table 2. *Cont.*

Reference	Sample Characteristics	Intervention Characteristics	Study Design and Duration	Evaluated Markers	Results
[25] Brazil	n: 81 hemodialysis patients Age: 52 (SD 15.2) y BMI: 24.9 (SD 4.4) kg/m^2	One unit/day of BN (~5 g with 290.5 µg Se)	Clinical trial 12 weeks	Antioxidant status	↑ Se in plasma and erythrocytes ↑ GSH-Px activity in erythrocytes (began to be within normal)
[26] Brazil	n: 37 morbidly obese women Age: 34.5 (SD 6.8) y BMI: 45.2 (SD 4.2) kg/m^2	One unit/day of BN (~290 µg of Se)	Clinical trial 8 weeks	Antioxidant status Glucose Anthropometry DNA damage	↑ Se in plasma and erythrocytes and GPx activity in all genotypes ↓ DNA damage in those with the Pro/Pro genotype. vs. baseline ↑ DNA damage in those with genotype Leu/Leu vs. Pro/Read ↔ weight, BMI, blood glucose
[27] Brazil	n: 37 morbidly obese women Age: 34.5 (SD 6.8) y BMI: 45.2 (SD 4.2) kg/m^2	One unit/day of BN (~290 µg of Se)	Clinical trial 8 weeks	Antioxidant status Lipid profile markers Glucose Anthropometry	↑ Se in plasma and erythrocytes ↑ GPx activity ↑ HDL-c ↓ TC/HDL-c and LDL-c/HDL-c ratio ↔ weight, BMI, total cholesterol, LDL-c, VLDL-c, TC, fasting glucose
[28] Brazil	n: 21 hemodialysis patients Age: 54.2 (SD 15.2) y BMI: 24.4 (SD 3.8) kg/m^2	One unit/day of BN (~5 g with 290.5 µg Se)	Clinical trial 12 weeks	Antioxidant status Anthropometry, body fat, Kidney function markers Minerals	↑ Se in plasma ↓ urea nitrogen Follow-up after 12 months: ↓ Se in plasma and urea nitrogen ↔ BMI, body fat, WC, creatinine, minerals
[29] Brazil	n: 40 hemodialysis patients Age: 53.3 (SD 16.1) y	One unit/day of BN (~5 g with 290.5 µg Se)	Clinical trial 12 weeks	Antioxidant status Lipid metabolism markers Inflammation Oxidative stress and DNA damage	↑ Se, GPx activity and HDL-c in plasma ↓ TNF, IL-6, 8-OHdG, 8-isoprostane, LDL-c, Castelli index I and II ↔ total cholesterol, TG
[30] Brazil	n: 29 hemodialysis patients Age: 51 (SD 3.3) y BMI: 23.6 (17.7–40.3) kg/m^2	One unit/day of BN (~5 g with 290.5 µg Se)	12-month follow-up after 3 months of BN consumption	Antioxidant status Lipid metabolism markers Inflammation Oxidative stress and DNA damage	↓ Se and GPx activity in plasma ↑ TNF, IL-6, 8-OHdG, 8-isoprostane ↔ total cholesterol, TG, LDL-c, HDL-c
[31] Brazil	n: 40 hemodialysis patients Age: 53.3 (SD 16.1) y	One unit/day of BN (~5 g with 290.5 µg Se)	Clinical trial 12 weeks	Antioxidant status Thyroid function markers	↑ Se in plasma, GPx activity, T3 and T4 levels ↔ TSH
[32] Brazil	n: 130 healthy subjects Age: 29.8 (SD 9.2) y BMI: 23.3 (SD 3.3) kg/m^2	One unit/day of BN (3 to 4 g with ~300 µg of Se)	Clinical trial 8 weeks	Glucose and lipid metabolism markers	↓ glucose at 4 and 8 weeks and total cholesterol at 8 weeks
[33] Brazil	n: 130 healthy subjects Age: 29.8 (SD 9.2) y BMI: 23.3 (SD 3.3) kg/m^2	One unit/day of BN (3 to 4 g with ~300 µg of Se)	Clinical trial 8 weeks	Antioxidant status	↑ mRNA expression of GPX1 in subjects with genotype in rs713041 ↑ Selenoprotein P mRNA expression in A allele carriers in rs7579 before and after consumption
[34] Brazil	n: 130 healthy subjects Age: 29.8 (SD 9.2) y BMI: 23.3 (SD 3.3) kg/m^2	One unit/day of BN (3 to 4 g with ~300 µg of Se)	Clinical trial 8 weeks	Antioxidant status	GPx1 activity: ↓ at 4 weeks but did not differ from baseline at 8 weeks GPx3 activity: ↑ at 4 weeks but did not differ from baseline at 8 weeks Se in plasma and erythrocytes: ↑ at 4 and 8 weeks Selenoprotein P: ↑ in 8 weeks

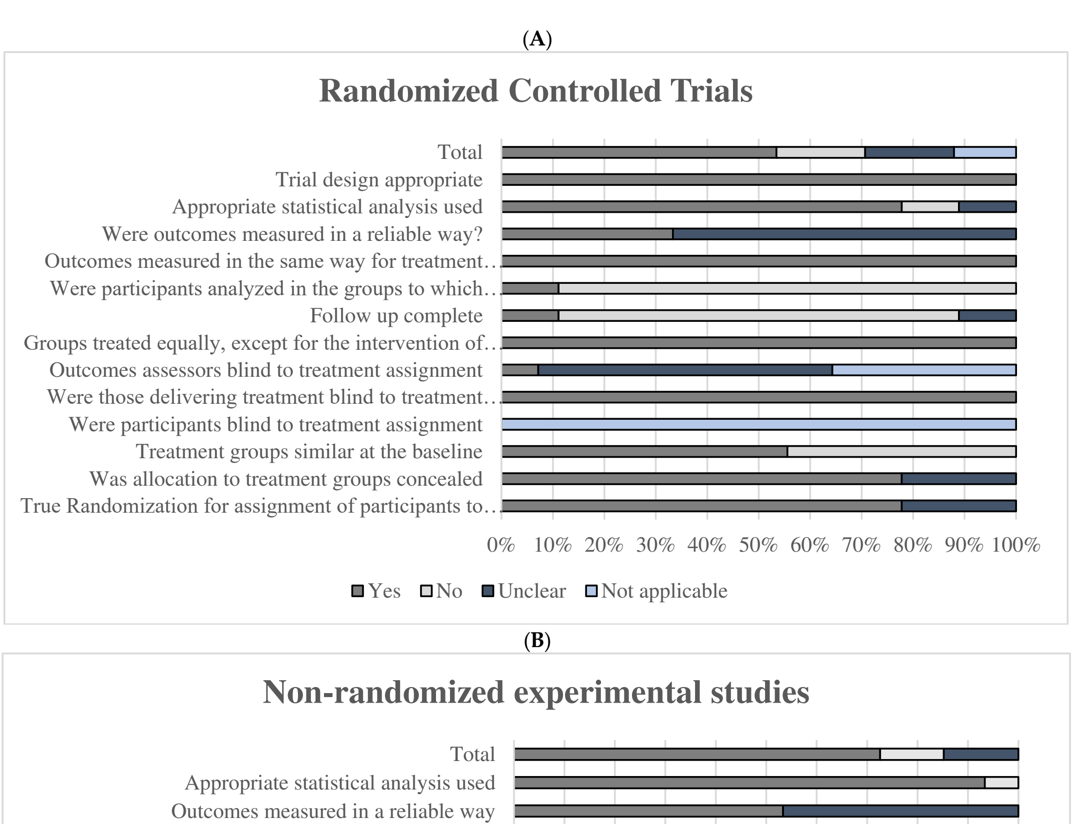
Table 2. *Cont.*

Reference	Sample Characteristics	Intervention Characteristics	Study Design and Duration	Evaluated Markers	Results
[35] Brazil	n: 60 subjects with type 2 diabetes Men: Age: 62 (SD 9) y BMI: 30.2 (SD 3.2) Women: Age: 66 (SD 8) BMI: 32.6 (SD 4.1)	One unit of BN/day (~3.7 g with 213.67 µg of Se)	Clinical trial 24 weeks	Antioxidant status Anthropometry DNA damage Glucose metabolism markers	↑ Se, waist circumference, glycemia ↓ DNA damage, both basal and cell-induced oxidative damage ↔ BMI, HbA1c
[36] Brazil	n: 32 patients using statins	G1: one unit/day of BN (~5 g with 290 µg of Se) for subjects classified as having high concentrations of creatine kinase G1: one unit/day of BN (~5 g with 290 µg of Se) for subjects classified as having normal creatine kinase concentration	Clinical trial 12 weeks	Antioxidant status Oxidative stress Lipid metabolism marker	G1, G2: ↓ concentrations of protein kinase, MDA, SOD vs. baseline G1, G2: ↑ Se in plasma and erythrocytes, GPx vs. baseline ↔ total cholesterol and mRNA expression of selenoproteins
[37] Brazil	n: 32 patients using statins Age: 50.1 (SEM 7.6) y BMI: 31.1 (SEM 3.8) kg/m^2	One unit/day of BN (~5 g with 290 µg of Se)	Clinical trial 12 weeks	Antioxidant status Anthropometry Oxidative stress Lipid metabolism marker	↑ erythrocyte GPx activity in all genotypes for the rs1050450 polymorphism in the GPx ↑ erythrocyte GPx activity for those with CC genotype for the rs3877899 polymorphism in the SELENOP and all genotypes for rs7579 polymorphism in the SELENOP ↓ creatine kinase in all genotypes for the rs1050450 polymorphism in the GPx ↓ creatine kinase for those with CC genotype for the rs3877899 polymorphism in the SELENOP and GG genotype for rs7579 polymorphism in the SELENOP

Legend: ↑, increased; ↓, decreased; ↔ unchanged; G, group; SD, standard deviation; BN, Brazil nut; Se, selenium; BMI, body mass index; Nrf2, nuclear factor erythroid 2-related factor 2; NQO1, NAD(P)H: quinone oxidoreductase 1; NF-κB, nuclear factor kappa B; MDA, malondialdehyde; IL-6, interleukin 6; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglycerides; VLDL-c, very-low-density lipoprotein cholesterol; PON 1, paraoxonase 1; Apo-A1, apolipoprotein A-1; Apo B, apolipoprotein B; GSH-Px, glutathione peroxidase; GPx, glutathione peroxidase; TNF, tumor necrosis factor; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone; DNA, deoxyribonucleic acid; HbA1c, glycated hemoglobin; SOD, superoxide dismutase; 8-OHdG, 8-hydroxydeoxyguanosine; SELENOP, selenoprotein P.

Of the twenty-four, nine publications were the results of randomized clinical trials. Of these, two studies were placebo-controlled [14,15], seven were controlled [16–22], and sixteen were non-randomized clinical trials. Only two non-randomized studies were controlled [23], whereas all others were uncontrolled [14,15,24–37]. Regarding the evaluation of biases using the checklist proposed by the JB, 64% of the answers were "yes", 15% were "no", 16% were "unclear", and 5% were "not applicable". This means that most of the studies evaluated in this review had a low risk of bias and, therefore, met the methodological requirements of study design, conduct, and analysis (Figure 2).

(A)



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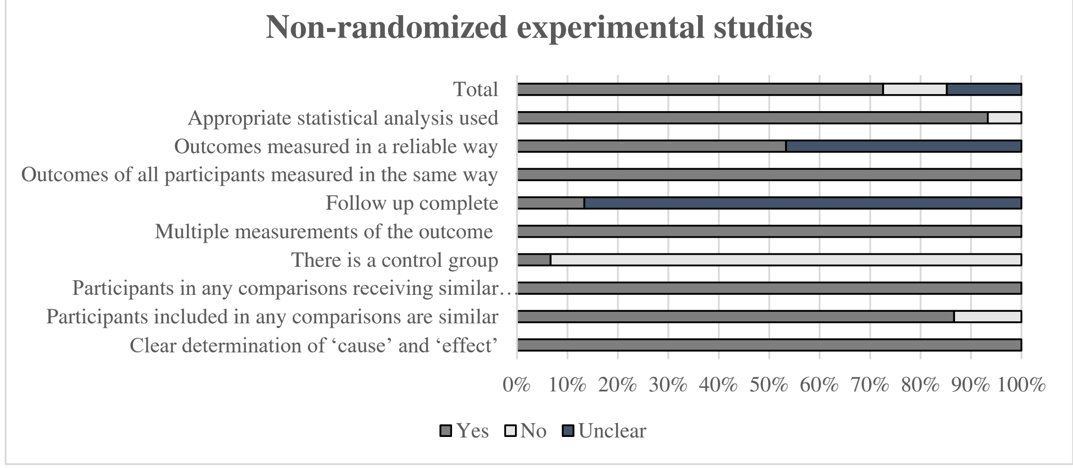

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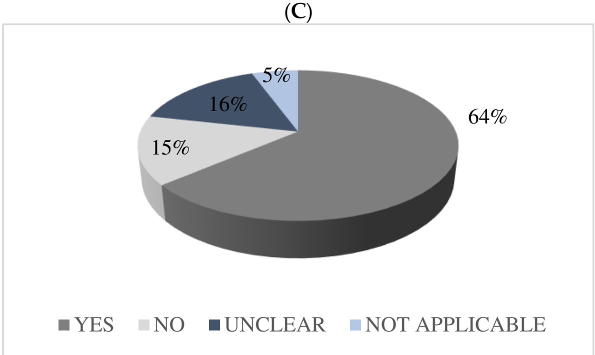

Figure 2. Assessment of studies' bias risk following the *Joanna Briggs Institute (JBI) Reviewer's Manual*. (**A**) Risk of bias assessment of randomized clinical trials (n = 9); (**B**) Risk of bias assessment of non-randomized clinical trials (n = 15); (**C**) Total score.

Regarding sample characteristics, studies involving healthy subjects or those with comorbidities, such as obesity, chronic kidney disease, mild cognitive impairment (MCI), type two diabetes, dyslipidemia, and coronary artery disease, were included (Figure 3). We highlight that the health classification indicated in this systematic review is similar to that of other researchers. For example, Thomson et al. (2008) and Strunz et al. (2008) included overweight subjects in their analyses. However, we included the study in the

category of healthy subjects based on the eligibility criteria described by the authors of the original articles. In other words, we evaluated subjects without diseases diagnosed by their respective studies.

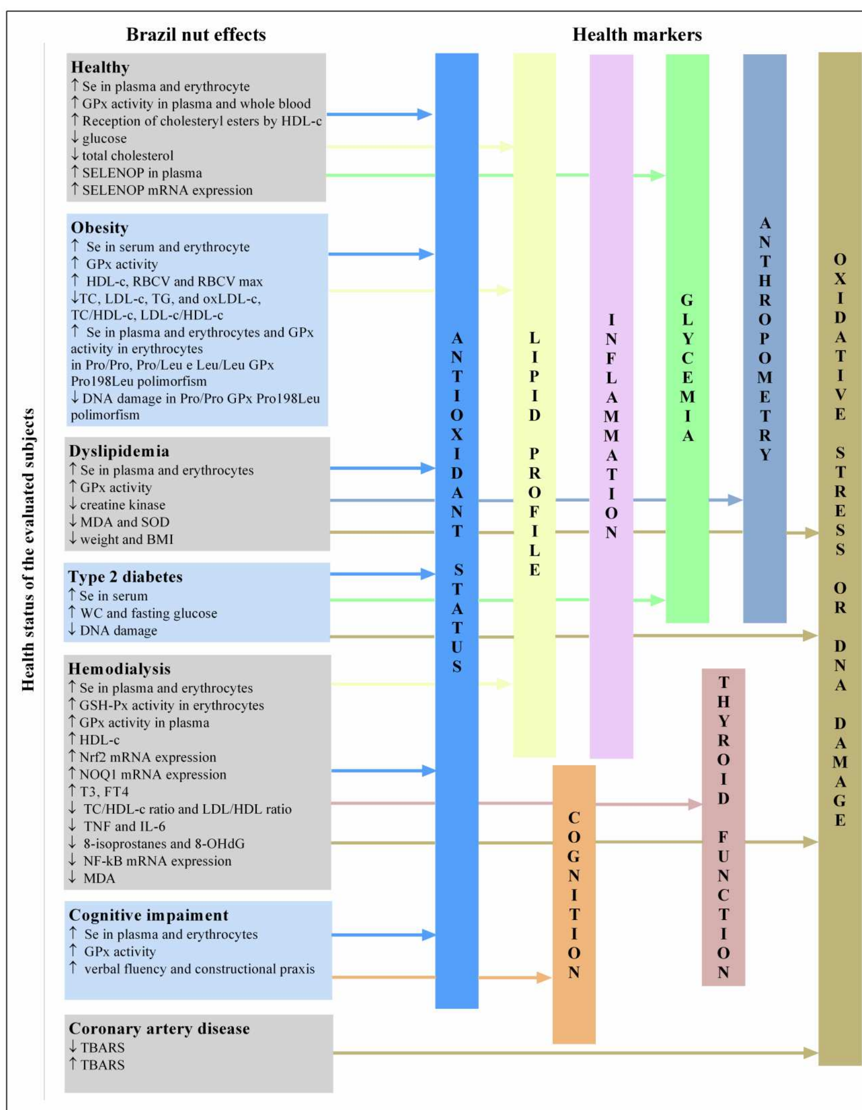

Figure 3. Summary of the effects of Brazil nuts consumption on human health. The effects of regular BN consumption were grouped according to the health status of the subjects evaluated in the studies included in the review. These effects were then linked to a larger category of health markers.

BNs (whole seed) were consumed in a free-living habitual diet in all studies. The Se concentration in BNs varied from 48 μg in six units [18] to 1.261 μg only in one unit [19].

Legend: GPx, glutathione peroxidase; HDL-c, high-density lipoprotein cholesterol; SELENOP, selenoprotein P; RBCV, red blood cell velocity; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglycerides; oxLDL-c, oxidized low-density lipoprotein cholesterol; DNA, deoxyribonucleic acid; MDA, malondialdehyde; SOD, superoxide dismutase; BMI, body mass index; WC, waist circumference; Nrf2, nuclear factor erythroid 2-related factor 2; NQO1, NAD(P)H: quinone oxidoreductase 1; T3, triiodothyronine; FT4, free thyroxine; TNF, tumor necrosis factor; interleukin 6; 8-OHdG, 8-hydroxydeoxyguanosine; NF-κB, nuclear factor kappa B; TBARS, thiobarbituric acid reactive substances.

3.3. BNs Consumed by Healthy Subjects (Subjects without Diagnosed Diseases)

Regular (15 days to 12 weeks) consumption of BNs (1–11 units/day; 48 µg to 862.65 µg of Se) improved the antioxidant statuses (increased Se in plasma and erythrocytes, GPx, GPx3, SELENOP, and SELENOP mRNA expression), and lipid profiles (decreased TC and increased HDL-c cholesteryl reception) of healthy subjects and reduced their fasting glucose [16,17,21,30,32,38] (Figure 3). Similarly, acute consumption of 1–10 units/day of BNs (156 µg to 1560 µg of Se) associated with a normocaloric diet improved the antioxidant statuses (increased Se in plasma) and lipid profiles (increased HDL-c, decreased LDL-c, and atherogenic indices) in 24 postprandial hours up to 30 days after a single day of consumption [38,39]. Considering that the recommended plasma Se range is between 60–100 µL, only one study with healthy subjects observed that the participants were Se-deficient at baseline (Figure 4A). In this study, Se-deficient subjects displayed an increased reception to cholesteryl esters by HDL-c after BN consumption [24].

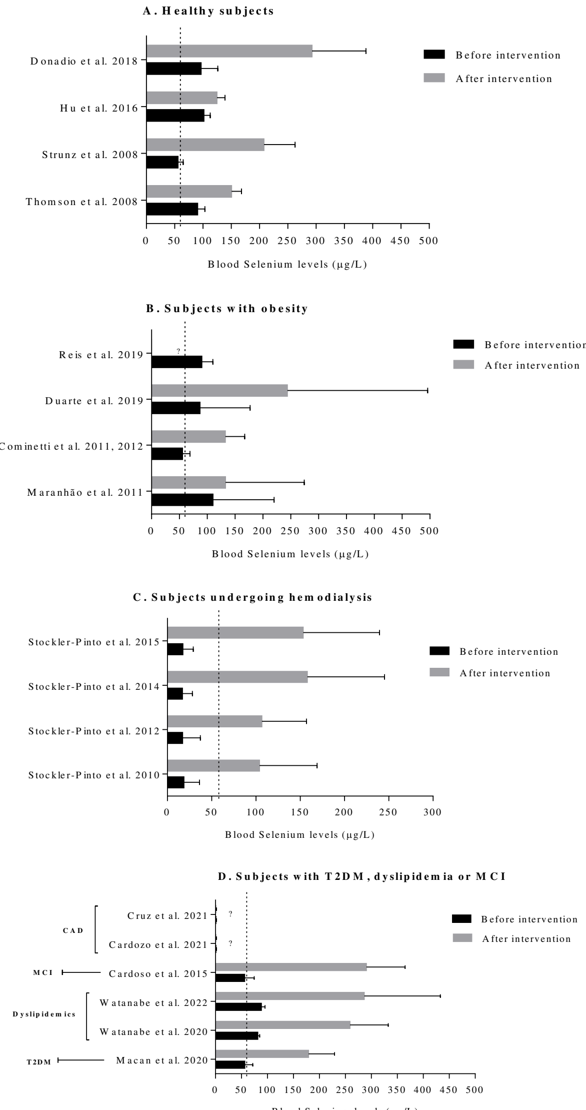

Figure 4. Blood selenium levels (plasma or serum or total blood) before and after Brazil nut supplementation in (**A**). healthy subjects [14,18,24,34], (**B**). subjects with obesity [15,19,20,26,27], (**C**). subjects undergoing hemodialysis [25,28–31], and (**D**). subjects with type 2 diabetes mellitus (T2DM) or using statins or mild cognitive impairment (MCI) or with coronary artery disease (CAD), ?, not informed [16,17,21–23,35–37].

Oxidative stress and related diseases, such as cardiovascular diseases (CVDs), are favored when antioxidant status is inferior to oxidizing agents in the body. Considering the importance of primary health care for disease prevention and health promotion, including BNs in the diet can help maintain the antioxidant status equilibrium by Se and related proteins [40,41]. In response to BN consumption, increased plasma SELENOP [34] and its expression [18] were observed in healthy subjects, confirming that SELENOP levels are a biomarker of Se status. Furthermore, an increase in GPx activity in the plasma and whole blood was observed in adult New Zealanders after 12 weeks, indicating the importance of Se in the production of antioxidant enzymes [14].

There is no evidence concerning the mechanisms involved in the beneficial effects of BN consumption on human lipid profiles. However, in experimental studies, Se supplementation downregulated Apo B and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase expression in hypercholesterolemic rats [42]. This finding may potentiate a beneficial effect of BN consumption on the lipid profile, as HMG-CoA participates in the cholesterol biosynthesis pathway, while Apo B is the main protein component of LDL-c. In another experimental study, co-supplementing rats fed a high-fat diet with Se and magnesium decreased blood and liver TG levels and TC/HDL-c and TG/HDL-c ratios while increasing antioxidant enzymes. Furthermore, co-supplementation inhibited hepatic lipogenesis gene expression, decreased HMG-CoA reductase, and increased cholesterol 7α-hydroxylase (CYP7A1) and lecithin cholesterol acyltransferase (LCAT) in the liver [43]. LCAT removes cholesterol from the blood and tissues and may help explain the increase in HDL-c and cholesteryl ester reception by HDL-c after BN consumption.

BN consumption decreased fasting glucose levels in healthy subjects [32]. In a study of non-diabetic subjects, supplementation with 200 µg of Se (as Se yeast) for six weeks reduced glycated hemoglobin but not fasting glucose [44]. In an observational study of participants with adequate Se status, a 10 µg/L increase in Se was associated with a 1.5% increase in insulin and a 1.7% increase in HOMA-IR [45]. Se status is an important determinant of the effect that Se supplementation will have on the body. In contrast, pistachio consumption showed beneficial effects on glucose metabolism markers in prediabetic subjects. The authors hypothesized that a synergistic effect of unsaturated fats, polyphenols, and carotenoids in the food matrix improved insulin sensitivity through the PI3K-AKT pathway [46].

Some studies have also explored the effect of polymorphisms in specific genes on the responses of healthy subjects to BN consumption, highlighting the influence of genetics on food consumption responses. TC decreased in the GA + AA and GG groups in subjects with polymorphism rs7579 in the SELENOP1 gene. However, allele A carriers tended to have higher cholesterol levels after consumption, mainly after four weeks of intervention. In addition, allele A carriers of polymorphism rs3877899 in the SELENOP1 gene had lower glucose levels at baseline and after eight weeks of intervention [32]. T allele (CT + TT) of the rs1050450 polymorphism in the GPx1 gene tended to have lower GPx activity than CC carriers. In contrast, subjects with polymorphism rs7579 in the SELENOP1 gene had increased plasma SELENOP1 concentrations after eight weeks of intervention, but with no difference between the GG and GG + AA alleles [34]. GPx1 mRNA expression increased in subjects with the CC genotype for rs1050450 after BN consumption, while it did not change in T allele carriers. SELENOP mRNA expression was higher in allele A carriers for rs7579 than in GG subjects after BN consumption [33,34]. SELENOS and SELENOF mRNA expression levels remained unchanged after BN consumption.

3.4. BN Consumption by Subjects with Obesity

Different effects were found in subjects with obesity who consumed BN depending on the amount of Se present in the BN. Consumption of three–five units/day of BNs (mean Se: 108.5 µg) increased serum Se levels compared to baseline. TC, TGs, and ox-LDL levels reduced after BN consumption, while red blood cell velocity increased compared to a placebo in adolescents with obesity after 16 weeks of consumption (Figure 3). No changes

were observed in anthropometric and glucose metabolism markers, CRP, GPx-3, and 8-epi-PGF2α levels [15]. Another study with women with obesity observed an improvement in plasma Se, GPx activity, and HDL-c, and a decrease in atherogenic indices after participants consumed one unit/day of BN (290 μg of Se) for eight weeks [27]. However, the BNs had higher Se contents compared to the study performed by Maranhão et al. (2011) [15], and the subjects were Se-deficient at baseline (Figure 4B). Moreover, improvements in plasma Se and GPx activity were observed in the Pro/Pro, Pro/Leu, and Leu/Leu genotypes of the Pro198Leu glutathione peroxidase polymorphism. In contrast, DNA damage decreased only in Pro/Pro genotype carriers versus the baseline, which was significantly lower than that in carriers of the Leu/Leu genotype [26].

As previously discussed, no human studies have explained the relationship between Se and lipid metabolism. However, animal studies have noted a reduction in HMG-CoA reductase after Se supplementation and consequently decreased endogenous cholesterol production [42]. The BN is a food matrix and, in addition to Se, also contains unsaturated fats, such as monounsaturated fatty acids (MUFAs), which have recognized functions in TC, TGs, and LDL-c in healthy subjects [47,48]. The benefits of the BN in reducing ox-LDLs, known oxidative stress marker and a risk factor for atherosclerosis [49], are easier to explain because Se is a non-enzymatic antioxidant and contributes to the selenoproteins' formation, adding to the redox balance by scavenging free radicals [50]. Excessive concentrations of these free radicals in the body can oxidize LDL-c, stimulating inflammation and leading to endothelial dysfunction in the intimal artery.

Weight and BMI did not decrease after regular BN consumption in subjects with obesity [15], suggesting that BN consumption cannot modulate weight when consumed in the context of a habitual diet, without energy restriction, for example. In addition, weight and BMI also did not increase. Studies have shown that despite the high-calorie density and high-fat content of nuts, habitually consuming nuts is not related to weight gain [51].

On the other hand, despite an increase in antioxidant status markers, women with obesity supplemented with one unit/day of BN containing approximately three times the upper limit (UL) of Se (400 μg) for eight weeks had increased pro-inflammatory cytokine expression and decreased GPx1 expression compared to the control group. Furthermore, increased miR-454-3p and miR-584-5p expression levels were observed [19,20]. The findings of this study reinforce that Se deficiencies and overload are harmful to health. It has been hypothesized that excessive Se consumption may play a pro-oxidant role by inducing reactive oxygen species (ROS), activating the Akt-NF-κB pathway, and further stimulating leukocytes and pro-inflammatory cytokine genes [52]. Simultaneously, the decrease in GPx expression can be explained by the fact that from a specific concentration of Se in the blood, GPx activity reaches its maximum [53]. Moreover, these studies note the beneficial health effects of consuming BN with Se contents below the tolerable UL for this population with high adiposity, reinforcing the need for attention regarding the choice of BN consumed based on Se content.

In summary, improvements in antioxidant status and lipid metabolism markers were observed after the minimal consumption of BNs containing an average Se of 108.5 μg in subjects with obesity (Figure 3). Se doses above the UL have shown adverse effects in women with obesity.

3.5. BN Consumption by Subjects with Dyslipidemia, Type 2 Diabetes, or Coronary Artery Disease

An increase in inflammatory cytokines and oxidative stress is expected in dyslipidemia, diabetes mellitus, and CVD etiology and progression. In this context, Se deficiency is related to an increased risk of CVDs and mortality, possibly due to reduced selenoprotein synthesis and antioxidant capacity feeding back inflammation [54,55].

BN is a source of Se, MUFAs, polyphenols, and phytochemicals. However, a single unit of BN does not contain significant amounts of MUFAs and bioactive compounds. The nutrient that stands out in its composition is the Se because a single unit of BN can contribute five times more than the daily requirement for this mineral [10]. For this reason,

we believe that most of the benefits achieved from BN intake are related to Se content (Table A2).

Consumption of one unit/day of BN (290 µg of Se) for 24 weeks improved antioxidant statuses (increased GPx activity and Se in the plasma and erythrocytes and reduced MDA and creatine kinase) in subjects using statins, while SELENOP mRNA expression was not affected [36] (Figure 3). Statins inhibit cholesterol synthesis via the mevalonate pathway, an important pathway for selenoprotein synthesis. Therefore, subjects who use statins are at an increased risk of developing Se deficiency and oxidative stress [56,57]. BN is a source of Se and may help maintain the nutritional status and oxidative balance of Se in patients using statins. Despite being instructed to maintain their usual diet, these subjects had reduced body weights and BMI. It is unlikely that the reductions in body weight and BMI were related to BN consumption. The authors reported that the effect of Se on BMI occurred under suboptimal Se conditions, which was not the case for the participants [36]. In addition, caloric restriction is necessary for weight loss, regardless of Se intake. However, studies have shown a relationship between selenium and adipogenesis [58], including increased lean mass and muscle mass [59]. Regarding the presence of polymorphisms, BN consumption increased GPX1 mRNA expression only in subjects with the rs1050450 CC genotype. SELENOP mRNA expression was significantly lower in subjects with the rs7579 GG genotype before and after the intervention.

No significant changes in Nrf2, NF-κB, NQO1, or peroxisome proliferator-activated receptor (PPARβ/δ) mRNA expression levels or markers of lipid metabolism and inflammation were found in subjects with coronary artery disease after three months of intervention with one unit/day of BNs (~290.5 µg of Se) compared to the control group. Controversial results regarding changes in TBARS were observed in the two articles on the effect of BN consumption in individuals with coronary artery disease [21,22] (Figure 3). The authors did not provide the baseline values of plasma Se values. This missing information is important to clarify the discussion because subjects with a normal Se status may not benefit from Se supplementation [55,60]. Another hypothesis is that these patients had multiple metabolic disorders and, therefore, required higher Se doses or extended interventions to benefit from BN consumption.

The consumption of one unit/day of BN (213.67 µg of Se) increased serum Se and decreased DNA damage ex vivo in subjects with type 2 diabetes treated with oral glucose-lowering medication and insulin [35] (Figure 3). Again, BN consumption had benefits related to oxidative stress in a different population and was closely associated with Se status and selenoprotein synthesis.

Nevertheless, the subjects had increased waist circumferences and fasting blood glucose levels compared to the baseline, but the HbA1c levels remained unaltered [35]. Plasma Se and fasting glucose levels did not correlate. Considering that all the subjects were overweight and instructed to maintain their regular diet, these results can be attributed to the unbalanced diet. BN consumption was unable to improve glucose metabolism markers. Other nuts, especially pistachios, have been associated with better glucose control. This nut can modulate the insulin signaling pathway (via PI3K-AKT), favoring glycemic control [46]. However, higher doses (>40 g/day or 20% of total energy) may be needed [61].

Indeed, few interventions regarding BN intake have included participants with diabetes, dyslipidemia, or coronary artery disease. Hence, the evidence is limited. In this population, as in others, the effects of daily BN intake seem to be related to Se metabolism. Therefore, people with low plasma Se levels probably benefit more from BN consumption. Further investigations are necessary to understand the pathways involved.

3.6. BN Consumption by Subjects Undergoing Hemodialysis

A range of benefits could be observed in patients undergoing hemodialysis who regularly consumed one unit/day of BN (290 µg of Se) for three months. These benefits included enhanced non-enzymatic (increased Se in plasma and erythrocytes) and enzymatic (increased GSH-Px activity in erythrocytes and GPx activity in plasma) antioxidant defense

systems, in line with the increased Nrf2 and NQO1 mRNA expression. In addition, the studies also showed improvements in inflammation (reduced Nf-κB mRNA expression, TNF, and IL-6 levels), oxidative stress (reduced malondialdehyde and 8-isoprostanes), and DNA damage (decreased 8-OHdG). There were also improved lipid profiles with an increased HDL-c and decreased atherogenic indices. Furthermore, elevated T3 and free thyroxine (FT4) levels were observed [19,34,35,37] (Figure 3). The same group of researchers in Brazil published all of these results. As Figure 4C presents, subjects undergoing hemodialysis were Se-deficient before BN supplementation (Figure 4).

Dialysis is a treatment for end-stage renal disease caused by chronic kidney disease, a debilitant disease associated with altered immune response, systemic inflammation, and oxidative stress. In addition to high oxidant production, subjects with chronic kidney disease have impaired antioxidative systems, favoring an oxidizing state [62]. These processes are responsible for the metabolic disorders observed in subjects with chronic kidney disease, such as dyslipidemias, who become susceptible to developing atherosclerosis, CVDs, and other complications [63]. Faced with a weakened state of health, subjects undergoing hemodialysis benefited greatly from BN consumption, a food matrix with a valuable nutritional profile, especially antioxidant compounds such as Se.

Although inflammation and oxidative stress are natural mechanisms in the body, they lead to the overproduction of pro-inflammatory mediators and oxidant agents when unregulated. For example, uremic toxins, such as indoxyl sulfate, produced by subjects with chronic kidney disease, are one of the agents responsible for activating NF-κB. Thus, pro-inflammatory cytokines, such as IL-6 and IL-1, are produced, and TNF, oxygen, and reactive hydrogen species feed a vicious cycle of stimulating the production of pro-inflammatory molecules and consequent inflammation exacerbation. Additionally, uremic toxins are linked to malondialdehyde and peroxynitrite production and also advanced glycation end products. These are related to NF-κB activation and a reduction in the activity of the transcription factor Nrf2 and, consequently, the underregulated expression of antioxidant enzymes [62]. These events favor a state of oxidative stress, making DNA susceptible to oxidative damage, as marked by an increase in 8-OH-dG.

Subjects with chronic kidney disease and end-stage renal disease are deficient in Se due to reduced intestinal absorption, loss during hemodialysis, and other causes. This worsens the depleting state of antioxidant agents. Therefore, BN supplementation seems to be interesting, improving the antioxidant, inflammatory, and oxidative stress status. However, 8-isoprostanes, 8-OHdG, IL-6, and TNF levels were significantly increased, whereas Se levels and GPx activity decreased 12 months after the intervention was interrupted [28,30], highlighting the importance of regular BN consumption. In addition, the kidneys participate in thyroid hormones' metabolism, which tends to be impaired in subjects with chronic kidney disease. As Se participates in thyroid hormone formation, BN supplementation increased T3 and FT4 levels in hemodialysis subjects.

3.7. BN Consumption by Subjects with MCIs

Consumption of one unit/day of BN (288.75 µg of Se) for 24 weeks increased Se levels in plasma and erythrocytes, plasma GPx activity, verbal fluency, and construction praxis in older adults with MCIs (Figure 3). In addition, Se status was positively correlated with the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) score, with higher scores indicating better cognitive performance [16].

Cognitive impairments and neurodegenerative diseases are related to oxidative stress caused by damage to the central nervous system. For this reason, Se deficiency can compromise the redox balance and reduce cognitive performance [64]. The authors have pointed out that most of the sample had Se deficiency at baseline, which seems to be a determining factor in achieving benefits from Se supplementation. However, in a population of older adults with adequate Se intake (85% of all participants), plasma Se levels were not associated with cognitive performance or neurotrophic factors [65].

Genetic factors also influence the responses to BN intake. For example, single nucleotide polymorphisms (SNPs) in genes encoding selenoproteins seem to negatively contribute to the protective effect against oxidative stress. In this sense, in subjects with MCIs, consuming BN daily (288.75 µg of Se) for 24 weeks increased GPx1 mRNA expression in carriers of a variant allele (CT + TT) for rs105045. Conversely, GPx1 mRNA expression was significantly reduced in rs7579 A-carriers and rs3877899 GG carriers [17].

The SNPs also affected SELENOP mRNA expression in MCI patients. While carriers of a variant allele (CT + TT) for rs1050450 showed increased SELENOP mRNA expression after treatment, no differences were observed for CC carriers [17]. Further studies are needed to clarify how polymorphisms would influence Se markers.

Studies on the effect of BN intake on MCIs are scarce. However, the available evidence shows superior benefits in people with low plasma Se values. In addition, Se deficiency can be a risk factor for cognitive impairment and polymorphisms that affect Se metabolism and utilization.

3.8. Future Perspectives

Currently, we know that BN consumption benefits human health, and seems to vary according to different routes of action and the health status of the sample evaluated. In addition, these findings refer to BN consumption in a free-living habitual diet without restrictions or adaptations. We emphasized above that individuals on hemodialysis benefited greatly from BN consumption. Many markers were evaluated in this sample of subjects undergoing hemodialysis, which does not mean that other studies did not observe the same benefits. This suggests that more studies are required to evaluate such markers also in other samples, with or without diseases. Considering that caloric restriction is one of the most reputable approaches in the literature for weight management, studies evaluating the effects of nut consumption in the context of caloric restriction are scarce. A recent study showed the superior effects of consuming of a mix of Brazilian nuts (30 g cashew nuts + 15 g BN) with a 500-calorie restricted diet in improving body composition and reducing the soluble adhesion molecule vascular cell adhesion protein 1 (VCAM- 1) [66]. Thus, it would be interesting to conduct studies in this field using BN. In addition, as Se is a powerful antioxidant, studies evaluating longevity markers are of great relevance alongside those examining the intestinal microbiota and their response to adequate Se blood levels compared to inadequate levels. In view of the evidence regarding the interaction between nutrients and the intestinal microbiota, nuts have been investigated as modulators of the microbiota, since it is a food matrix rich in nutrients. However, to date, no study has investigated the effect of BN on the microbiota. Studies comparing the effect of BN consumption with isolated Se supplementation would also be of great interest because of limited evidence in this field.

4. Conclusions

Nowadays, scientific evidence supports the health benefits of regular BN consumption in a free-living habitual diet. This seems to vary according to the health status of the subjects, the Se content of the BNs, the Se levels of the participants before the intervention, and the presence of some polymorphisms. The benefits of BN consumption were found in different action pathways.

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Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Search strategy.

PubMed	Search: ((((Brazil nut[Title/Abstract]) OR (Brazil nuts[Title/Abstract])) OR (Bertholletia excelsa[Title/Abstract])) OR (Bertholletia excelsa Humn. & Bonpl.[Title/Abstract])) OR (Bertholletia excelsa H.B.K[Title/Abstract]) Filters: Clinical Trial, Humans Sort by: Most Recent ("brazil nut"[Title/Abstract] OR "brazil nuts"[Title/Abstract] OR "bertholletia excelsa"[Title/Abstract] OR (("Bertholletia"[MeSH Terms] OR "Bertholletia"[All Fields] OR ("Bertholletia"[All Fields] AND "excelsa"[All Fields]) OR "bertholletia excelsa"[All Fields]) AND "humn"[All Fields] AND "bonpl"[Title/Abstract]) OR "bertholletia excelsa h b k"[Title/Abstract]) AND ((clinicaltrial[Filter]) AND (humans[Filter])) Translations Bertholletia excelsa: "bertholletia"[MeSH Terms] OR "bertholletia"[All Fields] OR ("bertholletia"[All Fields] AND "excelsa"[All Fields]) OR "bertholletia excelsa"[All Fields]
Embase	'brazil nut'/exp OR 'brazil nut' OR 'para nut' OR 'paranut' OR 'brazil nuts' OR 'bertholletia excelsa' OR 'bertholletia excelsa humn. & bonpl.' OR 'bertholletia excelsa h.b.k' AND ('clinical trial'/de OR 'human'/de)
Scielo	(ti:(Brazil nut)) OR (ti:(Brazil nuts)) OR (ti:(Bertholletia excelsa)) OR (ti:(Bertholletia excelsa Humn. & Bonpl.)) OR (ti:(Bertholletia excelsa H.B.K)) OR (ti:(castanha-do-brasil)) OR (ti:(castanha-do-pará))

Table A2. Brazil nut composition of studies included in the systematic review.

Studies	Quantity of BN	Selenium (µg)	Calories (Kcal)	Carbohydrates (g)	Proteins (g)	Lipids (g)	PUFA (g)	MUFA (g)	SFA (g)	Fiber (g)
[14]	2 units (~8.2 g)	53 (20–84)								
[15]	3 to 5 units (15–25 g)	108.5 (27)	124 (31)	5.2 (1.3)	16.8 (0.2)	10.1 (2.5)	4.2 (1.0)	5.5 (1.4)	3.0 (0.7)	
[16,17]	1 unit (~5 g)	288.75	35.74	0.54	0.81	3.27				
[18]	6 units	48								
[19]	1 unit (~5 g)	1261.4	35.31	0.79	0.78	3.25				
[20]	1 unit (~5 g)	1261.4	35.31	0.79	0.78	3.25				
[21]	1 unit (5 g)	290.5	36.7	0.45	0.75	3.53				
[22]	1 unit (5 g)	290.5	36.7	0.45	0.75	3.53				
[24]	11 units (45 g)	862.65	295.17	5.52	6.44	29.89	9.26	11.06	6.81	3.38
[25,28,29,31]	1 unit (5 g)	290.5	36.7	0.45	0.75	3.53				
[26,27]	1 unit (5 g)	290								
[23]	1 unit (5 g)	290.5	36.7	0.45	0.75	3.53				
[32–34]	1 unit (3–4 g)	300–400	21.99–29.32	0.45–0.6	0.39–0.52	2.07–2.76				
[35]	1 unit (3.7 g)	213.67	26.45	0.4	0.6	2.49				
[36,37]	1 unit (5 g)	290,00	34.8	0.65	0.55	3.58				
USDA [67]	1 unit (5 g)	96,00	33	0.585	0.715	3.36	1.22	1.2	0.805	0.375

Composition of Brazil nut (BN) was evaluated through Association of Official Analytical Chemists (AOAC) and carbohydrate obtained by difference. Selenium concentrations were determined through inductively coupled plasma mass spectrometry.

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5.3 ARTIGO ORIGINAL 1 – A ser submetido à revista British Journal of Nutrition (FI: 3.334)

Effect of Brazil nut (*Bertholletia excelsa* h.b.k.) within an energy-restricted diet on adiposity and lean mass indicators in women at cardiometabolic risk: a controlled, parallel study (Brazilian Nuts Study)

Alessandra da Silva[a]

Brenda Kelly de Souza Silveira[a]

Brenda Vieira Machado de Freitas[a]

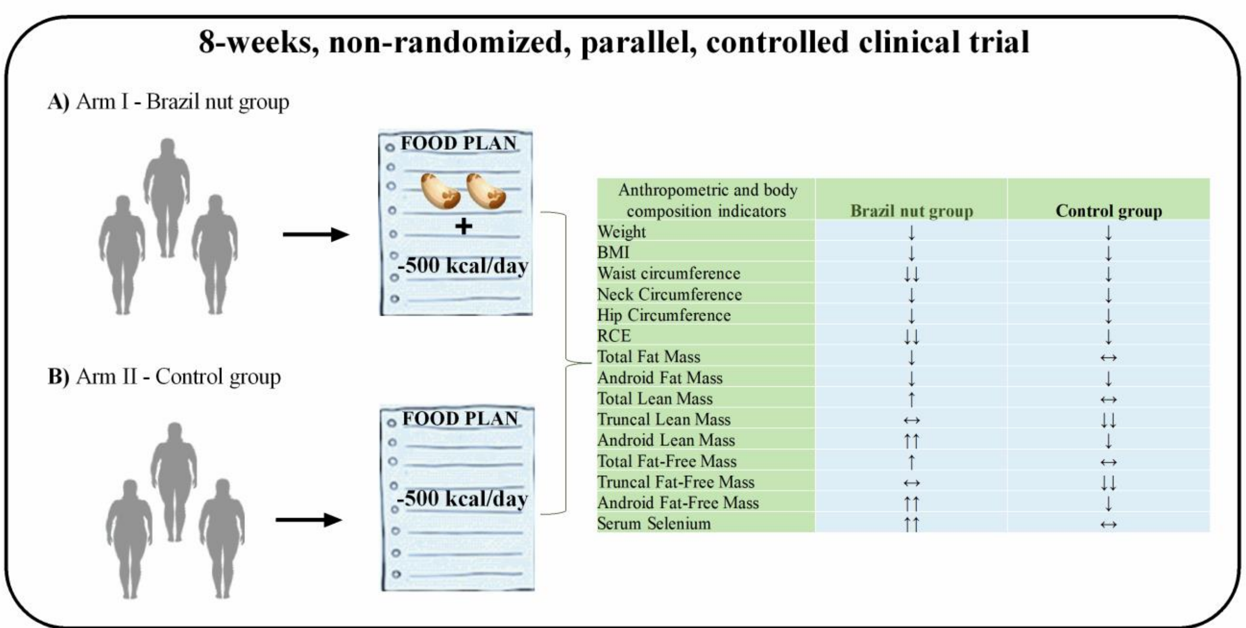
Karina Waskow[a]

Helen Hermana M. Hermsdorff[a]

Josefina Bressan[a]

[a] *Laboratory of Energy Metabolism and Body Composition. Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.*

Corresponding author: Alessandra da Silva. Laboratory of Energy Metabolism and Body Composition. Department of Nutrition and Health, Universidade Federal de Viçosa. Avenida PH Rolfs, s/n. Viçosa – MG, Brazil. 36570-900. Phone: +55 31 36125220. E-mail: alessandra.silva2@ufv.br



Graphical Abstract

Abstract

Background/objectives: Brazil nut (BN) has recognized bioactivity within a habitual diet, mainly through anti-oxidative and anti-inflammatory pathways. Despite this evidence, no study has evaluated the effect of BN consumption associated with obesity treatment. Therefore, we aimed to assess the effect of BN within an energy-restricted diet on anthropometry and body composition among women at cardiometabolic risk.

Subjects/Methods: This is an eight-week, non-randomized, parallel, controlled nutritional intervention study. The women were allocated into two groups: control energy-restricted diet (-500 kcal/d) free of BN (n=24) and energy-restricted diet (-500 kcal/d) with 2 units/day of BN (~ 347 µg of selenium) (n=25). Body composition was assessed using dual-energy X-ray absorptiometry.

Results: We analyzed 49 women [33.9 (SD 7.5) years old, 33.4 (SD 4.3) kg/m²]. Both groups had similar weight loss and serum selenium increased 276.7% in BN group. The BN group had a more pronounced reduction in waist circumference and waist-to-hip ratio and presented the most preserved percentages of lean and fat-free mass in relation to the control group. In addition, more preserved lean and fat-free mass were associated with higher variation in serum selenium levels.

Conclusions: The consumption of 2 units/day of BN in an 8-week weight loss intervention promoted a reduction in anthropometric measurements and improved body composition in women at cardiometabolic risk. Our results suggest that regular consumption of BN in energy-restricted diets may be a strategy to reduce central adiposity, preserve lean mass and, consequently, help obesity management.

Trial registration: RBR-8zfn5c and RBR-3ntxrm.

Key words: body fat; free-fat mass; obesity; oilseeds; selenium; weight loss

Introduction

Obesity is a chronic condition with increasing prevalence and incidence worldwide and an independent risk factor for other non-communicable chronic conditions with public health concerns [1–3]. Moreover, central adiposity, also known as abdominal obesity and even android obesity, reflects visceral fat accumulation associated with chronic diseases [4]. Seventy-four percent of all deaths worldwide are attributed to chronic non-communicable diseases and efforts should be directed toward controlling the high mortality rate [5].

Although obesity has a multifactorial etiology, biologically, the excess calories ingested in parallel with decreased energy expenditure lead to weight gain and increased adiposity [6]. In this sense, energy restriction is currently the best-established approach in the literature for weight loss and consequent obesity control [7]. However, it is associated with failure due to difficulty in adherence and loss of lean mass concomitant with weight loss stands out as one of the main challenges of this strategy [8,9].

In turn, functional foods alone, such as nuts, do not lead to weight loss, and they can be beneficial in controlling satiety and increasing thermogenesis [10–12]. The consumption of Brazilian nuts and almonds combined with an energy-restricted diet did not lead to greater weight loss but improved body composition, especially in maintaining lean mass [13] and decreasing fat mass [14].

In this sense, BN is a nut native to the Amazon biome, and like other nuts, it is a food with concentrated bioactive compounds such as fibers, proteins, phytochemicals, and unsaturated fats [15], in addition to it is one of the richest sources of selenium. This mineral is essential in redox homeostasis, the immune system, and endogenous cholesterol production [16]. Studies also suggest that selenium participates in adipogenesis [17] and, therefore, may influence body composition.

Several studies have investigated the effects of regular consumption of BN in the context of the usual diet on health, including elevation of plasma selenium, increased selenoproteins and HDL-c, improvement of inflammation, and oxidative stress [18–24]. However, these benefits vary between studies and seem to depend on the health status of the subjects evaluated, the levels of selenium in the blood before the intervention with BN, and even the presence of some polymorphisms in selenoproteins. Despite all this evidence, most studies are not controlled, the selenium content and the intervention time differ between studies. Furthermore, no study has evaluated the potential effect of BN consumption in the context of caloric restriction; neither study explored the possible relationship between selenium consumed through BN and body composition markers.

Thus, we evaluated the effect of BN intake within an energy-restricted diet on anthropometry and body composition. Second, we evaluated anthropometry and body composition according to weight loss, diet, and variation in serum selenium levels.

Subjects and Methods

Study design and ethical approval

The present study is a non-randomized, controlled parallel eight-week nutritional intervention trial conducted in free-living conditions with women at cardiometabolic risk.

The study occurred in the Department of Nutrition and Health of the Universidade Federal de Viçosa (UFV), Minas Gerais State, Brazil), with enrollment between June 2019 and September 2021. The study protocol followed Resolution CSN 466/2012 and was approved by the local ethical committee of the UFV (CAAE: 92004818.0.0000.5153 e 21448719.0.0000.5153). All participants were informed about the objectives and study procedures. Those that accepted the study conditions provided written informed consent. This study also is registered in the Brazilian Registers of Clinical Trials – REBEC (protocol: RBR-3ntxrm e RBR-8zfn5c).

Subjects, recruitment, and sample calculation

Adult women (20-55 years) with overweight (BMI ≥ 27 kg/m^2 and <30 kg/m^2), waist circumference ≥ 80 cm, and body fat percentage ≥ 32% associated with at least one another component of metabolic syndrome: triglycerides ≥150 mg/dL, high blood pressure arterial (≥130/85 mmHg) or high fasting glucose (≥100 mg/dL); or women with obesity (BMI ≥ 30 kg/m^2), with or without metabolic complications were included. Non-inclusion criteria comprised pregnant, lactate, or menopausal women; athletes; vegans; smoking; women with a history of HIV, illness or digestive, liver, kidney, cardiovascular, thyroid, cancer, inflammatory diseases, and eating disorders; history of drug and/or alcohol abuse; aversion or allergy to nuts; infectious episode in the last month; use of anti-inflammatory drugs, corticosteroids, antibiotics, and others that may affect energy appetite and metabolism; body weight instability (5% of usual weight) in the last 3 months; regular consumption of any quantity of BN; alcohol consumption higher than 21 units (168g) per week; dental problems that interfere with chewing; regular use of vitamin, mineral, and omega-3 supplements.

Participants were recruited in the city of Viçosa, Minas Gerais, Brazil and in neighboring cities. Advertisements in social media (Instagram®, Facebook®, and WhatsApp®) and local radio and TV were the recruitment methods. After an initial screening, the women who met the essential eligibility criteria (age, BMI, no pregnancy, menopausal, and medical/supplement use) were invited to a face-to-face visit to evaluate their health history, physical activity level, and anthropometry.

The primary outcome measure was anthropometry and body composition. The sample size was calculated on GPower software 3.1.9.7 version, using data from a similar intervention study entitled “The effect of almonds on anthropometric measurements and lipid profile in overweight and obese females in a weight reduction program: A randomized controlled clinical trial” [25]. The sample size was estimated considering an effect size d of approximately 0,74

kg (weight mean difference of -3.68 (SD 2.82) kg for the almond-enriched diet group and -1.27 (SD 3.62) kg for the nut-free diet group), the statistical power of 95%, two-sided α, and power of 80%. The estimated number of participants required in each group to answer our research question is at least 36 (considering 20% loss to follow-up).

Dietary interventions

Women were allocated by convenience into two groups: control, which was instructed to consume an energy-restricted diet (-500 kcal/day) without any type of nuts, or the Brazil nut group (BN), which was asked to follow the energy-restricted diet (-500 kcal/day) containing approximately 8g (two units) of BN daily. Participants were monitored via WhatsApp® and in a face-to-face consultation 30 days after the start of the intervention. In the 30-day consultation, a 24-hour food recall was applied to check compliance, questions regarding physical activity, medication use, difficulties, and progress of the prescribed food plan. All women were instructed to maintain their lifestyle and medications during the study and to inform the research team of any changes in the type or dosage of the ongoing medication.

Energy-restriction

Total energy intake was estimated for each participant using the Estimated Energy Requirement (EER) for adult women with overweight/ obesity [40]; formula: 448 – (7.95 × age [y]) + physical activity × (11.4 × weight [kg] + 619 × height [m]). Then, 500 kcal/day was deducted from the dietary prescription to achieve a loss of 2 kg per month. Participants received an individualized eating plan with five nutritionally-balanced menus, each with five meals (breakfast, morning snack, lunch, afternoon snack, and dinner). The average distribution of carbohydrates, protein, and lipids was 50.0%, 20.0%, and 30.0% of daily energy, respectively, in accordance with recommendations of obesity management guidelines [7]. An example of a prescribed menu for the control and BN group containing ~1,800 kcal/day is presented in the Supplementary material (**Chart 1**).

For the BN group, the diets included the energy provided by the daily portion of 8 g (2 units/day) of BN. To balance the diets in macronutrients, total, polyunsaturated, saturated, and total fats, the control group was instructed to consume a sauce based on soybean oil and lemon (2:1 ratio, respectively). On the other hand, the BN group was oriented to consume a sauce based on canola oil and lemon (2:1 ratio, respectively). Sauces were provided by the research team and handed out to women fortnightly.

Brazil nut

The BN used in the study was donated by ECONUT® (https://econut.com.br/). The BN is organic, grown at Fazenda Aruanã, located in the municipality of Itacoatiara, state of Amazonas, Brazil. All BN were received in modified atmosphere packages and portioned (two units) in laminated packages, vacuum sealed (Selovac Sealer model 200 B), and stored in a freezer at -20ºC until distribution to the participants.

The amount of two units of BN used in this study was defined based on its selenium content not exceeding the Tolerable Upper Intake Level (UL) of the mineral, which is 400 µg/day. Furthermore, evidence has shown the beneficial effects of BN consumption with a minimum selenium content of 290 µg for women with obesity [19,26]. Each BN unit used in our study had 173.6 µg of selenium, measured by inductively coupled plasma atomic emission spectrometry determination of elements in food using Microwave Assisted Digestion [44]. Then, we doubled the number of BN to approximate the amount of selenium contained in the nuts from previous studies, which did not exceed the UL.

We also assessed the lipid profile of our BN by gas chromatography following the protocol proposed by Folch et al. 1957 and Hartman and Lago 1973 [27]. Monounsaturated, polyunsaturated, and saturated fatty acids represent 18.7 %, 48.2 %, and 33.1% of total fat in BN, respectively (**Table S1, supplementary material**).

Data collection

The participants were instructed to go to the Health Service of the Federal University of Viçosa, fasting for 10 to 12 hours before and at the end of the nutritional intervention period for blood collection and assessment of body composition using Dual-energy X-ray absorptiometry (DEX). Anthropometry, physical activity practice, and food intake were evaluated at the Laboratory of Energy Metabolism and Body Composition (LAMECC).

Anthropometry and body composition

At the first and last consultations, body weight (kg), height (m), waist (WC), hip, and neck circumferences (cm) were measured. Body weight was assessed by Inbody 230 (Biospace Corp., Seoul, South Korea). The height was measured using a stadiometer (Seca 206 model, Hamburg, Germany). BMI was obtained through the ratio between weight in kg and squared height in meters. Waist circumference was measured at the umbilicus waist at the end of normal expiration using an inelastic tape (precision 0.1 cm). Hip and neck circumferences were measured utilizing an inelastic tape at the maximum posterior extension of the gluteus and in the middle of the neck, respectively. Waist-to-height ratio (WHtR) was obtained through the ratio between WC and height in cm. Deep-Abdominal-Adipose-Tissue Index (DAAT, cm^2) was calculated through the equation for women: - 278 + [- 0.86 x weight (kg)] + [5.19 x WC (cm)] [29].

DEX (Lunar Prodigy Advance DXA System, GE Lunar) was used for body composition assessment. To ensure the reliability of the DEX exam, the participants were instructed on the exam days: to wear light clothes, remove any metal accessories (earrings, piercings, rings, etc.), not to be menstruating, not to do activities that require effort and to consume caffeinated foods 1 day before the exam, and total fasting for at least 10 hours. Values in kilograms of fat mass (FM), fat-free mass (FFM), lean mass (LM), and total mass were obtained from the total body and regions such as trunk, android, and gynoid. Posteriorly, the percentages were calculated in

relation to total body measurements (**Table S2, supplementary material**). The android area is between the ribs and the pelvis, while the gynoid region includes the hips and upper thighs and overlaps the leg and truncal regions. Body mass changes refer to the Δ value obtained through the final assessment minus baseline.

Biological samples, serum selenium assessment, and metabolic markers

Blood samples were collected at the beginning and end of the intervention period to biochemical analyses. The, serum triglycerides (mg/dL) and high-density lipoprotein cholesterol (HDL-c, mg/dL) were assessed by enzymatic colorimetric method.

Visceral adiposity index (VAI) was calculated through the formula for women: [WC (cm) / (36.58 + 1.89 x BMI (kg/m²))] x (triglycerides (mmol/L) / 0.81) x (1.52 / HDL (mmol/L)) [30]. According to standardized protocols, serum selenium (μ/L) was determined using inductively coupled plasma mass spectrometry.

Dietary assessment

To assess food consumption, participants were instructed to complete three-day food records (two nonconsecutive weekdays and one weekend day) before the start of the nutritional intervention period. A dietitian checked all reported food and its respective quantities. In the 60-day consultation, a 24-hour food recall was applied by a dietitian. REC24h-ERICA® software [28] was used to enter the food record. The software has a database composed of a list of items included in the database of food and beverage purchases from the *Pesquisa de Orçamentos Familiares* (POF – Brazilian Household Budget Survey). The food items that were not contained in the database were added by the interviewers. Then, estimated energy and nutrients intake were obtained.

Statistics Analyses

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 21.0 for Windows. The distribution of quantitative variables was performed

following the principles of the Gaussian distribution. Thus, the Shapiro-Wilk test was performed for each group of interventions. Continuous variables were described as mean, standard deviation, or median, and 95% confidence interval. Parametric variables were compared within and between groups using the paired t-test and *Student's t-test*, respectively. Non-parametric variables were compared within and between groups using the Wilcoxon test and the Mann-Whitney, respectively. Analysis of covariance (ANCOVA) adjusted by baseline value was used to compare means between groups when the variable differed between groups at baseline. The level of significance in two-tailed tests was set at 5%.

Results

Two hundred and two women were recruited between August 2018 and September 2021, but one hundred and forty-six did not meet the inclusion criteria or showed no interest in participating. A total of fifty-six women initiated the study, and forty-nine concluded. Twenty-nine women were allocated to the control group and twenty-seven to the BN group. The dropout in the follow-up was 17.2% in the control group and 7.4% in the BN group. 100% of the dropout were for personal reasons (**Figure 1**). Although we started the study with a smaller sample size than required (36 women per group), we identified a power of 99% considering the mean difference in weight loss in the BN group (effect size = -1.7305), bilateral α of 5 % and sample size of the group.

Women included in the study were 33.9 (SD 7.5) years old and BMI of 33.4 (SD 4.3) kg/m². Hip circumference, android FM (kg and %) and gynoid FM (kg) were higher in the control group compared to the BN group at baseline, but all other variables were similar between groups (**Table 1**). Weight, BMI, WHtR, WC, NC, HC, DAAT, and percentage of android fat mass decreased in both groups (**Table 1 and Figure 2A**). Total fat mass (%) was only reduced in BN group compared to baseline (**Figure 2B**). However, women allocated to the BN group presented significantly higher reductions in WC and WHtR compared to the

control group (**Table 1 and Figure 2A**). At the same time, the truncal lean mass (%), android lean mass (%), truncal fat-free mass (%), and android fat-free mass (%) decreased more in the control group, compared to the BN group (**Figure 2C and 2D**). Moreover, total lean mass (%) and total fat-free mass (%) increased in the BN group compared to the baseline values (**Figures 2C and 2D**).

Approximately 86% of participants had low serum selenium (<100 μg/L) before the study. After 8-wk intervention, serum selenium increased 276.7% (199.4%-327.4%) (median and 95% CI) in the BN group (p≤0.001) and 5.2 % (-7.7%-50,4%) in the control group. In the control group, 91.7% of the women remained with low serum selenium, while no women with low serum selenium were observed in the BN group.

Although we expected weight loss in all participants, 16.3% (total sample n = 8; control group n = 5 and BN group n = 3) did not lose weight. When we analyzed only women who lost weight after the eight weeks of intervention, weight, BMI, WHtR, WC, NC, HC, and DAAT decreased in both groups. Similar to the finding observed when all women that completed the study were analyzed, total fat mass (%) decreased while an increase in total lean mass (%) and total fat-free mass (%) was observed after BN consumption. In addition, the lean mass (kg and %) and fat-free mass (kg and %) of the trunk and android regions decreased in the control group compared to the BN group (**Table 2**). The mean change in selenium and energy intake for the entire sample was 160.2 (SD 180.56) μg and -252.41 (662.29) kcal, respectively. In turn, the mean change in selenium and energy intake only for participants who lost weight was 170.23 (SD 167.32) μg and -212.06 (520.18) kcal, respectively.

Of the participants who lost weight, 43.9% (36.8% in the control group and 50% in the BN group; p=0.531) reached the goal of weight ≥ 4kg in 8-wk energy restriction. After categorizing women according to weight loss, women who consumed BN and lost ≥ 4 kg presented lower WC, HP, and HtR than women who lost < 4kg. Besides, they showed a lower

total fat mass (%) and higher serum selenium compared to women allocated to the control group. Lastly, the control group has higher reduction in the percentage of lean mass (truncal, gynoid, and total) and fat-free mass (truncal, android, gynoid, and total) compared to the BN group (**Table S3, supplementary material**).

To assess the potential relationship between the change in serum selenium and adiposity markers, we divided the sample by the median change in serum selenium. Then, those participants with high serum selenium after an 8-wk intervention had a lower reduction of truncal lean mass and truncal fat-free mass, while lean mass and fat-free mass of the android region decreased in those women with the lowest median serum selenium variation compared to those with the highest median of serum selenium. (**Table S4, supplementary material**).

Regarding nutrient intake during intervention, intake of carbohydrates and SFA significantly decreased in the control group after the intervention, and MUFA and SFA decreased while fiber and selenium intake increased after BN consumption, compared to baseline. Intake of energy, macronutrients, MUFA, PUFA, SFA and fiber did not differ between groups after the intervention. On the contrary, selenium intake was higher in the BN group compared to the control group (**Table 3**). Physical activity levels did not change in either group during the intervention period.

Discussion

In this study, a diet enriched with two units of BN in the context of a balanced energy-restricted diet resulted in similar weight loss to the control group (-2.7% vs -3.9%, p=0.154) in women at cardiometabolic risk but reduced significantly more central adiposity indicators (waist circumference and waist-to-height ratio) as well as preserved lean mass and fat-free mass of the truncal and android regions.

Many randomized [19,21,31–33], non-randomized [23,34–37], controlled and non-controlled clinical trials that evaluated the possible effects of regular BN consumption in a

habitual diet are available in the literature [18]. The beneficial effects of BN consumption, such as improving the antioxidant status through increasing blood selenium levels, is a consensus among studies [18,38,39]. Other benefits include improved lipid profile [19,20,31,34], oxidative stress, inflammation [34,40], and increased selenoproteins, such as glutathione peroxide [21,22,41]. However, to the best of our knowledge, this is the first study that evaluates the consumption of BN in the context of an energy-restricted diet.

Studies with a similar design to our study (energy-restricted diet + nuts) can be found, but this is the first study that evaluated the consumption of BN without the combination of other nuts in the context of caloric restriction. In a 16-week randomized controlled clinical trial, consumption of 42 g/day of pistachios associated with a caloric deficit of 500 to 1000 kcal/day had no superior effects on weight reduction, BMI, waist circumference, and other cardiometabolic risk markers in adults with overweight/obesity [42]. In another study, consumption of 56g of conventional or high-oleic peanuts associated with a caloric deficit of 250 kcal/day also did not have superior effects to nut-free energy restriction on anthropometric and body composition markers in men with overweight/obesity after four weeks of intervention [43]. However, consumption of high oleic peanuts decreased total body fat (kg and %) and gynoid fat (%) while increasing gynoid and total lean mass (%) when compared to baseline. Interestingly, the group that received only energy restriction decreased total and trunk fat-free mass and total and trunk lean mass (kg) compared to baseline. On the other hand, consumption of 50g/day of almonds associated with 1000 kcal of caloric deficit reduced weight, BMI, waist circumference, waist-to-hip circumference ratio, markers of glucose and lipid metabolism, and diastolic blood pressure after 12-wk intervention compared to the nut-free group in women with overweight/obesity [25]. Different nuts, intervention time and restricted calories were evaluated in these studies, which makes comparison difficult. However, almonds have higher amounts of

monounsaturated fatty acids, which may help explain the better results observed in the study [25].

We observed greater preservation of lean and fat-free mass of the trunk and android regions after BN consumption than control, regardless of weight loss. Interestingly, in our study, those women of BN group who lost ≥ 4 kg also had a greater decrease in total fat mass (%) and greater preservation of lean mass and total lean mass and trunk, android and gynoid regions compared to women of control group who lost ≥ 4 kg. Similar to our findings, the consumption of a mix of Brazilian nuts (30g of cashew nuts + 15g of BN) associated with an energy-restricted diet decreased the fat mass (%) while increased lean mass and fat-free mass compared to control energy-restricted diet in women at cardiometabolic risk [13]. However, the fat mass (%) in the android region increased in the group that consumed the mixed nuts compared to the control group. Despite the profile of the participants evaluated in this study being similar to ours, the content of selenium present in the BN consumed in our study is approximately seven times higher than in the previous study of our group [13].

One of the explanations for the improvement in body composition in our study and others with a similar design but with other types of nuts is that, despite being foods rich in fat, nuts are not associated with weight gain [10,44]. Nuts is a food matrix, the nut cell wall restricts access to its lipid content, and therefore, not all the fat in the nuts is bioaccessible and much is lost in the feces [45]. Moreover, most of the fats in nuts are unsaturated and this fat profile is more rapidly oxidized and has a greater thermogenic effect than saturated fats, leading to less fat accumulation in the body [46,47]. Another hypothesis is that nuts affect satiety and thus reduce total energy intake [10]. Despite all these hypotheses, the consumption of unsaturated lipids was the same in the groups evaluated in our study. Furthermore, among the nutrients evaluated, selenium was the only one that increased after BN consumption compared to the control group.

Furthermore, we obtained more pronounced preservation of lean and fat-free mass of the trunk and android region in women with higher median variation in serum selenium. These results suggest a new hypothesis for improving body composition after BN consumption through increasing serum selenium. However, the results of the studies are still conflicting. One study observed a significant reduction in body fat mass (kg) and an increase in lean (kg) and muscle mass in subjects with overweight or obesity after twelve weeks of a balanced diet with a caloric deficit of 20 to 25% associated with supplementation of 240 µg/day of L-selenomethionine compared to the baseline [48]. In another observational study (n=3,214) subjects with obesity had lower selenium intakes than those with normal-weight. Besides, subjects with the highest dietary intake of selenium had the lowest values of BMI, waist circumference, and percentages of total body, android, gynoid, and trunk fat, suggesting that dietary selenium intake alone may be responsible for 9% to 27% of the total changes in body fat percentage [49]. Another study showed that serum selenium concentrations decreased with increasing BMI, waist circumference, and body fat percentage [50]. Overall, the relationship between selenium and obesity markers is inverse. Conflicting with these studies, in an observational study, waist circumference, BMI, visceral fat, body fat, and oxidative DNA damage were significantly lower in the first tertile of plasma selenium compared with the other selenium tertiles in individuals with type 2 diabetes [51]. Finally, a recent meta-analysis showed no differences in intake and plasma/serum selenium levels, while a decrease in glutathione peroxidase activity in subjects with overweight/obesity was observed compared to normal-weight [52].

Selenium is an essential precursor of selenoproteins, which are important in cellular redox homeostasis. Evidence suggests that selenium prevents adipogenesis by modulating selenoproteins gene expression and oxidative stress-related genes. The literature also indicates that both selenium deficiency and excess are harmful to health and may contribute to adipose

tissue dysfunction and the consequent manifestation of metabolic alterations. Indeed, it appears that selenium participates in adipocyte hypertrophy and adipogenesis. Selenium in the physiological range possesses an overall adipogenic effect by activating the peroxisome proliferator-activated receptor and the CCAAT enhancer-binding protein-α (C/EBP) signaling through positive modulation of PI3K/Akt and C/EBP-β/δ pathways, resulting in up-regulation of adipocyte-specific genes. On the other hand, selenium overload exerts antiadipogenic activity through suppression of peroxisome proliferator-activated receptor and C/EBPα expression through several mechanisms including reduction of C/EBPβ expression as well as activation of 5′AMP-activated protein kinase and transforming growth factor β [17,53]. Thus, according to these mechanisms, we could justify the better body composition in women with greater variation in serum selenium after eight weeks of nutritional intervention.

The main limitation of this study was the lack of randomization of participants between groups. Randomized controlled trials are at the top of the scientific evidence pyramid for conducting studies that investigate cause and effect. The non-randomization of the sample can lead to selection bias. Despite this, our study had a control group and the vast majority of variables did not differ between participants at baseline. We used appropriate statistics to correct the initial variability between groups for the variables that differed at baseline. Another limitation was that we added a sauce to the participants' diets to balance the macro and micronutrient diets. To avoid exposure bias, both groups received the sauce. A strength of our study is the use of DEX, the gold standard method to evaluate body composition. In addition, both physical activity and preparation for performing the DEX were monitored during the study. Furthermore, as highlighted throughout this manuscript, this is an innovative study because it is the first to assess the potential effects of BN consumption combined with an energy-restricted diet.

In conclusion, the consumption of 2 units/day of BN in an 8-week weight loss intervention promoted a reduction in central adiposity indicatos and preserved lean and fat-free mass in women at cardiometabolic risk. These findings suggest that the consumption of BN associated with a dietary obesity treatment may be a strategy to reduce central adiposity and preserve lean mass, and consequently, help in the management of obesity.

Studies investigating the strategy on clinical markers of cardiometabolic risk are encouraged.

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Author contributions: A.S, B.K.S.S, H.H.M.H, and J.B. designed the experiment. A.S, B.K.S.S, B.V.M.F, and K.W conducted the research and collected data. A.S, B.K.S.S, B.V.M.F, and K.W analyzed the data. A.S wrote the manuscript. A.S, B.K.S.S, B.V.M.F, K.W H.H.M.H, and J.B edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability statement: The data that support the findings of this study are available from the corresponding author, AS, upon reasonable request.

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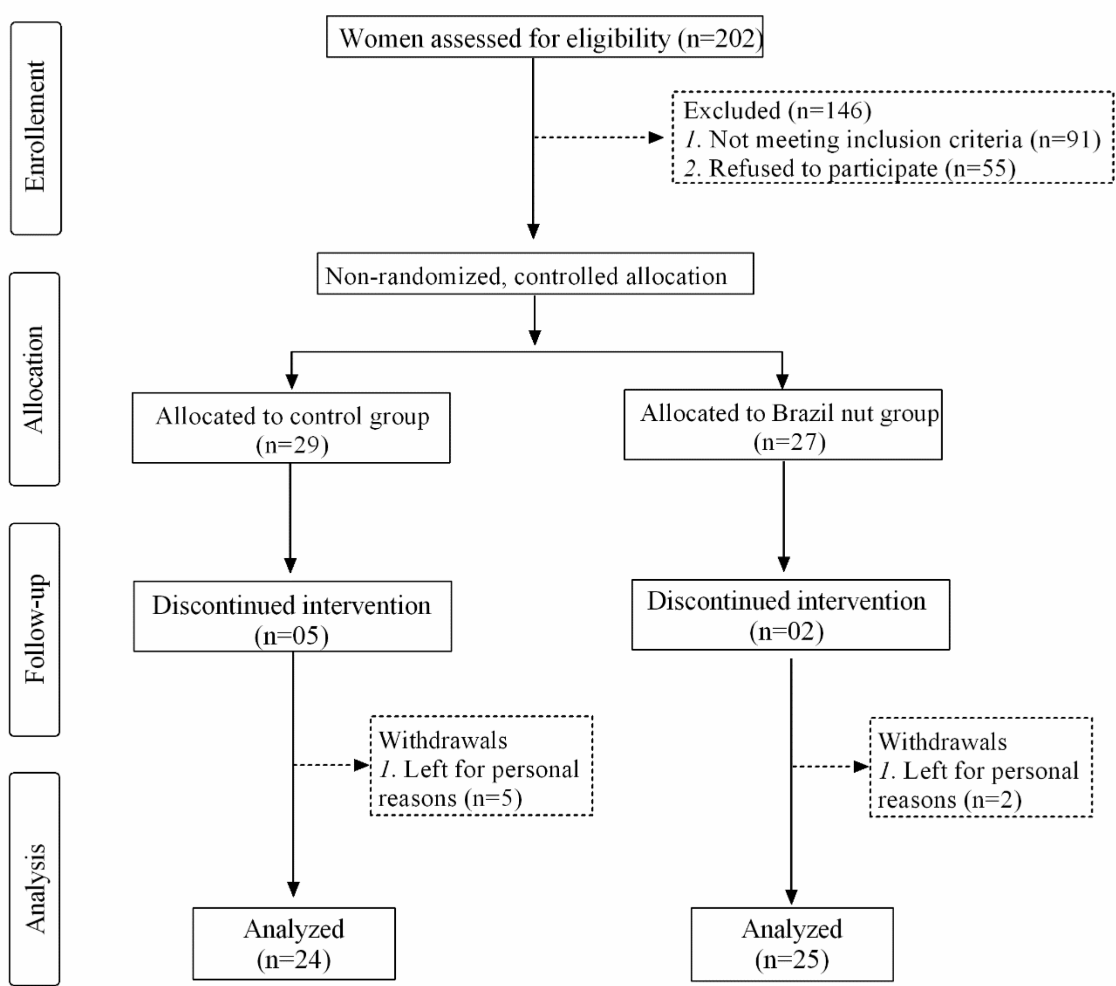
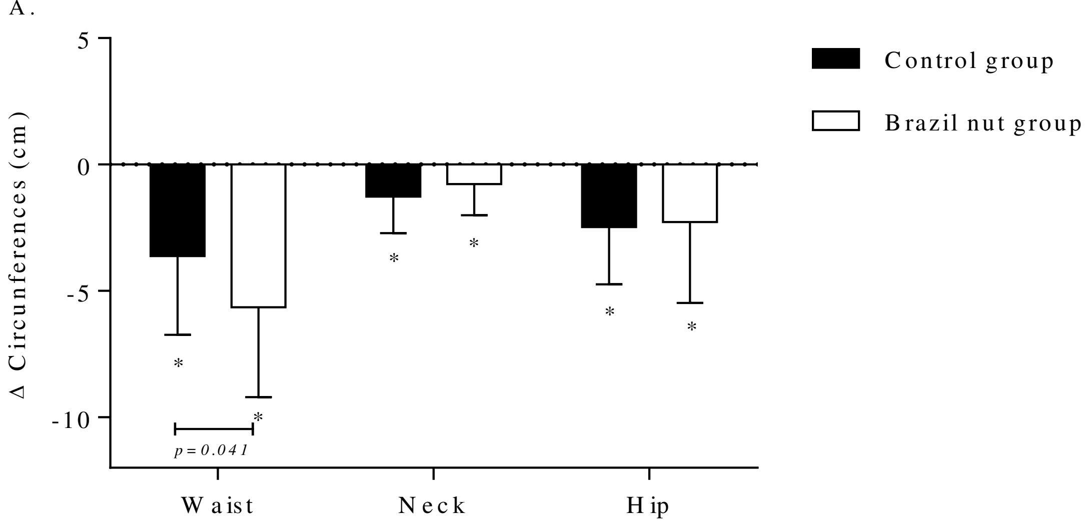
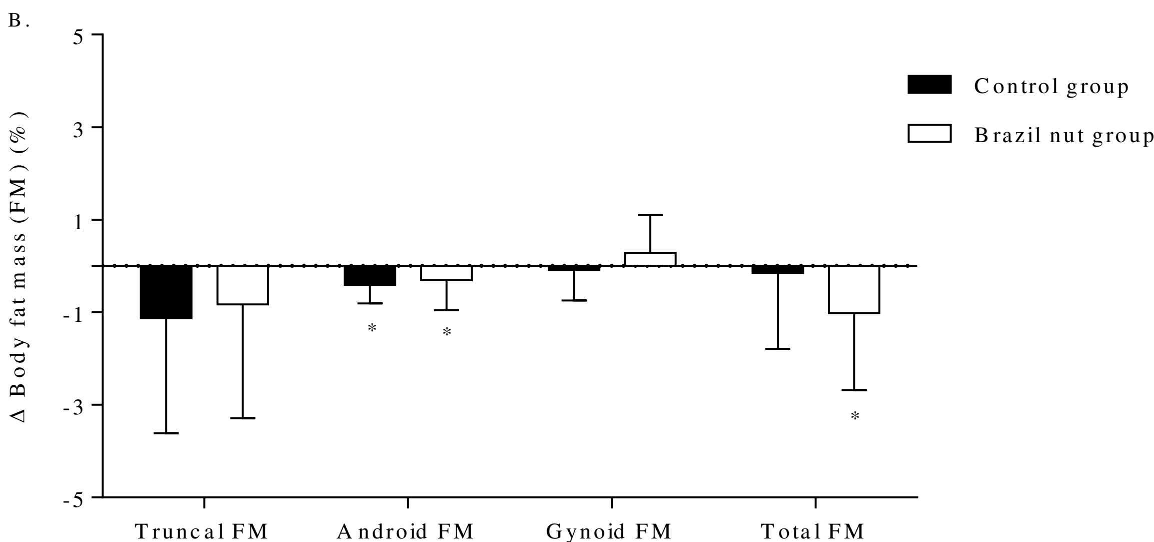
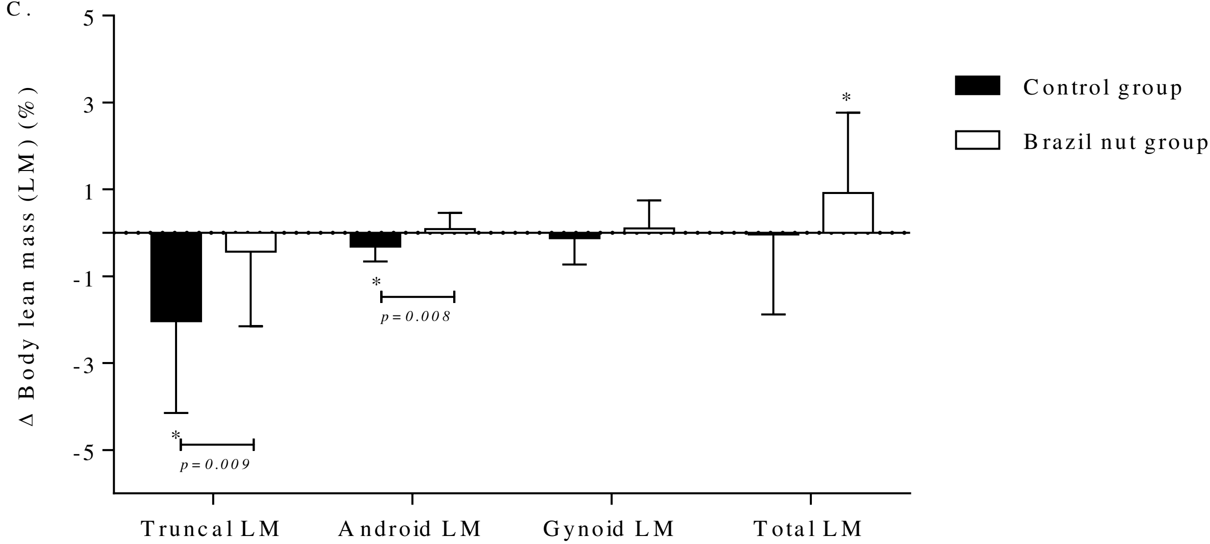


Figure 1: Study flow diagram.


A.
5
0
-5
-10
Δ Circunferences (cm)
p = 0.041
Waist
Neck
Hip
Control group
Brazil nut group


B.
5
3
1
-1
-3
-5
Δ Body fat mass (FM) (%)
Truncal FM
Android FM
Gynoid FM
Total FM
Control group
Brazil nut group



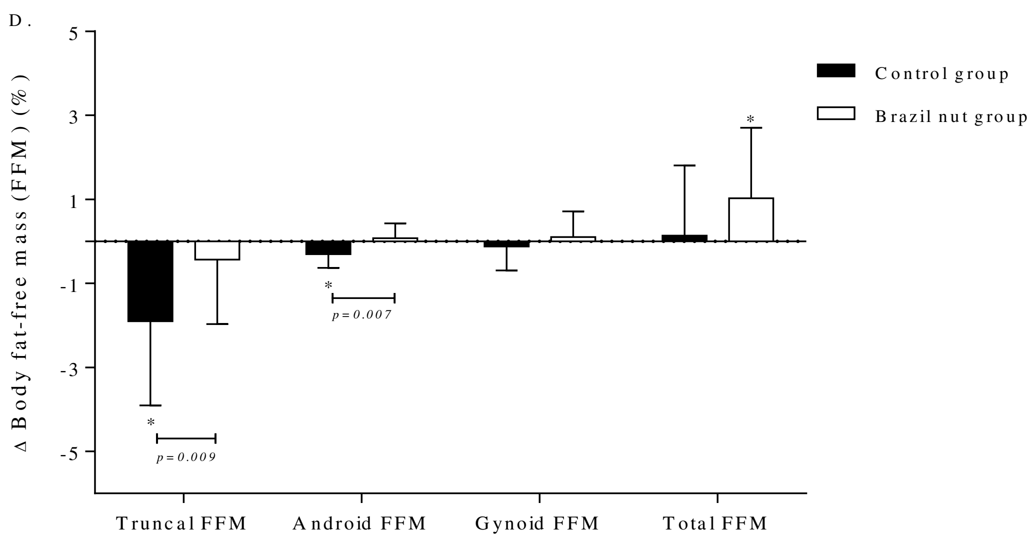


Figure 2: Effect of energy-restricted diet associated to Brazil nut consumption or not on **A)** waist circumference, neck circumference, and hip circumference; **B)** percentage of total fat mass and its compartments; **C)** the percentage of total lean mass and its compartments, and **D)** the percentage of total fat-free mass and its compartments. Δ = final assessment – baseline. *p<0.05 compared to baseline obtained through Paired *t*-test. *Student t-test* was performed for comparison between groups. HP and android FM was different between groups at baseline. Thus, the p-value for these markers was obtained through alnalysis of covariance (ANCOVA) analysis adjusted for HP and android FM at baseline, respectively.

Table 1: Change in anthropometry and body composition according to 8-wk energy-restricted diet (control vs. BN)

	Control group (n=24)		BN group (n=25)		Baseline p-values	Δ p-values
	Baseline	**Δ**	**Baseline**	**Δ**		
Age, y	32.83 (7.85)	-	34.92 (7.26)	-	0.339	-
Weight, kg	90.64 (15.76)	-2.49 (2.41) **	85 (9.33)	-3.25 (2.49) **	0.138	0.284
BMI, kg/m²	34.21 (5.12)	-0.94 (0.9) **	32.65 (3.2)	-1.28 (0.99) **	0.205	0.219
WHtR	**0.68 (0.08)**	**-0.02 (0.02) ** **	**0.67 (0.06)**	**-0.04 (0.02) ** **	0.504	**0.041**
VAI	2.25 (2.04)	0.04 (0.59)	1.99 (0.97)	0.21 (0.7)	0.576	0.393
DAAT, cm²	216.94 (52)	-16.64 (15.2) **	207.61 (38.23)	-24.45 (14,84) **	0.480	0.081
Conicity index	7.43 (0.56)	-0.02 (0.03) **	7.24 (0.34)	-0.04 (0.04) **	0.167	0.076
Δ Truncal FM, kg	23.41 (5.84)	-1.34 (2.08) *	21.72 (4.78)	-1.52 (1.61) **	0.303	0.748
Δ Android FM, kg	3.83 (0.8)	-0.29 (0.23) **	3.2 (0.66)	-0.29 (0.26) **	**0.008**	0.627
Δ Gynoid FM, kg	8.46 (2.15)	-0.32 (0.47) *	7.34 (1.1)	-0.27 (0.36) **	**0.045**	0.909
Δ Total FM, kg	44 (9.36)	-1.44 (2.05) *	39.92 (6.52)	-2.14 (1.67) **	0.102	0.230
Δ Truncal LM, kg	**20.72 (3.76)**	**-1.5 (1.46) ** **	**20.53 (3.07)**	**-0.57 (1.3) * **	**0.856**	**0.029**
Δ Android LM, kg	**3.02 (0.59)**	**-0.23 (0.18) ** **	**2.84 (0.46)**	**-0.02 (0.21)**	**0.264**	**0.002**
Δ Gynoid LM, kg	6.42 (1.19)	-0.26 (0.36) *	6.02 (1)	-0.07 (0.35)	0.236	0.103
Δ Total LM, kg	41.59 (6.11)	-1.3 (1.57) *	41.28 (5.19)	-0.78 (1.71) *	0.858	0.336
Δ Truncal FFM, kg	**21.7 (3.82)**	**-1.46 (1.39) ** **	**21.53 (3.04)**	**-0.57 (1.17) * **	**0.869**	**0.032**
Δ Android FFM, kg	**3.08 (0.58)**	**-0.23 (0.18) ** **	**2.9 (0.45)**	**-0.02 (0.21)**	**0.266**	**0.002**
Δ Gynoid FFM, kg	6.71 (1.23)	-0.25 (0.35) *	6.31 (1.01)	-0.07 (0.34)	0.247	0.101
Δ Total FFM, kg	44.43 (6.33)	-1.23 (1.41) *	44.06 (5.22)	-0.79 (1.57) *	0.838	0.304

Values are mean (SD). Δ = final assessment – baseline. * $p < 0.05$ or ** $p \leq 0.001$ are significant differences within-group (paired t-test). Baseline p-values and Δ p-values refer to the comparison between groups (*Student t-test*).

BMI, body mass index; WHtR, waist-to-height ratio; VAI, visceral adiposity index; DAAT, deep abdominal adipose-tissue; FM, fatty mass; LM, lean mass; FFM, fat-free mass.

Table 2: Change in anthropometry and body composition according to 8-wk energy-restricted diet (control vs. BN) only in women who had weight loss

	Control group (n=19)		BN group (n=22)		Baseline p-values	Δ p-values
	Baseline	**Δ**	**Baseline**	**Δ**		
Age, y	33.79 (7.19)	-	35.00 (7.59)	-	0.605	
Weight, kg	90.83 (14.59)	-3.36 (1.87) **	85.15 (9.45)	-3.85 (1.99) **	0.142	0.426
BMI, kg/m²	34.24 (5.18)	-1.26 (0.69) **	32.74 (3.37)	-1.51 (0.81) **	0.270	0.313
WC, cm	110.33 (11.11)	-4.39 (2.76) **	107.47 (8.24)	-5.67 (2.74) **	0.355	0.150
NC, cm	37.38 (2.7)	-1.60 (1.42) **	37.16 (2.1)	-0.80 (1.29) *	0.782	0.068
HC, cm	121.00 (11.24)	-3.10 (1.86) **	114.09 (6.51)	-2.77 (3.1) **	**0.020**	0.935
WHtR	0.68 (0.07)	-0.03 (0.02) **	0.67 (0.06)	-0.04 (0.02) **	0.548	0.154
VAI	1.89 (0.64)	-0.02 (0.66)	2.13 (0.93)	0.12 (0.68)	0.358	0.483
DAAT, cm²	216.25 (48.06)	-19.86 (13.96) **	206.53 (36.94)	-26.13 (13.22) **	0.474	0.155
Conicity index	7.44 (0.55)	-0.02 (0.02) **	7.25 (0.35)	-0.04 (0.03) **	0.145	0.191
Truncal FM, kg	23.8 (6.2)	-1.45 (2.18) *	21.6 (4.99)	-1.47 (1.58) **	0.236	0.969
Truncal FM, %	53.63 (6.05)	-1.11 (2.64)	54.27 (5.99)	-0.58 (2.26)	0.745	0.510
Android FM, kg	3.84 (0.85)	-0.29 (0.25) **	3.16 (0.66)	-0.27 (0.26) **	**0.009**	0.879
Android FM, %	8.77 (1.36)	-0.37 (0.4) **	7.97 (0.96)	-0.24 (0.64)	**0.042**	0.558
Gynoid FM, kg	8.53 (2.27)	-0.36 (0.48) *	7.29 (1.12)	-0.26 (0.37) *	0.052	0.500
Gynoid FM, %	19.18 (1.77)	-0.10 (0.69)	18.6 (1.97)	0.33 (0.8)	0.355	0.083
Total FM, kg	44.31 (10.06)	-1.64 (2.01) *	39.5 (6.54)	-2.23 (1.64) **	0.084	0.327
Total FM, %	49.46 (4.53)	-0.21 (1.74)	47.05 (4.4)	-0.96 (1.59) *	0.106	0.174
Truncal LM, kg	20.93 (3.6)	-1.63 (1.55) **	20.69 (3.11)	-0.74 (1.07) *	0.828	**0.044**
Truncal LM, %	50.1 (3.41)	-2.20 (2.25) **	49.86 (2.73)	-0.60 (1.55)	0.810	**0.014**
Android LM, kg	3.02 (0.53)	-0.24 (0.19) **	2.85 (0.46)	-0.03 (0.19)	0.304	**0.003**
Android LM, %	7.24 (0.62)	-0.33 (0.35) **	6.88 (0.48)	0.08 (0.35)	0.054	**0.001**
Gynoid LM, kg	6.46 (1.16)	-0.29 (0.37) *	6.05 (1.04)	-0.08 (0.36)	0.258	0.090
Gynoid LM, %	15.45 (1.12)	-0.17 (0.61)	14.54 (0.93)	0.16 (0.64)	**0.010**	0.625
Total LM, kg	41.69 (5.66)	-1.4 (1.67) *	41.45 (5.31)	-1.00 (1.48) *	0.893	0.443
Total LM, %	47.24 (4.41)	-0.005 (1.96)	49.61 (4.41)	0.82 (1.73) *	0.109	0.178

Truncal FFM, kg	21.94 (3.63)	-1.59 (1.47) **	21.69 (3.09)	-0.73 (0.98) *	0.814	**0.039**
Truncal FFM, %	49.13 (3.31)	-2.06 (2.13) **	48.99 (2.62)	-0.56 (1.44)	0.883	**0.015**
Android FFM, kg	3.08 (0.53)	-0.23 (0.19) **	2.91 (0.46)	-0.03 (0.19)	0.303	**0.003**
Android FFM, %	6.9 (0.57)	-0.31 (0.33) **	6.58 (0.45)	0.07 (0.34)	0.063	**0.001**
Gynoid FFM, kg	6.75 (1.19)	-0.28 (0.36) *	6.33 (1.05)	-0.07 (0.35)	0.255	0.083
Gynoid FFM, %	15.11 (1.08)	-0.18 (0.57)	14.27 (0.9)	0.16 (0.6)	**0.014**	0.535
Total FFM, kg	44.58 (5.81)	-1.33 (1.5) *	44.22 (5.37)	-0.99 (1.37) *	0.842	0.473
Total FFM, %	50.54 (4.53)	0.18 (1.76)	52.94 (4.39)	0.97 (1.61) *	0.107	0.159
Selenium, μg/L	65.05 (33.79)	-2.11 (45.05)	68.91 (27.14)	157.23 (85.52) **	0.691	**≤0.001**

Values are mean (SD). Δ = final assessment – baseline. * $p < 0.05$ or ** $p \leq 0.001$ are significant differences within-group (paired t-test). Baseline p-values and Δ p-values refer to the comparison between groups (*Student t-test*). For variables different between groups at baseline, Δ p-value was obtained through alnalysis of ANCOVA analysis adjusted by baseline value.

BMI, body mass index; WC, waist circumference; NC, neck circumference; HP, hip circumference; WHtR, waist-to-height ratio; VAI, visceral adiposity index; DAAT, deep abdominal adipose-tissue; FM, fatty mass; LM, lean mass; FFM, fat-free mass.

Table 3: Change in nutrient intake according to 8-wk energy-restricted diet (control vs. BN)

Energy and nutrients intake	Control group (n=24)	BN group (n=25)	p-value
Caloric deficit, kcal	743.83 (625.94875.73)	530.78 (325.05;795.17)	0.119
Total energy intake, kcal	-66.92 (-544.76;66.18)	-334 (-561.71;30.03)	0.544
Carbohydrate, g	-35.68 (-77.2;-4.53)*	-21.94 (-62.83;14.36)	0.528
Protein, g	-4.42 (-21.78;9.22)	-14.69 (-22.52;6.54)	0.481
Total fat, g	-0.4 (-18.6;9.55)	-2.77 (-21.87;0.2)	0.296
MUFA†, g	-2.67 (-8.97;2.98)	-3.22 (-7.87;0.21)*	0.811
PUFA, g	5.43 (-0.007;6.91)	0.36 (-2.83;2.79)	0.548
SFA, g	-5.92 (-10.53;-0.36)*	-4,79 (-9.38;-1.92)*	0.981
Fiber, g	0.85 (-5.36;5.62)	1.61 (0.54;6.82)*	0.576
Selenium, µg	11.45 (-28.96;18.98)	328.91 (304.71;361.81)**	**≤0.001**

Values are median (95% CI). Change is the final assessment – baseline. * $p < 0.05$ or ** $p \leq 0.001$ are significant differences within-group (Wilcoxon test). p-values refer to the comparison between groups (*Mann-Whitney*). For variables different between groups at baseline†, p-value was obtained through ANCOVA analysis adjusted by baseline value.

BN, brazil nut; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty mass.

Supplementary Material

Chart 1. Example of a prescribed menu for the control and Brazil nut group containing ~1,800 kcal/day.

Control group	Brazil nut group
Breakfast - 1 cup (60 ml) of coffee without sugar - 1 unit (50 g) of French bread - 1 slice (20 g) of Minas Cheese - 1 small (45 g) apple **Morning snack** - 1 unit (90 g) of strawberry yogurt - 2 units (12 g) of toast - 1 teaspoon (4 g) of butter **Lunch** - 4 tablespoons (100 g) of white rice - 1 medium shell (80g) of Carioca beans - 1 medium steak (100 g) grilled chicken breast - 2 tablespoons (60 g) of zucchini - 4 lettuce leaves (40 g) - 4 tablespoons (100 g) of carrots - 2 small (60 g) cauliflower sprigs - Sauce (12,5 g) (provided by the research team) - 3 small slices (150 g) of pineapple * A small quantity of vegetable oil (soybean) for cooking. **Afternoon snack** - 1 cup (90 ml) of fruit yogurt - 8 units (24 g) of tapioca flour biscuits - 1 medium (80 g) guava **Dinner** - 1 deep bowl (450 g) of pumpkin soup - Sauce (12,5 g) (provided by the research team) - 1 unit (50 g) of French bread - 1 teaspoon (4 g) of butter * For cooking, a small vegetable oil (soybean, corn, or rice).	**Breakfast** - 1 small cup (60 ml) of coffee or tea without sugar - 1 unit (50 g) of French bread - 1 slice (20 g) of Minas Cheese - 1 small (45 g) apple **Morning snack** - 2 units (~8g) of Brazil nut - 1 cup (150 ml) of coffee or tea without sugar - 1 small unit (35 g) of cheese bread **Lunch** - 4 tablespoons (100 g) of white rice - 1 medium shell (80g) of Carioca beans - 1 medium steak (100 g) grilled chicken breast - 3 tablespoons (100 g) of zucchini - 2 lettuce leaves (20 g) - 2 tablespoons (60 g) of carrots - Sauce (5 g) (provided by the research team) - 2 small slices (50 g) of pineapple * A small quantity of vegetable oil (soybean) for cooking. **Afternoon snack** - 1 cup (90 ml) of fruit yogurt - 8 units (24 g) of tapioca flour biscuits - 1 medium (80 g) guava **Dinner** - 1 deep bowl (450 g) of pumpkin soup - 1 unit (50 g) of French bread - 1 teaspoon (4 g) of butter * For cooking, a small vegetable oil (soybean, corn, or rice).

Table S1: Brazil nut composition

Nutrients	Brazil nuts (2 units ~8g)
Ashes (g)	0.2352
Humidity (g)	0.1560
Energy (kcal)	52,679
Carbohydrates (g)	0.2160
Proteins (g)	1.1872
Lipids (g)	5.2296
Fibers (g)	1.0336
Soluble fibers (g)	0.0448
Insoluble fibers (g)	0.9896
Palmitic fatty acid (g)	0.8079
Stearic fatty acid (g)	0.9225
Oleic fatty acid (g)	0.9826
Linoleic fatty acid (g)	1.0776
α-linolenic fatty acid (g)	1.4389
SFA (g)	1.7304
MUFA (g)	0.9826
PUFA (g)	2.5166
Selenium (µg)	347.20
Nitrogen (mg)	211.00
Phosphorus (mg)	40.00
Potassium (mg)	42.00
Calcium (mg)	22.00
Magnesium (mg)	24.00
Sulfur (mg)	20.00
Cooper (mg/kg)	0.1161
Iron (mg/kg)	0.2387
Zinc (mg/kg)	0.3900
Manganese (mg/kg)	0.1121
Sodium (mg/kg)	0.000048

Table S2: Equations used to calculate percentages of fat, fat-free mass, and lean mass for total body and regions

Body composition	**Equation**
FAT MASS (%)	
Percentage of trunk fat mass	Trunk fat mass (kg) x 100 / total body fat
Percentage of android fat mass	Android fat mass (kg) x 100 / total body fat.
Percentage of gynoid fat mass	Gynoid fat mass (kg) x 100 / total body fat
Percentage of total fat mass	Total fat mass (kg) x 100 / total body mass
FAT-FREE MASS (%)	
Percentage of trunk fat-free mass	Trunk fat-free mass (kg) x 100 / total fat-free mass
Percentage of android fat-free mass	Android fat-free mass (kg) x 100 / total fat-free mass
Percentage of gynoid fat-free mass	Gynoid fat-free mass (kg) x 100 / total fat-free mass
Percentage of total fat-free mass	Total fat-free mass (kg) x 100 / total body mass
LEAN MASS (%)	
Percentage of trunk lean mass	Trunk lean mass (kg) x 100 / total lean mass
Percentage of android lean mass	Android lean mass (kg) x 100 / total lean mass
Percentage of gynoid lean mass	Gynoid lean mass (kg) x 100 / total lean mass
Percentage of total lean mass	Total lean mass (kg) x 100 / total body mass

Table S3: Change in anthropometry, body composition, and serum selenium according to 8-wk energy-restricted diet (control vs. BN) and weight-loss variation (< or ≥ 4 kg) only in women who weight loss

	Control group (n=17)			**BN group (n=21)**		
	Weight loss ≥ 4kg					
	No (n=10)	**Yes (n=7)**	**p-value**	**No (n=10)**	**Yes (n=11)**	**p-value**
Age, y	34.08 (6.43)	33.29 (8.88)	0.823	35.55 (8.51)	34.45 (6.92)	0.745
Δ Weight, kg	-2.24 (1.06)	-5.29 (1.27)	**≤0.001**	-2.31 (1.05)	-5.39 (1.4)	**≤0.001**
Δ BMI, kg/m²	-0.87 (0.43)	-1.94 (0.5)	**≤0.001**	-0.89 (0.45)	-2.12 (0.59)	**≤0.001**
Δ WC, cm	-3.84 (1.83)	-5.26 (3.8)	0.297	-4.43 (2.47)	-6.92 (2.5)	**0.029**
Δ NC, cm	-1.58 (1.73)	-1.64 (0.85)	0.927	-0.30 (1.32)	-1.30 (1.08)	0.067
Δ HC, cm	-2.72 (2)	-3.69 (1.58)	0.298	-1.44 (3.3)	-4.10 (2.32)	**0.040**
Δ WHtR	-0.02 (0.01)	-0.03 (0.03)	0.341	-0.03 (0.02)	-0.04 (0.02)	**0.027**
Δ VAI	-0.12 (0.76)	0.13 (0.46)	0.436	0.36 (0.61)	-0.11 (0.69)	0.102
Δ DAAT, cm²	-18.01 (9.04)	-22.77 (19.99)	0.498	-20.99 (12.2)	-31.26 (12.66)	0.067
Δ Conicity index	-0.02 (0.02)	-0.02 (0.04)	0.935	-0.04 (0.05)	-0.04 (0.03)	0.913
Δ Truncal FM, kg	-1.68 (2.38)	-1.13 (2)	0.624	-0.82 (1.58)	-2.07 (1.39)	0.068
Δ Truncal FM, %	-1.46 (2.9)	-0.6 (2.34)	0.525	-0.43 (2.46)	-0.71 (2.17)	0.782
Δ Android FM, kg	-0.21 (0.28)	-0.40 (0.18)	0.158	-0.12 (0.2)	-0.40 (0.24)	**0.011**
Δ Android FM, %	-0.27 (0.48)	-0.53 (0.17)	0.188	-0.03 (0.69)	-0.43 (0.56)	0.154
Δ Gynoid FM, kg	-0.18 (0.37)	-0.61 (0.52)	0.074	-0.01 (0.27)	-0.50 (0.3)	**0.001**
Δ Gynoid FM, %	0.21 (0.73)	-0.56 (0.26)	**0.017**	0.54 (0.65)	0.14 (0.91)	0.271
Δ Total FM, kg	-1.6 (1.94)	-1.7 (2.26)	0.931	-1.15 (1.36)	-3.22 (1.23)	**0.002**
Δ Total FM, %	-0.75 (1.58)	0.55 (1.77) †	0.132	-0.30 (1.6)	-1.57 (1.39) †	0.069
Δ Truncal LM, kg	-1.11 (1.54)	-2.38 (1.3) †	0.096	-0.73 (1.04)	-0.76 (1.15) †	0.951
Δ Truncal LM, %	-2.01 (2.48)	-2.49 (2.02) †	0.680	-0.71 (1.68)	-0.51 (1.51) †	0.776
Δ Android LM, kg	-0.19 (0.21)	-0.31 (0.13) †	0.215	-0.003 (0.19)	-0.07 (0.2) †	0.447
Δ Android LM, %	-0.36 (0.41) ‡	-0.28 (0,28)	0.671	0.15 (0.39) ‡	0,03 (0.33)	0.443
Δ Gynoid LM, kg	-0.04 (0.14)	-0.65 (0.3) †	**≤0.001**	-0.02 (0.37)	-0.13 (0.36) †	0.492
Δ Gynoid LM, %	0,07 (0.61)	-0.54 (0.42) †	**0.038**	0.26 (0.68)	0.07 (0.61) †	0.524
Δ Total LM, kg	-0.45 (1.01)	-2.76 (1.52) †	**0.002**	-0.94 (1.54)	-1.06 (1.5) †	0.864
Δ Total LM, %	0.67 (1.78)	-0.97 (1.91) †	0.088	0.16 (1.77)	1.41 (1.54) †	0.101
Δ Truncal FFM, kg	-1.12 (1.52)	-2.25 (1.2) †	0.125	-0.67 (0.96)	-0.78 (1.04) †	0.816

Δ Truncal FFM, %	-1.91 (2.38)	-2.27 (1.87) †	0.747	-0.62 (1.61)	-0.52 (1.34) †	0.882
Δ Android FFM, kg	-0.19 (0.22) ‡	-0.3 (0.12) †	0.285	0.001 (0.18) ‡	-0.07 (0.2) †	0.412
Δ Android FFM, %	-0.34 (0.39) ‡	-0.28 (0.25) †	0.715	0.13 (0.37) ‡	0.02 (0.31) †	0.453
Δ Gynoid FFM, kg	-0.04 (0.14)	-0.63 (0.29) †	**≤0.001**	-0.01 (0.36)	-0.13 (0.35) †	0.444
Δ Gynoid FFM, %	0.06 (0.56)	-0.53 (0.4) †	**0.031**	0.25 (0.64)	0.08 (0.58) †	0.529
Δ Total FFM, kg	-0.46 (0.84)	-2.57 (1.36) †	**0.001**	-0.88 (1.42)	-1.08 (1.39) †	0.752
Δ Total FFM, %	0.75 (1.6)	-0.62 (1.76) †	0.114	0.31 (1.64)	1.57 (1.39) †	0.074
Δ Selenium, µg/L	-2.27 (53.43) ‡	-1.78 (24.91) †	0.984	140.64 (54.42) ‡	173.82 (108.59) †	0.376

Values are mean (SD). Δ = final assessment – baseline. P-values ≤ 0.05 indicate statistically significant differences within-group between weight loss categories (*Student's t-test*).

‡ refers to statistically significant differences between control and BN groups in those classified with weight loss values < 4kg obtained through *Student’s t-test*.

† refers to statistically significant differences between control and BN groups in those classified with weight loss values ≥ 4kg obtained through *Student’s t-test*.

BMI, body mass index; WC, waist circumference; NC, neck circumference; HP, hip circumference; WHtR, waist-to-height ratio; VAI, visceral adiposity index; DAAT, deep abdominal adipose-tissue; FM, fatty mass; LM, lean mass; FFM, fat-free mass.

Table S4: Anthropometric and body composition variations according to serum selenium (delta= 54.5 µg/L) after 8-wk energy-restricted intervention (n=48)

	Δ Serum Selenium Median, µg/L		
	54.50 (-138.00; 387.00)		**p-values**
Serum selenium, µg/L	2.20 (38.33)	165.70 (82.74)	**≤0.001**
Age, y	32.29 (7.46)	34.95 (7.19)	0.214
Δ Weight, kg	-2.53 (2.43)	-3.17 (2.52)	0.375
Δ BMI, kg/m²	-0.97 (0.91)	-1.23 (1)	0.348
Δ WC, cm	-3.75 (3.1)	-5.64 (3.71)	0.064
Δ NC, cm	-1.16 (1.37)	-0.76 (1.28)	0.315
Δ HC, cm	-1.98 (2.91)	-2.7 (2.65)	0.382
Δ WHtR	-0.02 (0.01)	-0.03 (0.02)	0.069
Δ VAI	0.008 (0.59)	0.25 (0.71)	0.216
Δ DAAT, cm²	-17.3 (15.1)	-24.29 (15.52)	0.129
Δ Conicity index	-0.02 (0.02)	-0.04 (0.04)	0.099
Δ Truncal FM, kg	-1.53 (1.8)	-1.54 (1.69)	0.993
Δ Truncal FM, %	-1.28 (2.25)	-0.87 (2.53)	0.580
Δ Android FM, kg	-0.3 (0.22)	-0.29 (0.27)	0.953
Δ Android FM, %	-0.39 (0.39)	-0.30 (0.68)	0.623
Δ Gynoid FM, kg	-0.31 (0.45)	-0.3 (0.36)	0.949
Δ Gynoid FM, %	0.05 (0.74)	0.18 (0.79)	0.563
Δ Total FM, kg	-1.7 (1.83)	-2.12 (1.73)	0.447
Δ Total FM, %	-0.42 (1.31)	-1.02 (1.73)	0.212
Δ Truncal LM, kg	-1.29 (1.41)	-0.6 (1.35)	0.111
Δ Truncal LM, %	-1.8 (2.22)	-0.51 (1.73)	**0.043**
Δ Android LM, kg	-0.19 (0.19)	-0.03 (0.22)	**0.015**
Δ Android LM, %	-0.27 (0.38)	0.06 (0.37)	**0.007**
Δ Gynoid LM, kg	-0.19 (0.31)	-0.08 (0.37)	0.336
Δ Gynoid LM, %	-0.04 (0.61)	0.06 (0.66)	0.586
Δ Total LM, kg	-1.07 (1.19)	-0.77 (1.81)	0.534
Δ Total LM, %	0.26 (1.49)	0.91 (1.92)	0.225
Δ Truncal FFM, kg	-1.28 (1.36)	-0.6 (1.21)	0.099
Δ Truncal FFM, %	-1.71 (2.09)	-0.51 (1.54)	**0.041**
Δ Android FFM, kg	-0.19 (0.19)	-0.03 (0.21)	**0.015**
Δ Android FFM, %	-0.26 (0.36)	0.05 (0.35)	**0.007**
Δ Gynoid FFM, kg	-0.18 (0.31)	-0.08 (0.35)	0.342
Δ Gynoid FFM, %	0.04 (0.58)	0.06 (0.62)	0.534
Δ Total FFM, kg	-1.07 (1.07)	-0.78 (1.64)	0.569
Δ Total FFM, %	0.41 (1.36)	1.02 (1.74)	0.209

Values are mean (SD). Δ = final assessment – baseline. Bold p-values indicate statistically significant differences between Δ serum selenium median (*Student's t-test*).

BMI, body mass index; WC, waist circumference; NC, neck circumference; HP, hip circumference; WHtR, waist-to-height ratio; VAI, visceral adiposity index; DAAT, deep abdominal adipose-tissue; FM, fatty mass; LM, lean mass; FFM, fat-free mass.

5.4 ARTIGO ORIGINAL 2 – A ser submetido à revista Nutrition, Metabolism & Cardiovascular Diseases (FI: 4.666)

Daily Brazil nut (*Berthollethia excelsa* h.b.k) improves lipid and liver function markers in women at cardiometabolic risk in energy-restriction condition: a controlled, parallel clinical trial (Brazilian Nuts Study)

Alessandra da Silva[1]

Brenda Kelly de Souza Silveira[1]

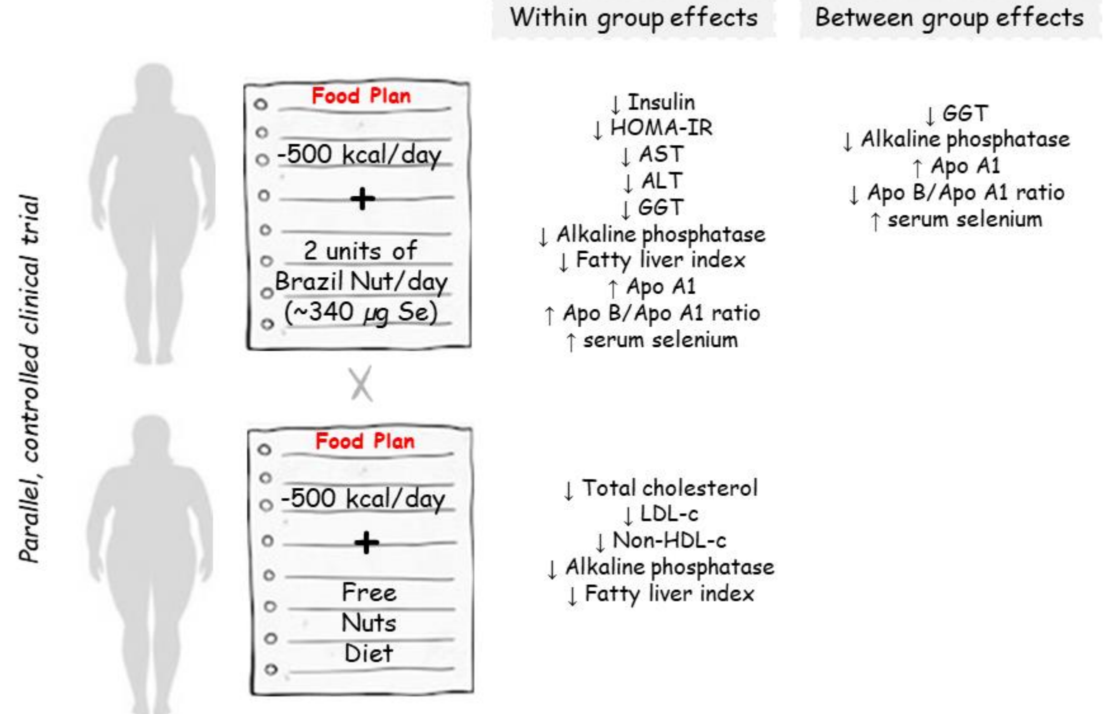
Brenda Vieira Machado de Freitas[1]

Helen Hermana M. Hermsdorff[1]

Josefina Bressan[1]

[1] Laboratory of Energy Metabolism and Body Composition. Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

Corresponding author: Josefina Bressan. Laboratory of Energy Metabolism and Body Composition. Department of Nutrition and Health, Universidade Federal de Viçosa. Avenida PH Rolfs, s/n. Viçosa – MG, Brasil. 36570-900. Phone: +55 31 36125220. E-mail: jbrm@ufv.br



Graphical abstract

Summary of the effects of energy-restricted diet associated to Brazil nut intake or not on cardiometabolic risk markers within groups and between groups.

Legend: ↓, decreased; ↑, increased; HOMA-IR, homeostatic model of insulin resistance assessment; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; Apo A1, Apolipoprotein A1; Apo B, Apolipoprotein B; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; FLI, fatty liver index.

Abstract

Background and objectives: The consumption of functional foods alongside the energy-restricted diet can be great allies in the weight loss process and improvement of cardiometabolic risk factors. Whether Brazil nut (BN) consumption in the context of energy restriction affects them remains to be answered. We aimed to evaluate the effect of BN intake within an energy-restricted diet on glucose, lipids, and liver metabolism markers in women at cardiometabolic risk. *Methods and Results*: This eight-week, non-randomized, parallel controlled nutritional intervention study enrolled women who were allocated into two groups with energy-restricted diet (-500 kcal): 1) free of BN (n=24) and 2) + 2 units/day of BN (approximately 347 μg of selenium) (n=25). Serum glucose, insulin, total cholesterol and lipoproteins, triglycerides, apolipoproteins, and liver enzymes were evaluated. BN consumption led to a mean increase of 27.4% in Apo A1 versus 6.3% in the control group. Reductions in gamma-glutamyl transferase and alkaline phosphatase enzymes were greater in the BN group than in the control group. The median increase in serum selenium was 276.7% in the BN group and 5.2% in the control group, with a positive correlation with the change in Apo A1 and the TyG index and a negative correlation with the Apo B/Apo A1 and alkaline phosphatase ratio. *Conclusion:* Consumption of BN within the energy-restricted diet improved serum selenium status and lipid and liver metabolism markers in women at cardiometabolic risk.

Trial registration: RBR-8zfn5c and RBR-3ntxrm.

Keywords: apolipoproteins; cardiometabolic risk; insulin resistance; nuts; selenium; weight loss

1. Introduction

Data from the National Health Survey (PNS) showed that about 60.3% of adult Brazilians had overweight, and 25.9% had obesity in 2019 [1]. Glucose impairment and increase in atherogenic molecules (increase in very low, low, and intermediate density lipoproteins), triglycerides and blood pressure are systemic effects caused by overweight/obesity [2], contributing to atherogenesis and associated heart diseases development [3].

Some systemic effects can be improved with even a modest 2-5% weight loss. [4]. Lifestyle change, which includes adherence to healthy eating habits and regular physical activity, is the basis of obesity treatment [5,6]. Despite limitations such as low long-term adherence and possible weight regains [7], among other limitations common to other weight loss strategies, a balanced energy-restricted diet is the most well-established current nutritional recommendation for weight loss available in the literature [5,8]. Parallel to energy restriction, the insertion of functional foods rich in fiber, unsaturated fats, and proteins can be great allies in the weight loss process.

Despite having all these characteristics in its composition, the consumption of nuts, especially Brazil nuts (BN) (*Bertholletia excelsa* h.b.k.)**,** is not associated with changes in body weight when consumed in the context of a habitual diet by subjects with obesity [9–11]. BN is a food matrix native to the Amazon region with well-established beneficial effects on improving antioxidant status, inflammation, lipid profile, and oxidative stress, mainly attributed to its high selenium content [13]. All these benefits vary between studies and the health status of the subjects evaluated and have been documented when BN is consumed in the context of the usual diet [14–19]. Whether BN consumption has all these benefits when placed in the context of energy restriction remains to be answered.

Thus, the aim of this study was to evaluate the effect of BN intake within an energy-restricted diet on markers of glucose, lipids, and liver metabolism in women at cardiometabolic risk. Afterward, we assessed the relationship of these markers according to weight loss and serum selenium variation.

2. Methods

2.1 Study design and ethical approval

The present study is a non-randomized, controlled parallel eight-week nutritional intervention trial conducted in free-living conditions with women at cardiometabolic risk.

The study was performed in the Department of Nutrition and Health of Universidade Federal de Viçosa-MG, Brazil, with enrollment between June 2019 and September 2021. The study protocol followed Resolution CSN 466/2012 and was approved by the local ethical committee of the Universidade Federal de Vicosa (CAAE: 92004818.0.0000.5153; UP protocol number: 2.832.601/2018). All participants were informed about the objectives and study procedures. Those that accepted the study conditions provided written informed consent. Furthermore, this study was registered on the Brazilian Registers of Clinical Trials – REBEC (protocol: RBR-8zfn5c).

2.2 Study population, recruitment, and sample calculation

Adult women (20-55 years old) with overweight (BMI ≥ 27 kg/m2 and <30 kg/m^2), waist circumference ≥ 80 cm, and body fat percentage ≥ 32% associated with at last one another component of metabolic syndrome: triglycerides ≥150 mg/dL, high blood pressure arterial (≥130/85 mmHg) or high fasting glucose (≥100 mg/dL); or women with obesity (BMI ≥ 30 kg/m^2), with or without metabolic complications were included. Non-inclusion criteria comprised pregnant, lactate, or menopausal women; athletes; vegans; smoking; women with a history of HIV, illness or digestive, liver, kidney, cardiovascular, thyroid, cancer, inflammatory diseases, and eating disorders; history of drug and/or alcohol abuse; aversion or allergy to nuts;

infectious episode in the last month; use of anti-inflammatory drugs, corticosteroids, antibiotics, and others that may affect energy appetite and metabolism; body weight instability (5% of usual weight) in the last 3 months; regular consumption of any quantity of BN; alcohol consumption higher than 21 units (168g) per week; dental problems that interfere with chewing; use of vitamin, mineral, and omega 3 supplements.

Participants were recruited in the city of Viçosa, Minas Gerais, Brazil, and in neighboring cities. Advertisements in social media (Instagram, Facebook, and WhatsApp) and local radio and TV were the recruitment methods. After an initial screening, the women who met the essential eligibility criteria (age, BMI, no pregnancy, menopausal, and medical/supplement use) were invited to a face-to-face visit to evaluate health history, physical activity level, and anthropometry.

2.3 Dietary interventions

Women were allocated by convenience into two groups: control, which was instructed to consume an energy-restricted diet (-500 kcal/day) without any type of nuts, or Brazil nut group (BN-group) was instructed to follow the energy-restricted diet (-500 kcal/day) containing approximately 8g (two units) of BN daily. Participants were monitored via WhatsApp and in a face-to-face consultation 30 days after the start of the intervention. In the 30-day consultation, a 24-hour food recall was applied to check compliance and questions regarding physical activity, medication use, difficulties and progress of the prescribed food plan. All women were instructed to maintain their lifestyle and medications during the study and to inform the research team of any changes in the type or dosage of the ongoing medication.

Energy-restricted diet

Total energy intake was estimated for each participant using the Estimated Energy Requirement (EER) for overweight or obese adult women [22]; formula: 448 – (7.95 × age [y]) + physical activity × (11.4 × weight [kg] + 619 × height [m]). Then, 500 kcal/day was deducted from the dietary prescription. Participants received an individualized eating plan with five nutritionally-balanced menus, each with five meals (breakfast, morning snack, lunch, afternoon snack, and dinner). The average distribution of carbohydrates, protein, and lipids were 50.0%, 20.0%, and 30.0% of daily energy, respectively, in accordance with the macronutrient distribution recommendations for obesity [22].

For the BN group, the diets included the energy provided by the daily portion of 8 g (two unities) of BN. To balance the diets in macronutrients, total, polyunsaturated, saturated, and total fats, the control group was instructed to consume a sauce based on soybean oil and lemon (2:1 ratio, respectively). On the other hand, the BN group was oriented to consume a sauce based on canola oil and lemon (2:1 ratio, respectively). Sauces were provided by the research team and handed out to women fortnightly.

Brazil nut

The BN used in the study was donated by ECONUT®. The BN is organic, grown at Fazenda Aruanã, located in the municipality of Itacoatiara, state of Amazonas, Brazil. All BN were received in modified atmosphere packages and portioned (two units) in laminated packages, vacuum sealed (Selovac Sealer model 200 B), and stored in a freezer at -20ºC until distribution to the participants.

The amount of two units of BN used in this study was defined based on its selenium content not exceeding the Tolerable Upper Intake Level (UL) of the mineral, which is 400 μg daily. In addition, evidence shows beneficial effects of BN consumption with a selenium content of 290 μg by obese women [23,24]. Each BN unit used in our study has 173.6 μg of

selenium. Then, we double the number of BN to approximate the amount of selenium contained in the nuts from previous studies, which does not exceed the UL.

The selenium content in the BN was measured by inductively coupled plasma atomic emission spectrometry determination of elements in food using Microwave Assisted Digestion [44]. The lipid profile of BN was performed by gas chromatography following the protocol proposed by Folch et al. 1957[45] and Hartman and Lago 1973 [46]. Monounsaturated, polyunsaturated, and saturated fatty acids represent 18.7 %, 48.2 %, and 33.1% of total fat in BN, respectively (**Supplementary Table 1**).

2.4 Data collection

The participants were asked to go to the Health Service of the Federal University of Viçosa, fasting for 10 to 12 hours before and at the end of the nutritional intervention period for blood collection and assessment of body composition using Dual-energy X-ray absorptiometry (DEX). Anthropometrical indicator as well as physical activity practice and food intake were evaluated at the Laboratory of Energy Metabolism and Body Composition (LAMECC).

Metavolic markers and serum selenium

Serum total cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), triglycerides, and glucose were analyzed by colorimetric method. Very low-density lipoprotein cholesterol (VLDL-c) was calculated using the Friedewald equation [25]. Serum glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase were analyzed using the enzymatic method. Serum insulin was determined by the chemiluminescence method. Serum Apolipoprotein E was determined by the immunonephelometry method. Apolipoproteins A1 and B were measured by the Turbidimetry method.

Total cholesterol minus HDL-c was calculated to obtain non-HDL-c. Total cholesterol/HDL, LDL/HDL, and Apo B/Apo A1 ratios were also estimated. HOMA-IR was calculated using the formula: fasting glucose (mg / dl) x fasting serum insulin (μU / ml) / 405 [26]. The triglyceride-glucose index (TyG index) was calculated using the formula: Ln [triglycerides (mg / dl) x fasting blood glucose (mg / dl) / 2] [27]. Fatty liver index (FLI) was estimated through the equation $(e^{0.953*loge\ (triglycerides) + 0.139*BMI + 0.718*loge\ (GGT) + 0.053*waist\ circumference - 15.745}) / (1 + e^{0.953*loge\ (triglycerides) + 0.139*BMI + 0.718*loge\ (GGT) + 0.053*\ waist\ circumference - 15.745}) * 100$ [28].

Serum selenium (μ/L) was determined using inductively coupled plasma mass spectrometry according to standardized protocols.

Dietary assessment

To assess food consumption, participants were instructed to complete three-day food records (two nonconsecutive weekdays and one weekend day) before the start of the nutritional intervention period. A dietitian checked all reported food and its respective quantities. In the 30-day and 60-day consultations, a 24-hour food recall was applied by a dietitian. ERICA® software adapted for the Brazilian population was used for energy and nutrients calculation. The caloric deficit achieved by participants was obtained through the EER minus the energy consumed reported in the 24-hour food recall applied to the 60-day intervention.

2.5 Statistics Analyses

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 21.0 for Windows. The distribution of quantitative variables was performed following the principles of the Gaussian distribution. Thus, the Shapiro-Wilk test was performed for each group of interventions. Continuous variables were described as mean, standard deviation, or median and 95% confidence interval. Parametric variables were compared within and between groups using the paired t-test and *Student's t-test*, respectively. For comparison of non-parametric variables within the group, the Wilcoxon test and the Mann

Whitney test were used for comparison between groups. Analysis of covariance (ANCOVA) adjusted by baseline value was used to compare means between groups when the variable differed between groups at baseline. Pearson correlation was performed to assess correlations between changes in serum selenium and biochemical variables. Intention-to-treat (ITT) analysis was not performed because this study was not randomized. The level of significance in two-tailed tests was set at 5%.

3. Results

A total of two hundred and two women expressed an interest in participating in the study, but one hundred and forty-six did not meet the inclusion criteria or dropped out before the start of the study. Thus, fifty-six women initiated the study, and forty-nine concluded. Twenty-nine women were allocated to the control group and twenty-seven to the BN group. The dropout in the follow-up was higher in the control group (17.2%) than in the BN group (7.4%). 100% of the dropout were for "personal reasons" (**Figure 1**).

Women included in the study was 33.9 (SD 7.5) years old and had BMI of 33.4 (SD 4.3) kg/m², which did not differ at baseline between the groups. The median increase in serum selenium was 276.7% in the BN group and 5.2 % in the control group. Weight decreased similarly between groups (control grouop: -2.7% vs BN group: -3.9%, p=0.154). The biochemical variables presented in this study did not differ between groups at baseline (**Table 1**). Insulin and HOMA-IR decreased while Apo A1 increased in the BN group compared to baseline. BN consumption led to a 27.4% mean increase in Apo A1 versus 6.3% in the control group (p-value ≤ 0.001). The Apo B/Apo A1 ratio increased in the BN group, but the final ratio was lower than the control group. On the other hand, total cholesterol, LDL-c, and non-HDL-c decreased in the control group compared to baseline.

Regarding liver enzymes, AST, ALT, and GGT decreased only in the BN group compared to baseline, being the changes in GGT enzyme were higher in the BN group

compared to the control group. Alkaline phosphatase and fatty liver index decreased in both groups, but the BN group showed a greater reduction in alkaline phosphatase than the control group (**Table 1**). These results remained after excluding participants who had no weight loss from the analyses, except for the GGT enzyme, which did not differ between groups.

We also observed that women with a weight loss of ≥ 4kg presented lower insulin, HOMA-IR, TyG index, and FLI after BN intervention compared to women who lost < 4kg. Additionally, participants in the BN group who lost ≥ 4kg presented lower insulin and higher levels of Apo A1 and plasma selenium compared to women who lost ≥ 4kg in the control group (**Table 2**)

Intake of energy, macronutrients, MUFA, PUFA, SFA and fiber did not differ between groups after the intervention (**data not shown**). On the contrary, selenium intake was higher in the BN group compared to the control group (**Figure 2B**). Furthermore, we observed a positive and significant relationship between serum selenium variation with the change in Apo A1 and the TyG index (**Figures 3A and B**) as well as a negative relationship with Apo B/Apo A1 ratio and alkaline phosphatase (**Figures 3C and D**).

4. Discussion

In this study, we evaluated the effects of consuming 2 units of BN within an eight-week energy restriction intervention. Our main findings were a higher increase in serum selenium and apolipoprotein A parallel to a decrease in liver enzymes and a lower Apo B/Apo A1 ratio after BN consumption compared to the group receiving an energy-restricted diet free of BN. Although many articles are found in the literature regarding the potential benefits of BN consumption [11,15,29–31], this is the first study to evaluate BN consumption in the context of an energy-restricted diet on markers of glucose, lipids, and liver function.

Only one study evaluated the effect of BN consumption on serum apolipoprotein levels, but no effect has been observed [15]. In this study, the participants were healthy and received

BN containing high amounts of selenium (862.65 µg) for fifteen days in the context of their usual diet. Although no increase in Apo A1 was observed as in our study, an increase in reception of cholesteryl esters by HDL-c was observed. Despite the selenium dose used in our study being lower than the dose used by Strunz et al. (2008), our study participants were at cardiometabolic risk and still received an energy-restricted diet associated with BN. These peculiarities may have been favorable to our study's increase in Apo A1. In another study, a mix of Brazilian nuts (30g of cashew nuts + 15g of BN) associated with an energy-restricted diet did not affect the apolipoproteins of women at cardiometabolic risk, but the selenium dose from the nuts used in this study was approximately seven times lower than ours [32].

The increase in serum selenium after BN consumption is not news [33]. All studies with BN that evaluated this mineral in the blood observed an increase [19], suggesting high absorption rate and bioavailability of selenium from these nuts. Furthermore, healthy subjects already show significant increases in plasma selenium 6 hours after BN consumption [34]. Corroborating our findings, we also observed a positive correlation between the variation in serum selenium and the variation in Apo A1. These results suggest a modulating role of selenium, obtained via BN, in the increase of Apo A1.

Unlike Apo B, which is the main apoprotein of atherogenic particles constituted by lipoproteins VLDL-c, IDL-c, and LDL-c, Apo A1 is the main apoprotein of HDL-c, which is antiatherogenic. In addition to being the main protein of the HDL-c molecule, Apo-A1 is also a cofactor of the Lecithin-Cholesterol Acyltransferase (LCAT) enzyme, which plays a role in cholesterol esterification [35]. A study of rats fed a high-fat diet and co-supplemented with selenium and magnesium showed a decrease in 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and an increase in cholesterol 7α-hydroxylase (CYP7A1) and LCAT in the liver [36]. HMG-CoA reductase participates in the cholesterol biosynthesis pathway while LCAT removes cholesterol from blood and tissues [35]. In a study with hypercholesterolemic

rats, selenium supplementation decreased Apo B and HMG-CoA reductase expression [37]. These data suggest a potential protective role of selenium in modulating lipid metabolism. No human studies have been found to confirm these findings.

A systematic review and meta-analysis showed that consumption of nuts generally did not affect Apo A1 levels but decreased Apo B, total cholesterol, triglycerides, and LDL-c. In addition, strong effects were observed in the decrease of Apo B in subjects with type 2 diabetes [38]. The profile of fats found in nuts, with a predominance of unsaturated fats, is one of the explanations for the beneficial effects observed in the consumption of nuts in the improvement of the lipid profile. Despite this, in our study, the profile of ingested fats did not differ between the groups evaluated. Selenium was the nutrient that differed between the groups and, therefore, may be responsible for the effects observed in our study. In addition, we have to consider that despite having similar weight loss to the group that received an energy-restricted diet free of BN, the group that consumed BN lost more waist circumference (data not shown). The loss of weight and fat in the abdominal region can be linked to an improvement in the metabolic profile [39]. Therefore, we suggest here that the consumption of BN potentiated the benefits of energy restriction.

Furthermore, we observed that the consumption of BN decreased more pronouncedly alkaline phosphatase and GGT compared to the control group. Only one study evaluated liver enzymes after BN consumption. Acute consumption of 5, 20, and 50 g of BN in the context of a usual diet did not change AST, ALT, alkaline phosphatase, and GGT in healthy subjects. Our result suggests that consumption of BN in the energy-restricted context may benefit liver health. Although not different between the two groups, the fatty liver index reduced in both groups after the intervention. The relationship between selenium and liver enzymes is not clear in the literature. In addition to being controversial, the studies are mostly with experimental models. The role of selenium also appears to depend on the amount in which it is present in the blood.

In a cross-sectional study with subjects with a median plasma selenium concentration of 213.0 μg/L, high plasma selenium levels were associated with higher ALT, AST, GGT, and higher odds of nonalcoholic fatty liver disease [40]. On the other hand, body selenium status and selenium intake were negatively associated with hepatitis, cirrhosis, and liver cancer in a meta-analysis [41]. In a study with rats induced to liver damage by the hepatotoxic agent carbon tetrachloride, a decrease in the activity levels of AST, ALT, and GGT enzymes parallel to the increase in plasma alkaline phosphatase was observed after selenium injection [42]. In another study, rats exposed to toxic doses of heavy metals showed a decrease in serum alkaline phosphatase activity, which was not influenced by selenium supplementation [43].

The mechanisms behind the connection between selenium and decreased liver enzymes are unclear. What is known is that alkaline phosphatase is a hydrolase involved in dephosphorylation and that GGT participates in the γ-glutamyl cycle, a pathway of glutathione synthesis and degradation [44,45]. In turn, glutathione peroxidase is a type of glutathione involved in the enzymatic defense system against free radicals that incorporate a selenocysteine residue in its active site. Selenocysteine has a selenium atom in its structure, making it necessary to maintain selenium levels to form enzymatic antioxidant defense systems [44].

Insulin and HOMA-IR decreased in the BN group compared to baseline after the intervention. However, the variation of these markers did not differ between groups and did not correlate with changes in serum selenium. Contrary to what we expected, a positive correlation was observed with the variation of the TyG index. Although not significant, triglycerides increased in both groups evaluated and showed a high variation within each group which may justify the elevation of the TyG index. Although studies suggest that high levels of selenium in the blood are related to an increase in triglycerides [40], we believe that other factors may have contributed to the rise in triglycerides, such as the profile of the carbohydrates consumed [46] and also the oils used in the sauces.

The main limitation of this study was the lack of randomization of participants between groups. Despite this, our study had a control group and the vast majority of variables did not differ between participants at baseline.

In conclusion, BN (approximately 347 µg of selenium) consumption within an energy-restricted diet for 8 weeks increased serum selenium and apolipoprotein A1 while decreased liver enzymes compared to an energy-restricted diet free of BN. These findings suggest that consuming BN within the energy-restricted diet may improve antioxidant status and lipid and liver function markers in women at cardiometabolic risk.

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Authors' contributions

The study was conceived and designed by AS, BKSS, HHMH, and JB. HHMH and JB coordinated the study. AS, BKSS and BVMF contributed to data collection. AS performed the statistical analysis and prepared the first draft of the manuscript. BKSS, BVMF, HHMH, and JB revised the paper and helped to write the final draft of the manuscript.

Consent for publication

The authors confirmed that all participants provided informed consent for publication.

Availability of data and material

The data evaluated in this article can be obtained by contacting the first author. email: alessandra.silva2@ufv.br

Declaration of competing interest

The authors declare no competing financial interests.

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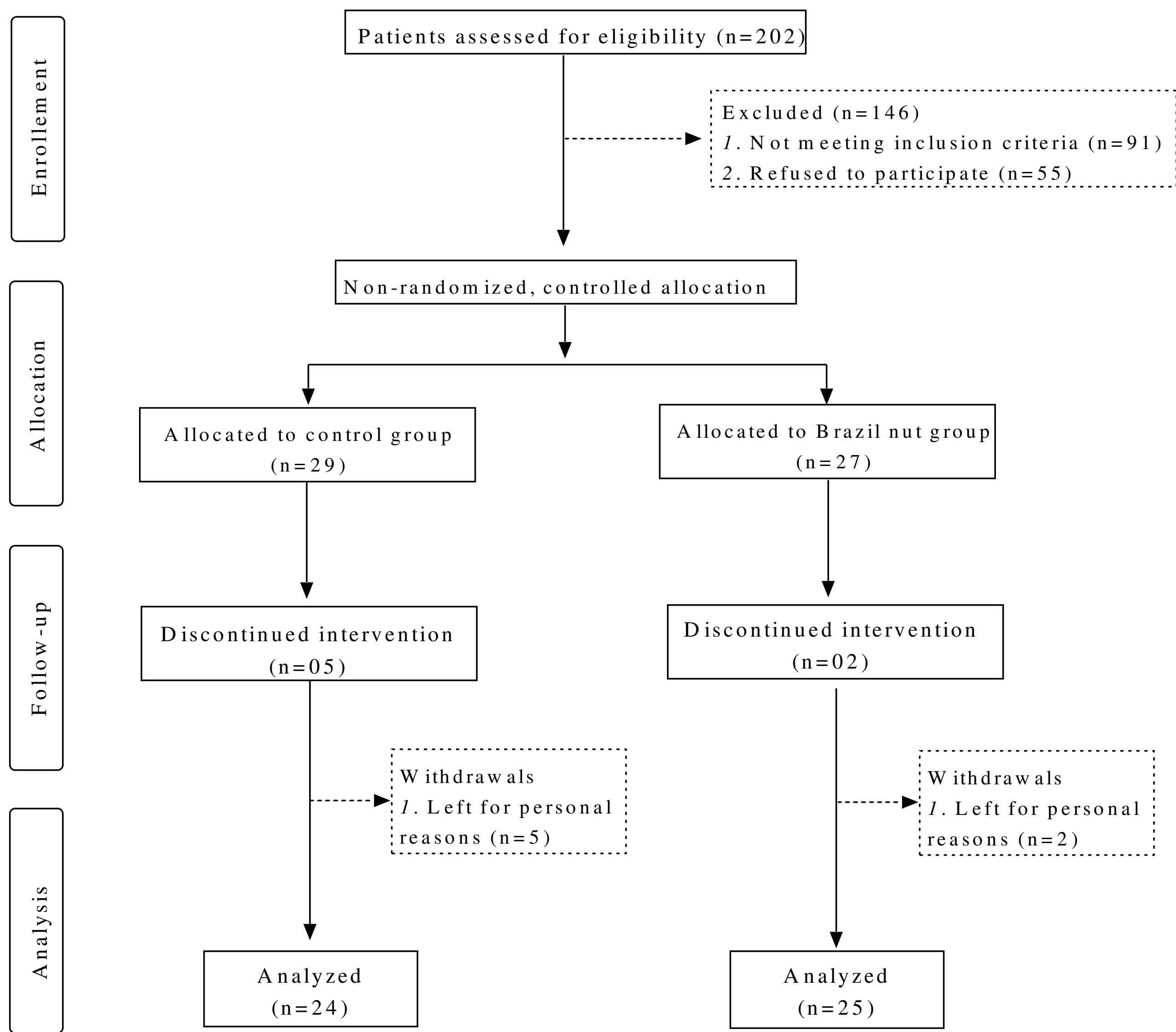
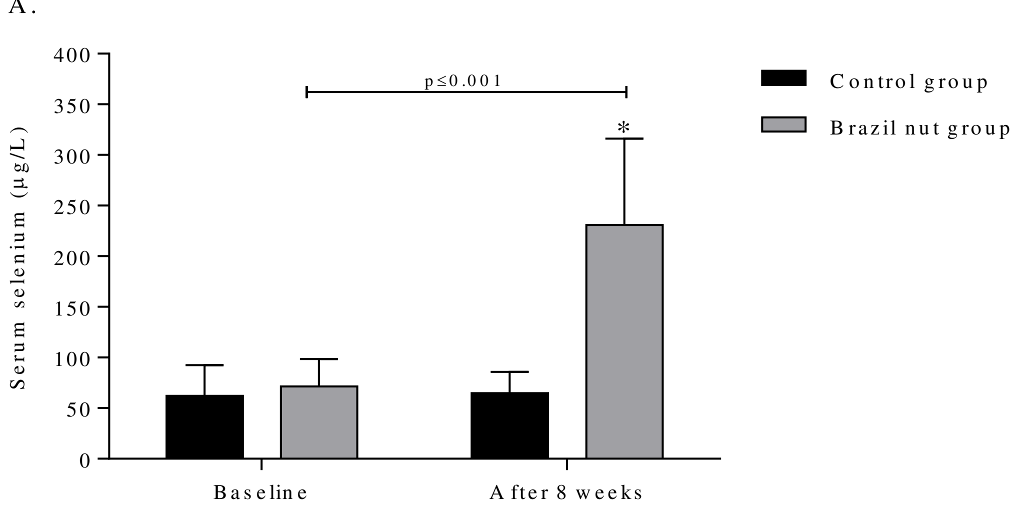


Figure 1: Study flow diagram.

A.



B.

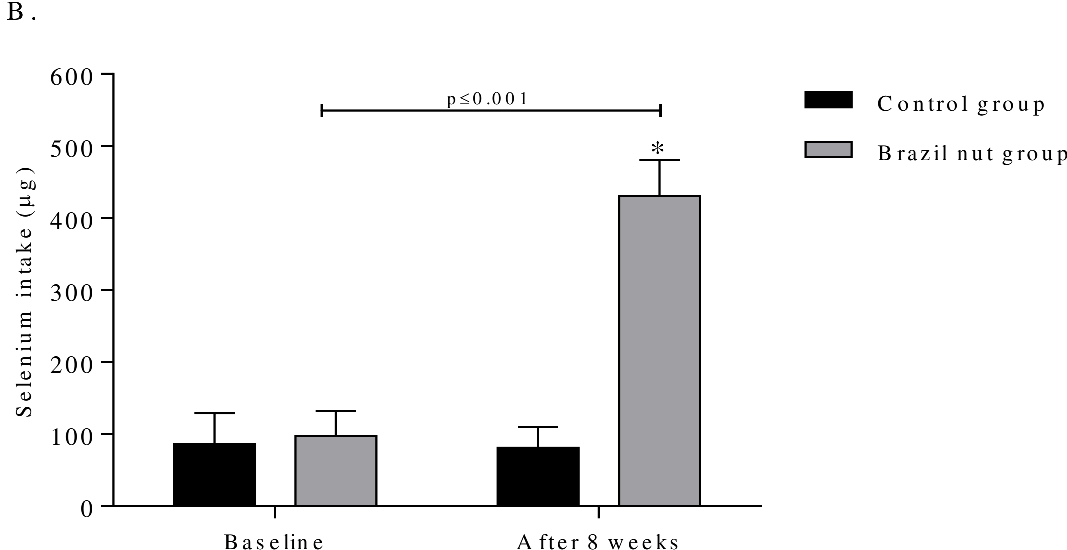


Figure 2: A. Serum selenium and **B.** selenium consumption, according to dietary 8-wk intervention groups. *p<0.05 compared to baseline obtained through Paired *t*-test. *Student t-test* was performed for comparison between groups.

Table 1: Change in cardiometabolic risk markers, according to 8-wk intervention (control vs. Brazil nut group)

	Control group (n=24)		**BN group (n=25)**		**Baseline p-value**	**Δ p-value**
	Baseline	**Δ**	**Baseline**	**Δ**		
Fasting glucose, mg/dl	92.61 (11.45)	1.39 (8.38)	93.8 (11.51)	2.32 (8.75)	0.721	0.709
Insulin, µUI/ml	11.13 (4.32)	-0.94 (4.49)	13.07 (5.62)	-2.98 (4.27) *	0.189	0.114
HOMA-IR	2.54 (1.05)	-0.19 (1.14)	3.01 (1.27)	-0.60 (1.03) *	0.170	0.192
TyG index	8.44 (0.50)	0.01 (0.33)	8.44 (0.49)	0.15 (0.41)	0.991	0.217
TC, mg/dl	177.63 (32.78)	-6.42 (11.33) *	187.2 (38.14)	-4.28 (23.96)	0.352	0.690
LDL-c, mg/dl	101.29 (28.86)	-5.88 (8.57) *	113.04 (36.71)	-3.84 (22.54)	0.220	0.677
HDL-c, mg/dl	47.88 (12.79)	-0.13 (8.39)	52.48 (15.63)	-2.56 (7.87)	0.266	0.300
VLDL-c, mg/dl	22.69 (17.02)	0.85 (6.83)	21.68 (9.62)	2.12 (8.99)	0.798	0.581
Triglycerides, mg/dl	113.33 (85.15)	4.50 (34.17)	109 (47.99)	9.76 (44.58)	0.826	0.646
Non-HDL-c	107.08 (30.79)	-7.19 (10.01) *	112.92 (36.72)	-3.67 (22.48)	0.550	0.481
TC/HDL-c	3.86 (0.82)	-0.18 (0.58)	3.8 (1.16)	0.00 (0.45)	0.835	0.223
LDL-c/HDL-c	2.24 (0.80)	-0.18 (0.44)	2.35 (0.98)	-0.06 (0.47)	0.650	0.372
Apo E, mg/l	42.83 (17.03)	0.47 (7.74)	46.01 (11.87)	0.23 (8.28)	0.454	0.917
Apo B, mg/dl	84.91 (15.06)	-2.13 (6.41)	94.04 (28.99)	-2.48 (16.45)	0.175	0.922
Apo AI, mg/dl	127.87 (26.07)	7.09 (23.94)	140.2 (38.14)	37.48 (20.58) **	0.201	**<0.001**
Apo B / Apo AI	0.68 (0.16)	0.95 (0.18)	0.71 (0.25)	0.79 (0.14) **	0.719	**0.001**
AST, U/L	24.58 (5.69)	0.67 (24.86)	26.17 (7.57)	-9.05 (6.42) **	0.413	0.065
ALT, U/L	16.67 (5.31)	-0.21 (7.78)	18.85 (7.61)	-3.21 (4.33) **	0.252	0.106
GGT, U/L	23.17 (10.25)	-2.08 (6.17)	36.85 (33.59)	-9.69 (17.11) *	0.062	**0.045**
Alkaline phosphatase, U/L	75.13 (19.10)	-5.63 (7.75) *	83.36 (26.99)	-16.88 (13.82) **	0.225	**0.001**
FLI	75.49 (16.57)	-7.11 (12.06) *	70.98 (20.61)	-7.78 (12.53) *	0.410	0.852

Values are mean (SD). Δ = final assessment – baseline. * $p < 0.05$ or ** $p < 0.001$ are significant differences within-group (paired t-test). Baseline p-values and Δ p-values refer to the comparison between groups (*Student t test*).

HOMA-IR, homeostatic model of insulin resistance assessment; TyG index, Triglyceride-glucose index; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; VLDL-c, very-low-density lipoprotein cholesterol; Apo AI, Apolipoprotein AI; Apo B, Apolipoprotein B; Apo E, Apolipoprotein E; AIP, atherogenic index of plasma; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; FLI, fatty liver index.

Supplementary Table 1: Brazil nut composition

Nutrients	Brazil nuts (2 units ~8g)
Ashes (g)	0.2352
Humidity (g)	0.1560
Energy (kcal)	52,679
Carbohydrates (g)	0.2160
Proteins (g)	1.1872
Lipids (g)	5.2296
Fibers (g)	1.0336
Soluble fibers (g)	0.0448
Insoluble fibers (g)	0.9896
Palmitic fatty acid (g)	0.8079
Stearic fatty acid (g)	0.9225
Oleic fatty acid (g)	0.9826
Linoleic fatty acid (g)	1.0776
α-linolenic fatty acid (g)	1.4389
SFA (g)	1.7304
MUFA (g)	0.9826
PUFA (g)	2.5166
Selenium (µg)	347.20
Nitrogen (mg)	211.00
Phosphorus (mg)	40.00
Potassium (mg)	42.00
Calcium (mg)	22.00
Magnesium (mg)	24.00
Sulfur (mg)	20.00
Cooper (mg/kg)	0.1161
Iron (mg/kg)	0.2387
Zinc (mg/kg)	0.3900
Manganese (mg/kg)	0.1121
Sodium (mg/kg)	0.000048

Supplementary Table 2: Change in cardiometabolic risk markers, according to 8-wk intervention (control vs. Brazil nut group)

	Control group (n=19)		BN group (n=22)		Baseline p-value	Δ p-value
	Baseline	Δ	Baseline	Δ		
Apo E, mg/l	43.96 (16.84)	-0.35 (7.02)	44.54 (9.23)	-0.45 (8.43)	0.714	0.967
Apo B, mg/dl	88.83 (13.8)	-2.78 (6.93)	96.32 (28.65)	-2.91 (17.27)	0.963	0.974
Apo A, mg/dl	125.89 (26.73)	7.28 (26.59)	142.18 (40.23)	37.91 (21.87) **	0.890	**0.001**
TC / HDL-c	4.00 (0.8)	-0.22 (0.65)	3.88 (1.17)	0.00 (0.48)	0.288	0.224
LDL-c / HDL-c	2.42 (0.78)	-0.20 (0.49)	2.40 (1)	-0.05 (0.5)	0.149	0.313
Apo B /Apo AI	0.72 (0.14)	0.94 (0.2)	0.72 (0.26)	0.78 (0.13) **	0.959	**0.008**
AST, U/L	24.53 (5.54)	0.21 (8.55)	26.60 (7.91)	-3.55 (4.52) *	0.102	0.122
ALT, U/L	16.42 (5.57)	0.68 (28.03)	19.92 (7.48)	-9.05 (6.85) **	0.345	0.097
GGT, U/L	23.47 (10.51)	-2.84 (6.54)	39.97 (34.69)	-11.10 (17.8) *	**0.044**	0.700
Alkaline phosphatase, U/L	75.89 (19.76)	-6.68 (8.2) *	84.36 (28.62)	-16.91 (14.68) **	0.285	**0.010**
FLI	75.76 (15.64)	-10.41 (11.39) *	73.70 (19.85)	-10.05 (10.07) **	0.723	0.915
Selenium, µg/L	65.05 (33.79)	-2.11 (45.05)	68.91 (27.14)	157.23 (85.52) **	0.691	**≤0.001**

Values are mean (SD). Δ = final assessment – baseline. * $p < 0.05$ or ** $p < 0.001$ are significant differences within-group (paired t-test). Baseline p-values and Δ p-values refer to the comparison between groups (*Student t-test*). For variables different between groups at baseline, Δ p-value was obtained through ANCOVA analysis adjusted by baseline value.

HOMA-IR, homeostatic model of insulin resistance assessment; TyG index, Triglyceride-glucose index; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; VLDL-c, very-low-density lipoprotein cholesterol; Apo AI, Apolipoprotein AI; Apo B, Apolipoprotein B; Apo E, Apolipoprotein E; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; FLI, fatty liver index.

Table 2: Change in cardiometabolic markers according to the intervention groups and weight variation (< or ≥ 4kg) only in women who weight loss

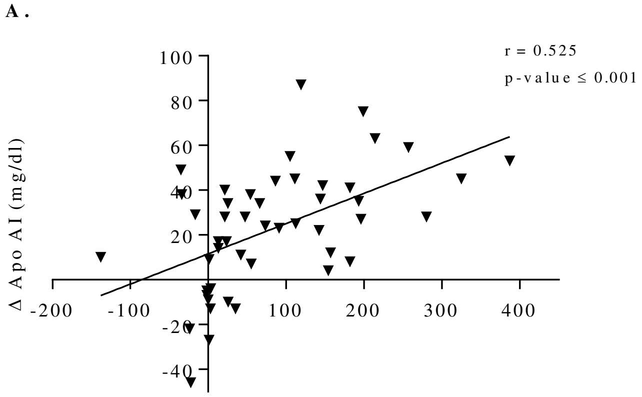
	Control group (n=17)			**BN group (n=21)**		
	Weight loss ≥ 4kg					
	No (n=10)	**Yes (n=7)**	**p-value**	**No (n=10)**	**Yes (n=11)**	**p-value**
Δ Glucose, mg/dl	0.17 (8.21)	-0.17 (10.68)	0.942	1.45 (8.39)	2.18 (7.6)	0.833
Δ Insulin, µUI/ml	-2.28 (4.64)	-0.40 (5.48) †	0.457	-1.43 (3.46)	-5.42 (4.17) †	**0.024**
Δ HOMA-IR	-0.58 (1.18)	-0.07 (1.26)	0.410	-0.32 (0.84)	-1.11 (1.02)	0.060
Δ TyG index	-0.09 (0.4)	0.02 (0.22)	0.560	0.19 (0.37)	-0.04 (0.32)	0.125
Δ TC, mg/dl	-9.33 (12.64)	-7.57 (9.61)	0.755	-1.82 (32.2)	-7.82 (17.58)	0.593
Δ LDL-c, mg/dl	-6.58 (10.66)	-7.43 (6.75)	0.853	-2.55 (28.7)	-3.82 (19.07)	0.904
Δ HDL-c, mg/dl	1.00 (8.64)	-2.86 (10.62)	0.400	-3.18 (10.34)	-2.45 (6.23)	0.844
Δ VLDL-c, mg/dl	-0.87 (8.54)	0.40 (2.14)	0.708	3.91 (9.21)	-1.55 (8.43)	0.163
Δ TG, mg/dl	-4.33 (42.78)	2.86 (10.38)	0.671	19.18 (45.28)	-8.73 (42.23)	0.150
Δ Non-HDL-c	-9.46 (12.74)	-5.28 (7.3)	0.441	-2.47 (28.7)	-3.61 (18.92)	0.913
Δ Apo E, mg/l	-2.18 (6.44)	3.32 (7.22)	0.120	0.99 (9.78)	-1.90 (6.99)	0.435
Δ Apo B, mg/dl	-4.33 (5.23)	0.33 (9.27)	0.186	0.64 (22.54)	-6.45 (9.49)	0.353
Δ Apo A, mg/dl	12.33 (27.09) ‡	-2.83 (24.58) †	0.266	40.72 (26.13) ‡	35.09 (17.44) †	0.559
Δ TC / HDL-c	-0.35 (0.57)	0.01 (0.74)	0.256	0.04 (0.52)	-0.04 (0.44)	0.699
Δ LDL-c / HDL-c	-0.26 (0.48)	-0.11 (0.53)	0.528	-0.06 (0.55)	-0.04 (0.47)	0.919
Δ Apo B / Apo AI	0.89 (0.18)	1.04 (0.2)	0.127	0.82 (0.18)	0.75 (0.06)	0.262
Δ AST, U/L	-2.17 (4.8)	4.29 (12.11)	0.326	-4.29 (6.02)	-2.82 (2.32)	0.074
Δ ALT, U/L	-6.08 (5.42) ‡	12.29 (45.33)	0.219	-11.65 (7.02) ‡	-6.45 (5.87)	0.458
Δ GGT, U/L	-5.25 (4.41)	1.29 (7.83)	**0.031**	-11.66 (20.87)	-10.55 (15.15)	0.887
Δ Alkaline phosphatase, U/L	-6.75 (7) ‡	-6.57 (10.58)	0.965	-19.73 (16.06) ‡	-14.09 (13.3)	0.381
Δ FLI	-13.20 (12.51)	-6.03 (8.37)	0.202	-4.99 (8.13)	-15.10 (9.52)	**0.014**
Δ Selenium, µg/L	-2.27 (53.43) ‡	-1.78 (24.91) †	0.984	140.64 (54.42) ‡	173.82 (108.59) †	0.376

Values are mean (SD). Δ = final assessment – baseline. p-values < 0.05 indicate statistically significant differences within-group between weight loss categories (*Student's t-test*).

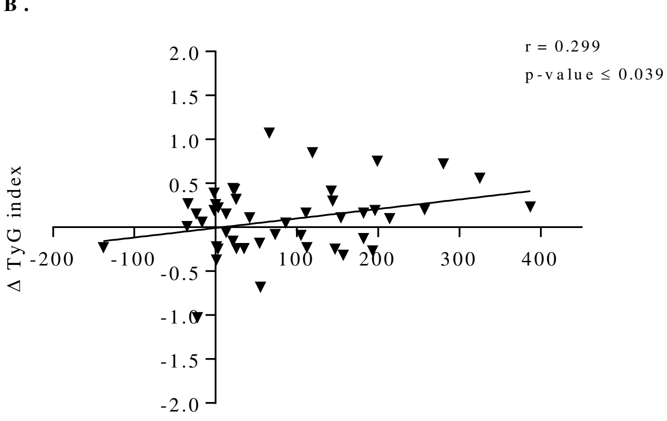
‡ refers to statistically significant differences between control group and BN group in those classified with weight loss values < 4kg obtained through *Student's t-test*.

† refers to statistically significant differences between control group and BN group in those classified with weight loss values ≥ 4kg obtained through *Student's t-test*.
HOMA-IR, homeostatic model of insulin resistance assessment; TyG index, Triglyceride-glucose index; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; VLDL-c, very-low-density lipoprotein cholesterol; Apo AI, Apolipoprotein AI; Apo B, Apolipoprotein B; Apo E, Apolipoprotein E; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; FLI, fatty liver index.

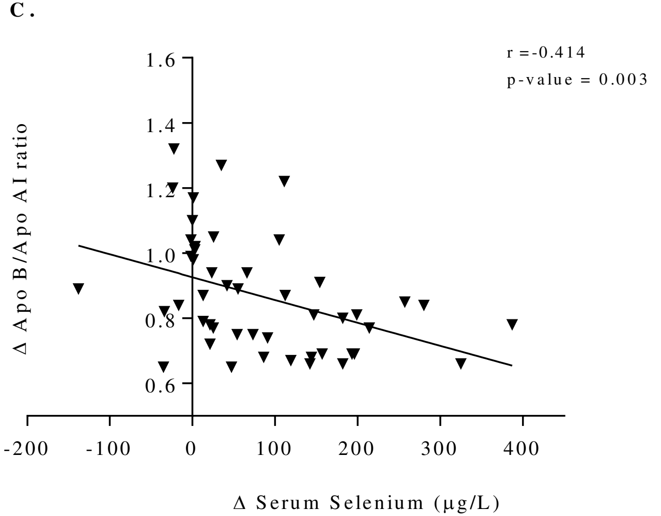
A.



B.



C.



D.

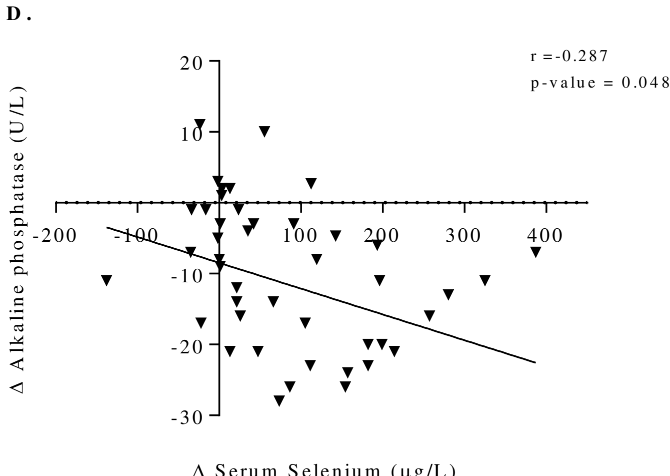


Figure 3. Correlations between variations in serum selenium and variations in **A.** Apo A1; **B.** TyG index; **C.** Apo B/Apo A1 ratio and **D.** alkaline phosphatase.

Δ = final assessment – baseline. p-values < 0.05 indicate statistically significant correlations (*Pearson correlation*).

5.5 ARTIGO ORIGINAL 3 – A ser submetido à revista Europen Journal of Clinical Nutrition (FI: 4,016)

Brazil nut (*Berthollethia excelsa* H.B.K.) intake within an energy-restricted diet is related to oxidative stress markers in women at cardiometabolic risk: a controlled, parallel clinical trial (Brazilian Nuts Study)

Alessandra da Silva[1]

Walmir da Silva[2]

Brenda Kelly de Souza Silveira[1]

Natália Aparecida Liberto Silva[1]

Helen Hermana M. Hermsdorff[1]

Josefina Bressan[1]

[1] *Laboratory of Energy Metabolism and Body Composition. Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.*

Corresponding author: Josefina Bressan. Laboratory of Energy Metabolism and Body Composition. Department of Nutrition and Health, Universidade Federal de Viçosa. Avenida PH Rolfs, s/n. Viçosa – MG, Brasil. 36570-900. Phone: +55 31 36125220. E-mail: jbrm@ufv.br

Abstract

Consumption of Brazil nut (BN) inserted in the habitual diet improved markers of antioxidant status and oxidative stress. Nevertheless, the effect of BN associated to an energy-restricted diet in these markers remains a gap in the literature. We aimed to assess the effects of an energy-restricted diet in association with BN on markers of antioxidant status, oxidative stress, and endothelial health. For this, women at cardiometabolic risk consumed for two months an -500 kcal energy-restricted diet + two units of BN (BN-group) or an -500 kcal energy-restricted diet free of nuts (CG). Superoxide dismutase (SOD), malondialdehyde (MDA), ferric reducing antioxidant of plasma (FRAP), and nitric oxide (NO) were assessed according to standard protocols. Health endothelial markers, GPX1, and OxLDL were estimated through ELISA kits. mRNA expression was evaluated by reverse-transcriptase quantitative real-time polymerase chain reaction (qRT-PCR). Plasma levels and mRNA expression of SOD decreased after BN consumption. MDA and ON decreased while FRAP increased in the BN-group compared to CG. GPX1, intercellular, and vascular adhesion molecules unchanged after interventions. An inverse relationship was observed between changes in serum selenium and changes in SOD and MDA. In conclusion, BN intake in the context of an energy-restricted diet improved markers of oxidative stress and antioxidant status.
Trial registration: RBR-8zfn5c and RBR-3ntxrm.

Keywords: oxidative stress; cardiometabolic risk; endothelial health; nuts; qRT-PCR; selenium

1. Introduction

Oxidative stress has been attributed as one of the main mechanisms behind the origin and progression of endothelial dysfunction and aging-related chronic diseases [1]. On its turn, obesity is characterized by high productions of oxygen and nitrogen reactive species allied to diminished antioxidant defense system [2]. Replacing the oxidant state by redox homeostasis has been the target of therapies against chronic diseases [3,4].

Redox homeostasis is achieved when oxidizing agents and antioxidants are in balance in the body [5]. Thus, exogenous sources of antioxidants such as minerals, vitamins, and non-nutrients together with endogenous antioxidants are of confirmed importance for combating free radicals that attack cell membranes, DNA, and signaling proteins [5,6]. Despite being limited, heterogeneous, and controversial, chronic consumption of nuts seemed to be effective to change some oxidative stress biomarkers in humans [7]. On the other hand, a meta-analysis showed a favorable effect of nut consumption on flow-mediated dilation, suggesting improvement in endothelial health [8].

The Brazil nut (BN) has been the subject of several studies due to its rich food matrix, with an emphasis on selenium [9]. Selenium participates in the formation of enzymatic and non-enzymatic defense systems and, therefore, has been associated with improvement in antioxidant status and oxidative stress [10,11]. On the other hand, the energy-restricted diet, which is one of the best-studied strategies for weight loss, has been associated with improved markers of oxidative stress [12,13] and endothelial health [14]. The consumption of 30g of cashew nuts + 15g of BN decreased the concentrations of soluble adhesion molecule VCAM-1 in women at cardiometabolic risk. However, to our knowledge, no study has evaluated the effects of BN consumption combined with an energy-restricted diet for two months on markers of antioxidant status, oxidative stress, and endothelial health in women at cardiometabolic risk.

Thus, the aim of this study was to evaluate the effect of BN intake within an energy-restricted diet on markers of antioxidant status, oxidative stress, and endothelial health in women at cardiometabolic risk. Afterward, we evaluated the relationship of these markers according to serum selenium variation.

2. Methods

Subjects

This study is a secondary analysis from a larger study whose primary objective was to evaluate the effects of consuming two BN units within a daily energy-restricted diet for two months on anthropometric and body composition markers in adult women at cardiometabolic risk (data no published yet). The study was approved by the Human Research Ethics Committee (CAAE: 92004818.0.0000.5153; UP protocol number: 2.832.601/2018) of the Universidade Federal de Viçosa, Minas Gerais, Brazil and was registered in the Brazilian Registers of Clinical Trials (REBEC) (protocol: RBR-8zfn5c). The study followed the Resolution CSN 466/2012 and all participants included signed the Free and Informed Consent Term.

Eligibility and non-inclusion criteria

For entering in the study the following eligibility criteria were considered: Adult women (20-55 years), with overweight (BMI $\geq$ 27 kg/m2 and $<$30 kg/m2), waist circumference $\geq$ 80 cm, and body fat percentage $\geq$ 32% associated with at last one another component of metabolic syndrome: triglycerides $\geq$150 mg/dL, high blood pressure arterial ($\geq$130/85 mmHg) or high fasting glucose ($\geq$100 mg/dL); or women with obesity (BMI $\geq$ 30 kg/m^2), with or without metabolic complications were included.

On the other hand, non-inclusion criteria were the following: pregnant, lactate, or menopausal women; athletes; vegans; smoking; women with a history of HIV, illness or digestive, liver, kidney, cardiovascular, thyroid, cancer, inflammatory diseases, and eating disorders; history of drug and/or alcohol abuse; aversion or allergy to nuts; infectious episode

in the last month; use of anti-inflammatory drugs, corticosteroids, antibiotics, and others that may affect energy appetite and metabolism; body weight instability (5% of usual weight) in the last 3 months; regular (daily) consumption of any quantity of BN; alcohol consumption higher than 21 units (168g) per week; dental problems that interfere with chewing; use of vitamin, mineral, and omega 3 supplements.

Dietary intervention

The study was performed in the Department of Nutrition and Health of Universidade Federal de Viçosa-MG, Brazil, with enrollment between June 2019 and September 2021. Advertisements in social media, local radio and TV were the recruitment methods. Participants allocated to the Brazil nut group (BN-group) received an -500 kcal energy-restricted diet with two units of BN (~8 g) daily for two months, while the control group (CG) received a nut-free -500 kcal energy-restricted diet. Participants were instructed to consume a -500 kcal energy-restricted diet aiming to lose at last four kilograms at the end of the intervention period. Energy requirements and macronutrient distribution were prescribed based on the recommendations of the obesity control guidelines [15]. The calories from the BN were accounted for within the food plan for the BN-group. BN contained 1.18 g of protein, 0.21 g of carbohydrates, and 5.22 g of lipids, with a total of 52.67 kcal and 247.2 µg of selenium. BN were donated by ECONUT® and were portioned (two units) in laminated packages, vacuum sealed (Selovac Sealer model 200 B), and stored in a freezer at -20ºC until distribution to the participants.

Blood collection and RNA extraction

Venous blood samples from the antebrachial vein were collected after 12 h of overnight fasting at the beginning and end of the intervention period. Vacuum tubes precoated with EDTA or heparin were used. Plasma samples were obtained after centrifugation at 3500 ×rpm for 10 min at 4ºC and stored at -80°C until analysis.

After removing plasma from EDTA tubes, peripheral blood mononuclear cells (PBMC) samples were obtained after the addition of Ficoll® solution and successive washes with saline solution. PBMC samples were immersed in the Tri Reagent® and the RNA was isolated following TRIzol® Reagent user guide (Invitrogen, ThermoFisher Scientific®). Briefly, chloroform was added to the sample homogenized with TRIzol® and the supernatant mixed with isopropanol. Then, successive washes were performed with ethanol and finally the RNA was reconstituted in ultrapure water. The absorbance ratio (A260/A280) indicated an ideal purity for extracted RNAs with values ranging from 1.8 to 2.0. The quality and integrity of extracted RNAs were also verified using an agarose gel 1.2% stained with ethidium bromide.

Assessment of plasma oxidative stress and endothelial markers

Superoxide dismutase (SOD) activity was evaluated by its ability to catalyze the reduction of superoxide radical (O_{22}) to hydrogen peroxide (H_2O_2) and O_2, using pyrogallol. SOD was read in a spectrophotometer at 570 nm and expressed in unit per milliliter of plasma [16].

Plasma antioxidant capacity was evaluated by the ferric reducing ability of plasma (FRAP) based on the reduction of Fe^{3+} to Fe^{2+} in the plasma by nonenzymatic antioxidants and subsequent complexation of Fe^{2+} with 2,4,6-tri (2-pyridyl) s-triazine to form a Fe^{2+} 22,4,6-tri (2-pyridyl)-s-triazine (2-pyridyl)-s-triazine chromophore [17]. The absorbance of the chromophore at 595 nm was read in a spectrophotometer and FRAP was expressed in micromoles per milliliter.

Malondialdehyde (MDA) was determined according to the method described by Buege et al. using a thiobarbituric acid reactive [18]. The absorbance of the chromophore at 535 nm was read in a spectrophotometer and MDA was expressed in micromoles per milliliter.

Nitric oxide (NO) concentration was determined using the Griess reagent according to the protocol proposed by Grisham et al. (1996) [19]. The plates were read in a

spectrophotometer at 570 nm, and NO was expressed in micromoles per milliliter. All markers were assessed in triplicate and mean values was used.

Plasma levels of GPX1, OxLDL, ICAM-1, and VCAM-1 were estimated using commercially available Human GPX1 (Glutathione Peroxidase 1), Human OxLDL (Oxidized Low-Density Lipoprotein), Human ICAM-1/CD54 (Intercellular Adhesion Molecule 1), and Human VCAM-1/CD106 (Vascular Cell Adhesion Molecule 1) ELISA assay kits (Elabscience, USA), respectively. All analysis were performed according to the manufacturer's instructions and the optical density (OD value) of each well was read at 450 nm.

100 µl of plasma, 100 µl of 5-fold diluted plasma, 100 µl of 100-fold diluted plasma, and 100 µl of 2-fold diluted plasma samples were required for GPX1, OxLDL, ICAM-1, and VCAM-1 analysis, respectively. GPX1 and OxLDL were expressed in pg/ml while ICAM-1 and VCAM-1 were expressed in ng/ml. The values of OxLDL, ICAM-1, and VCAM-1 were corrected by the dilution factor.

mRNA expression

The mRNA expression of SOD, GPX1, catalase, and nuclear factor erythroid 2-related factor 2 (Nrf2) were evaluated from PBMC using reverse-transcriptase quantitative real-time polymerase chain reaction (qRT-PCR). RNA was extracted with Tri Reagent® as briefly detailed above. The cDNA was synthesized with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). PowerUp™ SYBR™ Green Master Mix (Applied Biosystems) was used to gene expression assays. b-globin mRNA expression was used as control gene. PCR amplification was carried out using the ABI Prism 7500 Sequence Detection System (Applied Biosystems®) under standard mRNA expression cycling conditions.

Amplification conditions for all reactions were: 95°C for 5 minutes, 40 denaturing cycles at 95 °C for 15 seconds each, and annealing and extension at 60 °C for 60 seconds. After 40 amplification cycles, the dissociation curve was obtained by the additional step with a

gradual temperature elevation from 60 °C to 94 °C (0.01 °C/s). Quantitative Real-Time PCR data were obtained as Ct values that were used subsequently in statistical analysis. Triplicate samples were made for each gene. Technical replicates were considered appropriate with Coefficient of Variation (CV) values reaching a maximum of 10%.

The expression of genes was normalized against b-globin, and the expression level was calculated using the ΔΔCT (delta delta threshold cycle) method. The following primers sequences were used: SOD1 (Forward: 5'GGTGTGGCCGATGTGTCTATT3'; Reverse: 5'CTGCTTTTTCATCGACCACCA3'); GPX1 (Foward: 5'GCGGCGGCCCAG TCGGTGTA3'; Reverse: 5'GAGCTTGGGGTCGGTCATAA3'); catalase (Foward: 5'TCCGGGATCTTTTTAACGCCATTG3'; Reverse: 5'TCGAGCACGGTAGGGACAGTTCAC3'); Nrf2 (Forward: 5′-TTCAGCCAGCCCAGCACATC-3; Reverse: 5′-CGTAGCCGAAGAAACCTCATTGTC-3), and b-globin Foward: GCTTCTGACACAACTGTGTTCACTAGC; Reverse: CACCAACTTCATCCACGTTCACC).

Statistics Analyses

Shapiro-Wilk test was performed for each group of intervention to assessment of normality. Paired *t*-test was used to compare quantitative variables before and the intervention. *Student's t test* was performed to compare quantitative variables between groups and between serum selenium median categories. Results were described as mean and standard deviation. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 21.0 for Windows. The level of significance in two-tailed tests was set at 5%.

3. Results

In the present study, 48 adult women were evaluated, 23 from the CG and 25 from the BN-group. Serum selenium was higher in BN-group compared to controls while weight loss percentage was similar between groups (data not shown).

Plasma and PBMC samples from the GC were lack for some markers (GPX1, SOD, OxLDL, ICAM-1, VCAM-1, and mRNA expression) for this reason the groups were unbalanced (CG, n=8). Despite this, variances were not different between groups. Plasma levels of GPX1, Ox-LDL, ICAM-1, and VCAM-1 did not differ at baseline, from baseline, and between groups after the intervention period. We observed that SOD expression decreased in the BN-group after the intervention period compared to baseline. The expression of NRf2, GPX1, and catalase genes did not change over time and between groups (**Table 1**).

When evaluating all of the individuals who finished the intervention, we observed that overall plasma levels of SOD and MDA decreased compared to baseline (**Figures 1A and 1B**). However, when stratifying by intervention group, we observed that SOD, MDA, and NO decreased only in BN-group compared to baseline (**Figures 1A, 1B, and 1D**). Furthermore, the decrease in MDA and NO was statistically significant compared to GC. Additionally, FRAP was higher in BN-group compared to CG after the intervention (**Figure 1C**).

To investigate the presence of a possible relationship between serum selenium and markers of antioxidant status, oxidative stress, and endothelial health, we divided the all sample by the serum selenium variation median. Thus, we observed that women with a greater increase in selenium after the intervention period showed greater reductions in SOD and MDA compared to those with a lower serum selenium variation (**Table 2**).

4. Discussion

In this study, we observed that consumption of two units per day of BN included in a 8-wk energy-restricted intervention decreased plasma SOD1 and SOD1 mRNA expression compared to baseline, and reduced MDA and NO while increased plasma reducing ferric antioxidant compared to controls.

SOD1 is an antioxidant enzyme located throughout the cell cytoplasm, nucleus, and in the lumen mitochondrial [20]. This enzyme prevents that superoxide radical damage cellular

structures by converting superoxide radical into oxygen and hydrogen peroxide [21]. Only one study with subjects unisg statins evaluated the effect of BN consumption (one unity/day for 12 weeks) on SOD plasma activity, in which SOD reduced compared to baseline [22]. The production of antioxidant enzymes is stimulated by both the presence of free radicals [23] and even selenium [7]. In this sense, the stimulation of these two factors, can activate the Nrf2 transcription factor pathway, improving the expression of antioxidant response element (ARE) genes. By Keap-1/Nrf2 dissociation, Nrf2 migrates to the nucleus where it binds to ARE genes, triggering the transcription of antioxidant enzymes such as SOD, GPX, catalase, among others [7].

In our study, Nrf2 mRNA expression unchanged after the intervention period. On the other hand, patients on hemodialysis increased the expression of the marker combined with a decrease in Nuclear factor kappa B mRNA expression after consuming one unit of BN during twelve weeks [24]. The first hypothesis that could justify our finding is that the health status of our participants did not require the expression of Nrf2 and consequent increase in the production of antioxidant enzymes. Subjects with chronic kidney disease are in a profound state of oxidative stress, which perhaps contributed to an increase in the body's response and BN consumption to combat this state [25]. Second, in addition to constituting antioxidant enzymes such as glutathione peroxidases, selenium is a non-enzymatic antioxidant [10,26]. Thus, we observed an increase in FRAP after the intervention period, suggesting an increase in plasma antioxidant potential to combat the possible presence of free radicals. This hypothesis helps us to explain the observed inverse relationship between the change in serum selenium levels and the change in plasma SOD levels as well as the decreasing in SOD plasma activity and mRNA expression.

We also observed a decrease in MDA in BN-group compared to CG and this is a notable result because this is a marker of lipid peroxidation. According to this result, one unit of BN in

the context of the usual diet decreased the MDA in individuals undergoing hemodialysis [24] and using statins [22]. Free radicals attack polyunsaturated fatty acids present, for example, in cell membranes, producing end products such as MDA. Cell damage results in the activation of the inflammation cascade and also in the production of free radicals, forming a vicious cycle with impairment of cell function. A study showed that subjects with abdominal obesity had higher serum levels of MDA compared to normal-weight subjects while no difference was observed for subjects without abdominal obesity [27]. In another study, intra-abdominal fat was positively correlated with MDA and negatively correlated to SOD [28]. Abdominal obesity is a cardiometabolic risk due to its relation with visceral adiposity, pro-inflammatory cytokines and free radicals' production. In the Brazilian Nuts Study, we also bserved that participants in the BN-group presented a statistically greater reduction in waist circumference allied with a more preserved abdominal fat-free mass and lean mass in the android region compared to CG (data not published yet). These results may justify the reductions in MDA levels.

We also observed greater MDA reductions in women with higher median serum selenium variation, suggesting a protective role for selenium allied to waist circumference loss in lipid peroxidation. Unfortunately, studies evaluating the relationship between selenium and MDA are scarce. What is suggested is that as an antioxidant, selenium will inhibit lipid peroxidation and consequent decrease in MDA formation [7]. Thus, lower levels of MDA will be detected in plasma [7]. When oxidized, LDL molecules also produce MDA. However, OxLDL levels remained unchanged after intervention period. Contrary to this result, the consumption of 15-25g of BN during sixteen weeks decreased OxLDL of adolescents with obesity showing promising cardiovascular benefits for this population [29]. A peculiarity of this study compared to ours is that the adolescents studied did not have selenium deficiency at baseline, unlike the women evaluated in our study [9,29]. Thus, they may have benefited in this regard. In addition, the intervention time was twice as long as we used. Additionally, most of

the women evaluated in our study did not have comorbidities other than obesity and, in this sense, would not have anything to improve with regard to OxLDL.

NO decreased while ICAM-1 and VCAM-1 were unchanged after interventions. Corroborating our results, subjects undergoing bariatric surgery decrease NO after three and twelve months of surgery, suggesting the role of weight loss in improvement of the marker [30]. However, NO was unchanged in controls suggesting the impact of BN in the modulation of the marker. BN is a food matrix with high lipid and selenium content, but the selenium really stands out due to the small quantity of BN consumed daily. NO is a free radical that participates in numerous functions in the body depending on its precursor [31]. The NO synthase (NOS) 3 isoform shows anti-obesogenic and insulin-sensitizing effects while NO derived from NOS1 promotes hyperphagia. In turn, NOS2-derived nitrogen oxides are related to insulin resistance and inflammation [31]. In this study, NO was estimated through Grishan reaction, which quantifies nitrate and nitrite [32]. When oxidized, NO produces nitrogen dioxide and dinitrogen trioxide with deleterious action in cells.

To date, no studies evaluated these markers after BN consumption [9]. A previous study showed a decrease in VCAM-1 after women at cardiometabolic risk consumed a Brazilian nut mix (15g of BN + 30g of cashew nuts) [33]. In this study, authors discuss the possible role of oleic acid and selenium in the inhibition of expression adhesion molecules in endothelial cells. However, the mechanism behind these associations remains a gap in the literature [33].

This study has some limitations. The first is the lack of control group data for some markers. Despite having a smaller number of participants compared to BN-group, we still had some controls. In addition, before versus after comparisons are also of interest. The second is the lack of randomization. However, the evaluated variables did not differ between groups at baseline. Despite the limitations, this is the first clinical trial to investigate the effects of consuming BN, a nutritious fruit from the Amazon region, associated with an energy-restricted

diet on markers of antioxidant status, oxidative stress, and endothelial health in women at cardiometabolic risk. In addition, we performed RT-qPCR, which is a gold standard analysis in the nutrigenomics field.

5. Conclusion

In conclusion, daily BN intake increased plasma antioxidant capacity and decreased MDA and NO compared to controls in 8-wk energy-restricted diet. In addition, it reduced plasma and mRNA expression of SOD in women at cardiometabolic risk. These results suggest the beneficial effects of consuming BN allied with a balanced regime for weight loss on oxidative stress and antioxidant status.

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Authors' contributions

The study was conceived and designed by AS, BKSS, HHMH, and JB. HHMH and JB coordinated the study. AS and BKSS collect the data. AS, WS, and NASL performed all laboratorial analysis. AS performed the statistical analysis and prepared the manuscript. WS, BKSS, NASL, HHMH, and JB revised the paper and helped to write the final draft of manuscript.

Consent for publication

The authors confirmed that all participants provided informed consent for publication.

Availability of data and material

The data evaluated in this article can be obtained by contacting the first author. email: alessandra.silva2@ufv.br

Declaration of competing interest

The authors declare no competing financial interests.

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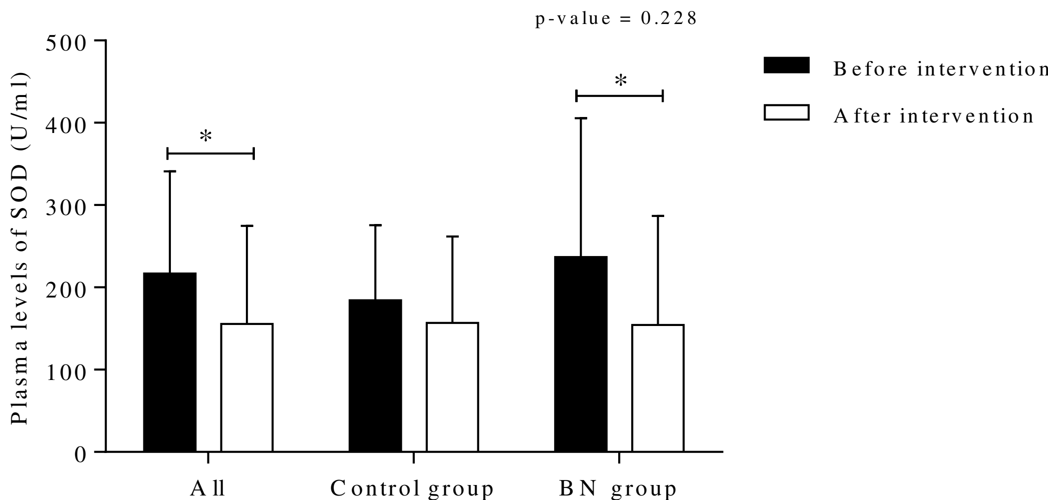
Table 1: Oxidative stress and endothelial health markers according to 8-wk energy-restricted intervention (control vs. BN-group)

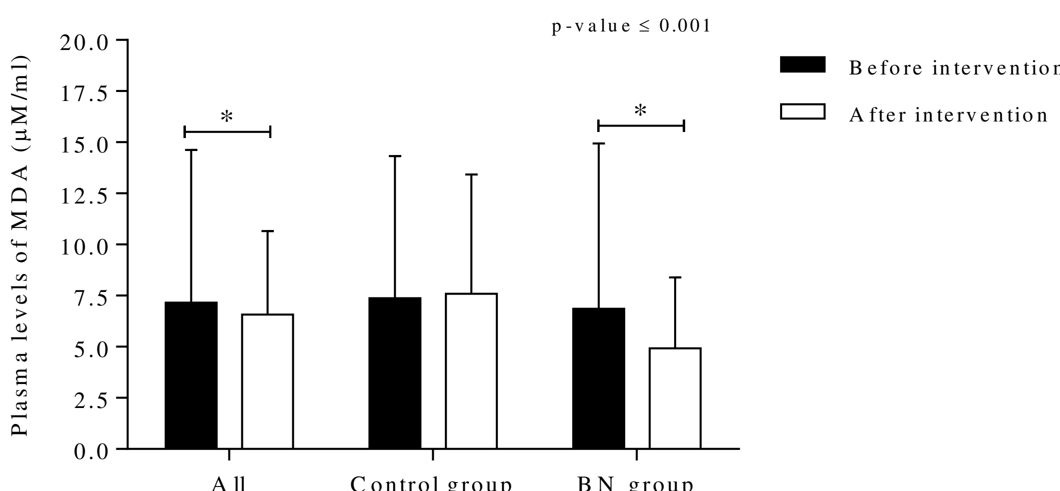
	Control group (n=8)		p-value	BN-group (n=25)		p-value
	Baseline	**Endpoint**		**Baseline**	**Endpoint**	
GPX1, pg/ml	20.91 (2.58-32.54)	13.12 (0.03-44.39)	0.674	30.77 (2.87-140.85)	21.64 (1.79-103.79)	0.130
Ox-LDL, pg/ml	2140.6 (1315.5-2777.65)	2383.8 (1525.5-3136.82)	0.575	2844.19 (1399.42-3716.83)	2573.06 (1769.49-3908.99)	0.253
ICAM-1, ng/ml	99.96 (75.48-294.51)	117.53 (79.71-205.28)	0.779	102.71 (73.25-202.02)	104.22 (70.06-194.11)	0.221
VCAM-1, pg/ml	90.15 (44.63-198.8)	71.67 (39.46-171.52)	0.327	74.42 (22.58-259.97)	77.15 (7.01-220.14)	0.459
SOD mRNA expression	0.61 (0.3-3.97)	0.56 (0.18-2.47)	1.000	0.73 (0.008-6.57)	0.36 (0.05-2.58)	**0.008**
NRf2 mRNA expression	0.92 (0.2-8.33)	0.57 (0.17-1.94)	0.401	0.99 (0.07-6.86)	0.91 (0.07-7.42)	0.689
GPX1 mRNA expression	3.79 (0.05-16.76)	1.14 (0.03-2.27)	0.345	1.05 (0.13-7.11)	1.05 (0.04-6.51)	0.615
Catalase mRNA expression	1.09 (0.38-3.6)	0.7 (0.22-1.36)	0.173	0.8 (0.06-18.05)	1.17 (0.07-7.4)	0.550

Values are mean (SD). P-values < 0.05 indicate statistically significant differences within-group (*Paired t test*).

FRAP, ferric Reducing Antioxidant Power; GPX1, glutathione peroxidase 1; ICAM-1, intercellular adhesion molecule 1; MDA, malondialdehyde; Nrf2, nuclear factor erythroid 2-related factor 2; ox-LDL, oxidized low-density lipoprotein; SOD, superoxide dismutase; VCAM-1, vascular cell adhesion molecule 1.



B.



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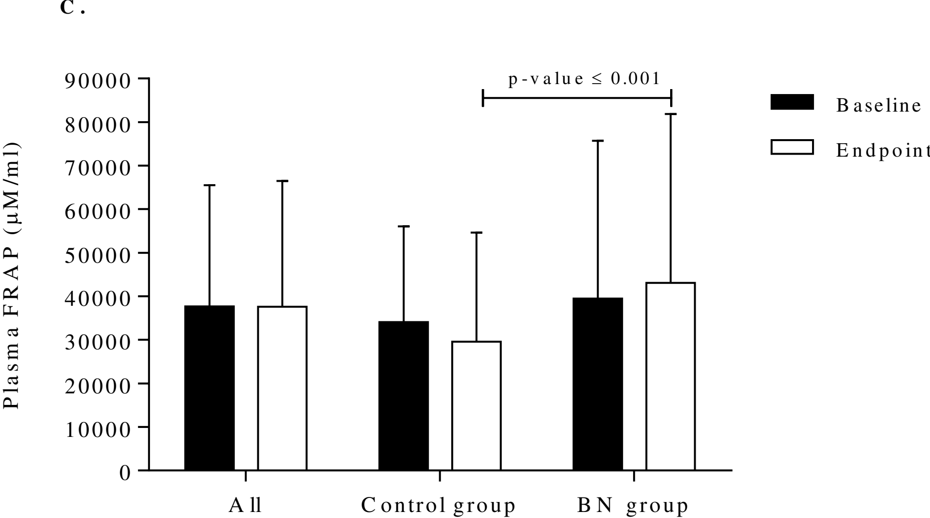

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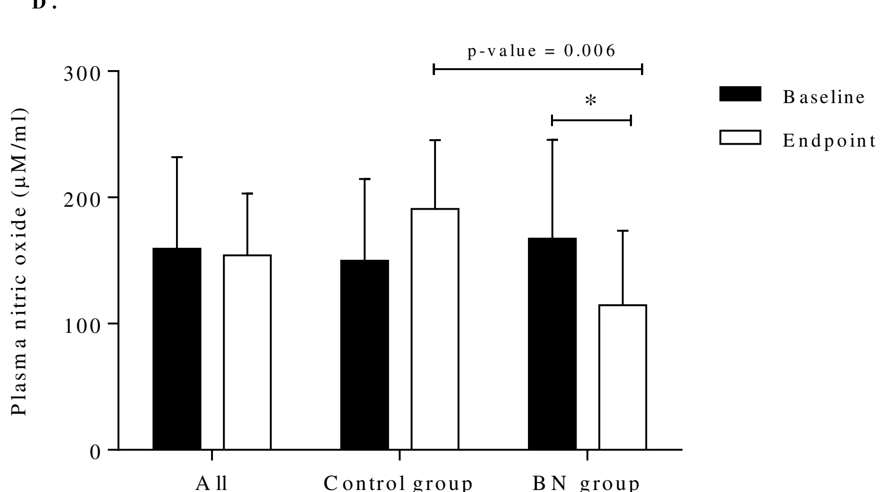


Figure 1: Effect of the 8-wk energy-restricted intervention (control vs. BN-group) on plasma **A.** superoxide dismutase (SOD), **B.** malondialdehyde (MDA), **C.** ferric reducing antioxidant power (FRAP), and **D.** nitric oxide.

*p<0.05 refers to final evaluation compared to baseline obtained through Paired *t*-test.

P-value refers to *Student t-test* for comparison between groups.

Table 2: Oxidative stress and endothelial health markers according to variation in serum selenium median regardless of intervention group

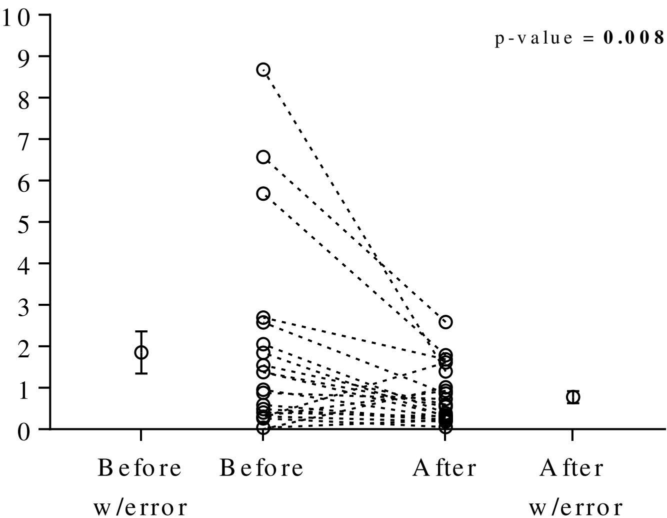
	Δ Plasma Selenium Median, µl		
	54.50 (-138.00; 387.00)		**p-value**
Δ Plasma selenium, µl	2.20 (38.33)	165.70 (82.74)	**≤0.001**
Δ MDA*, µM/ml	-1.19 (3.34)	-4.77 (3.32)	**0.001**
Δ SOD*, U/ml	-31.50 (74.86)	-98.41 (118.37)	**0.024**
Δ FRAP*, µM/ml	-1708.01 (12946.78)	3278.64 (15149.53)	0.235
Δ GPX1[a], pg/ml	-9.43 (28.68)	-9.96 (37.66)	0.969
Δ ox-LDL[a], pg/ml	18.94 (1066.13)	-117.08 (853.18)	0.699
Δ Nitric oxide*, µM/ml	14.44 (116.9)	-53.61 (125.96)	0.068
Δ SOD mRNA expression[b]	0.32 (1.93)	-1.12 (1.97)	0.078
Δ NRf2 mRNA expression[b]	-0.15 (3.21)	-0.26 (1.91)	0.912
Δ GPX1 mRNA expression[b]	-1.60 (5.34)	-0.21 (2.96)	0.382
Δ Catalase mRNA expression[b]	-0.39 (1.57)	-1.73 (6.17)	0.400
Δ ICAM-1[a], ng/ml	-1.91 (38.09)	-6.29 (22.89)	0.684
Δ VCAM-1[a], pg/ml	-16.36 (55.69)	-16.53 (81.51)	0.995

Values are mean (SD). Δ = final assessment – baseline. Bold p-values indicate statistically significant differences according to Δ serum selenium median (*Student's t-test*).

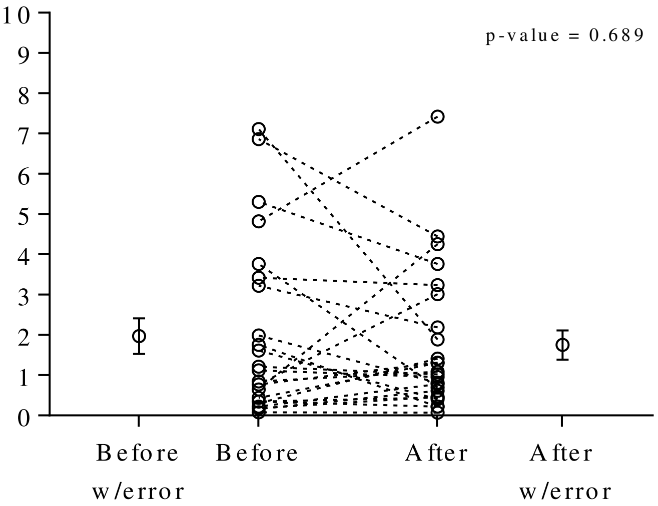
FRAP, ferric reducing antioxidant power; GPX1, glutathione peroxidase 1; ICAM-1, intercellular adhesion molecule 1; MDA, malondialdehyde; Nrf2, nuclear factor erythroid 2-related factor 2; ox-LDL, oxidized low-density lipoprotein; SOD, superoxide dismutase; VCAM-1, vascular cell adhesion molecule 1.

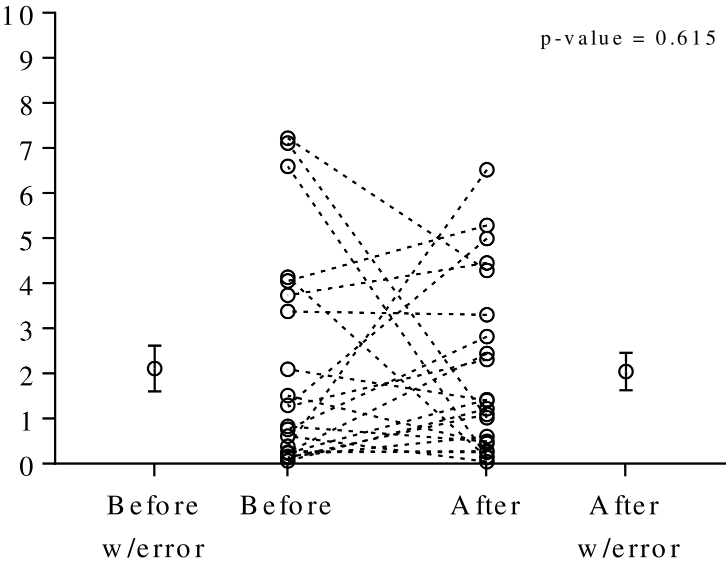
*n=48; [a] n=33; [b] n= 28

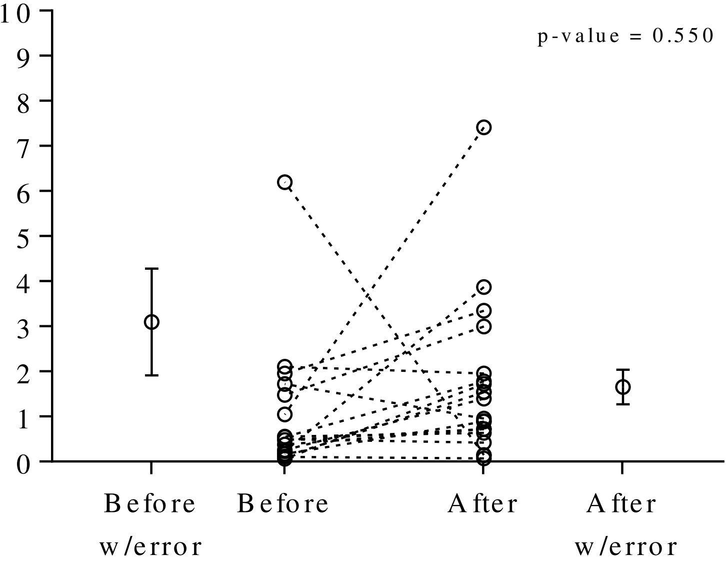
A. SOD mRNA expression



B. Nrf2 mRNA expression







Supplementary Figure 1: mRNA expression of **A.** superoxide dismutase (SOD), **B.** nuclear-eritroid-factor-2 (Nrf2), **C.** Glutathione peroxidase 1 (GPX1), and **D.** catalase enzyme according to 8-wk energy-restricted intervention with daily BN consumption. P-values < 0.05 indicate statistically significant differences within-group (*Paired t test*).

5.6 ARTIGO ORIGINAL 4 – A ser submetido à revista Nutrition (FI: 4.893)

Telomere length and telomerase after Brazil nut (*Berthollethia excelsa* h.b.k.) consumption within an energy-restricted diet: a controlled, parallel clinical trial (Brazilian Nuts Study)

Alessandra da Silva[1]

Walmir da Silva[2]

Helen Hermana M. Hermsdorff[1]

Josefina Bressan[1]

[1] Laboratory of Energy Metabolism and Body Composition. Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

Corresponding author: Josefina Bressan. Laboratory of Energy Metabolism and Body Composition. Department of Nutrition and Health, Universidade Federal de Viçosa. Avenida PH Rolfs, s/n. Viçosa – MG, Brasil. 36570-900. Phone: +55 31 36125220. E-mail: jbrm@ufv.br

ABSTRACT

Background: No study has evaluated the potential effects of nuts combined with an energy-restricted diet on longevity markers. **Objective:** To evaluate the effect of an energy-restricted diet associated or not with the consumption of Brazil nut (BN) on longevity markers and in 8-hydroxydeoxyguanosine (8-OHdG), a DNA damage marker, in women at cardiometabolic risk. We also investigated the relationship between dietary and serum selenium with changes in longevity markers. **Methods:** Forty-eight women with overweight or obesity were enrolled in a parallel, controlled clinical trial. Women consumed an energy-restricted diet of -500 kcal with either 2 BN units (BN-group, n = 25) or not (GC, n = 23) for eight weeks. Quantitative real-time polymerase chain reaction was used to analyze leucocyte telomere length. Plasma levels of telomerase and 8-OHdG were assessed through ELISA kits. Serum selenium was evaluated through inductively coupled plasma mass spectrometry method. All markers were assessed at baseline and after interventions. **Results:** Telomere length and 8-OHdG did not differ from baseline and between groups after the intervention period. Telomerase levels increased in the BN-group compared to the CG. In general, 48% of women had positive changes in telomere length. Greater reductions in weight, BMI, waist circumference, and waist-to-height ratio were found in women who increased telomere length in BN-group compared to CG. Significant correlations were observed between changes in telomere and telomerase with changes in intake and serum selenium levels. **Conclusion:** Consuming an energy-restricted diet with BN did not affect absolute and relative telomere length but increased plasma telomerase levels in women with overweight or obesity.

Trial registration: RBR-8zfn5c

Keywords: longevity; nuts; oilseeds; selenium; telomerase; telomeres

1. Introduction

Obesity is a growing epidemic condition characterized by increased oxidative stress and inflammation [1], which are the suggested mechanism behind telomere length attrite [2]. Although studies are still controversial, evidence suggests a relationship between obesity and telomere shortening [3,4], that is one of the cellular ageing pathways [5]. Telomeres are tandem TTAGGG repeats of DNA complexed with shelterin complex proteins among others that cap the ends of the chromosomes aiming to protect the genomic DNA against possible damage and, thus, providing stability and integrity to the chromosomes [6,7]. On its turn, telomerase is a ribonucleoprotein enzyme that add telomeric repeat sequences to the ends of chromosomes, hence elongating them to compensate for their attrition [2].

A robust body of evidence have been suggested that unhealth lifestyle could accelerate telomere shortening [8] and thus lead to premature ageing-related diseases [9]. Contrary, health lifestyle such as a high Mediterranean diet adherence and the consumption of some antioxidants, fruits, and vegetables were associated with longer telomeres. Nevertheless, there is a lack of clinical trials to confirm these associations [10,11].

Weight loss is associated with telomere lengthening and maintenance [12,13]. However, studies are heterogeneous and conflicting [14,15]. Studies have also shown a link between the calorie-restricted diet, a well-established nutritional approach to weight loss, and longevity including telomere lengthening and maintenance [16–18]. In its turn, the effect of consuming nuts, a group of oilseeds a with a rich variety of nutrients and phytochemicals, on telomere length has also been investigated. However, studies are also limited and controversial [19–22].

To date, no study has investigated the effects of nut consumption combined with energy restriction on telomere length, telomerase enzyme levels, and genes related to these markers. Beneficial effects of Brazil nut (BN) consumption such as improvement of oxidative stress, inflammation, serum lipids, and antioxidant status, among others, have been reported in a range

of studies conducted in subjects with different health status [23]. The studies highlight the possible contribution of selenium in greater amounts in BN compared to other nuts as one responsible for these beneficial effects [24]. Selenium participates in the formation of antioxidant and immune defense systems [25] and has been suggested as an ally to longevity [25], although studies are still limited.

Thus, we aimed to evaluate the effect of an energy-restricted diet associated or not with the consumption of two units of BN for eight weeks on telomere length, plasma levels of telomerase, DNA damage, and expression of genes related to such markers in women with cardiometabolic risk. Furthermore, we investigated the women's characteristics according to variations in telomere length and whether dietary and serum selenium variations were associated with changes in telomere length and telomerase.

2. Methods

Study characteristics and ethical aspects

The results of the present study are a secondary analysis of a study whose primary objective was to evaluate the effect of consuming an energy-restricted diet associated with the consumption of two units per day of BN or not on anthropometric and body composition indicators (data not published yet) in women at cardiometabolic risk.

This is a parallel controlled clinical trial carried out in adult women with cardiometabolic risk in free-living conditions. Data collection was performed in the Department of Nutrition and Health of Universidade Federal de Viçosa-MG, Brazil, with enrollment between June 2019 and September 2021. The study protocol followed the Resolution CSN 466/2012 and was approved by the local ethical committee of the Universidade Federal de Viçosa (CAAE: 92004818.0.0000.5153; UP protocol number: 2.832.601/2018). The study is also registered with the Brazilian Registers of Clinical Trials – REBEC (protocol: RBR-8zfn5c). All participants provided written informed consent before entering the study.

Study population

Eligibility criteria for entering in the study were the following: Adult women (20-55 years) with overweight (BMI ≥ 27 kg/m2 and <30 kg/m2), waist circumference ≥ 80 cm, and body fat percentage ≥ 32% associated with at last one another component of metabolic syndrome: triglycerides ≥150 mg/dL, high blood pressure arterial (≥130/85 mmHg) or high fasting glucose (≥100 mg/dL); or women with obesity (BMI ≥ 30 kg/m²), with or without metabolic complications were included. Non-inclusion criteria comprised pregnant, lactate, or menopausal women; athletes; vegans; smoking; women with a history of HIV, illness or digestive, liver, kidney, cardiovascular, thyroid, cancer, inflammatory diseases, and eating disorders; history of drug and/or alcohol abuse; aversion or allergy to nuts; infectious episode in the last month; use of anti-inflammatory drugs, corticosteroids, antibiotics, and others that may affect energy appetite and metabolism; body weight instability (5% of usual weight) in the last 3 months; regular consumption of any quantity of BN; alcohol consumption higher than 21 units (168g) per week; dental problems that interfere with chewing; use of vitamin, mineral, and omega 3 supplements.

Participants were recruited in the city of Viçosa, Minas Gerais, Brazil and in neighboring cities. Advertisements in social media, local radio and TV were the recruitment methods.

A total of 25 participants were included in the BN-group to answer the primary objectives with a power of 99% considering the mean difference in weight loss in the BN-group (effect size = -1.7305), bilateral α of 5 % and sample size of the group. In addition, a power of 71% considering an effect size of 0.5238980 in telomere length in the BN-group after intervention period was obtained. Samples and power calculation were performed in GPower software 3.1 version.

Interventions

Participants were instructed to consume an energy-restricted diet (-500 kcal/day) accompanied by two units per day of BN (BN-group) or an energy-restricted diet (-500 kcal/day) free of nuts. Total energy intake was estimated for each participant using the Estimated Energy Requirement for adult women with overweight or obesity [26] and 500 kcal/day was deducted from the dietary prescription to achieve a minimum weight loss of two kilograms per month. The distribution of macronutrients was within the recommended ranges according to the macronutrient distribution recommendations of the obesity control guidelines [26].

For the BN group, the diets included the energy provided by the daily portion of 8 g (two unities) of BN. Energy, total fats, and selenium from two units of BN were 52.7 kcal, 47 kcal, and 347.2 µg of selenium. The BN used in the study were donated by ECONUT® and were portioned (two units) in laminated packages, vacuum sealed (Selovac Sealer model 200 B), and stored in a freezer at -20ºC until distribution to the participants.

Data collection

Before and after the intervention period, face-to-face interviews were realized at the Laboratory of Energy Metabolism and Body Composition (LAMECC) to collect information about physical activity, food intake, and anthropometric assessment. In the morning, after overnight fasting, body composition assessment through Dual-energy X-ray absorptiometry (DEX) and blood collection were realized at the Health Service of the Federal University of Viçosa. All women were instructed to maintain their lifestyle and medicine use during the study and to inform the research team of any changes in the type or dosage of the ongoing medication.

Anthropometry and body composition

At the first and last consultations, body weight (kg), height (m), waist (WC), hip, and neck circumferences (cm) were measured. Body weight was assessed by Inbody 230 (Biospace

Corp., Seoul, South Korea). The height was measured using a stadiometer (Seca 206 model, Hamburg, Germany). BMI was obtained through the ratio between weight in kg and squared height in meters. Waist circumference was measured at the umbilicus waist at the end of normal expiration using an inelastic tape (precision 0.1 cm). Hip and neck circumferences were measured utilizing an inelastic tape at the maximum posterior extension of the gluteus and in the middle of the neck, respectively. Waist-to-height ratio (WHtR) was obtained through the ratio between WC and height in cm. Deep-

DEX (Lunar Prodigy Advance DXA System, GE Lunar) was used for body composition assessment. Values in kilograms of fat mass (FM), fat-free mass (FFM), lean mass (LM), and total mass were obtained from the total body and regions such as trunk, android, and gynoid.

Serum, plasma, DNA, and RNA preparation

Blood samples were collected at the beginning and end of the intervention period after a 12-hour overnight fast. Serum samples were obtained from blood collected in TRACE tubes without additives for selenium dosage. Plasma samples were obtained from blood collected in EDTA tubes following centrifugation at 3500 ×rpm for 10 min at 4ºC. Samples were storage at -80°C until analysis of telomerase and 8-OHdG levels.

After removing plasma from EDTA tubes, peripheral blood mononuclear cells (PBMC) samples were obtained after the addition of Ficoll® solution and successive washes with saline solution. Part of the PBMC samples were immersed in the Tri Reagent® and stored at -80 C until RNA extraction. PBMC samples without the Tri Reagent® were also stored for later DNA extraction.

Genomic DNA was isolated from the PBMC using the DNAzol Reagent® (Invitrogen™, USA) and storage at -80°C until use for telomere length quantification. First, PBMC was homogenized in DNAzol Reagent®, and then the genomic DNA was precipitated with ethanol. Following ethanol washes, DNA was solubilized in ultrapure water, quantified

using a NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific®), and diluted down to the final concentration of 20 ng/µl.

RNA was isolated following TRIzol® Reagent user guide (Invitrogen, ThermoFisher Scientific®). Briefly, chloroform was added to the sample homogenized with TRIzol® and the supernatant mixed with isopropanol. Then, successive washes were performed with ethanol and finally the RNA was reconstituted in ultrapure water.

Assessment of telomere length

The absolute telomere length of the leukocyte of each DNA sample was measured using a quantitative real-time polymerase chain reaction (qPCR). This is a validated optimized method proposed by O'Callaghan and Fenech (2011) [27] based on the Cawthon method for measuring relative telomere length [28] but modified by introducing an oligomer standard to measure absolute telomere length.

Briefly, this method performs separated reactions for the genes TEL, 36B4, and b-globin. The primer sequences for absolute telomere length quantification were teloF (CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGG TTTGGGTT) and teloR (GGCTTGCCTTACCCTTACCCTTACCC TTACCCTTACCCT). Sigle copy gene primers were 36B4F (CAGCAAGTGGGAAGGTGTAATCC) and 36B4R (CCCATTCTATCATCAACGGGTACAA). Primers used as endogenous standard were b-globinF (GCTTCTGACACAACTGTGTTCACTAGC) and b-globinF (CACCAACTTCATCCACGTTCACC). Primers used for standard curve were Telomere standard (TTAGGG-14) and 36B4 standard (CAGCAAGTGGGAAGGTGTAATCCGTCTCCACAGACAAGGCCAGGACTCGTTTGT ACCCGTTGATGATAGAATGGG). All primers were from Sigma Aldrich® and were purified by the manufacturer through the use of HPLC. Each sample was run in triplicate on

96-Well PCR Plate (ABI 7300 Sequence Detection System, Applied Biosystems). The mean of cycle threshold values was used.

The master mix contained a PowerUpTM SYBRTM Green (Applied Biosystems), telomere primer pairs, the DNA sample, and ultrapure water to complete the final volume. Absolute telomere length was calculated by plotting the sample's fluorescence signal for the TEL gene on a standard curve. Relative telomere length was calculated as the ratio of the telomere DNA length from the standard curve to the 36B4 DNA length.

The PCR cycling conditions (for both telomere and 36B4 amplicons) were: 10 min at 95°C, followed by 40 cycles of 95°C for 15 sec, 60°C for 1 min, followed by a dissociation (or melt) curve [27].

Assessment of mRNA expression

The mRNA expression of proteins comprising the shelterin and telomerase enzyme complex were evaluated from PBMC using quantitative real-time PCR (qPCR). RNA was extracted with Tri Reagent® as briefly detailed above. The cDNA was synthesized with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). PowerUp™ SYBR™ Green Master Mix (Applied Biosystems) was used to gene expression assays. b-globin mRNA expression was used as control gene. PCR amplification was carried out using the ABI Prism 7500 Sequence Detection System (Applied Biosystems) under standard mRNA expression cycling conditions. The expression of genes was normalized against b-globin, and the expression level was calculated using the ΔΔCT (delta delta threshold cycle) method.

Assessment of serum selenium, plasma levels of telomerase and 8-OHdG

Serum selenium (μ/L) was determined through inductively coupled plasma mass spectrometry method according to standardized protocols. Plasma levels of telomerase and 8-OHdG were estimated using commercially available Human TE (Telomerase) and 8-OHdG (8-

Hydroxydeoxyguanosine) ELISA assay kits (Elabscience, USA), respectively, following the manufacturer's instructions.

50 µl of plasma samples were required for 8-OHdG analysis and 100 µl of 100-fold diluted plasma samples for telomerase analysis. The optical density (OD value) of each well was read at 450 nm. Telomerase and 8-OHdG levels were established using the standard curve and expressed in ng/ml. For telomerase, the values were corrected by the dilution factor.

Dietary assessment

Energy, macronutrients, and selenium were estimated using three food records applied at baseline and a 24-hour food recall applied at the end of the intervention period. All food consumption analyzes were performed using the ERICA® software [29]. The software has a database composed of a list of items included in the database of food and beverage purchases from the *Pesquisa de Orçamentos Familiares* (POF – Brazilian Household Budget Survey). The food items that were not contained in the database were added by the interviewers.

Statistics Analyses

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 21.0 for Windows. The Shapiro-Wilk test was performed to evaluate the normality of quantitative variables. Student's t-test was performed to assess quantitative variables according to changes in absolute telomere length and between intervention groups. Data were presented as mean and standard deviation. Pearson correlation was used to evaluate the correlation between serum and dietary selenium changes with changes in telomere length, telomerase, and 8-OHdG levels. Pearson Chi-square was used to compare proportions of women who increased telomerase levels across negative and positive variations of telomere length. The level of significance in two-tailed tests was set at 5%.

3. Results

A total of forty-eight adult women with overweight/obesity were analyzed in the present study. 47.9% (n=23) of participants had positive changes in telomere length, of which 47.8% (n=11) are from CG and 52.2% (n=12) from BN-group (p-value = 1.000). The variation in absolute and relative telomere length and in 8-OHdG did not differ after the intervention period and between the evaluated groups (**Figures 1A and 1C**). However, we observed positive changes in plasma telomerase levels in BN-group compared to CG (**Figure 1B**), which remained significant even after excluding a possible outlier (**Supplementary figure 1**). The absence of a relationship was observed between increasing telomerase levels and changes in absolute telomere length (**Supplementary figure 2**).

When we categorized the participants according to the direction of variation in telomere length, we observed that waist circumference and waist-to-height ratio decreased only in the BN-group and in women who showed an increase in telomere length after the intervention period. On the other hand, a lower consumption of calories, proteins, and lipids was observed in the group of women classified with positive variation in telomere length and belonging to the CG (**Table 1**).

Analyzing only the women who had decreased telomere length after the intervention period, we observed that the percentages of lean mass and fat-free mass of the trunk and android regions were more preserved in the BN-group compared to the CG. In turn, when only women who increased telomere length after the intervention period were analyzed, we observed that weight, BMI, waist circumference, and waist-to-height ratio decreased more in BN-group compared to CG. Furthermore, the BN-group presented a more preserved percentage of lean mass and fat-free mass in the android region compared to the CG. We also observed that the BN-group increased selenium consumption and plasma levels of selenium, and telomerase

enzyme when compared to CG, regardless of the direction of telomere length variation (**Table 1**).

Variation in serum levels and consumption of selenium was not associated with variation in absolute telomere length (**Figures 2A and 2B**). However, after excluding two visible discrepant values of variation in absolute telomere length, a negative and significant correlation between the markers was observed (**Supplementary Figures 3A and B**). In turn, the variation in serum levels and consumption of selenium was positively correlated with the variation in the levels of the enzyme telomerase (**Figures 2B and 3B**).

To better explore the effect of interventions on longevity markers, we evaluated the expression of genes related to shelterin complex and telomerase enzyme. However, no amplification was observed in PCR analysis (**Supplementary Table 1**).

4. Discussion

In this study, we observed that an energy-restricted diet accompanied or not by 2 units per day of BN for 8 weeks did not impact the relative and absolute telomere length in women at cardiometabolic risk. Evidence suggests that telomere shortening is inversely associated with longevity and is linked to ageing-related chronic diseases [5,30]. Despite being a natural process of the organism and related to ageing, studies suggest that inflammation and oxidative stress are associated to the acceleration of telomere attrition [31–36]. Thus, nutrition could impact telomere length by modulating these mechanisms [10,11].

Caloric restriction is one of the most scientifically supported approaches to weight loss management [26,37]. In addition, it has been linked to lifespan regulation through mechanisms such as a decrease in TOR, IIS, JNK signaling, decrease in oxidative stress and increase in the AMPK cascade, sirtuin, autophagy, TNF inactivation, and activation of the FOXO transcription factor [17]. Both caloric restriction and the consumption of nuts, especially BN, have shown to have positive effects on the reduction in markers of inflammation [38–40] and oxidative stress

[41,42]. Consumption of two units per day of BN associated to an energy-restricted diet decreased tumor necrosis factor, and pro-inflammatory interleukins in women at cardiometabolic risk (data not shown). In another study, consumption of one unit per day of BN containing approximately 290.5 µg of selenium for 12 weeks decreased markers of oxidative stress and inflammation, including nuclear factor-κB (NF-κB) mRNA expression in hemodialysis patients [43]. NF-κB is a protein with a dual function, being responsible for the transcription of pro-inflammatory genes and also for regulating the expression of TERT, that is a protein of the telomerase complex whose function is to guarantee the stability of telomeres [35].

In addition, the selenium present mainly in BN acts as an antioxidant with modulation in immune system [44,45]. On the other hand, studies have shown conflicting results regarding weight loss and telomere length. In a study with subjects with obesity undergoing bioenteric intragastric balloon, weight loss was positively associated to telomere lengthening [13]. In contrast, postmenopausal women and adults with overweight or obesity had no changes in telomere length after 12 months of dietary weight loss accompanied or not by aerobic exercise and weight-loss program with or without mindfulness training, respectively [14,15].

Studies that evaluated the effect of nut consumption on telomere length are available in the literature; however, to our knowledge, this is the first study to evaluate the effect of nut consumption associated with an energy-restricted diet on telomere length and telomerase levels. Three of the four studies available showed no effect of nut consumption on telomere length [19,20,22]. In agreement with our results, regular consumption of almonds (15% of energy for 3 months) [20], pistachios (57g for 4 months) [19], and walnuts (30-60g for 2 years) [21] had no impact on telomere length in subjects with overweight/obesity, pre-diabetic, and cognitively healthy elderly, respectively. In contrast, regular consumption of a mix of nuts in a Mediterranean dietary context for five years increased telomere shortening compared to low-

fat dietary intake in subjects at cardiometabolic risk [22]. Heterogeneous studies were observed, which highlights the need for further studies in the field.

A significant increase in plasma levels of the telomerase enzyme was observed in the BN-group compared to the GC. Studies evaluating the effect of nut consumption on telomerase levels, activity, and even expression are limited. Pistachio consumption increased the expression of the TERT and WRAP53 genes, which are important enzymes related to the activity of the telomerase enzyme [19]. When telomeres shorten, telomerase goes into action in an attempt to add the "TTAGGG" sequences to the end of the chromosomes and thus prevent the telomere length from reaching a critical level and compromising the life of the cell [2]. In our study, the prevalence of participants who increased telomerase levels was similar between those who increased and decreased telomere length. In this sense, we understand that the increase in telomerase observed was more than a standardized response of the organism in an attempt to repair the telomere length, but an effect of the diet containing BN (**Figure 4**).

Interestingly, changes in telomere length and telomerase levels were negatively and positively related to changes in serum selenium, respectively. Studies evaluating the relationship between telomeres/telomerase and selenium are scarce. What is supposed is that by participating in the enzymatic and non-enzymatic antioxidant defense systems [44] and by the important functions in the modulation of the immune system [45], selenium could positively impact the telomere length and, thus, be associated with longevity. What we didn't expect was to observe an inverse relationship between the change in serum selenium levels and the change in telomere length. In a cross-sectional study of middle-aged and older adults from the National Health and Nutrition Examination Survey (NHANES), each 20 µg increase in dietary selenium intake was associated with 0.42% longer telomere length [46]. Curiously, this association observed by the authors did not remain significant in a subgroup analysis of participants with obesity and most of the women evaluated in our study were with obesity. Author's suggests

that due to increased levels of inflammation and oxidative stress in obesity, this, in turn, could weaken the protective effect of dietary selenium intake on telomere length [46]. These observations may suggest a possible interaction between selenium, obesity, and telomere length.

Obesity is a chronic condition that increased the oxidative stress and inflammation, which have a negative influence on telomere length. In addition, the G-rich telomeric sequence is relatively less capable of DNA repair, resulting in accelerated telomere loss during the cell cycle and subsequent replicative senescence [47]. In fact, a meta-analysis showed that individuals with normal-weight had longer telomeres than individuals with obesity. Despite a higher heterogeneity between studies, the authors observed a negative association between obesity, particularly central obesity, and telomere length [3]. In another study, adults with obesity had shorter telomere lengths than adults without obesity [4].

Plasma levels of 8-OHdG unchanged after the intervention in both groups. Contrary to our result, the consumption of one unit of BN per day for 12 weeks decreased 8-OHdG in hemodialysis patients, while the levels of this marker increased in plasma after 12 weeks of interruption of BN consumption [48,49]. The health status of the individuals evaluated in the previous study [48] compared to ours may help justify the observed results. Patients on hemodialysis are in a state of exacerbated oxidative stress and inflammation and in this case may have benefited more from the intervention with BN [50]. Reactive oxygen and hydrogen species are produced naturally by our body's metabolic processes. However, it can lead to damage to cellular structures such as DNA and membrane damage when in excess [51]. BN consumption associated with an energy-restricted diet decreased malondialdehyde, a marker of lipid peroxidation, and increased plasma ferric reducing antioxidant power in women at cardiometabolic risk (data not shown). Furthermore, a systematic review showed that although

more study is needed in the field, nut consumption seems to modulate other important markers of oxidative stress [42].

We also observed in our study a higher reduction in waist circumference and waist-to-height ratio in women who received BN and had an increase in telomere length compared to those who decreased telomere length. This result suggests a modulatory role of BN consumption on telomere length and a relationship between central body fat accumulation and telomere attrition. Of women who increased telomere length, greater reductions in weight, BMI, waist circumference, and waist-to-height ratio while more preserved lean mass and fat-free mass of the android region were observed in BN-group compared to controls. These results suggest a positive relationship between increased telomere length and better body composition. Of the women who reduced telomere length, those on the BN-group also had better results compared to the CG.

The accumulation of fat in the abdominal region is related to metabolic complications such as insulin resistance, accumulation of ectopic fat, metabolic syndrome and risk of developing cardiovascular diseases [52]. The production of free radicals and inflammatory markers are a result of structural and functional changes in adipocytes, which could contribute to cellular senescence. In a cross-sectional study, a negative relationship was observed between telomeres and waist circumference, total body fat, and visceral adipose tissue, suggesting a link between abdominal obesity and telomere attrition [53].

The main limitation of this study was the lack of randomization. Despite this, all markers assessed here did not differ between groups at baseline. Another limitation may be the short intervention time. Telomeres are structures involved in the aging process and perhaps in studies with longer intervention times more consistent results could be observed. Furthermore, our study had approximately 70% power, so studies with larger samples are encouraged to confirm our results. In addition, activity of telomerase was not evaluated. On the other hand, plasma

telomerase levels were evaluated and reflect the organism's stimulus to the production and consequent activity of the enzyme. We also evaluated absolute and relative telomere length, which facility the comparison of the results between studies. This study filled an important gap in the literature, as it is the first to evaluate the effect of consuming a nut, especially BN, associated with an energy-restricted diet on cell longevity markers.

5. Conclusion

BN consumption in the context of an energy-restricted diet did not impact telomere length but increased plasma telomerase compared to control group in women at cardiometabolic risk. Associations also were observed between serum selenium variation with telomere length and telomerase levels. In addition, women that consumed BN had more anthropometric and body composition advantagens than the controls, regardless of telomere length variation. The results observed here opens new views of future investigations about the relationship between obesity, telomere length, nuts, and selenium.

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Authors' contributions

The study was conceived and designed by AS, HHMH, and JB. HHMH and JB coordinated the study. AS and WS performed all laboratory analyzes. AS performed the statistical analysis and prepared the first draft of manuscript. AS, WS, HHMH, and JB revised the paper and helped to write the final draft of the manuscript.

Consent for publication

The authors confirmed that all participants provided informed consent for publication.

Availability of data and material

The data evaluated in this article can be obtained by contacting the first author. email: alessandra.silva2@ufv.br

Declaration of competing interest

The authors declare no competing financial interests.

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Table 1: Characteristics of participants according to negative or positive change in absolute telomere length

	CG (n=23)			BN-group (n=25)		
	Change in absolute telomere length					
	Decreased (n=12)	**Increased (n=11)**	**p-value**	**Decreased (n=13)**	**Increased (n=12)**	**p-value**
Δ Telomere length, kb per diplod genome	-15.48 (18.48)	9.61 (4.45)	**0.001**	-15.96 (10.27)	6.10 (6.23)	**≤0.001**
Age	31.66 (7.37)	32.81 (7.75)	0.719	36.92 (8.28)	32.75 (5.49)	0.155
Δ Weight, kg	-3.27 (2.6)	-1.49 (1.96) ‡	0.080	-2.87 (2.45)	-3.65 (2.56) ‡	0.445
Δ BMI, kg/m²	-1.23 (0.97)	-0.56 (0.72) ‡	0.077	-1.13 (1.02)	-1.42 (0.98) ‡	0.484
Δ WC, cm	-4.64 (3.09)	-2.30 (2.94) ‡	0.086	-4.22 (3.39)	-7.2 (3.16) ‡	**0.033**
Δ NC, cm	-0.90 (0.66)	-1.52 (2)	0.372	-0.96 (1.01)	-0.55 (1.45)	0.418
Δ HC, cm	-3.08 (1.66)	-1.59 (2.59)	0.132	-1.90 (3.86)	-2.67 (2.4)	0.561
Δ WHtR	-0.02 (0.02)	-0.01 (0.01) ‡	0.100	-0.02 (0.02)	-0.04 (0.01) ‡	**0.038**
Δ Truncal FM*, %	-1.64 (2.59)	-1.05 (2.04)	0.591	-1.21 (2.82)	-0.48 (2.12)	0.485
Δ Android FM*, %	-0.27 (0.45)	-0.55 (0.30)	0.138	-0.19 (0.58)	-0.4 (0.71)	0.453
Δ Gynoid FM*, %	-0.002 (0.87)	-0.16 (0.34)	0.605	0.55 (0.63)	0.02 (0.91)	0.118
Δ Total FM*, %	-0.5 (1.06)	-0.21 (1.68)	0.657	-1.12 (1.6)	-0.93 (1.77)	0.789
Δ Truncal LM*, %	-2.39 (2.6) †	-1.65 (1.62)	0.476	-0.19 (1.8) †	-0.64 (1.68)	0.543
Δ Android LM*, %	-0.35 (0.44) †	-0.3 (0,21) ‡	0.758	0.14 (0.38) †	0,03 (0.36) ‡	0.509
Δ Gynoid LM*, %	-0.2 (0.63)	0.01 (0.61)	0.469	0.06 (0.77)	0.13 (0.53)	0.803
Δ Total LM*, %	0.26 (1.11)	0.11 (1.99)	0.843	1.06 (1.85)	0.78 (1.91)	0.725
Δ Truncal FFM*, %	-2.27 (2.5) †	-1.54 (1.4)	0.456	-0.25 (1.63) †	-0.59 (1.49)	0.609
Δ Android FFM*, %	-0.34 (0.43) †	-0.28 (0.19) ‡	0.741	0.12 (0.35) †	0.02 (0.35) ‡	0.539
Δ Gynoid FFM*, %	-0.2 (0.61)	-0.006 (0.55)	0.443	0.07 (0.71)	0.12 (0.52)	0.835
Δ Total FFM*, %	0.43 (1.07)	0.25 (1.77)	0.781	1.13 (1.62)	0.92 (1.77)	0.773
Δ Plasma telomerase, ng/ml	-5.4 (10.81) †	-6.67 (7.36) ‡	0.757	5.91 (12.37) †	4.25 (9.7) ‡	0.068
Δ Plasma Selenium, µg/L	-2.27 (53.43) †	-1.78 (24.91) ‡	0.984	189.46 (100.13) †	127 (54.6) ‡	0.376
Δ 8-OHdG, ng/ml	-0.38 (4.68)	-1.06 (3.62)	0.702	0.42 (5.79)	-0.95 (5.31)	0.543
Δ Selenium intake, µg	24.72 (38.78) †	-27.97 (55.89) ‡	**0.022**	338.06 (30.69) †	328.89 (83.51) ‡	0.747

Δ Energy, kcal	120.36 (420.44)	-567.84 (767.6)	**0.021**	-142.76 (463.03)	-378.05 (789.37)	0.421
Δ Carbohydrates, g	-9.46 (55.75)	-70.53 (96.)	0.096	-15.98 (54.06)	-31.73 (107.79)	0.682
Δ Proteins, g	12.02 (19.13)	-21.05 (40.26)	**0.029**	-5.85 (21.69)	-9.94 (40.08)	0.778
Δ Lipids, g	13.81 (24.5)	-21.58 (30.33)	**0.009**	-5.22 (22.48)	-15.92 (25.7)	0.325

Values are mean (SD). Δ = final assessment – baseline. P-values ≤ 0.05 indicate statistically significant differences within-group according to negative or positive variations in absolute telomere length (*Student's t-test*).

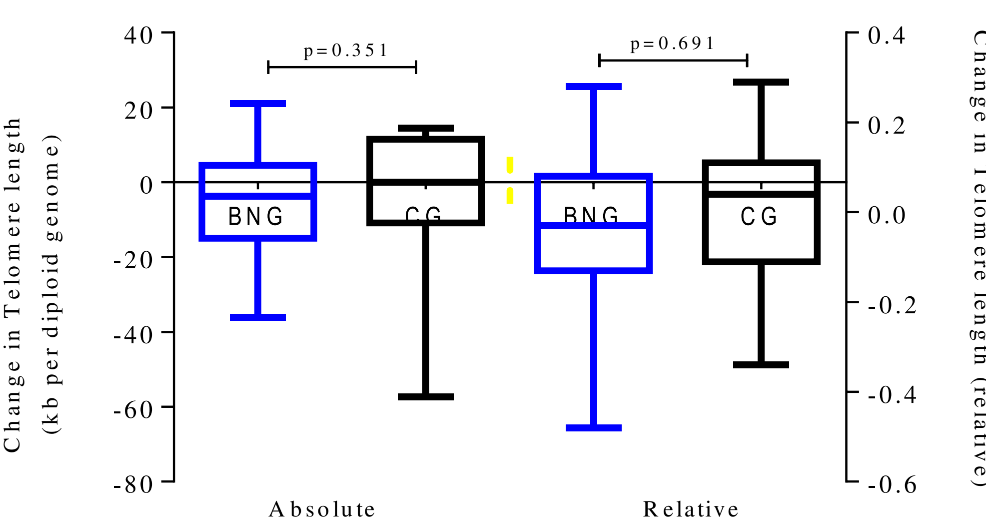
† refers to statistically significant differences obtained through *Student's t-test* between CG and BN-group in women who decreased absolute telomere length.

‡ refers to statistically significant differences obtained through *Student's t-test* between CG and BN-group in women who increased absolute telomere length.

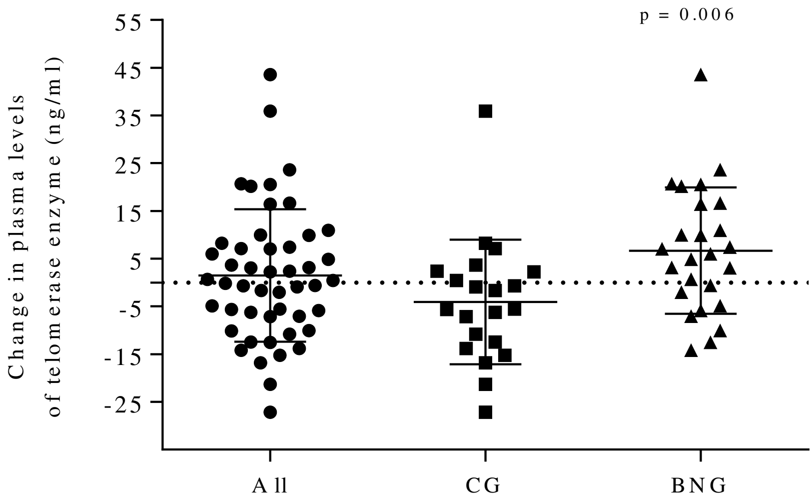
CG, control group; BN-group, brazil nut group; BMI, body mass index; WC, waist circumference; NC, neck circumference; HP, hip circumference; WHtR, waist-to-height ratio; FM, fatty mass; LM, lean mass; FFM, fat-free mass; 8-OHdG, 8-hydroxyguanosine.

*Total sample size = 42. CG, n = 19 (decreased telomere length, n = 10; increased telomere length, n = 9); BN-group, n = 23 (decreased telomere length, n = 11; increased telomere length, n = 12).

A.



B.



C.

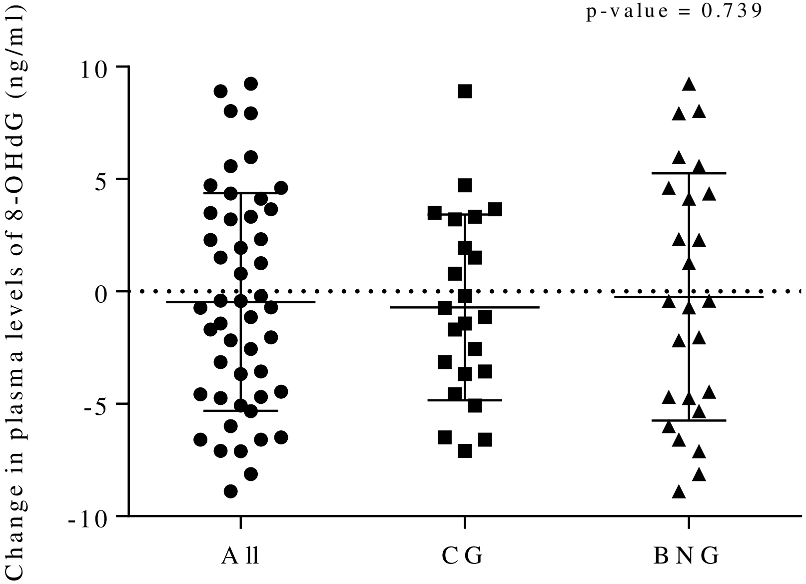
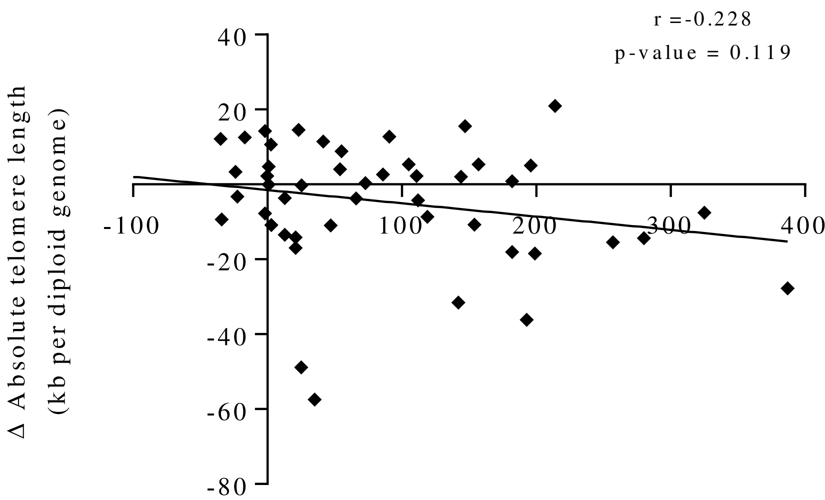


Figure 1: Changes in **A.** Absolute and relative leukocytes telomere length, **B.** Plasma telomerase levels, and in C. Plasma 8-hydroxyguanosine (8-OHdG) levels after 8 weeks of an energy-restricted diet with and without Brazil nut.

CG, control group; BN-group, Brazil nut group. p-values obtained through *Student's t test*.

A.



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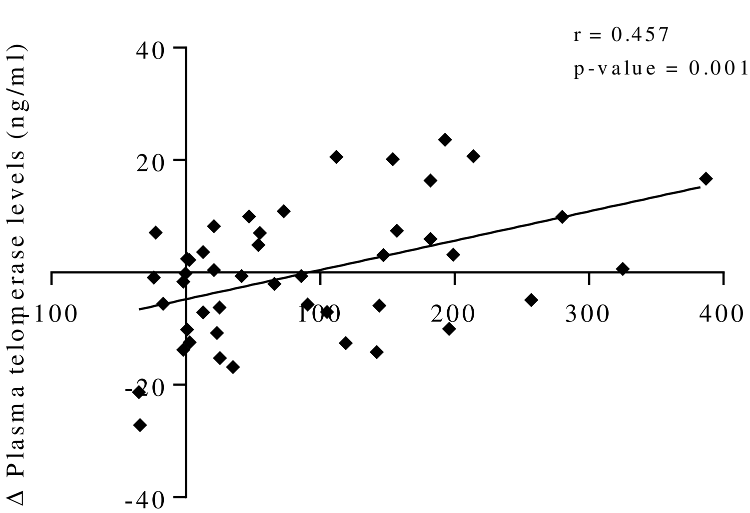


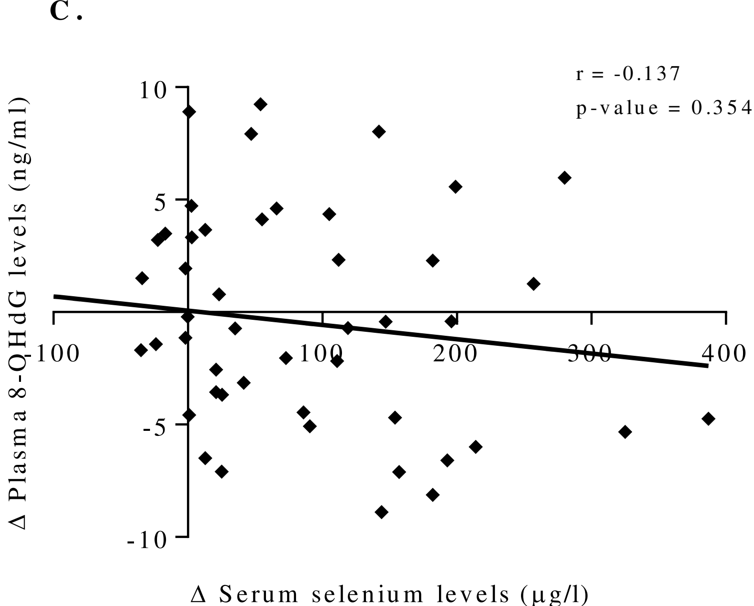
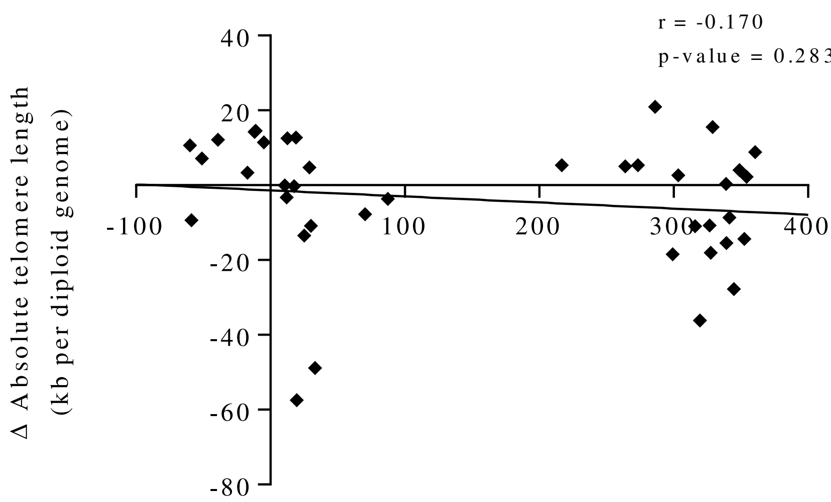


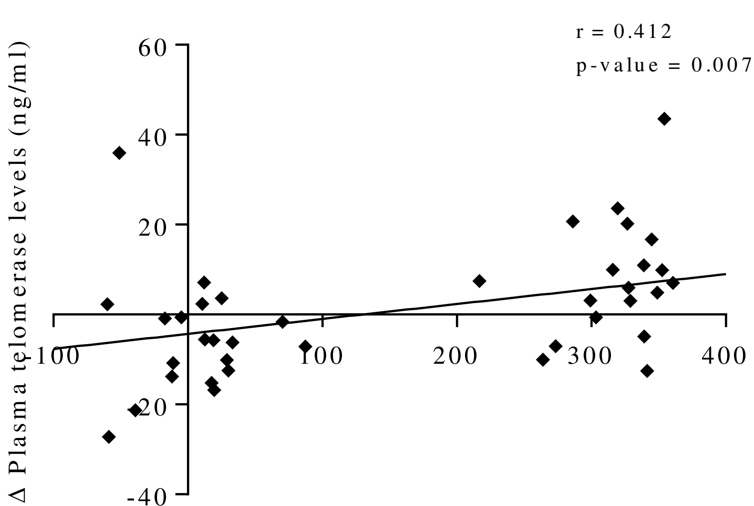
Figure 2: Correlations between changes in serum selenium levels with changes in **A.** Absolute telomere length, **B.** plasma telomerase levels, and in **C.** 8-hydroxyguanosine (8-OHdG) in women with overweight/obesity

r and p-values obtained through *Pearson* correlation.

A.



B.



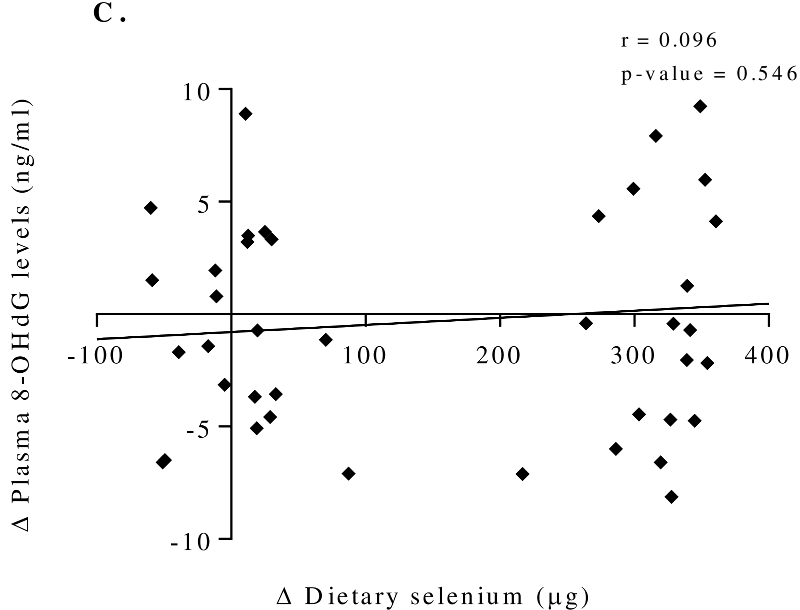
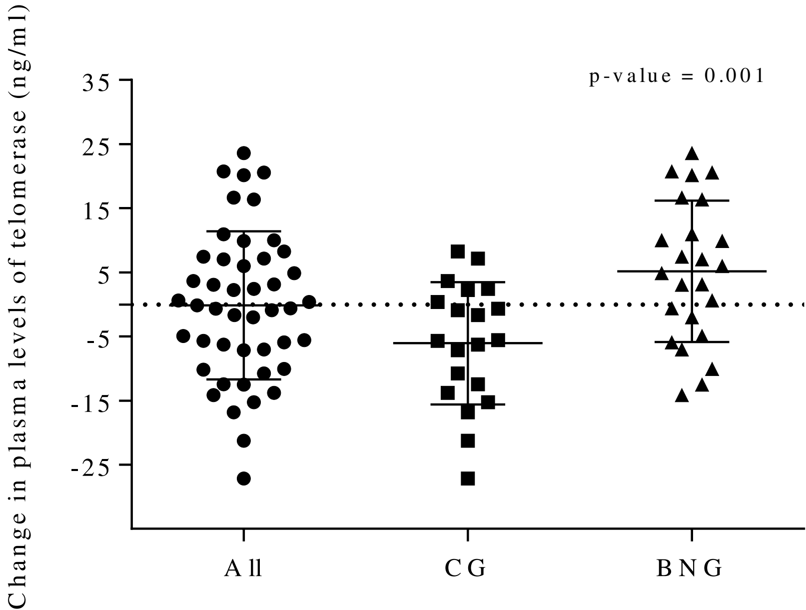


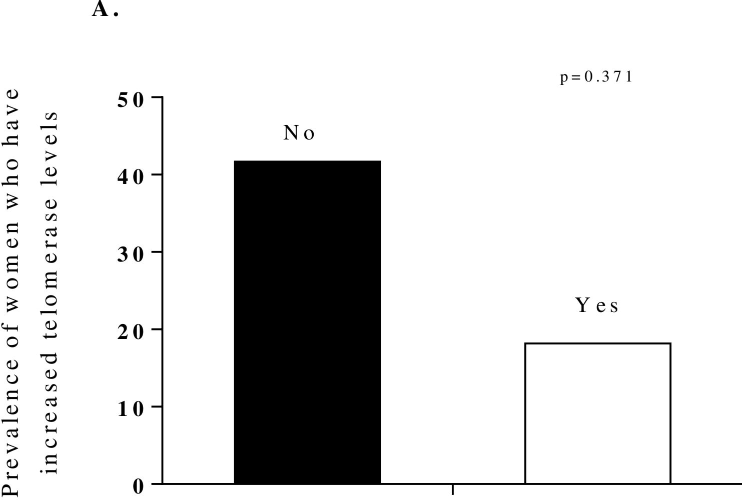
Figure 3: Correlations between changes in selenium intake with changes in **A.** Absolute telomere length, **B.** plasma telomerase levels, and in **C.** 8-hydroxyguanosine (8-OHdG) in women with overweight/obesity

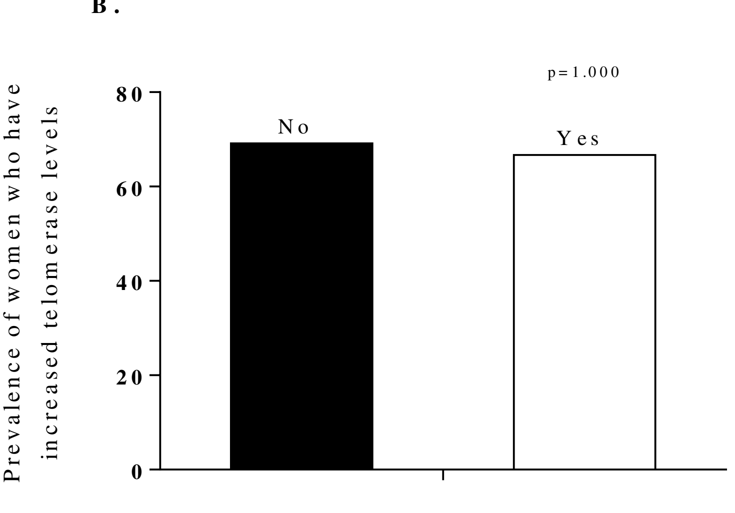
r and p-values obtained through *Pearson* correlation.



Supplementary Figure 1: Changes in plasma levels of telomerase after 8 weeks of an energy-restricted diet with and without Brazil nut.

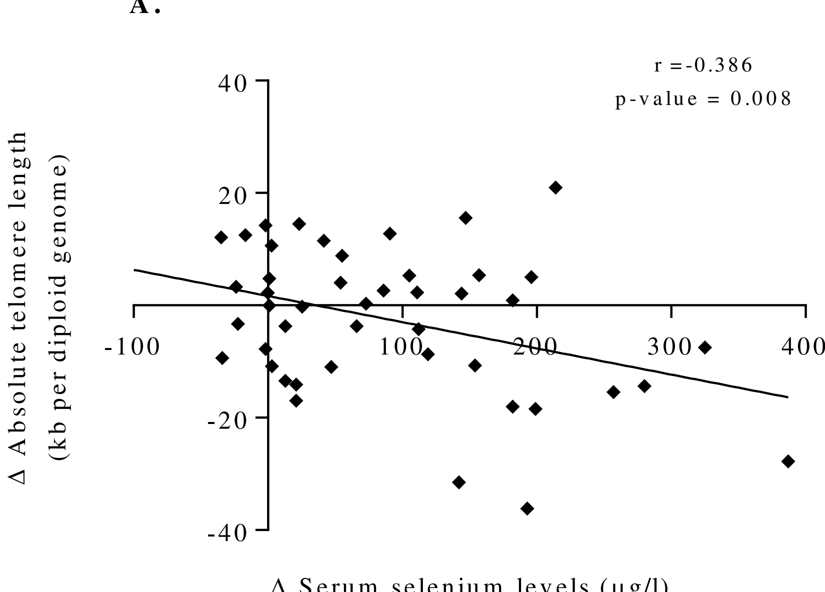
CG, control group; BN-group, Brazil nut group. p-values obtained through *Student's t test*.



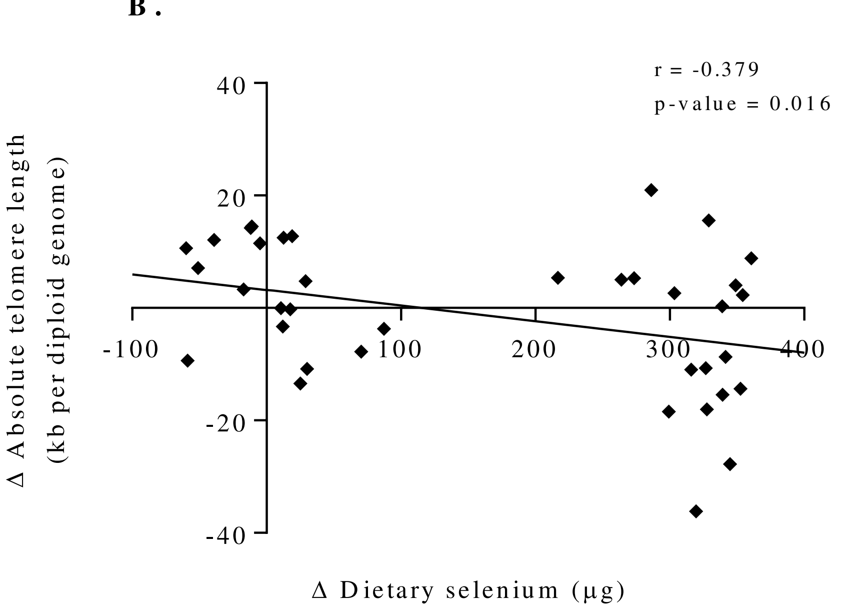


Supplementary Figure 2: Relationship between the prevalence of women who have increased telomerase levels with changes in telomere length from **A.** control group and **B.** Brazil nut group p-values obtained through *Pearson* Chi-Square.

A.



B.



Supplementary Figure 3: Correlations between changes in absolute telomere length with changes in **A.** serum selenium levels and **B.** dietary selenium in women with overweight/obesity after excluding outliers in Δ absolute telomere length.

r and p-values obtained through *Pearson* correlation.

Supplementary Table 1: Effect of nutritional intervention on the expression of genes related to shelterin complex and telomerase enzyme

Genes	Primers	Cycle Threshold (Ct) values
TINF2	Foward: GAGCCAGTACCAGGGTAGGA; Reverse: AGAGAAGGGAGCAGGGAGAG	40
ACD or TPP1	Foward: TACCAGGCGGGAGATGAAGA; Reverse: GGAGATAGAACTCTGCGGGC	40
NHP2	Foward: AATAGAGGCTGGGGGTGGAT; Reverse: CCCCCATCTACCCTGGAAGA	40
TERC	Foward: TCCAGAGGCCCTCTTCTCTC; Reverse: ATCTCCAGGGCCTTCCTCAT	40
TERT	Foward: TTCTCCCCTGGGTCCCTATG; Reverse: ATAGCCCATGGGTCTGAGGT	40
TRF1	Foward: TGGCAGGGGAGATACCATGA; Reverse: GGACGACTCCATACTGAGCG	40
TRF2	Foward: ACTGACAGAAGCAGTGGTCG; Reverse: GCTGTTCCACTTGCCTTTGG	40
WRAP53	Foward: AGGGGGAGCAGGTGGAATAT; Reverse: CAGGGACCACAGTGGGTAAC	40
POT1	Foward: GCCTGTGCTGTGCGATAATG. Reverse: CGAGGCTGAGGTGAGCTATG	40
NOP10	Foward: ACCTTACCCTCCCATCCTCC; Reverse: AAGATGCCATGAACCCCAGG	40

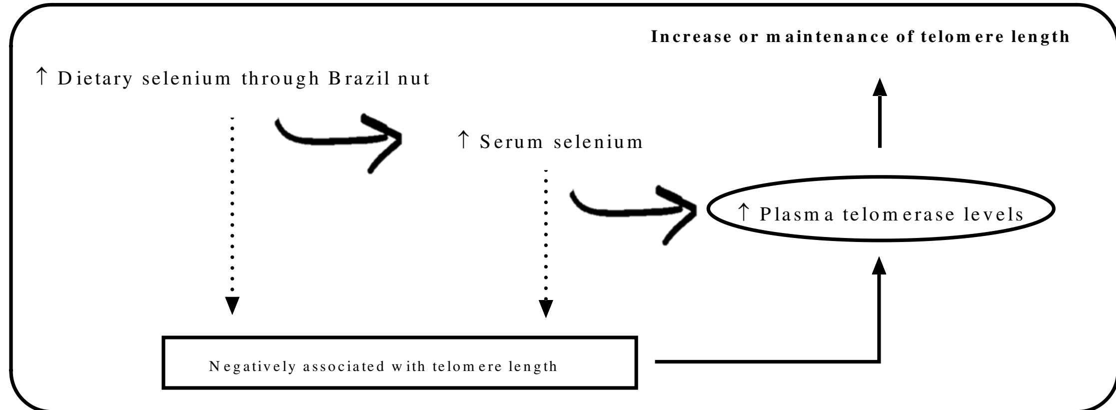


Figure 4: Scheme of the mechanisms by which the increase in plasma levels of the telomerase enzyme could be justified after consumption of an energy-restricted diet + 2 units of Brazil nuts a day for 8 weeks in women at cardiometabolic risk.

Consumption of 2 units of BN (~347 µg of selenium) led to increased serum selenium levels. Both consumption and serum levels of selenium were negatively associated with change in telomere length after the intervention period. Furthermore, a higher prevalence of women who reduced telomere length was observed in the group that received BN. Thus, we hypothesize that telomeric attrition stimulated telomerase production and consequent increase in plasma levels. This is a natural mechanism of the body in an attempt to avoid telomere attrition. However, we did not observe a relationship between an increase in the prevalence of women who had increased telomerase levels with those who had a decrease or increase in telomere length. These results may suggest that the increase in telomerase levels is not simply a conditional mechanism of the organism, but an effect of the intervention.

6. CONCLUSÕES GERAIS

Após consumirem 2 unidades ao dia de castanha-do-brasil por 8 semanas no contexto de dieta restrita em calorias, concluimos que mulheres em risco cardiometabólico tiveram igual consumo energético e de macronutrientes, perda de peso e comprimento telomérico comparado ao grupo controle. Por outro lado, apresentaram maior consumo e níveis séricos de selênio, aumento da atividade antioxidante total do plasma pelo método de redução do ferro, aumento de Apo A e também dos níveis plasmáticos da enzima telomerase comparado ao grupo controle. Além disso, o consumo de castanha-do-brasil potencializou a redução da circunferência da cintura, da relação cintura-quadril, de enzimas hepáticas, de óxido nítrico e de malondialdeído em paralelo a percentuais de massa magra e livre de gordura mais preservados em relação aos controles.

A variação dos níveis sérico de selênio se associou positivamente a variações em marcadores antropométricos, composição corporal, risco cardiovascular e com os níveis da telomerase. Os achados aqui resportados podem embasar novos mecanismos pelos quais o consumo de castanha-do-brasil exerce efeitos benefícos a saúde humana, bem como a inter-relação entre selênio e marcadores de adiposidade, aterogênese, estresse oxidativo e longevidade.

7. CONSIDERAÇÕES FINAIS

Os resultados e discussões apresentados nos artigos que compõem esse trabalho contribuem para o entendimento dos potenciais efeitos à saúde do consumo de castanha-do-brasil aliado a restrição calórica. O consumo regular de castanha-do-brasil no contexto da dieta habitual mostrou resultados promissores na saúde de diferentes amostras com diferentes status de saúde. Os trabalhos mostram resultados unânimes quanto ao aumento dos níveis séricos de selênio e portanto melhora do status antioxidante. Os resultados também apontam para uma considerável melhora no perfil lipídico, inflamação e estresse oxidativo de indivíduos em hemodiálise e abre caminhos para que indivíduos com outras comorbidades sejam investigados.

Até o momento, apenas quatro estudos avaliaram o efeito do consumo de castanhas no comprimento telômérico e em nenhum deles a castanha estudada foi a castanha-do-brasil. Nossos resultados corroboram com esses estudos no que diz respeito a ausência de efeito sobre o comprimento telomérico. No entanto, desperta a necessidade de mais investigações diante do aumento dos níveis plasmáticos da enzima telomerase que observamos no grupo que recebeu castanha-do-brasil. As castanhas são ricas em gorduras insaturadas e nós revisamos sistematicamente o efeito do consumo de ômega-3 no comprimento telomérico e na atividade da telomerase. Apesar dos resultados apontarem ausência de efeito do consumo de ômega-3 nos telômeros, o seu consumo modulou de maneira diferente os níveis e a atividade da telomerase e destaca a importância de mais investigações no campo alimentação versus longevidade.

De modo geral, o consumo de castanha-do-brasil incorporada a uma dieta para redução de peso trouxe benefícios a mulheres adultas em risco cardiometabólico. Ressaltamos a necessidade de estudos com outros perfis de amostras e maiores tempo de intervenção para confirmar os achados aqui mostrados.

APÊNDICE A - TCLE

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE)

A Sra. está sendo convidada como voluntária a participar da pesquisa "Efeito do consumo diário de castanha-do-brasil (*Bertholetia excelsa H.B.K)* sobre redução do peso e composição corporal, metabolismo energético, apetite, ingestão alimentar, reguladores metabólicos e marcadores genéticos". Nesta pesquisa nós pretendemos avaliar o efeito de uma dieta reduzida em calorias associada ou não ao consumo diário de amêndoas de castanha-do-brasil sobre a perda de peso e regulação metabólica em mulheres adultas com excesso de peso e risco cardiometabólico. O motivo que nos leva a estudar a castanha-do-brasil é o grande valor nutricional desse alimento e seus prováveis benefícios para a saúde.

Esse estudo terá duração do 8 semanas e a coleta dos dados será realizada ao início, durante e ao final do estudo. Ao longo desse período a Sra. seguirá uma dieta reduzida em calorias, a qual poderá ou não ser acompanhada da ingestão de castanha-do-brasil, que será fornecida quinzenalmente pelos pesquisadores. Além disso, enquanto participar do estudo a Sra. deverá manter o nível de atividade física habitual e evitar o consumo de bebida alcoólica.

Ao iniciar e ao finalizar sua participação no estudo, a Sra. deverá comparecer ao laboratório em uma ocasião, onde será realizado o teste de permeabilidade intestinal e as demais avaliações do estudo. Em ambos os dias (inicial e final) será necessário a realização de jejum noturno de 10-12 horas. Nas noites anteriores à realização do jejum, a Sra. deverá consumir um jantar padrão (sanduíche e suco), fornecido pelos pesquisadores. No dia em que comparecer ao laboratório, será realizada a coleta de sangue em jejum e sem seguida será realizado o teste de permeabilidade intestinal, onde a Sra. fará a ingestão de uma bebida contendo açúcares.

Nesse dia também será realizada a avaliação do peso, altura, gordura corporal. Nessa ocasião, como parte dos procedimentos da pesquisa, a Sra. responderá a questionários estruturados contendo perguntas sobre sua história clínica e socioeconômica, hábitos alimentares, nível de atividade física, comportamento frente ao alimento e qualidade do sono, com tempo e aplicação previsto em 1 hora. Ademais, as amostras de fezes deverão ser entregues ao início e ao final do estudo, em um dia de sua escolha. A partir dos dados e amostras coletadas durante o estudo, serão realizadas análises sanguíneas completas, avaliação de micro-organismos intestinais e atividade de genes relacionados com inflamação, estresse oxidativo e metabolismo.

Todos os procedimentos invasivos indispensáveis ao estudo serão realizados por profissionais treinados, minimizando ao máximo eventuais riscos e desconfortos. A coleta de sangue será realizada por um técnico em enfermagem, utilizando apenas materiais descartáveis, sendo possível uma sensação incômoda ou dolorida na hora de inserir a agulha e formação de hematomas no local da entrada da agulha algumas horas após o teste. O técnico em enfermagem será orientado a ser o mais preciso possível para evitar estes incômodos. No caso de eventuais complicações no momento da punção venosa, serão prestados os primeiros socorros no local pelo técnico em enfermagem e, caso haja necessidade, a Sra. será encaminhada para a Divisão de Saúde para demais cuidados. O uso da bioimpedância elétrica para avaliação da gordura corporal é desaconselhado a pessoas que possuam marca-passo ou quaisquer aparelhos eletrônicos semelhantes, pinos, placas metálicas e gestantes. Na ausência dessas restrições, a realização do exame de bioimpedância elétrica não está associada a prejuízos à saúde. Os alimentos fornecidos no estudo serão elaborados com matéria prima de boa procedência e

qualidade, os quais serão bem acondicionados visando manutenção da qualidade nutricional e microbiológica.

Para evitar qualquer tipo de constrangimento no momento da aferição das medidas e preservar a sua privacidade a avaliação antropométrica será realizada em uma sala fechada, silenciosa, por um profissional treinado. Durante a aplicação dos questionários, a Sra. poderá deixar de responder a uma ou a um conjunto de perguntas caso sinta-se constrangida, sem que isso traga qualquer alteração na relação de tratamento por parte dos pesquisadores. Da mesma forma, as amostras biológicas de fezes e urina serão coletadas e entregues em frascos adequados, fornecidos pelos pesquisadores, e sem identificação nominal.

Quanto aos benefícios, a Sra. terá seu estado nutricional avaliado e receberá um plano alimentar individualizado, visando a redução de peso corporal. Além disso, tanto o consumo de castanhas quanto a dieta restrita em calorias estão associados a benefícios à saúde. Ainda, a Sra. terá acesso aos seus dados de avaliação antropométrica, composição corporal e exames bioquímicos. Ao final do estudo, a Sra. receberá um novo plano alimentar individualizado, visando a manutenção de hábitos alimentares saudáveis e a adequação dos marcadores bioquímicos que se apresentarem fora dos níveis de normalidade.

Para participar deste estudo a Sra. não terá nenhum custo, nem receberá qualquer vantagem financeira. Apesar disso, diante de eventuais danos, identificados e comprovados, decorrentes da pesquisa, a Sra. tem assegurado o direito à indenização. A Sra. tem garantida plena liberdade de recusar-se a participar ou retirar seu consentimento, em qualquer fase da pesquisa, sem necessidade de comunicado prévio. A sua participação é voluntária e a recusa em participar não acarretará qualquer penalidade ou modificação na forma em que a Sra. é atendida pelos pesquisadores. Os resultados da pesquisa estarão à sua disposição quando finalizada. A Sra. não será identificada em nenhuma publicação que possa resultar. Seu nome ou o material que indique sua participação não serão liberados sem a sua permissão.

Este termo de consentimento encontra-se impresso em duas vias originais, sendo que uma será arquivada pelo pesquisador responsável, no "Laboratório de Metabolismo Energético e Composição Corporal (LAMECC - UFV)" e a outra será fornecida à Sra.

Os dados e instrumentos utilizados na pesquisa ficarão arquivados com o pesquisador responsável por um período de "cinco anos" após o término da pesquisa. Depois desse tempo, os mesmos, serão destruídos.

Os pesquisadores tratarão a sua identidade com padrões profissionais de sigilo e confidencialidade, atendendo à legislação brasileira, em especial, à Resolução 466/2012 do Conselho Nacional de Saúde, e utilizarão as informações somente para fins acadêmicos e científicos.

Eu,____, contato ___________________________, fui informada dos objetivos da pesquisa **"Efeito do consumo diário de castanha-do-brasil (*Bertholetia excelsa H.B.K)* sobre redução do peso e composição corporal, metabolismo energético, apetite, ingestão alimentar, reguladores metabólicos e marcadores genéticos"** de maneira clara e detalhada, e esclareci minhas dúvidas. Sei que a qualquer momento poderei solicitar novas informações e modificar minha decisão de participar se assim o desejar. Declaro que concordo o em participar. Recebi uma via original deste termo de consentimento livre e esclarecido e me foi dada a oportunidade de ler e esclarecer minhas dúvidas.

Pesquisadores Responsáveis:

Dra. Josefina Bressan – (31) 36125220

Dra. Helen Hermana Miranda Hermsdorff – (31) 36125220

Alessandra Silva – (32) 984547578
Brenda Kelly Souza Silveira – (31) 36125220

Endereço: Av. P.H. Rolfs s/n. Laboratório de Metabolismo Energético e Composição Corporal (LAMECC), Departamento de Nutrição e Saúde, Centro de Ciências Biológicas II, sala 50, 6º andar. Campus Universitário. Viçosa/MG.
Telefone: (31) 36125220
E-mail: castanhasbrasileiras@gmail.com

Em caso de discordância ou irregularidades sob o aspecto ético desta pesquisa, a Sra. poderá consultar:
CEP/UFV – Comitê de Ética em Pesquisa com Seres Humanos
Universidade Federal de Viçosa
Edifício Arthur Bernardes, piso inferior
Av. PH Rolfs, s/n – Campus Universitário
Cep: 36570-900 Viçosa/MG
Telefone: (31) 3612-2316
E-mail: cep@ufv.br
www.cep.ufv.br

Viçosa, ______ de ______________ de 20___.

______________________________ ______________________________

Assinatura do Participante Assinatura do Pesquisador

APÊNDICE B - Registro Alimentar de 24 Horas

ESTUDO
castanhas brasileiras

REGISTRO ALIMENTAR

I. IDENTIFICAÇÃO

Data de aplicação: ____/____/____ Pesquisador responsável: ______________________________

Iniciais (nome): ______________________________ Grupo:______ Nº ID:______

Data: _____/_____/_____ ( ) seg ( ) ter ( ) qua ( ) qui ( ) sex ( ) sáb ( ) dom

	ALIMENTOS	MEDIDA CASEIRA	QUANT (g)
Horário: Local:			
Horário: Local:			
Horário: Local:			
Horário: Local:			
Horário: Local:			
Horário: Local:			

ANEXO A - Parecer do Comitê de Ética

UNIVERSIDADE FEDERAL DE VIÇOSA - UFV

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Estudo Castanhas Brasileiras
Pesquisador: Josefina Bressan
Área Temática:
Versão: 2
CAAE: 92004818.0.0000.5153
Instituição Proponente: Departamento de Nutrição e Saúde
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.832.601

Apresentação do Projeto:
O presente protocolo foi enquadrado como pertencente à Área Temática: Ciências da Saúde

Conforme resumo apresentado no formulário online da Plataforma:

Estudos epidemiológicos têm demonstrado consistentemente os efeitos benéficos à saúde atribuídos ao consumo de amêndoas, contudo, poucos estudos clínicos randomizados controlados têm sido conduzidos com o objetivo de investigar esses efeitos. A castanha de caju (Anacardium accidentale L.) e a castanha-do brasil (Bertholetia excelsa H.B.K), são excelentes fontes de nutrientes e fazem parte da cultura alimentar brasileira, apesar disso, integram o grupo das nuts menos estudadas. Dessa forma, o presente trabalho propõe o desenvolvimento de um estudo clínico de intervenção nutricional o qual fornecerá diariamente por oito semanas amêndoas de castanhas brasileiras a mulheres com excesso de peso e risco cardiometabólico. As voluntárias serão distribuídas aleatoriamente em três grupos experimentais paralelos: Grupo controle: Restrição calórica (- 500 kcal/dia) sem consumo de amêndoas (n= 26); Grupo teste 1: Restrição calórica (-500 kcal/dia) + mix de amêndoas de castanhas brasileiras (30g de castanha de caju + 15g castanha-do-Brasil) (n= 26); Grupo teste 2: Restrição calórica (-500 kcal/dia) + amêndoas de castanha do-Brasil (15g castanha-do-Brasil) (n= 26) e serão avaliados os efeitos do consumo das amêndoas sobre o risco cardiometabólico, composição corporal, metabolismo energético,

UNIVERSIDADE FEDERAL DE VIÇOSA - UFV

Investigador	PROJETO_DE_PESQUISA.docx	20/06/2018 17:12:47	Josefina Bressan	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_CEP.docx	20/06/2018 17:12:09	Josefina Bressan	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

VICOSA, 21 de Agosto de 2018

Assinado por:
Maria da Conceição Aparecida Pereira Zolnier
(Coordenador)

Endereço: Universidade Federal de Viçosa, Avenida PH Rolfs s/n, Edifício Arthur Bernardes
Bairro: Campus Universitário **CEP:** 36.570-900
UF: MG **Município:** VICOSA
Telefone: (31)3899-2492 **E-mail:** cep@ufv.br

UNIVERSIDADE FEDERAL DE VIÇOSA - UFV

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO DO CONSUMO DIÁRIO DE CASTANHA-DO-BRASIL (BERTHOLETIA EXCELSA H.B.K) SOBRE REDUÇÃO DO PESO E COMPOSIÇÃO CORPORAL, METABOLISMO ENERGÉTICO, APETITE, INGESTÃO ALIMENTAR, REGULADORES METABÓLICOS E MARCADORES GENÉTICOS

Pesquisador: Josefina Bressan

Área Temática:

Versão: 1

CAAE: 21448719.0.0000.5153

Instituição Proponente: Departamento de Nutrição e Saúde

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.649.033

Apresentação do Projeto:

O presente projeto foi enquadrado como pertencente à Área Temática: Ciências da Saúde

Conforme resumo apresentado no formulário online da Plataforma: Nos últimos anos o consumo de castanhas tem se destacado por seu efeito cardioprotetor atribuído a melhora do perfil lipídico, efeito antioxidante, anti-inflamatório e controle do peso corporal. Apesar das evidências, parte dos estudos realizados com esses alimentos é de natureza observacional havendo carência de estudos clínicos controlados bem delineados e que tenham avaliado o efeito da castanha-do-Brasil. Nessa perspectiva, o presente estudo tem como objetivo avaliar o efeito da ingestão diária de castanha de caju castanha-do-brasil (Bertholetia excelsa H.B.K) associado a uma dieta hipocalórica sobre a perda de peso e regulação metabólica em mulheres adultas com excesso de peso e risco cardiometabólico. Trata-se de um estudo clínico, controlado, com 52 voluntárias adultas distribuídas em dois grupos experimentais paralelos: 1) Grupo controle: restrição calórica (- 500 kcal/dia) sem consumo de castanhas e 2) Grupo teste: restrição calórica (- 500 kcal/dia) + castanha-do-brasil (15g). O período de intervenção terá duração de oito semanas, ao longo das quais todas as voluntárias seguirão a dieta prescrita. Ao início e ao final da intervenção (dia experimental inicial e dia experimental final), as participantes comparecerão ao laboratório pela

UNIVERSIDADE FEDERAL DE VIÇOSA - UFV

Continuação do Parecer: 3.649.033

Declaração de Instituição e Infraestrutura	Uso_instalacoes.pdf	19/09/2019 14:06:47	Josefina Bressan	Aceito
Folha de Rosto	Folhaderosto.pdf	19/09/2019 14:06:27	Josefina Bressan	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_DE_PESQUISA.docx	18/09/2019 21:50:49	Josefina Bressan	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.docx	18/09/2019 21:47:46	Josefina Bressan	Aceito
Orçamento	ORCAMENTO.docx	18/09/2019 21:45:47	Josefina Bressan	Aceito
Cronograma	CRONOGRAMA.docx	18/09/2019 21:33:25	Josefina Bressan	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

VICOSA, 18 de Outubro de 2019

Assinado por:
Maria da Conceição Aparecida Pereira Zolnier
(Coordenador(a))

Endereço: Universidade Federal de Viçosa, Avenida PH Rolfs s/n, Edifício Arthur Bernardes
Bairro: Campus Universitário **CEP:** 36.570-977
UF: MG **Município:** VICOSA
Telefone: (31)3612-2316 **E-mail:** cep@ufv.br

ANEXO B - Questionário Internacional de Atividade Física (IPAQ)

ESTUDO
castanhas brasileiras

QUESTIONÁRIO: IPAC CURTO (Folha 1/2)

I. IDENTIFICAÇÃO

Data de aplicação: ____/____/____ Pesquisador responsável: ________________________________

Iniciais (nome): ____Grupo:______ Nº ID:______

Nós estamos interessados em saber que tipos de atividade física as pessoas fazem como parte do seu dia a dia. Este projeto faz parte de um grande estudo que está sendo feito em diferentes países ao redor do mundo. Suas respostas nos ajudarão a entender que tão ativos nós somos em relação à pessoas de outros países. As perguntas estão relacionadas ao tempo que você gasta fazendo atividade física na **ÚLTIMA** semana. As perguntas incluem as atividades que você faz no trabalho, para ir de um lugar a outro, por lazer, por esporte, por exercício ou como parte das suas atividades em casa ou no jardim. Suas respostas são MUITO importantes. Por favor responda cada questão mesmo que considere que não seja ativo. Obrigado pela sua participação!

Para responder as questões lembre que:

- atividades físicas **VIGOROSAS** são aquelas que precisam de um grande esforço físico e que fazem respirar MUITO mais forte que o normal
- atividades físicas **MODERADAS** são aquelas que precisam de algum esforço físico e que fazem respirar UM POUCO mais forte que o normal

Para responder as perguntas pense somente nas atividades que você realiza **por pelo menos 10 minutos contínuos** de cada vez.

1a Em quantos dias da última semana você **CAMINHOU** por pelo menos 10 minutos contínuos em casa ou no trabalho, como forma de transporte para ir de um lugar para outro, por lazer, por prazer ou como forma de exercício?

dias _____ por **SEMANA** ( ) Nenhum

1b Nos dias em que você caminhou por pelo menos 10 minutos contínuos quanto tempo no total você gastou caminhando **por dia**?

horas: ______ Minutos: _____

2a. Em quantos dias da última semana, você realizou atividades **MODERADAS** por pelo menos 10 minutos contínuos, como por exemplo pedalar leve na bicicleta, nadar, dançar, fazer ginástica aeróbica leve, jogar vôlei recreativo, carregar pesos leves, fazer serviços domésticos na casa, no quintal ou no jardim como varrer, aspirar, cuidar do jardim, ou qualquer atividade que fez aumentar **moderadamente** sua respiração ou batimentos do coração **(POR FAVOR NÃO INCLUA CAMINHADA)**

dias _____ por **SEMANA** ( ) Nenhum

2b. Nos dias em que você fez essas atividades moderadas por pelo menos 10 minutos contínuos, quanto tempo no total você gastou fazendo essas atividades **por dia**?

horas: ______ Minutos: _____