ANA PAULA SILVA CALDAS

EFEITO DO CONSUMO DE CASTANHAS BRASILEIRAS SOBRE A COMPOSIÇÃO CORPORAL, FUNÇÃO ENDOTELIAL E ESTRESSE OXIDATIVO DE MULHERES EM RISCO CARDIOMETABÓLICO

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.

Orientadora: Josefina Bressan

Coorientadora: Helen Hermana Miranda Hermsdorff

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Assentimento:

Ana Paula Silva Caldas
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Josefina Bressan
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Dedico esse trabalho aos meus pais, Paulo e Alzerina, pelo amor e apoio irrestrito.

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*“*Mas tudo o que é precioso é tão difícil quanto raro*”.*

(Benedictus de Spinoza)

RESUMO

CALDAS, Ana Paula Silva, D.Sc., Universidade Federal de Viçosa, março de 2021. **Efeito do consumo de castanhas brasileiras sobre a composição corporal, função endotelial e estresse oxidativo de mulheres em risco cardiometabólico**. Orientadora: Josefina Bressan. Coorientadora: Helen Hermana Miranda Hermsdorff.

O consumo de castanhas tem se destacado por seu efeito cardioprotetor associado a melhora do perfil lipídico, controle do peso corporal, efeito antioxidante e anti-inflamatório. No entanto, a maioria dessas evidências é fruto de estudos conduzidos com amêndoas de castanhas pouco presentes na alimentação brasileira ou a partir de estudos observacionais. Nessa perspectiva, o presente estudo tem como objetivo avaliar o efeito da ingestão diária de uma mistura de amêndoas de castanhas brasileiras (castanha-do-Pará, Bertholletia excelsa H.B.K e castanha de caju, Anacardium occidentale L.) associado a uma dieta com restrição calórica sobre a composição corporal, função endotelial e estresse oxidativo de mulheres em risco cardiometabólico. Trata-se de um estudo clínico, randomizado, controlado, com 40 mulheres distribuídas aleatoriamente em dois grupos, 01) Controle: restrição calórica (- 500 kcal/dia) sem consumo de amêndoas de castanhas (n= 19) e 2) Mix de nuts: restrição calórica (- 500 kcal/dia) associada ao consumo diário de um mix de amêndoas de castanhas brasileiras (15 g de amêndoa de castanha-do-Pará + 30 g de amêndoa de castanha de caju) (n= 21). O período de intervenção durou oito semanas, ao longo das quais todas as voluntárias seguiram a dieta prescrita. Ao início e ao final da intervenção, todas as participantes foram submetidas a avaliação antropométrica e da composição corporal, pressão arterial sistólica e diastólica e Índice Tornozelo Braquial (ITB). Ainda, foram coletadas amostras de sangue em jejum e analisados os marcadores de risco cardiometabólico (CT, LDL-c, HDL-c, TGL, Apo A1, ApoB, ApoE, glicose de jejum, insulina de jejum e índice TyG), marcadores sistêmicos da função endotelial (ICAM-1, VCAM-1, e ON), marcadores de estresse oxidativo (SOD, MDA, FRAP, CAT, GPx e LDLox), marcadores da função hepática (GGT, AST, ALT e fosfatase alcalina) e selênio plasmático. As análises estatísticas foram realizadas utilizando o software SPSS versão 22.0, considerando-se um nível de significância de α= 5%. A normalidade das variáveis foi avaliada pelo teste de Shapiro-Wilk. O teste t pareado ou o teste de Wilcoxon foram utilizados para avaliar o efeito do tempo nos tratamentos. Para avaliar as diferenças entre os grupos, foram utilizados o teste t de

amostras independentes ou teste U de Mann-Whitney. Quando apropriado, o teste de ANCOVA foi empregado para a comparação de médias entre os grupos utilizando os valores basais como variáveis de ajuste. Os dados foram apresentados como média ± erro padrão da média. Após o período de intervenção, houve redução do peso, do IMC e dos perímetros da cintura e do quadril estatisticamente semelhante entre os grupos. Contudo, as participantes do grupo mix de nuts apresentaram melhora da composição corporal com maior redução do percentual de gordura (0,1 ± 0,3 vs. -1,3 ± 0,4; p= 0,019) e menor perda de massa magra nos compartimentos corporais em comparação às mulheres alocadas no grupo controle. Ainda, após o consumo do mix de nuts houve melhora do selênio plasmático (8,9 ± 7,3 vs. 35,4 ± 7,2; p= 0,010), além de redução da molécula de adesão VCAM-1 (24,3 ± 14,6 vs. -25,8 ± 10,4; p= 0,010), sugerindo redução da inflamação endotelial. No entanto, os marcadores de risco cardiometabólico, estresse oxidativo e função hepática permaneceram inalterados após o período de intervenção. Assim, os dados do presente estudo demonstraram que o consumo de amêndoas de castanhas brasileiras associado a uma dieta com restrição calórica pode ser estimulado, uma vez que promove melhora da composição corporal, da concentração plasmática de selênio e da molécula de adesão VCAM-1, os quais em conjunto podem contribuir para a redução do risco cardiovascular.

Palavras-chave: Amêndoa de castanha-do-Brasil. Amêndoa de castanha de caju. Selênio. Gordura corporal.

ABSTRACT

CALDAS, Ana Paula Silva, D.Sc., Universidade Federal de Viçosa, March, 2021. **The effect of Brazilian nut consumption on body composition, endothelial health, and oxidative stress of cardiometabolic risk women**. Adviser: Josefina Bressan. Co-adviser: Helen Hermana Miranda Hermsdorff.

Currently, nuts intake has been associated with a cardioprotective effect due to improvement in lipid profile and body weight control, besides antioxidant and anti-inflammatory effects. However, most of this evidence results from studies conducted with nuts that are little present in Brazilian food or from observational studies. Thus, the present study aims to evaluate the effect of Brazilian mixed nut intake (Brazil nut, Bertholletia excelsa H.B.K and cashew nut Anacardium occidentale L.) associated with an energy-restricted diet on body composition, endothelial function, and oxidative stress makers of women at cardiometabolic risk. This is a randomized controlled 8-week clinical trial, with 40 women allocated in two groups, 01) Control: energy-restricted diet (-500 kcal/day) absent in nuts (n=19), or 2) Mixed nuts: energy-restricted diet (-500 kcal/day) associated with daily intake a Brazilian mixed nut (15 g of Brazil nut + 30 g of cashew nut) (n=21). Over the intervention period, all women followed the prescribed diet. At the beginning and final intervention, all participants were submitted to anthropometric, body composition, systolic and diastolic blood pressure, and ankle-brachial index evaluation. Also, fasting blood samples were collected for evaluation of cardiometabolic risk markers (CT, LDL-c, HDL-c, TGL, Apo A1, ApoB, ApoE, glucose, insulin, and TyG index), systemic markers of endothelial function (ICAM-1, VCAM-1, and NO), oxidative stress (SOD, MDA, FRAP, CAT, GPx, and LDLox), liver function (GGT, AST, ALT, and Alkaline phosphatase), and plasma selenium. Statistical analyses were performed using the software SPSS versão 23.0, adopting a statistically significant level of α= 5%. The Shapiro-Wilk normality test was used to check for the normal distribution of the data. To determine the effect of time on treatments was performed the paired t-test or Wilcoxon test. For the between-group evaluation, an independent-samples t-test or Mann-Whitney U test was used. When appropriate, the ANCOVA test was used to compare means considering basal values as covariates. Data were expressed as mean ± SEM. After the intervention, there are reductions of body weight, BMI, waist, and hip circumferences statistically similar between groups. However, women in the mixed nut group showed improvement in body composition

supported by a higher reduction in body fat (%) (0.1 ± 0.3 vs. -1.3 ± 0.4; p= 0.019) and lower lean mass loss in the body compartments in comparison to those allocated in the control group. Furthermore, after mixed nuts intake there is an improvement in the plasma selenium concentration (8.9 ± 7.3 vs. 35.4 ± 7.2; p= 0.010), besides reduction in the adhesion molecule VCAM-1 (24.3 ± 14.6 vs. -25.8 ± 10.4; p= 0.010), suggesting a reduction of endothelial function. Nonetheless, the markers of cardiometabolic risk, oxidative stress, and liver function remain unchanged after 8-week. Thus, the data of the present study demonstrate that Brazilian nuts intake associated with an energy-restricted diet might be recommended since promoting the improvement of body composition, selenium status, and plasma VCAM-1 concentration, which together can contribute to the reduction of cardiovascular risk.

Keywords: Body Fat. Brazil nut. Cashew nut. Selenium.

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LISTA DE ABREVIATURAS E SIGLAS

ABI	Ankle-brachial index
AMPK	Proteína Quinase Ativada por Monofosfato de Adenosina, do inglês AMP-activated protein kinase
ApoA	Apolipoproteína A
ApoB	Apolipoproteína B
ApoE	Apolipoproteína E
CAT	Catalase
COX2	Ciclo-oxigenase-2
DCV	Doença cardiovascular
DEXA	Absorciometria de dupla energia de raio-X, do inglês Dual-energy X-ray Absortiometry
DHGNA	Doença hepática gordurosa não alcoólica
DM2	Diabetes mellitus tipo 2
eNOS	Óxido nítrico-sintase endotelial
EROs	Espécies reativas de oxigênio
FMD	Flow-Mediated Vasodilatation
FRAP	Poder de redução do íon ferro, do inglês Ferric Reducing Antioxidant Power
GPx	Glutationa peroxidase
HbA1c	Hemoglobina Glicada
HDL-c	Lipoproteína de alta densidade
ICAM-1	Intercellular Adhesion Molecule 1
IL-10	Interleucina 10
IL-18	Interleucina 18
IL-6	Interleucina 6
ITB	Índice tornozelo-braquial
LDL-c	Lipoproteína de baixa densidade
LDL-ox	LDL oxidada, do inglês Oxidized Low-Density Lipoprotein
MDA	Malondialdeído
NAD^+	Nicotinamida Adenina Dinucleotídeo

Nrf2	Nuclear factor erythroid 2-related factor 2
ON	Óxido nítrico
PAD	Pressão arterial diastólica
PAS	Pressão arterial sistólica
Sirt1	Sirtuína 1
SOD	Superóxido dismutase
TBARS	Ácido tiobarbitúrico
TGF-β	Fator de transformação do crescimento β, do inglês Transforming growth factor beta
TGL	Triglicerídeos
TNF-α	Fator de necorse tumoral alfa
TyG	Índice triglicerídeo/glicose
VCAM-1	Vascular cell adhesion protein 1
VLDL	Lipoproteína de muito baixa densidade

SUMÁRIO

1 INTRODUÇÃO

As doenças cardiovasculares (DCV) são as principais causas de incapacidade e morte precoce em países industrializados e em muitos países desenvolvidos. Cerca de 17,7 milhões de mortes por ano, 31% de todas as mortes globais, são decorrentes de DCV (WORD HEALTH ORGANIZATION, 2020). O desencadeamento dessas doenças - que se manifestam principalmente como infartos e derrames – devem-se essencialmente ao uso do tabaco, a dieta pouco saudável, a inatividade física e o uso abusivo do álcool. Esses hábitos predispõem ao sobrepeso e a obesidade e elevam a pressão e a glicose sanguínea, que em conjunto são prejudiciais à saúde cardiovascular (ROS, 2009; WORD HEALTH ORGANIZATION, 2020)

Desde 1998, a American Heart Association classificou oficialmente a obesidade como o principal fator de risco modificável para as DCV (ECKEL; KRAUSS, 1998). O excesso de adiposidade aumenta o risco de DCV por estimular os fatores de risco intermediários – dislipidemia, diabetes e hipertensão (DESPRÉS, 2012). A dislipidemia característica da obesidade manifesta-se principalmente pela elevada concentração de triglicerídeos (TGL) em jejum e pós-prandial em combinação com o aumento das lipoproteínas de baixa densidade (LDL) e redução do lipoproteínas de alta densidade (HDL) (KLOP; ELTE; CABEZAS, 2013). Ao mesmo tempo em que promove aumento das concentrações plasmáticas de ácidos graxos livres, o excesso de adiposidade também está associado à secreção de adipocinas pró-inflamatórias com efeito adverso sobre a ação da insulina, predispondo ao surgimento do diabetes (WESTPHAL, 2008). O excesso de peso e suas comorbidades também afetam a saúde endotelial e levam ao aumento da pressão arterial (HALL et al., 2012). Estudos de metanálise mostram que a redução do peso corporal, mesmo que modesta, reduz o risco de hipertensão e DCV de maneira significativa (JONES, 1996; NETER et al., 2003).

A alimentação saudável continua sendo um dos pilares da prevenção às DCV (ROS, 2009). Tradicionalmente utilizada como estratégia terapêutica para o manejo do excesso de peso, a restrição calórica também tem sido associada com promoção da saúde cardíaca e redução do risco cardiovascular, uma vez que impacta no melhor controle do peso corporal e do metabolismo glicêmico e lipídico, além de reduzir a inflamação e o estresse oxidativo (BRANDHORST; LONGO, 2019; SCIARRETTA et al., 2020). Durante muitos anos, dietas que tinham como alvo os lipídios plasmáticos

foram as principais formas de intervenção nutricional. Contudo, o consumo de alguns alimentos e nutrientes específicos, bem como padrões alimentares, também estão associados ao efeito cardioprotetor (BRANDHORST; LONGO, 2019; BROWN; HU, 2001; NETTLETON et al., 2006). Nas últimas décadas vários estudos epidemiológicos têm demonstrado que o consumo de nuts está associado à redução do risco cardiovascular, tornando-as um dos principais alimentos com alegação cardioprotetora da atualidade (GUASCH-FERRÉ et al., 2017; LARSSON et al., 2018; LIU et al., 2020; O'NEIL et al., 2011; SABATÉ; ANG, 2009)

Compreende-se por nuts todos os frutos secos que possuem uma semente envolvida por um epicarpo rígido – nozes, avelã, amêndoa, pistache, amendoim, pinhão, amêndoa da castanha de caju, macadâmia, noz-pecã e amêndoa da castanha-do-Pará (ROS, 2010). Em geral, as nuts são densamente calóricas e fornecem de 5,6 a 6,5 kcal/g, em decorrência do alto teor de lipídios (45 – 75% do peso), constituído principalmente por gordura monoinsaturada (ácido graxo oleico). As nuts também são complexas matrizes alimentares fontes de proteínas, fibras, vitaminas (vitamina E e B6, ácido fólico, e niacina), minerais (magnésio, potássio e cobre), fitoesteróis (estigmasterol, campesterol e sitosterol) e compostos fenólicos (catequinas, resveratrol etc.) (VADIVEL; KUNYANGA; BIESALSKI, 2012).

A American Heart Association (AHA) recomenda desde os anos 2000 o consumo diário de 30g de nuts baseado em suas alegações cardioprotetoras (KRAUSS et al., 2000). A inserção desses alimentos a uma dieta saudável parece melhorar o perfil de ácidos graxos plasmáticos em pacientes com diabetes mellitus tipo 2 (DM2), reduzir as concentrações plasmáticas de colesterol total e LDL, bem como aumentar a taxa de HDL:colesterol total, reduzir a inflamação e promover melhora da função arterial em indivíduos com dislipidemia (MUKUDDEM-PETERSEN; OOSTHUIZEN; JERLING, 2005; ROS et al., 2004; VADIVEL; KUNYANGA; BIESALSKI, 2012; ZHAO et al., 2004; ZIBAEENEZHAD, 2005). De acordo com a revisão sistemática publicada em 2015 por Martin et al., embora haja evidências de benéficios cardiovasculares atribuídos ao consumo de nuts baseados em suas características nutricionais, poucos estudos clínicos considerados bem controlados foram conduzidos até o momento. Além disso, o efeito do consumo das amêndoas tipicamente brasileiras, amêndoa da castanha-do-Pará (Bertholletia excelsa H.B.K) e

amêndoa da castanha de caju (Anacardium occidentale L.) e) tem sido pouco investigado.

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2 REVISÃO DE LITERATURA

2.1 Obesidade, doença cardiovascular e função hepática

A obesidade tem sido caracterizada como uma epidemia global com graves consequências para a sociedade moderna. De acordo com estimativas da Organização Mundial da Saúde (OMS), mais da metade da população adulta global apresenta sobrepeso ou obesidade (WHO, 2015). A prevalência da obesidade aumentou rapidamente em muitas regiões do mundo, e se as tendências atuais continuarem, em 2025 a obesidade atingirá 18% nos homens e mais de 21% nas mulheres, impondo um desafio aos indivíduos, sociedade e sistemas de saúde (DI CESARE et al., 2016). No Brasil, segundo a Pesquisa de Vigilância de Fatores de Risco e Proteção para Doenças crônicas por Inquérito Telefônico (Vigitel) de 2019, a prevalência de excesso de peso foi de 57,1% para homens e 53,9% para mulheres, enquanto a frequência de adultos com obesidade foi de 19,9% para homens e 21,0% em mulheres (MINISTÉRIO DA SAÚDE DO BRASIL, 2020).

O excesso de peso e a obesidade estão diretamente associados ao desenvolvimento de DCV. A presença da obesidade, induz direta e indiretamente o aumento da morbimortalidade associada a essas doenças. Os efeitos diretos envolvem as adaptações estruturais e funcionais do sistema cardiovascular induzidas pela obesidade para acomodar o excesso de peso corporal, bem como os efeitos das adipocinas secretadas pelo tecido adiposo sobre a inflamação e homeostase vascular. Todo esse processo leva a um ambiente pró-inflamatório e pró-trombótico. Os efeitos indiretos são mediados por fatores concomitantes de risco para DCV, como resistência à insulina, DM2, adiposidade visceral, hipertensão e dislipidemia (KOLIAKI; LIATIS; KOKKINOS, 2018). Dessa forma, a obesidade está associada com a prevalência da maioria das doenças cardiovasculares, incluindo hipertensão, doença arterial coronariana, insuficiência cardíaca e fibrilação atrial (KOLIAKI; LIATIS; KOKKINOS, 2018; MATHEW et al., 2008).

As DCV são desencadeadas principalmente pela aterosclerose (FROSTEGÅRD, 2013). O excesso de peso corporal e especialmente o acúmulo de gordura na região abdominal acelera a progressão da aterosclerose décadas antes das primeiras manifestações clínicas da doença arterial coronariana. Mais que a quantidade total de gordura corporal, a integridade e funcionalidade do tecido adiposo configuram aspectos fundamentais na determinação do risco cardiometabólico

(BASTIEN et al., 2014; KOLIAKI; LIATIS; KOKKINOS, 2018). Na obesidade, a expansão do tecido adiposo conduz a hiperplasia e hipertrofia dos adipócitos, seguida por diminuição da vascularização a qual promove hipóxia localizada e necrose isquêmica, levando os adipócitos à morte. Este evento, por sua vez, estimula a infiltração de macrófagos ativados e é considerado um dos fatores indutores de um ciclo de resposta inflamatória característico da obesidade, denominado inflamação subclínica (KOLIAKI; LIATIS; KOKKINOS, 2018). Uma das mais significantes modulações pró-inflamatórias induzidas pela obesidade é a polarização dos macrófagos residentes no tecido adiposo em direção a um fenótipo pró-inflamatório (M1), que pode ativar vias inflamatórias e prejudicar a sinalização da insulina (LUMENG; BODZIN; SALTIEL, 2007). As complexas interações entre diferentes tipos de células dentro do tecido adiposo inflamado podem contribuir para o seu impacto global nas complicações relacionadas à obesidade (KOLIAKI; LIATIS; KOKKINOS, 2018).

Além do estímulo à inflamação, a obesidade também aumenta o risco cardiovascular por induzir o aumento do estresse oxidativo (FURUKAWA et al., 2004). Esse efeito deve-se em partes à expressão prejudicada de enzimas antioxidantes como a superóxido dismutase (SOD), a glutationa peroxidase (GPx) e a catalase (CAT) no tecido adiposo, o que leva ao aumento da produção de espécies reativas de oxigênio (ERO) (LOVREN; TEOH; VERMA, 2015). O acúmulo de tecido adiposo tem sido associado ao aumento de marcadores do estresse oxidativo, os quais prejudicam a sinalização insulínica e induzem a inflamação, além de estarem associados ao processo aterosclerótico, favorecendo o desenvolvimento de DCV. Quando as partículas de LDL acumuladas na camada íntima sofrem ação das ERO ocorre a formação de LDL oxidada, a qual induz a ativação do sistema imune inato na íntima e a ativação endotelial. Uma vez ativado, o endotélio secreta moléculas de adesão tais como ICAM-1, VCAM-1, e-selectina e p-selectina e as células do musculo liso secretam quimiocinas que juntas atraem monócitos, linfócitos, mastócitos, e neutrófilos para a parede arterial dando início ao processo aterosclerótico (BERGHEANU; BODDE; JUKEMA, 2017; INSULL, 2009). Além da atuação direta na formação de partículas aterogênicas, as ERO também atuam por meio da inibição da síntese de óxido nítrico (ON), aumentando a instabilidade endotelial e predispondo ao desenvolvimento da aterosclerose (LOVREN; TEOH; VERMA, 2015).

Indivíduos com obesidade também apresentam risco de desenvolver alterações hepáticas. O excesso de tecido adiposo, especialmente na região abdominal, predispõe ao desenvolvimento de esteatose hepática não alcoólica, atualmente considerada a manifestação hepática da síndrome metabólica (HARRIS et al., 2019; STRANGES et al., 2004). A doença hepática gordurosa não alcoólica (DHGNA) tem se tornado a principal causa de doença hepática crônica no mundo alcançando 25% de prevalência global e associando-se a DCV clínica e subclínica (ZHANG et al., 2019). Vusirikala et al. (2020), mostraram que indivíduos com obesidade mesmo sem alterações metabólicas apresentavam maior risco de desenvolver DHGNA em comparação aos controles com peso normal (VUSIRIKALA et al., 2020). A característica marcante dessa doença é a esteatose hepática, quimicamente definida pelo conteúdo de triglicerídeos intra-hepáticos maior que 5% do volume do fígado ou peso do fígado ou histologicamente definido quando 5% ou mais dos hepatócitos contêm triglicerídeos intracelulares visíveis na ausência de causas secundárias como álcool e drogas (FABBRINI; SULLIVAN; KLEIN, 2010). Do ponto de vista sistêmico, níveis aumentados principalmente de alanina aminotransferase (ALT) e triglicerídeos, e secundariamente de gama-glutamiltransferase (GGT), parecem ser indicadores bioquímicos sensíveis da presença de esteatose hepática (STRANGES et al., 2004).

Recentemente, as enzimas hepáticas ALT e GGT, mesmo dentro da faixa normal, têm sido utilizados na predição de diabetes, DHGNA ou outros distúrbios metabólicos (FRASER et al., 2009; GOESSLING et al., 2008). No estudo conduzido por Xie et al. (2018), GGT e ALT mostraram associação positiva com marcadores de risco cardiometabólico como HOMA-IR, TGL, glicemia de jejum e perímetro da cintura. Além disso, quanto maior a concentração de GGT e ALT maior o risco de obesidade com fenótipo metabolicamente não saudável (XIE et al., 2018). As correlações documentadas entre GGT e ALT com marcadores de risco cardiovascular têm sido atribuídas ao estresse oxidativo, inflamação e resistência à insulina (RI), demonstrando a estreita associação entre excesso de peso, alterações metabólicas e função hepática (YAMADA et al., 2006).

2.2 Consumo das nuts e risco cardiometabólico

O consumo regular de nuts apresenta benéficios sobre diversos fatores de risco cardiovascular. Apesar da alta densidade calórica, o consumo de desses alimentos não está associado ao ganho de peso e pode reduzir o risco de obesidade em indivíduos saudáveis e com síndrome metabólica (SM) (VADIVEL; KUNYANGA; BIESALSKI, 2012). Um estudo transversal com 800 adolescentes espanholas não encontrou relação entre a frequência do consumo de nuts e o peso corporal (SORIGUER et al., 1995). Estudos clínicos, por sua vez, têm demonstrado a associação entre o consumo de nuts e redução do peso corporal (ALMARIO et al., 2001; GARG; BLAKE; WILLS, 2003; RAJARAM et al., 2001; WIEN et al., 2003). Entre os possíveis mecanismos envolvidos nesse efeito estão a ação das nuts sobre a saciedade, com consequente menor ingestão calórica de forma a compensar o aumento da disponibilidade de energia (AKHLAGHI et al., 2018; TAN; DHILLON; MATTES, 2014)

As alterações na homeostase glicêmica antecedem o DM2 e as DCV e desempenham um papel fundamental na patogênese dessas doenças (MCLAUGHLIN et al., 2004). Nos últimos anos, o consumo de nuts tem sido associado a um efeito antidiabético tanto em indivíduos saudáveis quanto naqueles com homeostase glicídica alterada (KIM; KEOGH; CLIFTON, 2017). Uma metanálise de 12 ensaios clínicos randomizados controlados conduzidos em indivíduos com DM2 avaliou o efeito do consumo de dietas suplementadas com diferentes tipos nuts (amêndoas, amêndoa de castanha-do-Pará, amêndoa de castanha de caju, avelãs, macadâmia, noz-pecã, pinhão, pistache e nozes) sobre a homeostase glicêmica (VIGUILIOUK et al., 2014). Esse estudo demonstrou que o consumo de nuts em uma dose média de 56g/dia melhorou o controle glicêmico em indivíduos com DM2, evidenciado pela redução da HbA1c e da glicemia de jejum, contudo, sem alteração o HOMA-IR (VIGUILIOUK et al., 2014). Em indivíduos com resistência à insulina que seguiram a dieta recomendada pela American Diabetes Association (ADA) acrescida de 60g/dia de amêndoas, houve redução de 23% nas concentrações de insulina, 25% no HOMA-IR e 18% no HOMA-β sem alterar a glicose de jejum, quando comparados à dieta controle sem amêndoas (WIEN et al., 2010). Em indivíduos saudáveis, o consumo de uma dieta mediterrânea suplementada com pistache em quantidade equivalente a 20% da necessidade energética diária resultou em redução de 8,8 ± 8,5

% na glicose de jejum (SARI et al., 2010). Kim; Keogh; Clifton. (2017) sugeriram que o potencial papel do consumo nuts na melhora do controle glicêmico está associado principalmente ao conteúdo de ácidos graxos monoinsaturados desses alimentos; fibras e polifenóis também têm sido associados ao efeito antidiabético das nuts devido sua capacidade de alterar a microbiota intestinal.

Embora auxiliem no controle do peso corporal e do homeostase glicídica, o efeito do consumo de nuts sobre a pressão arterial permanece controverso (DJOUSSÉ; RUDICH; GAZIANO, 2009). Pacientes com doença arterial coronariana que consumiram amêndoas (10g/dia) durante 12 semanas não apresentaram alteração na pressão arterial (JAMSHED et al., 2015). Similarmente, em indivíduos com excesso de peso, o consumo de 56g/dia de amêndoas também não afetou a pressão arterial (KATZ et al., 2012). Segundo uma metanálise conduzida por Mohammadifard et al. (2015), o consumo de pistache ou mistura de nuts apresenta efeito na redução da pressão arterial sistólica e diastólica. No entanto, a mais recente metanálise pulicada, concluiu a partir de 61 estudos clínicos que o consumo de nuts não modifica a pressão arterial sistólica ou diastólica (DEL GOBBO et al., 2015).

Apesar do alto teor de gorduras e da alta densidade calórica, estudos epidemiológicos demonstraram que o consumo de nuts pode modular o perfil lipídico de maneira benéfica (NASH; NASH, 2008; NASH; WESTPFAL, 2005; SABATÉ; ANG, 2009). Estudos clínicos com nozes (MA et al., 2010; RAJARAM et al., 2009; TAPSELL et al., 2009; TORABIAN et al., 2010), amêndoas (JENKINS et al., 2008), avelãs (MERCANLIGIL et al., 2007), pistache (GEBAUER et al., 2008), macadâmias (GRIEL et al., 2008) e amendoim (LOKKO et al., 2007) mostraram reduções de 4% a 11% do LDL-c, evidenciando a eficácia de diferentes tipos de nuts sobre o perfil lipídico. Além disso, uma metanálise avaliando 61 estudos clínicos concluiu que o consumo de nuts (amêndoas, nozes, pistache, amêndoa de castanha de caju, amêndoa de castanha-do-Pará, avelã, noz-pecã e macadâmia; 5-100g/dia por 3-26 semanas) também reduz colesterol total, ApoB e triglicerídeos (DEL GOBBO et al., 2015). Ademais, nuts são ricas em ácidos graxos monoinsaturados - um substrato mais resistente à oxidação – os quais podem ser incorporados pelas lipoproteínas e torná-las menos susceptíveis a processos oxidativos (REAVEN; WITZTUM, 1996). Berry et al. (1992) observaram menor taxa de oxidação do LDL-c plasmático em indivíduos saudáveis que

consumiram dieta enriquecida com amêndoa em comparação aos que consumiram dieta baixa em gordura isenta desse alimento (BERRY et al., 1992).

2.3 Consumo de nuts e função endotelial

O endotélio corresponde ao revestimento celular contínuo do sistema cardiovascular e principal regulador da homeostase vascular (HADI; CARR; AL SUWAIDI, 2005). Em situações fisiológicas, o endotélio mantém a circulação e o fluxo sanguíneo, regula o tônus vascular e modula a adesão de leucócitos e plaquetas e a transmigração de leucócitos, garantindo o funcionamento dos diferentes órgãos e tecidos corporais (DEANFIELD; HALCOX; RABELINK, 2007; HADI; CARR; AL SUWAIDI, 2005). Quando instalada, a disfunção endotelial ocorre na camada íntima das grandes e pequenas artérias, resultado de um processo inflamatório crônico acompanhado pela perda de fatores antitrombóticos e aumento nos produtos vasoconstritores e pró-trombóticos, além de aumento anormal da reatividade endotelial. Tais mudanças, além de configurarem uma das primeiras alterações detectáveis, também representam um importante contribuinte para as manifestações locais e sistêmicas da doença cardiovascular aterosclerótica (HIRASE; NODE, 2012).

A aterosclerose é o processo patológico predominante na origem das DCV e resulta da combinação entre anormalidades no metabolismo das lipoproteínas, estresse oxidativo e inflamação crônica (HERRINGTON et al., 2016; ROS, 2009). A formação da aterosclerose prevê que a injúria endotelial causada por fatores de risco cardiovascular tradicionais – tabagismo, hipertensão arterial e alteração do perfil lipídico – levem à retenção de LDL na camada íntima endotelial. Em seguida, essa lipoproteína é exposta a fatores que a transformam em formas oxidadas de LDL (MCLAREN et al., 2011). A presença de LDL-oxidada (LDL-ox) induz o endotélio a secretar citocinas que atraem células mononucleares circulantes dando início ao processo inflamatório do qual também fazem parte as moléculas de adesão, molécula de adesão intracelular 1 (ICAM-1), vascular cell adhesion 1 (VCAM-1) e E-selectina, e quimiocinas (MCLAREN et al., 2011; SINGH et al., 2002). Uma vez instalada, a aterosclerose pode causar infarto do miocárdio (IM), insuficiência cardíaca (IC), e acidente vascular cerebral (AVC) (GREAVES; GORDON, 2001).

Fatores dietéticos também apresentam um papel importante na modulação da oxidação, inflamação e, consequentemente da função endotelial (BARBOUR et al.,

2014; DAVIS; KATZ; WYLIE-ROSETT, 2007; ROS, 2004). O consumo de alimentos contendo ácidos graxos ômega-3, ácido fólico, vitaminas antioxidantes C e E, compostos fenólicos e L-arginina pode exercer efeitos benéficos sobre a reatividade vascular, seja diminuindo a ativação endotelial ou melhorando o FMD (do inglês, Flow-Mediated Vasodilatation) - técnica não invasiva padrão ouro para avaliação da função endotelial- em indivíduos saudáveis ou em pacientes com risco cardiovascular elevado (BROWN; HU, 2001; ELLINS; HALCOX, 2011). Existem evidências de que a dieta mediterrânica, caracterizada pelo elevado consumo de frutas, legumes, verduras leguminosas, sementes oleaginosas, peixe, azeite e consumo moderado de vinho, melhoram a saúde endotelial (DAVIS; KATZ; WYLIE-ROSETT, 2007).

Assim como padrões alimentares, o consumo de alimentos específicos também apresentam efeitos importantes na reatividade vascular (KRIS-ETHERTON et al., 2008). Estudos agudos demonstraram que dietas ricas em gordura saturada prejudicam a função endotelial, dado que uma única refeição rica em gordura saturada é geralmente seguida por disfunção endotelial transitória associada a lipoproteínas ricas em triglicerídeos (DE KONING; RABELINK, 2002; SANDERSON et al., 2004; WEST, 2001). Sejam agudos ou crônicos, esses efeitos prejudiciais podem ser minimizados pela administração de ácidos graxos poli-insaturados, especialmente da série n-3 e outros nutrientes presentes nas nuts, como as vitaminas antioxidantes (KRIS-ETHERTON et al., 2008). Recentemente, diversos estudos têm investigado o efeito do consumo das nuts sobre a saúde vascular (ADAMO et al., 2018; BHARDWAJ et al., 2018; HUGUENIN et al., 2015b). Segundo Salas-Salvado et al. (2014), além de reduzir a inflamação endotelial, as nuts também podem melhorar a função vascular devido ao seu conteúdo de L-arginina, um precursor de óxido nítrico (ON), o qual desempenha um papel essencial como vasodilatador endógeno.

Duas recentes metanálises demonstraram evidências dos benefícios do consumo das nuts para a saúde endotelial, especialmente sobre a resistência vascular. O consumo diário de 20-56 g de diferentes tipos de nuts (amendoim, nozes, pistache, amêndoa, amêndoa de castanha-do-Pará, pinhão, amêndoa de castanha de caju e avelã) por 4-56 semanas mostraram aumento do FMD, sugerindo melhora da função endotelial (NEALE et al., 2017; XIAO et al., 2018). No entanto, o efeito do consumo desses alimentos não resultou em diferenças significativas na adiponectina, TNF-α, IL-6, e nas moléculas de adesão ICAM-1 ou VCAM-1, sugerindo perda de

evidências a respeito do efeito do consumo de nuts sobre os marcadores sistêmicos relacionados à função endotelial (NEALE et al., 2017; XIAO et al., 2018). Em contrapartida, estudos clínicos demonstraram redução nas concentrações plasmáticas de ICAM-1, VCAM-1 e IL-6 após a inserção de nuts na dieta (ESTRUCH et al., 2006; ROS et al., 2004). Mena et al. (2009) verificaram redução de ICAM-1, IL-6 e expressão de ligantes inflamatórios por monócitos circulantes após três meses de suplementação com 30 g de uma mistura (15 g de nozes, 7,5 g de amêndoas e 7,5 g de avelãs) de nuts (MENA et al., 2009).

2.4 Consumo de nuts e estresse oxidativo

As espécies reativas de oxigênio (ERO) são produzidas em todos os organismos como subprodutos do metabolismo celular. Em quantidades moderadas, as ERO desempenham funções fisiológicas importantes estimulando a ativação de vias de sinalização em resposta a mudanças no ambiente intra e extracelular. Contudo, quando em altas concentrações as ERO provocam danos a biomoléculas como lipídios, proteínas e DNA, com potencial impacto em todo o organismo (BIRBEN et al., 2012; TU et al., 2019). Esse desequilíbrio entre a produção de ERO e sua eliminação do organismo por meio da ação de mecanismos antioxidantes é definido como estresse oxidativo. Em níveis continuamente aumentados, o estresse oxidativo pode levar a um processo inflamatório crônico, associado à gênese de diversas doenças crônicas, incluindo alterações na função endotelial, seguido de aterosclerose, a qual induz o surgimento de DCV, DM1 e 2, câncer, doenças neurológicas e pulmonares, Alzheimer, degeneração macular associada a idade, eventos cardiovasculares em doentes renais crônicos, entre outros (BEATTY et al., 2000; HE et al., 2015; HEITZER et al., 2001; KIM et al., 2015; MARITIM; SANDERS; WATKINS, 2003).

Para manter o equilíbrio redox, o organismo dispõe de um sistema de defesa antioxidante não enzimático, composto por vitaminas, oligoelementos e compostos bioativos, e um enzimático composto entre outras enzimas pela SOD, CAT e GPx, as quais metabolizam produtos oxidativos tóxicos e requerem como cofatores micronutrientes como ferro, selênio, zinco, cobre e manganês para uma ótima atividade catalítica (ANUJ et al., 2016; MASELLA et al., 2005) As enzimas superóxido dismutases (SOD1, 2 e 3) são um grupo de enzimas fundamentais que atuam como

primeira linha de defesa antioxidante em virtude da capacidade de converter radicais superóxido altamente reativos em peróxido de hidrogênio e oxigênio molecular. A catalase, por sua vez, catalisa a conversão do peróxido de hidrogênio em água e oxigênio molecular e a enzima glutationa peroxidase catalisa a redução de H_2O_2 em peróxido orgânico e água (AGUILAR; NAVARRO; PÉREZ, 2016).

Essa reposta antioxidante coordenada é regulada por meio da ativação do elemento de resposta antioxidante (ARE, do inglês, Antioxidant Responsive Elemento), localizado na região regulatória de cada gene, como resultado da ativação da via Nrf2-keap1 (KOBAYASHI; YAMAMOTO, 2005). O Nrf2 desempenha um papel central na regulação dos mecanismos de defesa celular contra o estresse provocado pelo ambiente. Em condições fisiológicas, essa proteína encontra-se principalmente no citosol ligada à proteína Keap1. Sob situações de estresse, ocorre a quebra da ligação Nrf2-Keap1 de maneira dose-dependente e a molécula livre de Nrf2 é transportada para o núcleo da célula onde se liga a pequenas proteínas denominadas MAF (do inglês, musculoaponeurotic fibrosarcoma oncogene homolog). Uma vez formado, o complexo Nrf2-MAF ativa a região gênica ARE, estimulando a expressão de genes que codificam enzimas de fase II da resposta antioxidante como hemeoxigenase 1, glutationa S-transferase, catalase, superóxido dismutase e NADPH quinona hidrogenase 1 (KOBAYASHI; YAMAMOTO, 2005; MASELLA et al., 2005; TU et al., 2019; VASCONCELOS et al., 2019).

Alguns fatores nutricionais podem ativar o Nrf2. A restrição calórica, seja crônica ou intermitente, parece ativar o Nrf2 induzindo efeitos benéficos sobre a saúde e a longevidade (VASCONCELOS et al., 2019). Além disso, ácidos graxos como EPA, DHA e polifenóis também induzem o aumento da resposta antioxidante celular por meio da ativação do Nrf2 (PANG et al., 2013; ZHOU et al., 2019). Ros (2009), sugere que os polifenóis, fitoesteróis e outros antioxidantes presentes nas nuts, podem prevenir danos oxidativos por neutralizar radicais livres e estimular a defesa antioxidante endógena. Alguns estudos têm sustentado essa afirmação por verificar melhora do status antioxidante após o consumo de uma única dose de avelã, pistache ou macadâmia (DURAK et al., 1999; GARG et al., 2007; KOCYIGIT; KOYLU; KELES, 2006). Vários estudos que avaliaram o consumo de amêndoa da castanha-do-Pará também verificaram benefícios antioxidantes evidenciados pelo aumento da atividade

da enzima GPx (COMINETTI et al., 2011a; RITA CARDOSO et al., 2016; STOCKLER-PINTO et al., 2010).

2.5 Castanhas brasileiras: Evidências clínicas de benefícios à saúde

O cajueiro (Anacardium occidentale L.) é uma árvore frutífera nativa das Américas Central e do Sul, sendo o Brasil considerado seu local de origem. Atualmente, diferentes espécies são cultivadas em regiões tropicais, e em algumas áreas subtropicais (DE PAIVA; DE BARROS; CAVALCANTI, 2009). Apesar de ser a terceira castanha mais produzida em todo o mundo, ainda existem poucos estudos que tenham investigado os efeitos do seu consumo na saúde humana (RICO; BULLÓ; SALAS-SALVADÓ, 2015). A castanha de caju (Anacardium occidentale L.) é fonte dos minerais magnésio (292 mg/100g) e potássio (660 mg/100g), de gorduras monoinsaturadas (23,5g /100g) e poli-insaturadas (7,8 g/100g), e dos antioxidantes flavonols, especialmente catequinas (5.7 mg/g) e epicatequinas (4.5 mg/g), e ácidos fenólicos (ácido elágico e ácido gálico) (PHENOL-EXPLORER, 2018; USDA, 2018).

Por sua vez, a amêndoa da castanha-do-Pará (*Bertholletia excelsa H.B.K"*) é uma espécie nativa da Amazônia. Foi descoberta originalmente crescendo em solos duros e bem drenados ao longo do rio amazonas em países como Brasil, Peru, Colômbia, Venezuela e Equador (CARDOSO et al., 2017; YANG, 2009). Assim como as demais nuts, a amêndoa da castanha-do-Pará também possui elevada densidade calórica (700 kcal/100g) devido ao alto teor de lipídios (25% de AGMI, 21% de AGPI, 15% de AGS). Além disso, é considerada uma boa fonte de micronutrientes, fitoesteróis, tocoferol e fenólicos, todos associados a diferentes benefícios para a saúde (YANG, 2009). Dentre esses micronutrientes, destaca-se o selênio, uma vez que essa amêndoa é considerada a principal fonte alimentar desse mineral, podendo acumular 100 –1000 mcg de selênio/ g^{-1} dependendo do solo da região de origem (CARDOSO et al., 2017). O selênio tem ação anti-inflamatória e antioxidante, e está associado a benefícios cardiovasculares (CARDOSO et al., 2017; DONADIO et al., 2017; JOSEPH, 2013; RAYMAN, 2012; ZULET et al., 2009).

Para melhor compreender o estado da arte a respeito dos estudos clínicos conduzidos com as amêndoas de castanha-do-Pará e caju, foi realizada uma busca sistemática na base de dados MEDLINE/PubMed em fevereiro de 2021, utilizando como descritores “Brazil nut” ou “*Bertholletia excelsa H.B.K"* para a amêndoa da

castanha-do-Pará e “cashew nut”, “cashews” ou “Anacardium occidentale L”, para a amêndoa da castanha de caju, aplicando-se os filtros “clinical trials” e “randomized clinical trial” e limitando a busca dos termos utilizados aos títulos e resumos. Ao todo, foram identificados 16 artigos com amêndoa da castanha-do-Pará e 16 artigos com amêndoa de castanha de caju. Após seleção e busca reversa, foram selecionados 16 artigos com amêndoa de castanha-do-Pará (16 artigos encontrados, 11 selecionados, 5 excluídos e 5 incluídos por busca reversa) (**APÊNDICE B**) e 7 artigos com amêndoa de castanha de caju (16 encontrados, 6 selecionados, 10 excluídos e 1 incluídos por busca reversa) (**APÊNDICE C**) que avaliaram o efeito do consumo dessas duas amêndoas sobre diferentes aspectos da saúde.

Mah et al. (2017), demonstraram que o consumo diário durante 28 dias de 28–64 g/dia de amêndoa da castanha de caju, reduziu as concentrações plasmáticas de colesterol total e LDL em homens e mulheres em risco cardiometabólico. Quando consumida em quantidade equivalente a 20% das necessidades energéticas, a amêndoa da castanha de caju não afetou os marcadores do perfil lipídico e pressão arterial de indivíduos com SM, contudo, promoveu aumento significativo da glicose de jejum (MUKUDDEM-PETERSEN et al., 2007b). Em pacientes com DM2, o consumo regular por 12 semanas de 30 g/dia de castanha de caju reduziu a pressão arterial sistólica e aumentou o HDL (MOHAN et al., 2018a).Tais benefícios parecem estar relacionadas a composição nutricional da castanha de caju, que apesar de possuir elevada densidade energética (≈ 600 kcal/100 g), apresenta alto teor de ácidos graxos insaturados (79.7 %), principalmente ácido oleico e linoleico (60,7 % e 17,8% do total de ácidos graxos, respectivamente), combinado a uma variedade de esteróis, vitaminas, aminoácidos e fibra alimentar (RICO; BULLÓ; SALAS-SALVADÓ, 2015).

Ainda, o consumo diário de castanha de caju em quantidade equivalente a 20% do valor energético total (63–108 g/dia) promoveu aumento da capacidade antioxidante total, mas não melhorou o perfil lipídico, resposta glicêmica e inflamatória em indivíduos com SM após 8 semanas de intervenção (DAVIS et al., 2007; PIETERS et al., 2005a). Segundo os autores, em virtude da forte influência da obesidade sobre os marcadores de risco cardiometabólico, o consumo de amêndoas tem benefícios limitados quando a perda de peso não é alcançada em indivíduos com excesso de peso (PIETERS et al., 2005a). Por fim, Schutte et al. (2006) e Baer and Novotny (2019), ao investigar o efeito da ingestão de amêndoa de castanhas de caju (42 – 103

g) ao longo de 8 semanas em indivíduos com SM e saudáveis, respectivamente, não observaram mudanças nas lipoproteínas, apolipoproteínas, resistência à insulina, pressão arterial e moléculas de adesão. Contudo, houve aumento na glicemia de jejum e TNF-α.

Assim como a amêndoa da castanha de caju, o efeito do consumo da amêndoa da castanha-do-Pará sobre a saúde também tem sido pouco investigado. Em sua totalidade, os estudos encontrados demonstram melhora do status do selênio após o consumo de 4 - 45 g/dia por um período de 15 dias a 6 meses. Os principais efeitos observados estão relacionados à melhora do perfil oxidativo, demonstrado pelo aumento da atividade da glutationa peroxidase (THOMSON et al., 2008). Em mulheres com obesidade mórbida, o consumo diário de 1 unidade de amêndoa castanha-do-Pará (~4 g) durante 8 semanas melhorou o estado nutricional do selênio e a atividade da glutationa peroxidase (COMINETTI et al., 2011b). Em pacientes saudáveis, um estudo agudo desenvolvido por Colpo et al. (2014), demonstrou que o consumo de 20-50 g de castanha-do-Pará promoveu redução nas concentrações plasmáticas de IL-1, IL-6, TNF-α, e IFN-γ e aumento de IL-10. Após 3 meses de intervenção, pacientes em hemodiálise também apresentaram redução da inflamação e do estresse oxidativo com o consumo diário de uma unidade de castanha-do-Pará, correspondendo a aproximadamente 5g/dia e contendo cerca de 290,5 µg de selênio/ dia (STOCKLER-PINTO et al., 2014). Nesses pacientes, também foi observado aumento da concentração plasmática de selênio, maior atividade da enzima glutationa peroxidase e redução de 8-isoprostano e danos oxidativos ao DNA. Além disso, houve aumento da concentração de HDL, contudo, colesterol total e LDL permaneceram inalterados (STOCKLER-PINTO et al., 2010, 2012, 2015a, 2015b). Entre indivíduos hipertensos e com dislipidemia, o consumo de 13 g/dia de farinha de amêndoa de castanha-do-Pará parcialmente desengordura associada a uma dieta normocalórica, promoveu redução do colesterol total, colesterol não-HDL e Apo A1 e não alterou os demais marcadores do perfil lipídico (LDL, HDL, TGL, ApoB, ApoB/ApoA), bem como pressão arterial, glicose de jejum e estresse oxidativo (CARVALHO et al., 2015; HUGUENIN et al., 2015a, 2015b). Ainda, o benefício do consumo da amêndoa de castanha-do-Pará sobre a função cognitiva também foi investigado. No estudo conduzido por Cardoso et al. (2016) em idosos com comprometimento cognitivo, houve melhora da fluência verbal após 6 meses de consumo diário de uma unidade de amêndoa de

castanha-do-Pará. Porém, nesse estudo nenhum benefício sobre os marcadores do estresse oxidativo foi observado. Em sua maioria, os benefícios observados após o consumo de amêndoa de castanha-do-Pará foram atribuídos ao aumento da concentração plasmática de selênio.

Duarte et al. (2019) e Reis et al. (2019) investigaram o efeito da adição de uma unidade de amêndoa de castanha-do-Pará (5 g) contendo elevada concentração de selênio (913,8 µg a 1687,4 µg por unidade) sobre marcadores inflamatórios, status do selênio e expressão de miRNAs em mulheres com obesidade. Após 2 meses de intervenção, houve melhora no selênio plasmático e eritrocitário, bem como aumento na atividade da GPx e expressão gênica da selenoproteína P, demonstrando melhora do estado nutricional do selênio. A resposta inflamatória também sofreu modulação. Embora em nível plasmático nenhuma alteração significativa nos marcadores inflamatórios (PCR, MCP-1, IL-6, IL-10, IL-1β, TNF-α, IFN-γ e fibrinogênio) tenha sido observada, houve aumento na expressão dos genes IL-6, IL-10, TNF-α, TLR2 e TLR4, as quais foram significativamente associadas com o aumento da concentração plasmática de selênio. Os autores discutem que a alta dose de selênio pode ter levado ao aumento da expressão de genes associados com vias pró-inflamatórias, mas que o aumento concomitante da IL-10 pode representar um mecanismo anti-inflamatório compensatório. Em relação à expressão de miRNAs circulantes, houve aumento do miR-454-3p e miR-584-5p após o consumo da amêndoa da castanha-do-Pará, ambos associados à ativação da via VDR/RXR, sugerindo o papel do selênio na homeostase óssea.

Vale ressaltar que alguns dos artigos publicados, tanto com amêndoa de castanha-do-Pará quanto com amêndoa da castanha de caju, são frutos de um mesmo estudo clínico, por vezes replicando resultados. Assim, de acordo com a literatura foram conduzidos somente 7 estudos clínicos com amêndoa de castanha-do-Pará, um deles utilizando um subproduto (farinha de amêndoa de castanha-do-Pará parcialmente desengordura) e 4 estudos com amêndoa de castanha-de-caju, destacando a necessidade de condução de mais estudos com esses alimentos.

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3 OBJETIVOS

3.1 Objetivo geral

Avaliar o efeito da ingestão diária de amêndoas de castanhas brasileiras associadas a uma dieta com restrição calórica sobre a composição corporal, a função endotelial e o estresse oxidativo de mulheres em risco cardiometabólico.

3.2 Objetivos específicos

- Caracterizar as voluntárias quanto ao risco cardiometabólico e variáveis antropométricas e da composição corporal;
- Avaliar o efeito da ingestão diária das castanhas sobre:
    - Marcadores de risco cardiometabólico (antropometria, composição corporal, CT, LDL-c, HDL-c, VLDL, TGL, Apo A, Apo B, Apo E, glicose de jejum, insulina de jejum e índice TyG);
    - Marcadores da função endotelial (ICAM-1, VCAM-1, óxido nítrico, pressão arterial e índice tornozelo-braquial);
    - Marcadores de estresse oxidativo (glutationa peroxidase, superóxido dismutase, malondialdeído, FRAP e LDL-ox);
    - Selênio plasmático;
- Comparar entre o grupo controle e o grupo teste os efeitos da intervenção sobre a composição corporal, a função endotelial e o estresse oxidativo.

4 METODOLOGIA GERAL

4.1 Aspectos éticos

Os procedimentos descritos no presente projeto de pesquisa estão de acordo com a Resolução CNS/466 de 2012 e Declaração de Helsinki, que tratam dos princípios éticos da beneficência e não maleficência na pesquisa clínica. O mesmo, encontra-se aprovado pelo Comitê de Ética em Pesquisa com Seres Humanos da Universidade Federal de Viçosa (CAAE: 92004818.0.0000.5153; N: 2.832.601) **(ANEXO 1)**. Todas as mulheres selecionadas foram informadas sobre os objetivos e procedimentos inerentes ao estudo e assinaram o Termo de Consentimento Livre e Esclarecido em duas vias (**APÊNDICE D**).

4.2 Delineamento do estudo

Trata-se de um estudo clínico, randomizado controlado, paralelo com oito semanas de duração, conduzido com mulheres em risco cardiometabólico. O estudo foi desenvolvido no Laboratório de Metabolismo Energético e de Composição Corporal (LAMECC) do Departamento de Nutrição e Saúde da Universidade Federal de Viçosa, Minas Gerais-Brasil.

4.3 Critérios de inclusão

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

- Mulheres adultas com idade de 20 a 55 anos;
- Excesso de peso (≥ 27 kg/m^2), perímetro da cintura elevado (≥ 80 cm) e excesso de gordura corporal (≥ 32%) associados a pelo menos um outro componente da Síndrome Metabólica: triglicerídeos ≥150 mg/dL, pré-hipertensão arterial (>130 e/ou >85 mmHg para pressões arteriais sistólicas e diastólicas, respectivamente) ou glicemia de jejum >100 mg/dL; ou mulheres com obesidade (BMI ≥ 30 kg/m^2), independente da presença de alterações metabólicas.

4.4 Critérios de exclusão

Foram excluídos do estudo participantes com:

1. IMC < 27 kg/m^2, perímetro da cintura < 80 cm, percentual de gordura corporal < 32%, e triglicerídeos <150 mg/dL e/ou pressão arterial sistólica <130 e/ou diastólica <85 mmHg e/ou glicemia de jejum <100 mg/dL;
2. Gestantes, lactantes, ou mulheres na menopausa;
3. Atletas;
4. Fumantes;
5. Com histórico de HIV, doença ou alterações digestiva, hepática, renal, cardiovascular, tireoide, câncer, doenças inflamatórias e desordens alimentares;
6. Com histórico de abuso de drogas e/ou álcool;
7. Com aversão ou alergia a castanhas;
8. Com episódio infeccioso no último mês;
9. Que faziam uso de medicamentos anti-inflamatórios, corticoides, antibióticos e outros que possam afetar o apetite e metabolismo energético;

10. Com instabilidade ponderal (5% do peso usual) nos últimos 3 meses;
11. Com consumo habitual de castanhas > 30 g/dia;
12. Com consumo de álcool > 21 unidades (≈168g) por semana;
13. Com hábitos alimentares restritivos: veganos e vegetarianos.
14. Com problemas dentários que interfiram na mastigação.

4.5 Cálculo amostral

Para estimar o tamanho amostral utilizou-se a variável principal peso corporal, adotando-se uma diferença esperada de 4 kg, com base na restrição calórica proposta, e um poder estatístico de 96% (α ≤ 0,05). Foi utilizado como referência os valores de baseline do estudo “Acute Effect of Coconut Oil Consumption does not Affect Postprandial Human Cytokines in Healthy Overweight Women” (MAYUMI et al., 2016). A amostra necessária foi estimada com base na fórmula proposta por mera; Thompson; Prasad. (1998), conforme apresentado abaixo:

$$n = 2 \times \left(\frac{t\alpha/2 \times \sigma}{E}\right)^2$$

tα/2 = 2.235

σ = 3.79

E= 4 kg

tα/2 = Valor da tabela de distribuição t (two-tailed), segundo o grau de liberdade
DP = desvio padrão
E = diferença que se deseja detectar no estudo

Assim, considerando uma taxa de desistência de 30% foram necessárias pelo menos 12 mulheres por grupo de intervenção.

4.6 Recrutamento e triagem

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

Após o primeiro contato, era realizada uma pré-triagem telefônica ou via e-mail. As participantes pré-selecionadas eram convidadas a uma visita presencial para confirmação da elegibilidade, onde respondiam a um questionário semiestruturado com questões referentes a história clínica e alimentar, dados sociodemográficos, antropométricos e de composição corporal, pressão arterial e exames bioquímicos (triglicerídeos e glicose de jejum), quando necessário. Se disponível, as participantes poderiam apresentar exames atuais (últimos 3 meses), caso contrário, eram encaminhadas a um Laboratório de Análises Bioquímicas local para realizar os exames gratuitamente.

Após confirmação dos critérios de inclusão, cada participante foi informada sobre todos os procedimentos inerentes ao estudo, e aquelas que estavam de acordo assinavam o Termo de Consentimento Livre Esclarecido (TCLE).

4.7 Run-in

Um período de run-in ou pré-tratamento de 7-10 dias foi aplicado para identificar e excluir participantes com probabilidade de não adesão ao protocolo do estudo. Nessa fase, as participantes foram orientadas a manter sua dieta habitual com restrição ao consumo de qualquer tipo de oleaginosas, frutos secos tipo berries (cranberry, blueberry, gojiberry e uvas passas), açaí, cacau, canela, azeite de oliva e bebidas alcoólicas. Ao final do período de run-in, as participantes tinham o peso aferido e aquelas que apresentarem variação superior a ± 1 kg, ou que reportaram o consumo de algum dos alimentos e/ou bebidas restringidos, foram consideradas más respondedoras e excluídas do estudo.

4.8 Randomização

Após completar o período de run-in, as participantes foram randomicamente alocadas na proporção de 1:1 em um dos quatro grupos de intervenção:

1) Controle: Restrição calórica (- 500 kcal/dia) sem consumo de castanhas (n= 19);

2) Mix de nuts: Restrição calórica (- 500 kcal/dia) contendo mix de castanhas (15g de amêndoa de castanha-do- Pará + 30g de amêndoa de castanha de caju) (n= 21);

Para a randomização foi empregado o método de Minimisation com o objetivo de garantir equilíbrio entre os grupos quanto a diferentes fatores prognósticos, definidos a partir do potencial para interferir nas variáveis de desfecho (ABRAMSON, 2011). No presente estudo, foram considerados como fatores prognósticos idade, IMC, e % de gordura. Para realizar a randomização utilizou-se o software WinPepi, versão 11.65 (Copyright J.H. Abransom, Aug, 23, 2016).

4.9 Intervenção

O período de intervenção teve duração de oito semanas e consistiu no seguimento de uma dieta restrita em calorias (-500 kcal) e isenta de amêndoas de castanhas pelas mulheres alocadas no grupo controle e dieta com restrição calórica (-500 kcal/ dia) contento um mix de 45g (15g de amêndoa de castanha-do-Pará + 30g de amêndoa de castanha de caju) de amêndoas de castanhas brasileiras por aquelas alocadas no grupo intervenção (**Figura 4.9.1**).

Ao início e ao final do estudo as participantes compareceram ao LAMECC em estado de jejum (10 -12h), onde foram submetidas a avaliação antropométrica e da composição corporal, aferição da pressão arterial e índice tornozelo-braquial e coleta de amostras de sangue. No dia anterior, todas as voluntárias consumiram um jantar padrão, o qual consistiu em um sanduíche de frango com suco de uva industrializado, para minimizar os impactos nos marcadores de jejum (**Ver sessão Jantar padronizado**). Nas quarenta e oito horas anteriores aos procedimentos iniciais e finais, todas as mulheres eram orientadas a evitar o consumo de alimentos termogênicos (café, alimentos e bebidas contendo cacau, canela, frutos secos, chás e bebidas alcoólicas) e a prática de atividade física intensa.

No primeiro dia de intervenção, as participantes receberam o plano alimentar, contendo cinco dietas restritas em calorias nutricionalmente balanceadas. Além disso, também foram orientadas a não alterar o padrão de atividade física durante o estudo e a reportar qualquer mudança no tipo/dosagem de medicamentos de uso contínuo. Ao longo do período de oito semanas, quinzenalmente as participantes compareciam a visitas de monitoramento nutricional para avaliação da adesão ao protocolo do estudo por meio da aferição do peso corporal e avaliação do consumo alimentar por meio da aplicação de recordatório alimentar de 24-horas. Na ocasião, o mix de amêndoas de castanhas também era distribuído. Cada participante recebia 15

pacotes, quantidade suficiente para consumo diário até a data da consulta seguinte. Quando necessário, foi fornecido um adicional de 20% para as participantes que relataram eventual distribuição a familiares. O controle do consumo do mix de amêndoas de castanhas foi feito por meio da contagem dos pacotes retornados. Além disso, ao início e final da intervenção foi realizada a dosagem de selênio plasmático. Para maior padronização, todas as participantes foram orientadas a ingerir as amêndoas de castanhas em substituição a colação. Além disso, durante o período de intervenção, foi vetado o consumo de qualquer outro tipo de amêndoa de castanha às participantes dos grupos mix. Ao grupo controle (CT), por sua vez, não foi permitido o consumo de nenhum tipo de amêndoa de castanha durante o período de intervenção.

Ainda, ao início do estudo todas as participantes receberam uma cartilha contendo informações referentes ao estudo como: objetivos, procedimentos, cuidados necessários nos dias anteriores ao comparecimento ao laboratório e orientações para o preparo de alimentos.

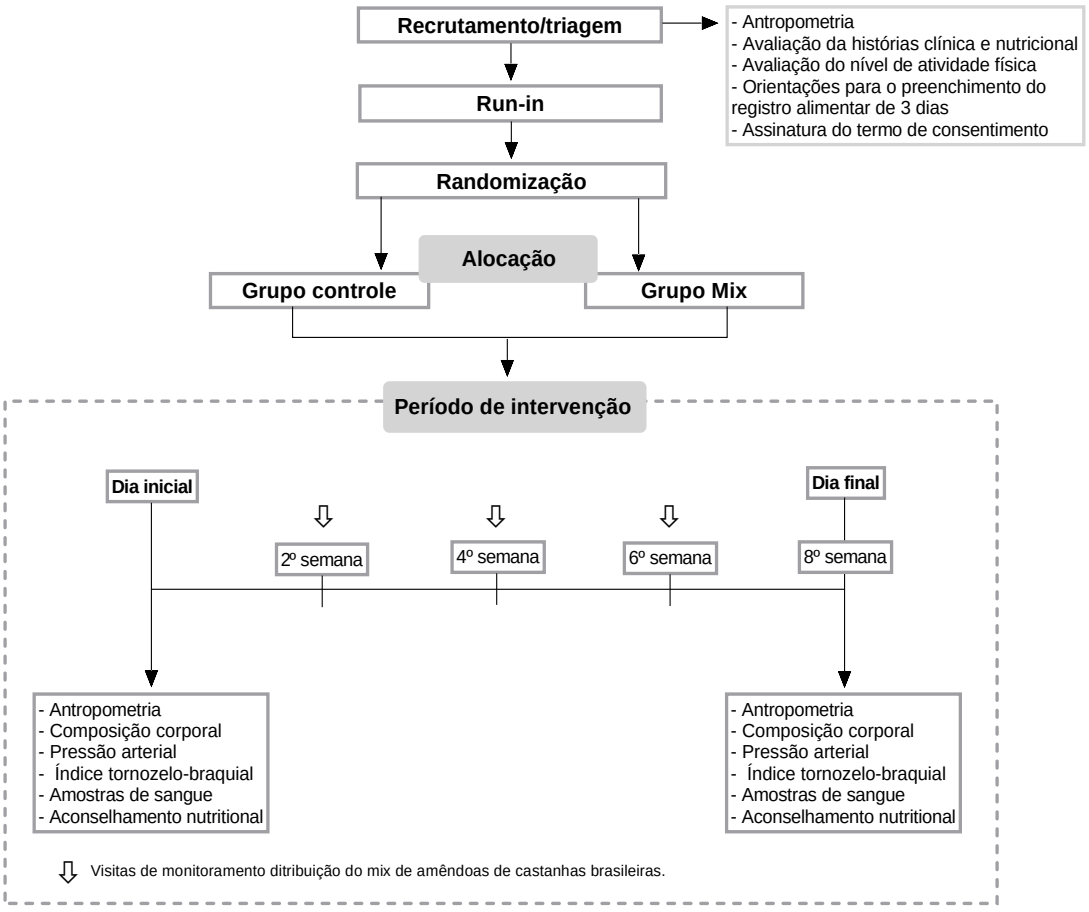

Figura 4.9.1: Fluxograma do estudo.

4.9.1 Plano alimentar

A todas as mulheres foi prescrito um plano alimentar contendo cinco dietas nutricionalmente balanceadas, cada dieta contendo cinco refeições (café da manhã, colação, almoço, lanche da tarde e jantar). A necessidade energética foi estimada a partir do Estimated Energy Requirement (EER) para mulheres adultas com excesso de peso e obesidade (IOM, 2002). Em seguida, 500 kcal foram subtraídas para a prescrição dietética. A média de distribuição de macronutrientes foi 45,4% de carboidratos, 22,0% de proteínas e 32,6% de lipídios. Para o grupo mix a dieta foi calculada contabilizando a energia fornecida pela porção diária do mix de amêndoas de castanhas. Devido ao alto teor de gordura incrementado à dieta a partir do mix de amêndoas de castanhas, foi recomendado ao grupo controle o consumo de duas colheres de sopa (duas vezes ao dia no almoço e no jantar) de molho de salada à base de óleo de soja e limão, na proporção de 2:1. O molho foi preparado pelos pesquisadores e suas calorias também foram contabilizadas na dieta prescrita ao grupo controle. Ambos, mix de amêndoas de castanha e molho de salada foram fornecidos quinzenalmente durante as visitas de monitoramento nutricional.

Todas as voluntárias receberam aconselhamento nutricional individualizado por nutricionistas treinadas. Ao longo do período de intervenção as participantes foram orientadas a utilizar apenas óleo de soja no preparo das refeições.

4.9.2 Mix de amêndoa de castanhas

As castanhas utilizadas no estudo foram doadas pela Embrapa Agroindústria Tropical – Fortaleza - Ceará (castanha de caju) e pela empresa Inovam Brasil® (castanha-do-brasil). Todas as castanhas recebidas foram selecionadas manualmente para eliminação daquelas que se apresentarem defeituosas e/ou danificadas. Em seguida, as castanhas selecionadas foram porcionadas em embalagens laminadas, seladas à vácuo (Seladora Selovac modelo 200 B) e acondicionadas em freezer a -20ºC até o momento da distribuição às voluntárias.

A quantidade de amêndoa de castanha-do-Pará contida no mix foi definida com o objetivo alcançar a necessidade diária de selênio. O teor desse mineral na amêndoa de castanha-do-Pará foi mensurado por espectrometria de emissão atômica por plasma acoplado indutivamente (FOOD AND DRUG ADMINISTRATRION, 2010) e cada porção de 15g contém aproximadamente 51µg de selênio. Para definir as 30g

de amêndoa de castanha de caju, utilizamos como referência estudos anteriores que investigaram os benefícios cardiovasculares do consumo de amêndoas de castanhas (ESTRUCH et al., 2013; MOHAN et al., 2018b; TAPSELL et al., 2004).

4.9.3 Jantar padronizado

Nos dia anteriores à coleta de sangue e demais procedimentos, todas as voluntárias receberam um jantar padronizado, o qual foi consumido em casa antes do jejum noturno. Essa refeição consistiu em um sanduíche de frango acompanhado de suco industrializado conforme especificado na tabela abaixo (**Tabela 4.9-1**).

Tabela 4.9-1. Composição nutricional do sanduíche de frango (jantar padrão).

Alimentos	Quantidade (g)	Energia (kcal)	Proteína (g)	Lipídios (g)	Carboidratos (g)	Fibra Alimentar (g)
Suco de uva Tial®	200	104	0	0	26	0
Frango, peito, sem pele, cozido	100	119	21,5	3	0	0
Pão, trigo, forma, integral	50	126,5	4,7	1,85	24,95	3,45
Requeijão Cremoso Integral	45	116,4	4,3	10,5	1,1	0
Cenoura, crua	24	8,16	0,31	0,04	1,84	0,76
Milho Verde	24	84,2	0,1	0	20,9	0,2
Molho de Tomate	12,5	5,2	0,12	0	0,95	0,18
Cebola	10	3,9	0,17	0,01	0,89	0,22
Óleo de Soja	1g	8,8	0	1	0	0
Sal	0,5	0	0	0	0	0
TOTAL	421	577,2	31,2	16,4	76.6	4,81

CHO: carboidratos; LIP: lipídios; PTN: proteínas.

4.9.4 Critérios de descontinuidade do estudo

Durante as 8 semanas de intervenção, as voluntárias que não apresentaram boa adesão ao protocolo do estudo (< 80%), que manifestaram qualquer reação adversa ao consumo das castanhas, que engravidaram ou que manifestaram sintomas da menopausa (diagnosticados pelo médico) foram excluídas do estudo.

4.10 Análise da composição das castanhas

4.10.1 Caracterização físico-química

Os teores de umidade, proteína, lipídios, cinzas e sólidos solúveis foram determinados, respectivamente, pelos métodos nº 934.01, nº 984.13, nº 930.05, nº

930.05 e nº 932.12 da AOAC (1995). A quantidade total de carboidratos foi calculada por diferença: 100 – (% água + % proteína + % lipídios + % cinzas).

4.10.2 Análise de minerais

Para a análise de minerais, as amostras foram digeridas conforme procedimentos descritos em Miyazawa et al. (2009), com algumas modificações. Em um tubo de digestão foram adicionados 1 g de amostra e 8 mL de solução de ácido nítrico e ácido perclórico (3:1, v/v) e mantido em temperatura ambiente por uma noite. Posteriormente, a digestão foi feita em bloco digestor a 200 ℃ por 4 horas. Após o resfriamento o extrato foi transferido para balão volumétrico de 50 mL, completado o volume com água deionizada e filtrado em papel quantitativo de filtragem lenta. Brancos foram preparados de maneira semelhante às amostras. A quantificação de fósforo, potássio, cálcio, magnésio, enxofre, sódio, cobre, ferro, zinco e manganês foi feito em espectrômetro de emissão óptica por plasma acoplado indutivamente (ICP-OES).

4.10.3 Perfil de ácidos graxos

O perfil de ácidos graxos foi analisado na fração lipídica das amêndoas de castanha-do-Pará e de caju por meio de cromatografia gasosa, a qual foi realizada no Laboratório BIOAGRO/UFV em Cromatógrafo a gás modelo CG Solution marca SHIMADZU, equipado com detector FID. Foram utilizados os seguintes padrões: ácido graxo palmítico (C16:0), ácido graxo esteárico (C18:0), ácido graxo oleico (C18:1), ácido graxo linoleico (C18:2) e ácido graxo linolênico (C18:3). Para registro e análise dos cromatogramas, o aparelho foi acoplado a um microcomputador, utilizando-se o programa GC Solution. Os compostos foram separados e identificados em uma coluna capilar Omegawax (30 m x 0,25 mm). Para a separação cromatográfica, 1 µL de amostra será injetado com auxílio de seringa de 10 µL (Hamilton®) em sistema Split = 10. O gás Nitrogênio foi utilizado como carreador com velocidade linear programada para 43.2 cm/s e os gases Hidrogênio e Ar sintético formaram a chama no detector. As temperaturas do Injetor e do Detector foram controladas isotérmicas em 220ºC e 240ºC. A temperatura inicial da coluna foi de 150℃ (mantida por 5 minutos), e

aumentando em 4℃ por minuto até atingir 220. O fluxo do gás de arraste na coluna foi de 0,8 mL/minuto.

4.11 Avaliações e mensurações

4.11.1 Padrão de Atividade Física

O padrão de atividade física foi avaliado por meio do questionário IPAQ (International Physical activity Questionnaire), versão curta, proposto por Ainsworth et al. (2000) e classificado de acordo com FAO/WHO/UNU (AINSWORTH et al., 2000; FAO/WHO/OUNU, 2001; PARDINI et al., 2001). Todas as voluntárias foram orientadas a manter o padrão de atividade física durante todo o estudo.

4.11.2 Avaliação do consumo alimentar

O consumo de alimentos foi avaliado por meio de registros alimentares por três dias não consecutivos (um final de semana e dois dias da semana). Os dados de consumo foram 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) (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA, 2010). Os alimentos que não estavam contidos no banco de dados foram adicionados pelos entrevistadores. As quantidades consumidas dos alimentos foram convertidas em medidas de massa (em gramas) e / ou volume (mililitros) para relacionar os dados de consumo de alimentos a uma tabela de composição nutricional no software SPSS (versão 22.0) e, em seguida, estimamos a ingestão de energia e nutrientes (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA - IBGE, 2011).

4.11.3 Antropométrica e composição corporal

O peso das voluntárias foi aferido utilizando-se balança eletrônica microdigital, capacidade de 150kg e precisão de 100g, vestindo roupas leves. A altura foi determinada por meio de antropômetro vertical milimetrado, com extensão de 2,2 m e escala de 0,5 cm. Em ambas as situações as voluntárias encontravam-se em pé, em posição firme, com os braços relaxados e cabeça no plano horizontal. O índice de massa corporal (IMC) que relaciona o peso (kg) e a altura (metros) ao quadrado também foi calculado.

O perímetro da cintura foi aferido com a voluntária em pé, usando uma fita métrica flexível e inelástica durante a expiração normal, sendo esta aferida sobre a cicatriz umbilical. Medidas do perímetro da cintura ≥ 80 cm foram adotadas para a classificação da obesidade abdominal (IDF, 2005). O perímetro do quadril foi aferido na região de maior proeminência entre os quadris e as nádegas também com a voluntária em pé.

A composição corporal foi avaliada por meio da técnica de DEXA (Dual-energy X-ray Absortiometry) com tecnologia fan-beam (Lunar Prodigy Advance DXA System, versão 13,31, GE Lunar), seguindo o protocolo recomendado pelo fabricante. Ademais, como método alternativo, a bioimpedância elétrica tetrapolar (Bioimpedância tetrapolar, Inbody, modelo Y230) também foi utilizada para a estimativa do percentual de gordura corporal total, porcentagem de massa magra e porcentagem de água corporal total, de acordo com as recomendações do fabricante.

4.11.4 Coletas de amostras biológicas

Ao início e ao final da intervenção, todas as voluntárias foram submetidas à coleta de sangue em jejum realizada por um profissional capacitado. Foram coletados 16 mL de sangue em tubos adequados para sorologia, tubos EDTA e tubos para análise de elementos traços. Os tubos permaneceram em repouso por 20 minutos a 4ºC e em seguida foram centrifugados à 3500 rpm por 15 minutos à 4 ℃ para retirada de soro, plasma e eritrócitos. O material coletado foi aliquotado em microtubos e armazenado em ultra-freezer (Thermo Scientific/Forma 900 Series®) a -80º C para posteriores análises.

4.11.5 Marcadores de risco cardiometabólicos

Parte das determinações bioquímicas do presente estudo foram realizadas no Laboratório de Análises Clínicas do Departamento de Nutrição e Saúde da Universidade Federal de Viçosa em autoanalisador (Mindray / BS-200® Chemistry Analyzer) de acordo com a metodologia dos kits comerciais (Bioclin®). Foram dosados por meio de testes colorimétricos enzimáticos a concentrações séricas de glicose, triglicerídeos, colesterol total, lipoproteína de alta densidade - colesterol (High Density Lipoprotein- cholesterol - HDL), lipoproteínas de baixa densidade – colesterol (Low Density Lipoprotein-cholesterol - LDL). A concentração da lipoproteína de muito baixa

densidade – colesterol (Very Low Density Lipoprotein-cholesterol - VLDL) foi calculada pela fórmula proposta por Friedewald; Levy; Fredrickson (1972). Os índices aterogênicos, colesterol total:HDL e LDL-c:HDL propostos por Castelli (1988), também foram calculados.

A determinação da concentração sérica de insulina foi realizada utilizando imunoensaio de eletroquimioluminescencia (electrochemiluminescence immunoassay - ECLIA em laboratório terceirizado. A concentração sérica de proteína C reativa ultrassensível (PCR-us) foi determinada utilizando-se kit comercial específico (Quibasa -Química Básica) empregando-se a técnica de imunoturbidimetria em auto-analisador (Mindray / BS-200® Chemistry Analyzer). Foram excluídos da análise dos dados valores de PCR-us superiores a 10 mg/dL por serem indicativo de inflamação aguda (PEARSON, 2003). As apolipoproteínas foram dosadas por meio do método de sistemas de Imunoquímica IMMAGE®. Para determinar o grau de resistência insulínica foi calculado os índice Triglyceride-glucose index (TyG), recentemente sugerido por Simental-mendía; Rodríguez-ramírez; Reyes-romero (2013), segundo a fórmula indicada a seguir:

TyG: Ln [(triglicerídeos de jejum (mg/dL)) x (glicemia de jejum (mg/dL))/2]

4.11.6 Marcadores da função endotelial

As moléculas de adesão solúveis ICAM-1, VCAM-1 foram analisadas em plasma por meio da técnica de ELISA utilizando kits comerciais (Elabscience Biotechnology Co., Ltd, USA) de acordo com as recomendações do fabricante.

A pressão arterial sistólica e diastólica das voluntárias foi aferida com o uso de um medidor de pressão automático Omron® modelo HEM-7113 após repouso de 10 min. A voluntária estava de bexiga vazia, na posição sentada, com pernas descruzadas, pés apoiados no chão, dorso recostado na cadeira e relaxadas. O braço foi posicionado na altura do coração, livre de roupas, apoiado com a palma da mão voltada para cima e o cotovelo ligeiramente fletido (VI DIRETRIZES BRASILEIRAS DE HIPERTENSÃO, 2010). Na primeira avaliação, as medidas foram obtidas em ambos os braços e, em caso de diferença, o braço com o maior valor foi utilizado como referência para as medidas subsequentes (WHO, 2007).

O ITB foi estimado após repouso durante 10 minutos com a voluntárias na posição supina. A pressão arterial sistólica (PAS) foi aferida em ambos os braços na

artéria braquial e na artéria pediosa e tibial posterior em ambos os tornozelos. Em seguida, o ITB foi determinado pela divisão entre o maior valor de PAS aferido no tornozelo e o maior valor de PAS braquial. O ITB foi calculado para cada perna e o menor valor foi adotado o como ITB geral da voluntária (KIM; WATTANAKIT; GORNIK, 2012). Para assegurar a correta ausculta da pulsação foi utilizado um Doppler vascular portátil (MEDMEGA®, DV 610B). A partir dos valores de ITB obtidos, as participantes foram classificadas em normais (1-1.4), limítrofe (0,91 – 0,99) ou em risco cardiovascular aumentado independente da presença de sintomas de Doença Arterial Periférica (≤ 0,90 e > 1.40) (ABOYANS et al., 2012)

A dosagem do óxido nítrico (ON) plasmático foi realizada utilizando-se o reativo de Griess para detecção do nitrito usado como indicador da síntese de ON de acordo com protocolo proposto Grisham; Johnson; Lancaster (1996). As análises de ON foram realizadas em amostras de plasma em triplicata. A concentração do óxido nítrico das amostras foi determinada utilizando curva padrão com concentrações conhecidas de nitrito de sódio que variaram de 0-125µM/L. Os valores finais foram expressos em µmol/L.

4.11.7 Marcadores de estresse oxidativo

Todos os marcadores de estresse oxidativo foram dosados em triplicata em amostras de plasma por meio de ensaios colorimétricos.

A determinação da Superóxido dismutase (SOD) baseou-se na capacidade desta enzima em catalisar a reação do superóxido ($O2^{\bullet-}$) em peróxido de hidrogênio (H^2O^2) diminuindo assim a razão de auto-oxidação do pirogalol (MARKLUND; MARKLUND, 1974). A leitura foi feita a 570nm e os valores expressos em U de SOD/L de plasma. A dosagem do malondialdeído (MDA) baseou-se na dosagem das espécies reativas ao ácido tiobarbitúrico (TBARS). Foi utilizado o método descrito por Buege e Aust, (1975) com modificações (BUEGE; AUST, 1975). A concentração de MDA na mostra foi determina a 535 nm a partir da curva padrão utilizando o TMPO (1,1,3,3-Tetrametoxipropano) como padrão, os pontos da curva variaram de 0-4 µM/L e os valores finais expressos em µmol/L. A atividade da glutationa peroxidase foi determinada por meio do kit comercial EnzymChrom™ seguindo as recomendações do fabricante. Para a determinação da capacidade antioxidante do plasma foi o utilizado o método do FRAP. Este ensaio tem como base a mensuração da habilidade

dos antioxidantes (redutores presentes no plasma) reduzirem, em condições de pH baixo, o complexo Fe^{+3}/tripiridiltriazina (TPTZ) para a forma ferrosa Fe^{+2}, o qual possui intensa cor azul. Foi utilizado o método inicialmente proposto por Benzie; Strain (1996) e a leitura realizada a 593 nm.

4.12 Análises estatísticas

As análises estatísticas foram realizadas utilizando-se o software do SPSS (versão 22.0, USA). Os testes de Shapiro-Wilk e Levene foram utilizados para avaliar a normalidade da distribuição e a homocedasticidade das variâncias, respectivamente. O teste t pareado ou o teste de Wilcoxon foram utilizados para avaliar o efeito do tempo nos tratamentos. O teste t de amostras independentes ou teste U de Mann-Whitney foram adotados para avaliar as diferenças entre os grupos. Quando apropriado, o teste de ANCOVA foi empregado na comparação de médias entre os grupos utilizando valores basais como covariáveis de ajuste. Os dados foram apresentados como média ± erro padrão da média. Foi adotado como critério de significância $\alpha = 5\%$

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5 RESULTADOS

5.1 Artigo 01: Beneficial effects of Brazilian nuts consumption in overweight women at cardiometabolic risk (Brazilian Nuts Study): study protocol of a randomized controlled trial

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Short title: Brazilian Nuts Study Protocol

Keywords: Brazil nut; cashew nut; obesity; cardiometabolic risk; selenium

ABSTRACT

Among tree nuts, Brazil nut (Bertholletia excelsa H.B.K) and cashew nut (Anacardium Occidentale L.) are considered the least studied. This study aims to evaluate the effect of daily consumption of Brazilian nuts (Brazil and cashew nuts) within an energy-restricted diet on cardiometabolic risk, energy metabolism, food intake, intestinal permeability, genetic, and chronobiological markers in overweight women at cardiometabolic risk. Here, we describe the design of the Brazilian nuts study. This is a randomized controlled parallel 8-week intervention trial conducted with women randomly allocated to 1) Control group: energy-restricted diet without nuts, or 2) Brazilian nut group (BN-group): energy-restricted diet containing 15 g of Brazil nuts + 30g of cashew nuts. The present investigation about the effect of Brazilian nuts appears promising and little explored. Through this randomized clinical trial, we will provide high-quality evidence about the beneficial effects of Brazilian nut consumption

on human health. **Trial registration:** Brazilian Registry of Clinical Trials: RBR-3ntxrm. Registered February 27, 2020. http://www.ensaiosclinicos.gov.br/rg/RBR-3ntxrm/.

Keywords: Brazil nut; cashew nut; obesity; cardiometabolic risk; selenium

INTRODUCTION

Defined as abnormal or excessive fat accumulation harmful to health, overweight and obesity are recognized as a severe public health concern. Since 1975, the prevalence of obesity more than doubled [1]. According to the World Health Organization (WHO), more than 1.9 billion people worldwide are overweight, of whom 650 million are obese. Obesity is closely associated with metabolic alterations, which act as a trigger for chronic non-communicable diseases, especially cardiovascular disease [2].

Towards body weight reduction, therapeutics approaches that target moderate calorie reduction (500 to 1,000 kcal/day) for a weight loss between 0.5 to 1.0 kg per week associated with the healthy dietary pattern are indicated for more effective weight loss [3,4]. Even a moderate 5% weight loss has considerable health benefits, such as reduction of intra-abdominal adipose tissue, intrahepatic triglyceride content, systolic blood pressure, and plasma triglyceride concentration, besides increase multi-organ insulin sensitivity and β cell function, leading for the reduction of cardiometabolic risk [5]. For this reason, new treatments for chronic disease have been studied to evaluate the effect of food-based strategies on metabolic disruption, body weight, and associated comorbidities [6,7].

Current evidence suggest that nuts consumption can modulate lipid profile, glycemic control, blood pressure, oxidative stress, appetite, microbiota, gene expression, suppressing the hunger and desire to eat, and increase the fullness sensation; all direct or indirectly linked to cardiometabolic risk [8–19]. Some nutrients from nuts as phytosterols, fiber, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) contribute to their healthy effect. Brazil and cashew nuts are traditional nuts consumed in Brazil and still considered least studied. Brazil nut (Bertholletia excelsa H.B.K) is a native species to the Amazon, discovered growing along the Amazon River in countries like Brazil, Peru, Colombia, Venezuela, and Ecuador [20,21]. Besides being the main food source of selenium (100-1000 mg of

selenium/ g-1) 21, Brazil nut also contains phytosterols, tocopherols, squalene, and phenolics, which are associated with health benefits related to its anti-inflammatory and antioxidant activities [20–23]. The cashew tree (Anacardium Occidentale L.) is native to Central and South America, being Brazil thought its country of origin [24–27]. In addition to its pleasant taste, cashew nuts have interesting nutritional properties, such as a high lipids content, predominantly MUFA and PUFA, that are associated with reduction of cholesterol, low-density lipoprotein concentrations, and cardiovascular events [26].

However, there is insufficient evidence about the benefits of the daily consumption of Brazilian nuts. In addition, their effect on intestinal permeability and chronobiological markers have never been investigated. The present randomized controlled parallel 8-week clinical trial aims to investigate the effect of daily consumption of Brazilian nuts (Brazil nut, Bertholletia excelsa H.B.K and cashew nut, Anacardium Occidentale L.) associated with an energy-restricted diet on weight loss and body composition, energy metabolism, food intake, cardiometabolic risk, intestinal permeability, and chronobiological and genetic markers in overweight women at cardiometabolic risk. We hypothesized that the consumption of Brazilian nuts within an energy-restricted diet will potentiate the benefits of energy restriction, one of the most traditional approaches in the management of obesity and its comorbidities.

METHODS

Study Design

The Brazilian Nuts Study was designed according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 checklist [28]. This is a randomized controlled parallel 8-weeks clinical trial, with overweight or obese women at cardiometabolic risk conducted in free-living conditions. The study will be carried out in the Department of Nutrition and Health of Universidade Federal de Viçosa-MG, Brazil. The study schedule is presented (**Table 5.1.1**).

Ethical aspects

The study protocol will be conducted following the guidelines of the Helsinki Declaration and was approved by the institutional review board of the Universidade Federal de Viçosa (registration number: CAAE: 92004818.0.0000.5153; N: 2.832.601/ 2018). All participants will be informed about objective and study procedures. Those

that accept the study conditions will be asked to provide written informed consent. Furthermore, this study was registered on the Brazilian Registers of Clinical Trials – REBEC (protocol: RBR-3ntxrm).

Eligibility criteria

Will be considered eligible to study adult women (20-55 years), with overweight (BMI ≥ 27 kg/m² and <30 kg/m²), high waist circumference (≥ 80 cm) and body fat ≥ 32%, associated with at least one another component of metabolic syndrome: triglycerides ≥150 mg/dL, high arterial blood pressure (≥130/85 mmHg) or fasting glucose (≥100 mg/dL); or obese women (BMI ≥ 30 kg/m2), regardless of the presence of metabolic complications. Exclusion criteria included: pregnant, breastfeeding or menopausal women; athletes; vegans; smoking; women with a history of diseases such as digestive illness; dysfunction in liver, kidney, or thyroid; cancer, HIV, inflammatory or cardiovascular diseases; 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 (Δ higher than 5% in the last three months); usual consumption of any type of nut greater than 30 g/day; daily consumption of Brazil nuts greater than 5 g/day; alcohol consumption greater than 21 units (168g) per week; dental problems that interfere with chewing; consumption of vitamin, mineral and omega 3 supplements.

Sample size

The sample size was calculated based on the primary outcome (body weight), with a significance level of α = 0.05 and 96% power, based on the two-sided t-test. We calculated the required sample size using the formula proposed by Mera; Thompson; Prasad (1998) [29], adopting 4 kg as weight-loss anticipated (based on the energy-restricted diet prescribed). Were estimated of nine women the sample size for study conduction. To compensate for potential dropouts, the sample was increased by 30% yielding a sample size of at least twelve women for each group.

Randomization

Participants will be allocated 1:1 in one of the two intervention groups: a) Control group: energy-restricted diet (- 500 kcal / day) without nut consumption (n = 19); or b) Brazilian nuts group (BN-group): energy-restricted diet (- 500 kcal / day) containing a daily Brazilian nuts portion (15 g of Brazil nuts + 30 g of cashew nuts) (n = 21). The minimization method will be used to ensure the balance of predefined prognostic factors between groups [30]. In the present study, age, BMI, and body fat percentage will be considered prognostic factors due to their potential to interfere in the outcome variables. The randomization will be performed by the researchers using the WinPepi software, version 11.65 (Copyright J.H. Abransom, Aug 23, 2016).

Recruitment

Recruitment and selection of participants will take place at Viçosa, Minas Gerais, Brazil. Advertisements in social media and flyers will be used as recruitment methods. The women who contact the researchers will receive a link to an online screening survey with essential eligibility criteria (age, body weight, height, pregnancy, menopausal, and medical/supplement use) to investigate if they are eligible for the study. Those who were pre-selected will be invited to a face-to-face visit for a complete screening with the evaluation of health history, physical activity level, anthropometric evaluation, and to give more details about the study procedures. During this visit, each selected woman will provide verbal and written consent, and subsequent experimental visits will be scheduled.

Run-in period

A run-in period or pre-treatment of seven to ten days will be applied to identify and exclude the women with a probability of non-compliance to study protocol. In this period, the women will be asked to maintain the habitual diet, with restriction to intake of any type of nuts, oleaginous seeds, dried fruit, cocoa, cinnamon, olive oil, and alcoholic beverages. At the final run-in period, the women will be weighed, and those who had bodyweight variation higher than ± 1 kg or that report the consumption of not permitted foods or beverages, will be considered "poor responders”, and will be excluded from the study.

Intervention

Following baseline measurements, women will be randomized to the control or BN-group. For eight weeks, the women in the control group will consume an energy-restricted diet (See section 2.8.1 energy-restricted diet) without any type of nuts, while those in the BN-group will follow the energy-restricted diet containing 45g (30g of cashew plus 15g of Brazil nut) of Brazilian nuts daily (Figure 1). At the beginning and the end of this period, the women will be submitted for an initial and final experimental day, respectively, to assess fasting and postprandial variables, fill out questionnaires about physical activity practice, food intake, eating behavior, sleep quality, and biological chronotype. On these occasions, the women will attend to Laboratory of Energy Metabolism and Body Composition (LAMECC) in a fasting state (10-12h). They will be asked to consume a control or a Brazilian nuts drink according to the allocation group (See section 2.8.2 Test drinks). At fasting, 1, 2, and 4 hours postprandial, vacuum blood collections will be performed by a nurse. Furthermore, in fasting and every 60 minutes counted from the ingestion of the test drinks, blood pressure and subjective appetite sensation will be assessed. The resting energy expenditure (REE), diet-induced thermogenesis (DIT), and substrate oxidation also will be estimated over five sections, each with 20 minutes, at fasting and postprandial state.

At the end of the morning, after the last blood collection (4 hours postprandial), a standard lunch - approximately 1kg of pasta with bolognese sauce and 200 mL of industrialized juice – will be served for ad libitum consumption. The food that remaining will be weighed to assess the impact of test drinks on satiety. Additionally, the women will be instructed to complete a food record (free-living) from the time they left the laboratory until the end of the day to assess the long-term effect of drinks on food intake.

The women also will attend to LAMECC to evaluate intestinal permeability in two other moments nonconsecutive with experimental days, at the initial and final of intervention (See section 2.10.11 Intestinal permeability and microbiota).

Once included in the study, women will receive a brochure elaborated by researchers describing study procedures and providing instructions to be followed during the intervention period. All women will be asked to inform any change in the type or dosage of medicines of continuous use. Also, every fifteen days, the women will attend LAMECC for face-to-face nutritional advisement visits. On these occasions,

body weight and adherence to the diet will be monitored. 24-h dietary recalls will be taken on each nutritional advisement visit to evaluate food consumption. Over the study, Brazilian nuts consumption will be checked by the return of not consumed nut packages. Besides, at the beginning and the end of the study, the plasma selenium concentrations will be assessed. All women will be asked to maintain their lifestyle during the study.

Energy-restricted diet

For all women will be provided an eating plan with five nutritionally-balanced menus, each with five meals (breakfast, elevenses, lunch, snack, and dinner). The total energy intake will be estimated using the Estimated Energy Requirement (EER) for adult women with overweight or obesity [31]; then, 500 kcal/day will be deducted for the dietary prescription. The average distribution of macronutrients was 22.0%, 32.6%, and 45.4% of daily energy from proteins, lipids, and carbohydrates, respectively. For the BN-group, the diets will be calculated including the energy provided by the daily portion of 45 g of Brazilian nuts. Due to the high-fat content from Brazilian nuts, the control group will be asked to consume two tablespoons (twice a day; at lunch and dinner) of a salad dressing based on soy oil and lemon (2:1 ratio, respectively), which was prepared in the Metabolic Kitchen of LAMECC and its calories also will be included in the total caloric value of control diets. Both Brazilian nuts packages and salad dressing bottles will be handed out to women fortnightly during the face-to-face nutritional visits.

All dietary advice will be individualized and provided by dietitians every two weeks. Throughout the study period, participants will receive instructions to use only soy oil to prepare meals consumed across the day.

Test drinks

At the initial and final experimental days, participants will consume a control (without nuts) or Brazilian nuts drink, according to the allocation group, to evaluate postprandial markers. Both drinks had similar nutritional composition and sensory aspects (**Table 5.1.2**). The nuts used to prepare of test drinks will be previously separated into individual portions and stored at -20˚C until the moment of use to ensure microbiological quality and avoid losses in nutritional quality.

Brazilian nuts

The nuts used in the study were donated by Embrapa Agroindustria Tropical - Fortaleza - Ceará (cashew nuts) and by the company Inovam Brasil® (Brazil nuts). After received, all nuts will be selected manually to eliminate those that were inadequate for consumption. Then, the chosen nuts will be portioned (15g of Brazil nut and 30 g of cashew nut) in laminated packages, vacuum sealed (Selovac Sealer model 200 B), and stored in a freezer at -20ºC until distribution to the participants.

The Brazilian nuts quantity was defined based on the selenium content of Brazil nut to meet daily selenium recommendation, each portion of 15g of Brazil nut provide approximately 51µ of Selenium; the 30g of cashew nut is supported by previously published studies evaluating cardiovascular benefits of tree nuts consumption [32–34]. The physic-chemical analyses of nuts were already performed in three repetitions. Moisture, ash, protein, and total dietary fiber were determined following recommendations of Association of Official Analytical Chemists [35]. For lipids evaluation were used ANKOM Technology Method [36]. Carbohydrate concentrations were estimated by the equation: [100 – (% moisture + % fat + % protein +% total dietary fiber + % ash)]. The total energy was estimated considering the conversion factors of 4 kcal·g-1 for protein and carbohydrate, and 9 kcal·g-1for lipids. Each portion of Brazilian nuts provided approximately 10.4 g of carbohydrates, 9.2g of proteins, 18.4 g of lipids, 2.6 g of fibers, and 261.5 kcal. For mineral analyses, samples were digested as proposed by Miyazawa et al. (2009) [37]. Following, P, K, Ca, Mg, S, Na, Cu, Fe, Zn, Mn, Se were determined using inductively-coupled plasma atomic emission spectrometry (**Table 5.1.3**) according to FDA recommendations [38]. Also, the lipid profile of Brazil and cashew nut was determined by gas chromatography following the protocol proposed by Folch et al. 1957 [39] and Hartman and Lago 1973 [40]. Unsaturated fatty acids represent 75.9 % of total fat in Brazil nut and 84.67 % in cashew nut (**Table 5.1.4**).

Study compliance and Side effect monitoring

The participant's compliance will be assessed through 24-hour dietary recalls, the return of Brazilian nuts packages, bodyweight evaluated in face-to-face meetings, and the dosage of plasma selenium concentrations after the intervention period. On

each visit, women will be encouraged to report any side effects associated with Brazilian nuts consumption, such as diarrhea, flatulence, bowel discomfort, bloating, nausea, the feeling of fullness, or others. Once any side effects are identified, the participant will be withdrawn from the study. Furthermore, during the intervention period, women who do not show adherence to the study protocol, who become pregnant, or who manifested symptoms of menopause (diagnosed by the doctor), will be excluded from the study.

Outcomes

Primary and secondary outcomes

The primary outcomes of the study were the difference (final - initial assessments) in body weight and waist circumference between intervention groups, which will be measured by standardized protocols. Both anthropometric measurements represent independent risk factors for cardiovascular disease [41,42].

The secondary outcome variables are direct or indirectly related to cardiometabolic risk, such as energy metabolism evaluated by indirect calorimetry; appetite by visual analogue scale (VAS), food intake by direct food weighing method, and 24-hour diet recall; lipid and glucose profile, plasma inflammatory, oxidative stress, and genetic markers on blood samples; endothelial function on blood samples and by standardized method; blood pressure by standardized method; and intestinal permeability in urine samples.

Measurements

Physical activity, anthropometry, and body composition

All measurements will be taken at baseline and after the 8-week intervention. Physical activity level will be evaluated by the International Physical Activity Questionnaire and classified according to FAO/WHO/UNU [43–45]. Height will be measured using a stadiometer (precision 0.5 cm) and waist circumference using an inelastic tape (precision 0.1 cm). Two measurements will be taken at the umbilicus waist at the end of normal expiration, and the mean calculated. Hip circumference also will be measured twice utilizing an inelastic tape at the maximum posterior extension

of the gluteus, and the average calculated. Body composition will be assessed by dual-energy X-ray absorptiometry (DEXA) (Lunar Prodigy Advance DXA System, GE Lunar) in a subsample (73.3% for the control group, n=11; 71.5% for the BN-group, n= 10) due to the equipment schedule availability.

Dietary assessment

At the beginning of the intervention, all women will be asked to fill a 24-hour dietary record for three nonconsecutive days (two weekdays and one weekend day) for evaluation of habitual diet. Over the intervention period, one 24h-dietary recall will be applied during each nutritional advisement visit to monitoring diet compliance. The consumption record will be entered in the REC24h-ERICA software [46], which has a database composed of a list of items included in the food and beverage purchase database from the Pesquisa de Orçamentos Familiares (POF – Brazilian Household Budget Survey) [47]. The food items that are not contained in the database will be added by the interviewers. The quantities consumed of the food will be converted into measures of mass (in grams) and / or volume (milliliters) to relate food consumption data to a nutritional composition table [48] into SPSS software (version 23.0, USA), and then, we will estimate the intake of energy and nutrients.

The consumption also will be evaluated by a direct method using the Food Weighing Method. For this, the meals will be weighed before and after serving to determine the amount consumed using a digital scale (Exact Basic®, model BS-3000A).

Satiety and palatability

To assess the subjective feelings of appetite and satiety, we will use a VAS questionnaire to evaluate pre- and postprandial hunger, fullness, desire to eat, and prospective food consumption during the test day. The VAS is composed of 100 mm lines where words describing extremes were anchored at each end (example: I have never been so hungry / I am not hungry at all), for a total of eight questions presented in random order. Subjects will be requested to make a vertical mark on each line that best matched how they are feeling at the time [49]. We will run six applications of the

VAS questionnaire over each experimental day in the following times: fasting, and 10, 60, 120, 180, and 240 minutes after the meal (test or control drink).

Furthermore, the palatability of the drinks will be evaluated regarding the visual appeal, smell, taste, lingering taste, and general palatability immediately after drinks intake, using the 100 mm VAS [49].

Scales will be scored by measuring the distance from the left side of the line to the mark with a ruler.

Energy metabolism

The energy metabolism measurement will be performed by indirect calorimetry in according to the manufacturer guidelines (Carefusion Vmax® Series, Califórnia, EUA). Each woman will be submitted to five sections of 20 min (one at fasting and four at postprandial state) on indirect calorimetry to estimate Resting Energy Expenditure (REE), Respiratory Quotient (RQ), and substrate oxidation. Diet-induced thermogenesis will be calculated as the increase in energy expenditure above the REE measured and expressed as a percentage of the test drinks calories [50]. To calculate substrate oxidation, urinary nitrogen will be analyzed by the Kjeldahl method [51] in the urine samples collected after overnight fasting and over 240 min after drinks intake. Fasting and postprandial substrate oxidations will be calculated using standard equations [52] and expressed as mg per minute.

Blood pressure

Blood pressure will be measured at fasting and each one hour after test drinks intakes. Women will be instructed to remain seated and resting for 10 min before the first blood pressure measurement. Also, they will be instructed to keep both feet on the floor without crossing legs, and the right arm was supported at heart level. In the first evaluation, blood pressure will be taken in both arms. If the measures are different, the arm with the highest value will be standardized for the following measurements. Blood pressure will be measured using an automatic monitor (Omron Healthcare, Inc., Model OMRON HEM 7200, USA). The average of two additional measurements will be used for participants with blood pressure in the hypertensive range.

The Ankle Brachial Index

The Ankle Brachial Index (ABI) evaluation will be conducted after the women stay in resting for 10 minutes in the supine position. The systolic blood pressure will be measured in both arms and both ankles in the dorsalis pedis and posterior tibial arteries. The ABI value will be determined by taking the higher pressure of the two arteries at the ankle, divided by the higher brachial systolic pressure. The ABI will be calculated for each leg, and the lower value will be adopted as the patient's overall ABI. To ensure the correct auscultation of the pulse, a portable vascular Doppler (MEDMEGA®, DV 610B) will be used. The estimated ABI will be classified as normal (1-1.4), borderline (0.91 - 0.99), or at increased cardiovascular risk regardless of the presence of symptoms of Peripheral Arterial Disease (≤ 0.90 and > 1.40) [53].

Biological Samples

An experienced nurse will collect the venous blood. The collection will be performed at fasting and postprandial (1h, 2h, and 4h) conditions at the beginning and the end of the intervention. The blood samples will be centrifuged to obtain EDTA plasma and serum for biochemical analyses, a buffy coat for DNA recovery, and PBMC for gene expression. PBMC will be isolated by Ficoll® Paque Plus (GE Healthcare Life Sciences, Chalfont St Giles, UK). Until analysis, samples will be preserved in 1.5 mL Eppendorf tubes and frozen at a temperature of - 80 ℃. Moreove r, extra aliquots of all samples will be stored at −80◦C for additional outcomes that might arise during or after the study completion.

In the experimental and permeability test days, the same protocol for urine sample collection will be used. The women will be asked to collect urine samples all night before the test day (for 12 hours, fasting urine) in a container, which was then be brought back into the lab. At the lab, all morning, the urine also will be collected (for 270 minutes, postprandial urine). After collection, the urine will be homogenized and aliquoted (30 mL). For avoiding microbial growth, Thimerosal will be added (0.007 g). Subsequently, the urine sample will be stored at -20ºC until the time of analysis. Also, all women will be required to collect stool samples at the initial and final intervention period. Each of them will receive detailed instructions about the method for material collection. A plastic holder will be used to collect feces into a sterilized screw-capped

collection container. The women will be instructed to store the samples refrigerated and deliver them up to 24 h after collection to the Laboratory. Following this, the stool samples will be processed and stored in cryotubes at − 80 °C for future microbiota analyses.

Metabolic markers

After the study conclusion, serum LDL-c, HDL-c, total cholesterol, triglycerides, glucose, AST, ALT, GGT, Alkaline phosphatase, and urinary creatinine will be carried out in the Laboratory of Clinical Analysis of the Department of Nutrition of Universidade Federal de Viçosa by colorimetric methods using Mindray BS-200 Chemistry Analyzer. The serum very-low-density lipoprotein cholesterol (VLDL-c) will be calculated using the Friedewald equations [54]. Homeostasis model assessment – insulin resistance (HOMA-IR) will be calculated as follows: fasting blood glucose (mg/dL) x fasting serum insulin (μU/mL)/ 405. To determination of apolipoproteins, plasma selenium, insulin, and cortisol plasma and serum samples will be sent to the appropriate laboratory. Plasma melatonin will be determined by ELISA (Elabscience Biotechnology Co., Ltd, USA).

To plasma determinations of hormones GIP, GLP1 and ghrelin and adipokines adiponectin, resistin, and PAI-I will be performed multiplex assay from Milliplex using Human Metabolic Hormone Magnetic Bead Panel (HMHEMAG-34K-03, Merck) and Human Adipokine Magnetic Bead Panel 2 (HADK1MAG-61K-03, Merck), respectively. Bead-complexes will be read on Luminex® 200TM and analyzed by MAGPIX® with xPONENT 4.2 software.

Inflammatory, oxidative stress, and endothelial function markers

Cytokines multiplex assay from Milliplex (MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel – Immunology Multiplex Assay HADK2MAG-61K-05, Merck) will be used to assess the levels of IL1-β, IL-6, IL-8, and TNF-α. Bead-complexes will be read on Luminex® 200TM and analyzed by MAGPIX® with xPONENT 4.2 software. The adhesion molecules ICAM-1 and VCAM-1 will be assessed by ELISA (Elabscience Biotechnology Co., Ltd, USA). High-sensitive C-reactive protein will be quantified by an automated analyzer system using commercial

assay kits (Mindray BS-200 Chemistry Analyzer). The oxidative stress markers will be evaluated by standardized colorimetric methods: nitric oxide [55], superoxide dismutase [56], malondialdehyde [57], Ferric Reducing Antioxidant Power [58], and catalase [59].

Genetic markers

Total RNA will be isolated from PBMC using TRI reagent (Sigma-Aldrich) following the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Total extracted RNA will then be reverse transcribed to complementary DNA (cDNA) using a cDNA synthesis kit. The gene expression will be determined using the Real-Time PCR technique and specific primers for metabolic regulation genes (SIRT1 and 3, and PGC1-α), inflammatory genes (NF-κB, and IκB), antioxidant genes (Nrf2, glutathione peroxidase, superoxide dismutase and catalase), and circadian clock genes (clock, Cryl, PerL, and Bmal). The relative expression levels of mRNA will be normalized by endogenous control glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Intestinal permeability and microbiota

For the intestinal permeability test, the women will attend to the laboratory after overnight fasting (12 h), then ingested 200 mL of a solution containing lactulose (10 g), mannitol (5 g), and sucrose (20 g). Following, the women remained in the laboratory for 270 minutes, in the first 120 minutes, they will not be allowed to eat any food or drink. After, they will be asked to drink 150 mL of water at 120 and 180 minutes. The entire volume of urine produced during the fasting (12 hours) and postprandial (270 minutes) state will be collected. Urinary sugars (lactulose and mannitol) excretions will be analyzed by HPLC, Dionex Ultimate 3000 Dual coupled to a Shodex RI-101 refractive index detector maintained at 40℃, and Phenomenex Rezex ROA ion exchange column, 300 x 7.8 mm maintained at 40℃ [60]. The permeability will be defined as % urinary excretion and excretion ratios for sugars from hours 0–270 minutes.

Eating behavior, chronotype, and sleep quality

The questionnaire The Three-Factor Eating Questionnaire (adapted version from the Brazilian population) [61] will be used to identify and characterize cognitive

restriction, emotional eating, and uncontrolled eating behaviors at the beginning and end of the intervention. Besides, the women will fill the questionnaire Morning-Eveningness Questionarie – MEQ [62] or evaluation of biologic chronotype at initial and final intervention. Also, will be applied Epworth Sleepiness Scale [63] and the Pittsburgh Sleep Quality Index to verify sleep quality [64].

Statistical Analyses

The database will be made after double data entry to identify and correct possible failures. Statistical analyzes will be performed using the SPSS software (version 23.0, USA). The Shapiro-Wilk normality test will be used to check for the normal distribution of the data, and the Levene test to assess the homoscedasticity of the variances. To determine the effect of time on treatments, we will perform the paired t-test or Wilcoxon test. To the between-group evaluation, independent-samples t-test or Mann-Whitney U test will be used. When appropriate, the ANCOVA test will be used to compare means considering covariates. Two-way ANOVA for repeated measures (ANOVA-RM) followed by the Tukey-Kramer test will be employed to check the effect of treatment, time, and the interaction between time and treatment on the postprandial variables. Data will be expressed as mean ± SEM or median (p25-p75th percentiles values), when appropriate. The α level of 5% will be considered significant.

DISCUSSION

The Brazilian nuts study was designed to a broad investigation of the beneficial effects of an energy-restricted diet containing a mixed nut (cashew and Brazil nut) on cardiometabolic risk, energy metabolism, food intake, intestinal permeability, and genetic markers in overweight women at cardiometabolic risk.

At the moment, Brazil nuts consumption has been associated to the antioxidant effect [65–68], improvement of lipid profile [66,68], the anti-inflammatory effect [69], but no effect on blood pressure, waist circumference, and BMI have been reported [66,68]. Regarding cashew nut, some evidence demonstrate improvement or no effect on lipid profile, glycemic control, and blood pressure after a long-term intervention (28 – 118 g/day) [70–72]. The evaluated studies have wide methodological variations (health and unhealthy subjects, different quantities of nuts, in different forms - flour, raw, toasted, salted,

unsalted, crushed) that limit the support the benefits of Brazilian nuts on health. Thus, there is little and controversial information regarding the health effect of Brazil and cashew nut, although just like the other nuts, they have high nutritional quality. The weight of the present trial concerns the investigation of several aspects of health, providing evidence about the consumption of nuts least investigated. Also, our findings could lead to a better comprehension of the Brazil and cashew nuts effect stimulating new studies.

Body weight is one of the most important independent risk factors for several chronic disease. Some different mechanisms previously associated with the effect of the nuts intake on body weight control, such as enhanced satiety, resting energy expenditure, and diet-induced thermogenesis, will be investigated in this study [12]. Similarly, traditional, and emerging cardiovascular risk factors also will be evaluated to strengthen the evidence about the health effect of Brazilian nuts. Besides the health effects traditionally attributed to the nuts, intestinal permeability, chronobiological, and genetic markers also will be evaluated. This RCT of rigorous methodological design will help to establish the effectiveness of the mixed nut consumption on these physiological systems, which are already affected by overweight. Hence, the analyses carried out in this study have great potential to help clarify how nuts may promote beneficial effects.

Circadian rhythms and intestinal permeability, two fundamental processes, have been consistently associated with obesity etiology and cardiometabolic disturbance [73–77]. Chronodisruption changes the core machinery of the molecular circadian clock, inducing metabolic alterations, which may lead to obesity and higher cardiometabolic risk [75]. On the other hand, high blood endotoxin levels caused by gut barrier dysfunction may trigger systemic inflammation, which is appointed as a link between increased intestinal permeability, endotoxemia, and obesity, in addition to a higher risk to develop atherosclerosis [77,78]. Some evidence suggests that both mechanisms may suffer nutrient modulation [79–81]. Thus, for the first time, intestinal permeability and chronobiological markers will be studied after nutritional intervention with Brazilian nuts. Through this comprehensive investigation, our findings will allow us to understand how Brazil and cashew nuts consumption can modulate essential physiological conditions associated with body fat excess by assessing the related mechanisms.

The possible limitations of this study include the frequency and duration of face-to-face visits required, which demands time availability and can contribute to a higher dropout rate. Also, the impossibility of blinding the intervention is a factor that could affect the assessment in both groups, since after receiving dietary advice, the women might try to introduce non-prescribed changes in your diets. However, because pre-prepared foods have not been provided, we will achieve a better representation of mixed nut consumption in free-live conditions. Finally, once the study was carried out with women, the results cannot be extended to the general population. On the other hand, Brazilian nuts study has several strengths. To our knowledge, this is the first study to investigate Brazilian nuts currently least investigated. Being an RCT, the results should provide high-quality evidence, and help to clarify the mechanisms involved in the health effect of the Brazil and cashew nut intake on body weight control and physiological systems harmfully affected by obesity. Besides, the regular face-to-face monitoring with a dietitian over the study, encouraged the women to follow the study protocol.

CONCLUSION

Brazilian Nuts Study is a short randomized clinical trial designed to evaluate the effect of Brazilian nuts consumption associated with an energy-restricted diet on cardiometabolic risk, energy metabolism, food intake, intestinal permeability, genetic, and chronobiological markers in overweight women. When complete, the present study should provide an understanding of how Brazil and cashew nuts consumption can modulate essential physiological conditions associated with body fat excess by assessing the related mechanisms, besides provide high-quality evidence about the beneficial effects of both nuts consumption on human health.

Trial status: Ongoing (data analysis).

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AUTHORS' CONTRIBUTIONS

All authors were involved in designing the study and drafting the protocol. All authors read and approved the final protocol.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This protocol was approved by the institutional review board of the Universidade Federal de Viçosa (registration number: CAAE: 92004818.0.0000.5153; N: 2.832.601/ 2018) and was conducted in accordance with the Declaration of Helsinki (approval number: IR.AJUMS.REC.1395.729). All participants were informed about objective and study procedures and sign an informed consent form. This investigation was registered on Brazilian Registers of Clinical Trials – REBEC (protocol: U1111-1236-1647).

DISCLOSURE STATEMENT

The authors declare that they have no competing interests.

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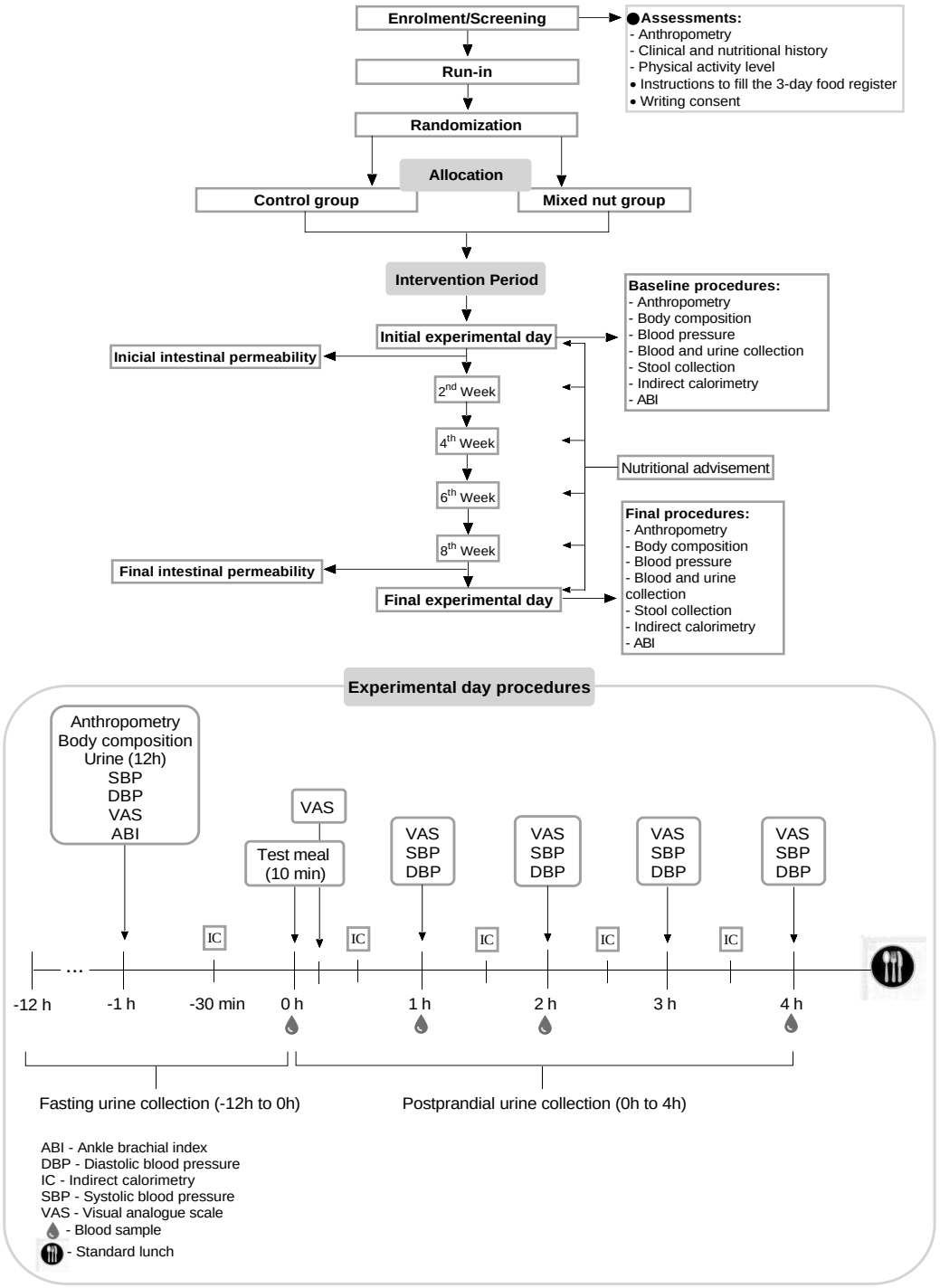

Figure 5.1.1 Study design and data collection scheme.

Table 5.1.1 Schedule of enrolment, interventions, and assessments.

Procedures and evaluations	Tool/ Biological sample	Enrolment	Run-in	Baseline	Follow up			Final assessments
				Initial experimental day	2nd week	4th week	6th week	Final experimental day
Enrolment								
Screening	Questionnaire	+						
Informed consent	TCLE	+						
Risk factors measurements (blood glucose and triglycerides)	Biochemical analyses	+						
Run-in								
Habitual diet (without nuts)	Nutritional guidance		+					
Allocation								
Randomization	Software		+					
Intervention								
Energy-restricted diet (- 500 kcal/day) containing daily portion of Brazilian nuts	-			+	+	+	+	+
Energy-restricted diet (- 500 kcal/day) without nuts	-			+	+	+	+	+
Nutritional counseling	Face-to-face visit		+	+	+	+	+	+
Nuts distribution	Face-to-face visit			+		+	+	+
Monitoring	Phone calls, and e-mails				+	+	+	
Measurements								
Anthropometry and body composition	Inbody®, DEXA, measuring tape, stadiometer	+	+	+	+	+	+	+
Blood pressure	Blood Pressure Monitor	+		+				+
Eating Behavior	Questionnaire	+						+
Physical activity	Questionnaire	+			+	+	+	+
Sleep quality	Questionnaire			+				+
Biological chronotype	Questionnaire			+				+
Food intake	24h food recall		+		+	+	+	+
Intestinal permeability	Urine		+					+
Energy metabolism	Indirect calorimetry			+				+
Biological samples	Blood, Urine, Feces			+				+
Satiety and appetite	Questionnaire			+				+
Postprandial food intake	Weighing food, 24h food			+				+

Table 5.1.2. Nutritional composition of control and test drinks.

	Quantity (g)	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)	Fiber (g)	Available carbohydrate (g)
Control drink							
Water	300	-	-	-	-	-	-
Skim powdered milk	40	143.7	13.8	0.37	21.2	0	21.2
Soybean oil	22.7	204.3	0	22.7	0	0	0
Sucrose	10	39.9	0.03	0	9.9	0	9.9
Whey protein concentrate	7	27.5	4.2	0.4	1.6	0	1.6
Starch corn	6	21.4	0.04	0.05	5.2	0.04	5.1
Synthetic hazelnut	4	-	-	-	-	-	-
Food coloring (drops)	4	-	-	-	-	-	-
TOTAL	385.7	437.03	18.14	23.5	38.04	0.04	37.9
Test drink							
Water	300	-	-	-	-	-	-
Cashews	30	182.1	5.5	13.8	8.7	1.1	7.6
Skim powdered milk	30	107.8	10.4	0.3	15.9	0	15.9
Brazil nut	15	103.4	2.2	9.5	2.2	1.2	1.07
Sucrose	11	43.9	0.04	0	10.9	0	10.9
TOTAL	386	437.3	18.2	23.6	37.8	2.3	35.5

Table 5.1.3. Nutritional composition of the Brazil nut and Cashew nut per 100-g serving.

Nutrients	Brazil nut	Cashew nut
Moisture (g)	1.2 ± 0.08	4.8 ± 0.02
Ash(g)	3.5 ± 0.05	2.6 ± 0.07
Lipids (g)	55.1 ± 0.7	34.1 ± 1.1
Protein(g)	17.6 ± 0.4	22.4 ± 0.9
Carbohydrates (g)	12.44 ± 0.8	28.8 ± 0.7
Fiber (g)	10,6 ± 0.0	7.4 ± 0.0
Soluble fiber (g)	2.9 ± 0.0	3.0 ± 0.0
Insoluble fiber (g)	7.7 ± 0.0	4.4 ± 0.0
Total energy value (kcal)	658.7 ± 11.4	540.9 ± 17.5
P (g)	0,67 ± 0.01	0.49 ± 0.009
K (g)	0.59 ± 0.01	0.64 ± 0.01
Ca (g)	0.23 ± 0.002	0.035 ± 0.002
Mg (g)	0.37 ± 0.007	0.25 ± 0.005
S (g)	0.23 ± 0.004	0.18 ± 0.003
Na (mg)	-	0.01 ± 0.001
Cu (mg)	1.94 ± 0.089	2.06 ± 01
Fe (mg)	3.32 ± 0.4	5.82 ± 0.2
Zn (mg)	5.66 ±0.3	6.90 ± 0.1
Mn (mg)	1.60 ± 0.03	1.83 ± 0.06
Se (µg)	340 ± 0.05	50 ± 0.0

The values are mean ± standard deviation.

Table 5.1.4. Percentage of fatty acids in relation to total fatty acids of the Brazil nut and Cashew nut.

Fatty Acids	Brazil nut	Cashew nut
Palmitic acid (C16:0)	14.0	8.19
Estearic acid (C18:0)	10.0	7.23
Oleic acid (C18:1n9)	36.3	67.0
Linoleic acid (C18:2n6)	39.5	17.5
Alpha-linolenic acid (C18:3n3)	0.1	0.07
Total SFA	24.0	15.4
Total MUFA	36.3	67.0
Total PUFA	39.6	17.6

Values are mean of triplicate.

5.2 Artigo 02: Mixed Brazilian nuts intake improve body composition and endothelial health in women at cardiometabolic risk (Brazilian Nuts Study): a nutritional intervention randomized controlled trial

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ABASTRACT

Several mechanisms have been proposed for the beneficial effect of nuts on health. However, Brazilian nuts remains least studied. We aim to evaluate the effect of a Brazilian mixed nut associated with an energy-restricted diet on body weight, body composition, cardiometabolic markers, and endothelial function in women at cardiometabolic risk. Brazilian nuts study is a randomized controlled parallel 8-week dietary intervention trial. Forty women were randomly allocated to 1) Control group: Energy-restricted diet without mixed nuts, n= 19 or, 2) Brazilian nut group (BN-Group): Energy-restricted diet containing daily 45 g of mixed nuts (15 of Brazil nuts + 30g of cashew nuts), n= 21. The mixed nuts provided 66 µ of selenium/day. At the beginning and final intervention anthropometry, body composition, and blood pressure were measured. Fasting blood sampling was obtained to evaluate lipid profile, glucose homeostasis, and endothelial function markers. After 8-week, plasma selenium concentration significantly increased in mixed nuts group (Δ = + 31.5 ± 7.8 µg/L; p= 0.001). Brazilian nuts intake reduced total body fat (-1.3 ± 0.4 %) parallel to preserving lean mass in BN-group compared to the control group. Besides, the soluble adhesion molecule VCAM-1 decreased (24.03 ± 15.7 pg/mL vs. -22.2 ± 10.3 pg/mL; p= 0.019) after Brazilian nuts intake. However, lipid profile and glucose homeostasis markers, apolipoproteins, and blood pressure remained unchanged after intervention. Thus, the

addition of Brazilian mixed nuts to an energy-restricted diet can be a healthy strategy to improve body composition, selenium status, and endothelial inflammation in cardiometabolic risk women.

Trial registration: Brazilian Registry of Clinical Trials: RBR-3ntxrm. Registered July 1, 2019. http://www.ensaiosclinicos.gov.br/rg/RBR-3ntxrm/.

Keywords: Brazil nut; cashew nut; obesity; weight loss; VCAM-1, body fat.

INTRODUCTION

Obesity is well-established as the main risk factor for cardiovascular diseases (CVD). The effects of obesity on CVD are associated with other metabolic risk factors such as insulin-resistance, hypertension, hypercholesterolemia, and hyperglycemia [1]. Recent estimates point out that up to 2025, 18% of men and 21% will be obese [2], which is evidence that women are most vulnerable to obesity pandemic [2,3].

Body weight is the major modifiable independent risk factor for CVD [4]. Weight loss of at least 3% from baseline is clinically relevant since this amount is associated with improvements in multiple cardiometabolic risk markers, including reducing insulin resistance, triglycerides, LDL, HDL, and non-HDL cholesterol [4–7]. Regardless of the cause of obesity, reduction in energy intake and increase of physical activity practice remains among the main cornerstones of obesity treatment [8]. However, long-term adherence to an energy-restricted diet is highly challenging, making it difficult to achieve substantial and sustained weight loss [9]. For this, foods that promote greater satiety can help reduce energy intake, increase compliance to weight-loss diets, and promote weight loss. Further, some foods' positive effects on managing obesity go beyond weight loss and might drive metabolic benefits [8,10].

In this regard, several studies have demonstrated that nuts consumption can modulate lipid profile, glycemic homeostasis, blood pressure, oxidative stress, and food intake [11–19]. In subjects with overweight or obesity, sensory and nutritional characteristics of nuts potentially modify the secretion of intestinal hormones, and consequently, the appetite sensation [18,19]. Some nutrients from nuts as unsaturated fatty acids, minerals, phytosterols, and fiber, contribute to their health effect. Nevertheless, most of this evidence was not from studies conducted with Brazilian nuts, currently considered poorly studied.

Brazil nut (Bertholletia excelsa H.B.K) is a native species to the Amazon considered the main food source of selenium (100-1000 mg of selenium/ g-1) [20,21]. Also, this nut contains phytosterols, tocopherols, squalene, and phenolics related to its anti-inflammatory and antioxidant activities [20–23]. The cashew tree (Anacardium occidentale L.) is native to Central and South America, being Brazil thought its country of origin [24–27]. Besides the pleasant taste, cashew nuts have valuable nutritional properties, such as high lipids content, predominantly MUFA, and PUFA, both associated with the reduction of cholesterol, LDL-c, and cardiovascular events [26].

At present, few human clinical trials have investigated the benefits of Brazilian nuts intake on cardiometabolic risk markers. In all founded studies, Brazilian nuts were included in a normal-caloric diet. The beneficial effect of these nuts on lipid profile and blood pressure is controversial, and no effect on glucose homeostasis was observed [28,29,38,39,30–37]. So, this study aimed to evaluate the effect of Brazilian mixed nuts associated with an energy-restricted diet on body weight, body composition, cardiometabolic markers, and endothelial function in women at cardiometabolic risk. We hypothesized that an energy-restricted diet containing Brazilian mixed nut would result in improvements in evaluated variables.

METHODOLOGY

Study design, participants, and recruitment

The Brazilian Nuts Study is a randomized controlled parallel 8-week nutritional intervention trial with women at cardiometabolic risk conducted in free-living conditions. Eligibility criteria included: adult women 20-55 years old, 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 obese women (BMI ≥ 30 kg/m2), regardless of the presence of metabolic complications. Non-inclusion criteria were 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;

usual consumption of nuts greater than 30 g/day; alcohol consumption higher than 21 units (168g) per week; dental problems that interfere with chewing; use of vitamin, mineral and omega 3 supplements.

Advertisements in social media and flyers were the recruitment methods. After an initial screening, the women who met the essential eligibility criteria (age, body weight, height, pregnancy, menopausal, and medical/supplement use) were invited to a face-to-face visit to evaluate health history, physical activity level, and anthropometry. The study occurred in the Department of Nutrition and Health of Universidade Federal de Viçosa-MG, Brazil. The study protocol followed the guidelines of the Helsinki Declaration and was approved by the institutional review board of the Universidade Federal de Viçosa (registration number: CAAE: 92004818.0.0000.5153; N: 2.832.601/ 2018). All participants were informed about objective and study procedures. Those that accepted the study conditions provided written informed consent. Furthermore, this study is registered on the Brazilian Registers of Clinical Trials – REBEC (protocol: RBR-3ntxrm).

Dietary intervention

Before the intervention, a run-in period of seven to ten days was applied to identify and exclude women with a probability of non-compliance to study protocol. After, women were randomly allocated into two groups: control, which consumed an energy-restricted diet (-500 kcal) without any type of nuts, or Brazilian nuts group (BN-group) that followed the energy-restricted diet (-500 kcal) containing 45g (30g of cashew plus 15g of Brazil nut) of Brazilian mixed nuts daily. At the beginning and end of the intervention period, the women visited the Laboratory of Energy Metabolism and Body Composition (LAMECC) to fasten blood sample collection, anthropometry, body composition evaluation, and fill out questionnaires about physical activity practice, food intake-behavior, and eating behavior.

For 48-hour before the procedures, all women were asked to avoid caffeine and alcohol and to maintain their habitual physical activity levels. Additionally, every fifteen days, the women attended the LAMECC for face-to-face nutritional advisement visits. On these occasions, body weight, 24-h dietary recalls, and physical activity practice questions were taken to monitoring study compliance (**Figure 5.2.1**). Over the study, we checked mixed nut consumption by the return of not consumed nut packages. Besides, at the beginning and the end of the study, the plasma selenium

concentrations were assessed. All women were asked to maintain their lifestyle during the study and inform of any change in the type or dosage of the medication for continuous use.

Energy-restricted diet

All women received an eating plan with five nutritionally-balanced menus, each with five meals (breakfast, morning snack, lunch, afternoon snack, and dinner). The total energy intake was estimated using the Estimated Energy Requirement (EER) for adult women with overweight or obesity [40]; then, 500 kcal/day were deducted for the dietary prescription. The average distribution of macronutrients was 22.0%, 32.6%, and 45.4% of daily energy from proteins, lipids, and carbohydrates, respectively, according to AMDR range. For BN-group, the diets were calculated, including the energy provided by the daily portion of 45 g of mixed nut. Due to the high-fat content of the mixed nut, the control group was asked to consume two tablespoons (twice a day; at lunch and dinner) of a salad dressing based on soy oil and lemon (2:1 ratio, respectively), which was prepared in the Metabolic Kitchen of LAMECC and its calories also included in the total caloric value of control diets. Both mixed nut and salad dressing were handed out to women fortnightly during the face-to-face nutritional visits.

All dietary advice was individualized and provided by dietitians every two weeks. Throughout the study period, participants received instructions to use only soy oil to prepare meals consumed over the day.

Mixed nut composition

The nuts used in the study were donated by Embrapa Agroindustria Tropical - Fortaleza - Ceará (cashew nuts) and Inovam Brasil® (Brazil nuts). After received, all nuts were manually selected to eliminate those that were inadequate for consumption. Then, the chosen nuts were portioned (15g of Brazil nut and 30 g of cashew nut) in laminated packages, vacuum sealed (Selovac Sealer model 200 B), and stored in a freezer at -20ºC until distribution to the volunteers.

The number of Brazilian nuts on the mixed nuts was defined based on the selenium content of Brazil nut to meet daily selenium recommendation; the 30g of cashew nut is supported by previously published studies evaluating tree nuts' cardiovascular benefits [41–43]. The selenium content in the Brazil nut was measured

by inductively-coupled plasma atomic emission spectrometry [44]. Each portion of 15g of Brazil nut provides approximately 51µ of Selenium. Also, we determined the lipid profile of Brazil and cashew nut by gas chromatography following the protocol proposed by Folch et al. 1957[45] and Hartman and Lago 1973 [46]. Unsaturated fatty acids represent 75.9 % of total fat in Brazil nut and 84.6 % in cashew nut (**Suplemmental table 5.2-1**).

Blood sampling

After overnight fasting (12 h), registered nurses collected venous blood samples from the antebrachial vein using vacuum tubes precoated with EDTA or heparin as an anticoagulant. After, blood samples were centrifugated (1500 g, 15 min, 4 Cº), aliquoted, and stored at -80 Cº until analysis.

Cardiometabolic risk markers

Serum LDL, HDL, total cholesterol, triglycerides, glucose were analyzed by the colorimetric enzymatic method using a commercial kit Bioclin® (Belo Horizonte, MG, Brasil) in the automatic analyzer (BS200 Mindray®, Nanshan, China). Insulin and high-sensitive C-reactive protein were quantified in fasting serum by automated analyzer systems using commercial assay kits. Apolipoproteins were assessed using Immunochemistry Systems MMAGE® (Beckman Coulter, Inc. EUA). The serum very-low-density lipoprotein cholesterol (VLDL) was calculated using the Friedewald equations [47]. Non-HDL cholesterol was calculated as total cholesterol – HDL cholesterol. The total-cholesterol:HDL and LDL:HDL ratios were also computed [48]. Insulin resistance was evaluated using TyG index, calculated by the formula Ln [fasting triglycerides (mg/dl)×fasting glucose (mg/dl)/2] [49]

Endothelial function markers

The adhesion molecules ICAM-1 and VCAM-1 were assessed using commercial ELISA kits following the manufacturer's recommendations (Elabscience Biotechnology Co., Ltd, USA). Nitric oxide (NO) concentration was determined in triplicate using the Griess reagent according to the protocol proposed by Grisham et al. (1996) [50].

Before ABI and blood pressure measurement, the women were instructed to remain seated and resting for 10 min. Ankle and brachial systolic blood pressures were measured using a hand-held Doppler machine (MEDMEGA®, DV 610B) and oscillometric blood pressure cuffs. Right and left ABI measurements were calculated by dividing the highest systolic blood pressure in each leg by the highest arm pressure. The estimated ABI was classified as normal (1-1.4), borderline (0.91 - 0.99), or at increased cardiovascular risk regardless of the presence of symptoms of Peripheral Arterial Disease (≤ 0.90 and > 1.40) [51]. In the first evaluation, blood pressure was measured in both arms. If the measures were different, the arm with the highest value was standardized for the following measurements. Blood pressure was measured using an automatic monitor (Omron Healthcare, Inc., Model OMRON HEM 7200, USA). The average of two additional measurements was used for participants with blood pressure in the hypertensive range.

Plasma selenium concentrations

According to standardized protocols, a commercial lab determined the plasma selenium level using the inductively coupled plasma mass spectrometry method.

Dietary assessment

All women completed one 3-day food record (two nonconsecutive weekdays and one weekend day), before the study baseline assessments. During the follow-up, every fifteen days, a 24-hour dietary recall was applied. Food records were analyzed using REC24h-ERICA software, adapted for the Brazilian population.

Anthropometry and body composition

Body weight was assessed by a bioelectrical impedance analysis device (Inbody 230, Biospace Corp., Seoul, Korea). Waist circumference was measured using an inelastic tape (precision 0.1 cm). Two measurements were taken at the umbilicus waist at the end of normal expiration, and the mean was calculated. BMI was defined as the ratio between weight in kg and squared height in meters. Hip circumference was also measured twice utilizing an inelastic tape at the maximum posterior extension of the gluteus, and the average was calculated. Additionally, body composition was assessed by dual-energy X-ray absorptiometry (DEXA) (Lunar Prodigy Advance DXA System, GE Lunar) in a subsample (control group: 73.3%, n=11; BN-group: 71.5%,

n=10) due to the equipment schedule availability. The DEXA analyses provided total and regional body fatness, including truncal, android, and gynoid composition. The truncal area included the neck, chest, abdominal, and pelvic areas. The android area is between the ribs and the pelvis areas, while the gynoid region includes the hips and upper thighs and overlaps both the leg and truncal area [52].

Randomization

The minimization method was employed for randomization to ensure the balance of predefined prognostic factors between groups [53]. In the present study, age, BMI, and body fat percentage were considered prognostic factors based on their potential to interfere with the outcome variables. The randomization procedure was performed using the WinPepi software, version 11.65 (Copyright J.H. Abransom, Aug 23, 2016) by the researchers.

Statistics Analyses

The sample size calculation considered the primary outcome (body weight), with a significance level of $\alpha = 0.05$ and 96% power, based on the two-sided t-test [54]. Adopting 4 kg as weight-loss anticipated (based on the energy-restricted diet prescribed). The number of nine women was the necessary sample size for study conduction. We increased more 30% to compensate potential dropouts, yielding a sample size of at least twelve women for each group.

In this study, the missing data ratio was 27.5%. Due to nature non-random of the missing data "missing not at random (MNAR)", the use of the multiple imputation method is not recommended [55]. Therefore, the intention to treat analyses (ITT) was not possible. All statistical analyses used the Statistical Package for the Social Sciences software Version 23.0 for Windows (SPSS, Chicago, IL, USA), and a p-value <0.05 was considered statistically significant. Figures displaying statistical analysis were produced using Prism 6 (GraphPad, La Jolla, CA, USA). The database was made after double data entry to identify and correct possible failures. We also used the Shapiro-Wilk normality test to check for the normal distribution of the data and the Levene test to assess the homoscedasticity of the variances. To determine the effect of time on treatments, we performed the paired t-test or Wilcoxon test to the between-

group evaluation, t-test, or Mann-Whitney U test to independent-samples comparisons. Data are expressed as mean ± SEM.

RESULTS

Forty women were randomized, and twenty-nine concluded the study. The drop out in the follow-up was higher in the BN-group (25.9%) than in the control group (22.2%). The main explanations were "personal reasons" (72.8%), "side effects" (18.1%), and "noncompliance to study protocol" (9.1%) (**Supplemental figure 5.2-1**). There was no difference between women that complete and those who did not complete the study (data not shown). Women included in the study had 31.4 ± 1.6 years and 33.4 ± 0.7 kg/m². At baseline, there was no significant difference between groups for cardiometabolic risk markers, endothelial function markers, anthropometric and body composition variables, and plasma selenium concentrations (**Table 5.2.1**; **Table 5.2.2**;**Suplemmental table 5.2-2**; **Figure 5.2.1**).

After 8-week dietary intervention, body weight, BMI, waist and hip circumference, waist-to-height and waist-to-hip ratio showed significant reductions compared with baseline, but no difference between groups (**Figure 5.2.2**). In contrast, the BN-group women exhibited body composition improvement compared to the control group. Mixed nuts intake significantly promoted a reduction in body fat (%) parallel to a rise in lean mass (%) and free fat mass (%). Besides, for body regions, truncal lean mass (kg and %) and free fat mass (kg and %) increase in the BN-group compared to the control group. However, the android fat mass was higher in the mixed nuts than in the control group (**Suplemmental table 5.2-2**). Regarding cardiometabolic risk factors, both groups showed a similar reduction in total cholesterol, LDL-c, and systolic blood pressure. Interestingly, VCAM-1 significantly reduced after mixed nut consumption (**Table 5.2.2**).

Basal plasma selenium was 57.4 ± 3.8 µg/L and 57.6 ± 4.1 µg/L in control and mixed nut, respectively. All women presented low plasma selenium (<100 µg/L) before this study [56]. After intervention, BN-group showed a higher increase in plasma selenium (∆ = + 35.4 ± 7.2 µg/L; p= 0.001) in comparison to control group (∆= +8.9 ± 7.3 µg/L; p= 0.157). Furthermore, 86.7% (n= 13) in the control group and 57.2% (n= 8) in the BN-group remained with plasma selenium <100 µg/L (**Table 5.2.2**).

Regarding food intake, compared with baseline, the energy intake decreased similarly, -201.9 ± 206.6 kcal and -287.7 ± 107.3 kcal in the control and BN-group, respectively (**Table 5.2.3**). Cholesterol intake reduced during the intervention on both groups, without a difference between them. Carbohydrate, protein, total fat, and fiber intake remained comparable between groups after intervention. As expected, at 8-week, MUFA intake was higher in the BN-group than in the control group (-1.0 ± 3.9 g vs. 1.9 ± 2.1 g; p= 0.009), while the PUFA intake was the opposite (6.6 ± 1.8 g vs. -1.1 ± 1.4 g; p= <0.001).

DISCUSSION

In this dietary intervention trial, the Brazilian nuts intake within an energy-restricted diet for 8-weeks promoted improvements in body composition and ICAM-1 reduction, suggesting improvement of endothelial inflammation, and enhanced plasma selenium concentrations in women at cardiometabolic risk.

Weight control is a primary strategy to reduce the cardiovascular disease burden [57]. At the same time, body fat reduction contributes to ameliorates metabolic alterations associated with overweight and obesity [58]. For the first time, an RCT evaluated the effect of Brazilian nuts intake within an energy-restricted diet. After the intervention, women allocated in the BN-group had a lower total fat mass (%) and the most preserved total lean and free fat mass than the control group. According to available trials, Brazilian nuts have never been part of an approach to body weight reduction. However, in studies with regular diets, including cashew [42] or Brazil nuts [28,29] in free-living conditions, there was no impairment in weight maintenance.

The effect of nuts on adiposity might be associated with the high content of unsaturated fatty acids. MUFA and PUFA are possibly more quickly oxidized and have a higher thermogenic effect than saturated fatty acids, carrying less fat accumulation [59,60]. Also, Moussavi et al. (2008) proposed that the consumption of a MUFA-rich diet could reduce body fat due to the energy expenditure enhancement, mediated, at least in part, through activation of the sympathetic nervous system [61]. However, the nuts effect on body composition, especially on fat content, remains a topic for further studies.

Although the Brazilian mixed nuts intake has promoted total fat mass (%) reduction, this study found no effect on metabolic markers. Few clinical trials have investigated the effect of Brazilian nuts on lipid profile. In a previous study, 16-week of

Brazil nut supplementation (15-25g/day) in adolescents with obesity reduced total cholesterol and LDL but did not affect HDL and triglycerides. In subjects with dyslipidemia and hypertension, the consumption of partially defatted Brazil nut flour for 12 weeks reduced the total cholesterol but did not change on LDL, HDL, and triglycerides (Carvalho et al., 2015). Concerning the four RCTs with cashew nuts, supplementation of this nuts (30-108g/day, for 8-12 weeks) did not affect total cholesterol, LDL, HDL, triglycerides, or VLDL [36–38,62], while only Mah et al. (2017) found a decrease in LDL and total cholesterol in dyslipidemic adults after ten weeks of cashew nut intake (32-64g/day). The evidence about Brazilian nuts effect on lipid profile still controversial. In the present study, 8-week mixed nuts intake did not change total cholesterol, LDL, HDL, VLDL, and triglycerides compared to the control group.

Apolipoproteins are structural and functional proteins of the lipoprotein particles that conduct the lipids to the organism's target organs and tissues [63]. Our investigation showed that 8-week mixed nut intake did not affect the concentrations of Apo AI, Apo B or Apo E, and Apo B/Apo AI ratio. This result supports the no effect observed on lipid profile markers. The lack of significant changes in apolipoproteins has also been observed in previous human studies with Brazilian nuts. The cashew nut consumption for 12 weeks did not modify Apo AI, Apo AII, and Apo B in healthy individuals [38]. The other two studies also did not observe changes in apolipoproteins after daily 45g of Brazil nut or 15g of partially defatted Brazil nut flour for 15 days and 12 weeks, respectively [28,33]. Furthermore, the literature suggests that for different mechanisms, some components of nuts - magnesium, fiber, α-linolenic acid, L-arginine, antioxidants, and MUFA - may protect against insulin resistance. However, like the apolipoproteins result, glucose homeostasis markers remained unchanged after daily mixed nuts intake [64].

Nuts are complex food matrices that contain macro and micronutrients previously associated with blood pressure regulation and endothelial function improvement. Unsaturated fatty acids have well-established macro and microvascular functions and can regulate blood pressure [65,66]. Nutrients and bioactive components in nuts, such as alpha-linolenic acid, L-arginine, fiber, and polyphenols, may modulate inflammation and the development of endothelial dysfunction [64]. Besides, micronutrients of nuts, such as magnesium, potassium, and calcium, may involve several regulation mechanisms of blood pressure [67–69]. Regardless of the benefits attributed to its nutrients, we no observed changes in NO plasma concentration,

systolic or diastolic blood pressure after Brazilian nuts intake. To our knowledge, only two clinical trials investigated the effect of Brazil nut on blood pressure, and similarly, no difference was observed [33,34]. For cashew nut, two studies [38,62] observe no effect on blood pressure regulation, and one [42] showed a reduction only in systolic blood pressure. Also, in the most recent metanalyses evaluating 61 RCTs about tree nuts effect on blood pressure, no effect was detected [70]. Thus, in the dietary context, merely the tree nuts intake for 8-weeks seems not to be enough to promote blood pressure benefits.

Concerning endothelial function, two recent meta-analyses showed a positive effect of tree nuts consumption on flow-mediated dilation (FMD), a traditional indicator of endothelial function [71,72]. However, the no effect on biomarkers of endothelial dysfunction, such as soluble cellular adhesion molecules (ICAM-1, VCAM-1), indicates a lack of consistent evidence for the effects of nut consumption on endothelial inflammation [71]. For Brazilian nuts, only one trial [38] evaluated the effect of cashew nut on ICAM-1 and VCAM-1, and no effect was verified. Regarding the Brazil nut, no available study assessed soluble adhesion molecules. Herein, we observe a significant reduction of 24.3% of VCAM-1 concentration after mixed nut intake, suggesting a beneficial effect of Brazilian nuts on endothelial inflammation.

The ABI was initially proposed as a noninvasive diagnostic method for lower-extremity peripheral artery disease. Later, it came to be used as an indicator of atherosclerosis, serving as a prognostic marker for cardiovascular events and functional impairment, even in the absence of symptoms [51,73]. This study is the first to evaluate the Brazilian nuts intake on ABI, but we did not find a significant ABI change in both groups. However, all women showed normal values for ABI at baseline, suggesting no peripheral arterial impairment to suffer modulation by the dietary intervention.

The beneficial effect of Brazil nut intake on selenium status has been demonstrated for all studies that could be found [28,29,77–79,30–34,74–76]. Toward the optimal activity of selenium-dependent proteins, such as the glutathione peroxidases and selenoprotein P, serum selenium values should be between 100 and 130 µg/L [80]. In our investigation, all women did have a low plasma selenium concentration at baseline. At 8-week, plasma selenium was significantly improved in the BN-group, although 57.2% of these women have remained with plasma Se below the normal values. This result can be explained by the Se quantity provided in the

mixed nut (66 µg by portion), which was below the quantity commonly provided by the long-term studies with Brazil nut (at last 200 µg od Se/day). Regarding the potential cardiovascular benefits of selenium, evidence show selenoproteins prevent oxidative modification of lipids, inhibit platelet aggregation, and reduce inflammation [81]. Thus, it is plausible to suggest that any plasma selenium improvement already contributes to reducing cardiovascular risk.

Despite the attempts to control the women's food intake, they did not achieve the planned energy restriction (-500 kcal/day), according to the evaluation of basal and final food intake by 24-hour food record. Nevertheless, the energy restriction was similar between groups after follow-up. The free live condition can explain this result since variations in food available might interfere with energy intake control, despite closely nutritional monitoring over the study. However, the decreases observed in cholesterol intake demonstrate an improvement in the diet quality independent of energy restriction.

This study has some limitations. Firstly, there was e a high percentage of lost to follow-up. Secondly, due to not blinded design, we have to consider that some changes in diet, of which we were not aware, might have happened. On the other hand, the present study's strength was the close control of lifestyle throughout the intervention period, forward to minimize its influence on the outcomes. Furthermore, this clinical trial represents the first scientific evidence about the effects of a mixed with Brazilian nuts with an energy-restriction diet on body composition, traditional cardiometabolic risk factors, and endothelial function in cardiometabolic risk women.

CONCLUSION

In this 8-week dietary intervention study, the mixed nut intake within an energy-restricted diet improves body composition, reduces the ICAM-1, an endothelial inflammation marker, and enhances selenium status. Thus, Brazilian nuts intake can potentially improve dietary strategies for obesity control and CVD prevention.

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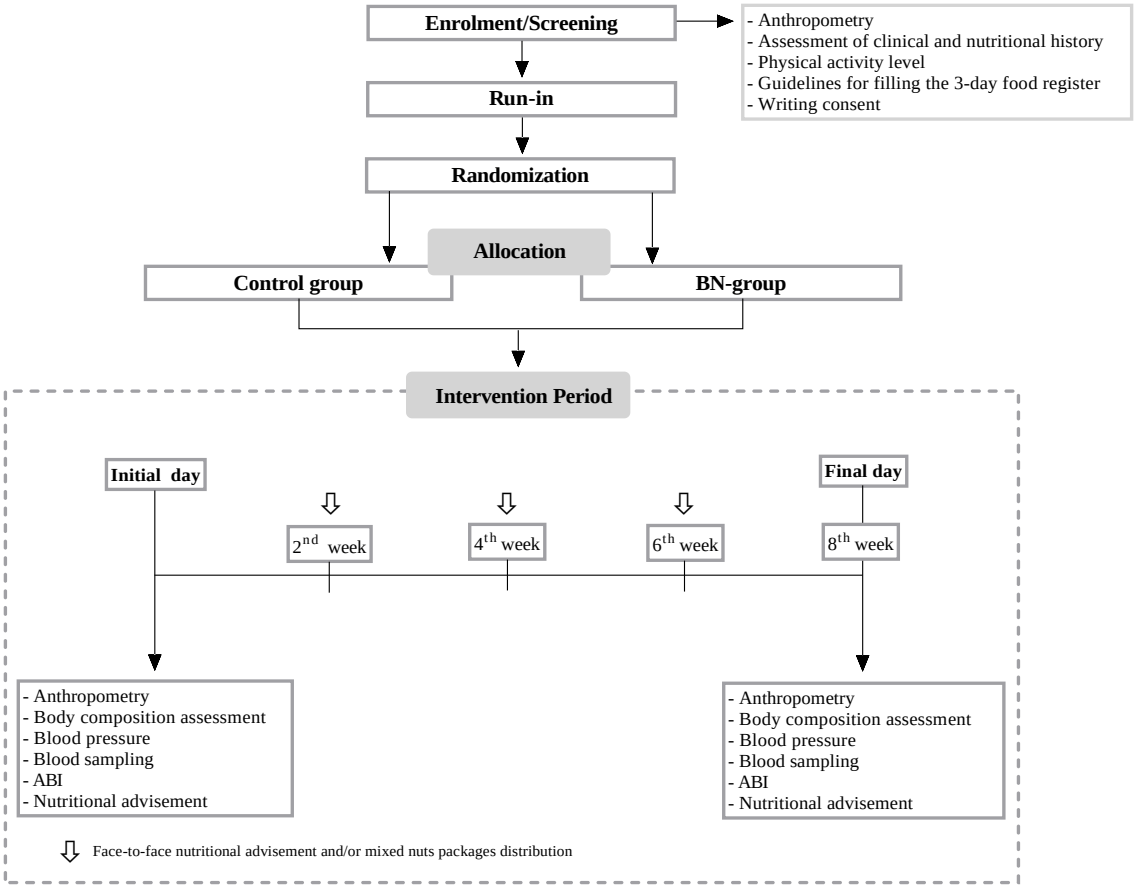


Figure 5.2.1. Study design schematic. ABI, Ankle brachial index.

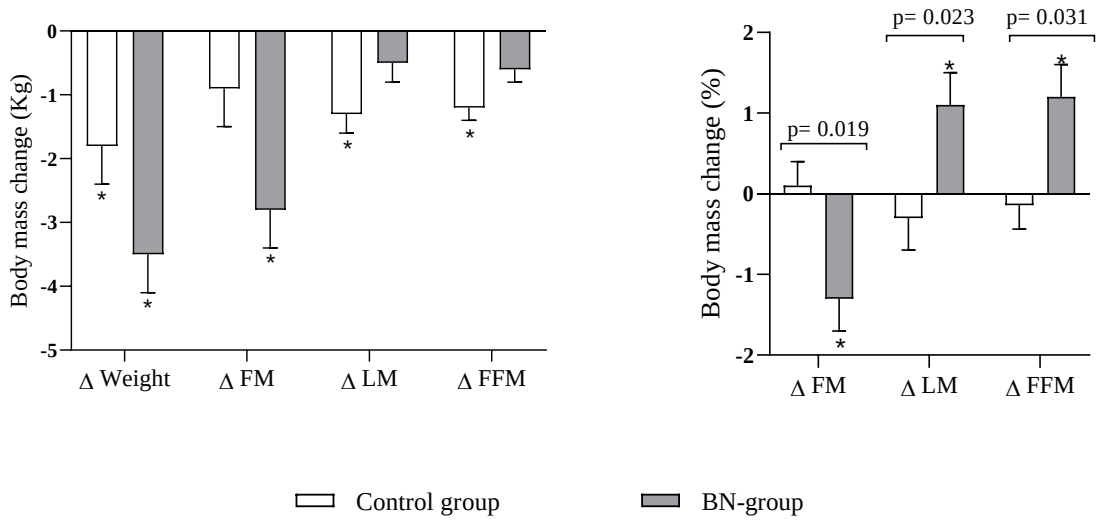
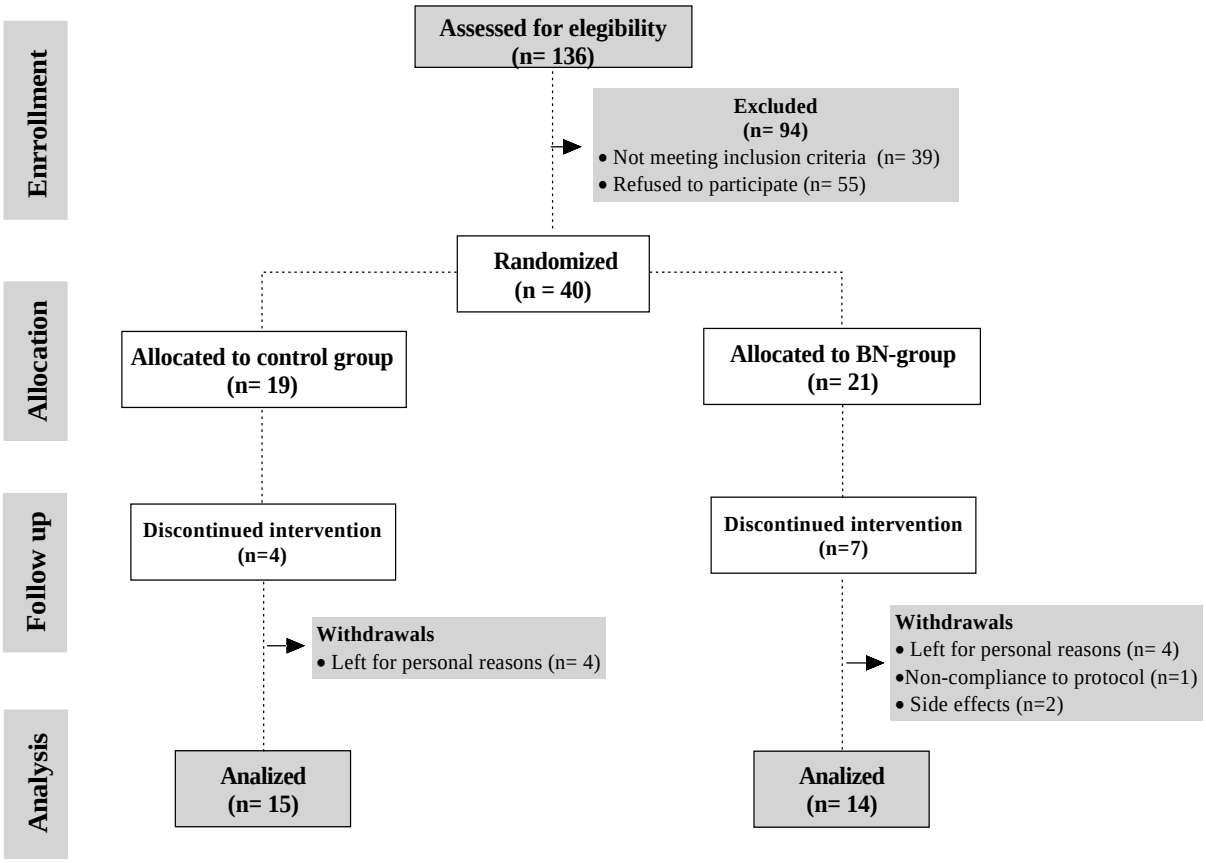


Figure 5.2.2. Body mass changes. Values are mean ± SEM. Δ = final – baseline assessment; FM, fat mass; LM, lean mass; FFM, free fat mass. *Significant difference within-group ($p < 0.05$; paired t-test or Wilcoxon test). p-values refer to between-groups comparison (independent-samples t-test or Mann-Whitney U test).



Supplemental figure 5.2-1. CONSORT flow diagram.

Table 5.2.1. Baseline anthropometric and body composition characteristics according to the diet groups.

	Control (n= 15)		BN-group (n= 14)			
	Baseline	Δ	Baseline	Δ	Baseline p-values	Δ p-values
Anthropometry						
Age (years)	31.6 ± 2.1	-	31.2 ± 2.7	-	0.972	
Body weight (kg)	87.9 ± 3.9	-1.8 ± 0.6*	90.5 ± 3.8	-3.5 ± 0.6**	0.644	0.073
BMI (kg/m²)	33.0 ± 1.0	-0.6 ± 0.2*	33.8 ± 1.2	-1.3 ± 0.2**	0.596	0.071
Waist (cm)	107.7 ± 2.6	-2.9 ± 0.6**	107.7 ± 2.8	-5.5 ± 1.1**	0.997	0.104
Hip (cm)	117.2 ± 2.6	-1.9 ± 0.6*	116.7 ± 2.8	-1.9 ± 0.8*	0.892	0.961
Neck (cm)	36.8 ± 0.8	-1.2 ± 0.4*	36.9 ± 0.6	-0.6 ± 0.2*	0.905	0.282
WHtR	0.6 ± 0.01	-0.01 ± 0.004**	0.65 ± 0.01	-0.034 ± 0.007**	0.874	0.064
WHR	0.9 ± 0.01	-0.01 ± 0.004*	0.92 ± 0.01	-0.032 ± 0.01*	0.837	0.073
Body composition (DEXA) [§]						
Total fat mass (kg)	42.04 ± 2.4	-0.9 ± 0.6	43.9 ± 2.7	-2.8 ± 0.6*	0.603	0.065
Total body fat (%)	48.08 ± 1.3	0.1 ± 0.3	48.7 ± 1.0	-1.3 ± 0.4*	0.698	**0.019**
Total lean mass (kg)	41.9 ± 1.8	-1.3 ± 0.3*	42.5 ± 1.3	-0.5 ± 0.3	0.826	0.106
Total lean mass (%)	48.6 ± 1.3	-0.3 ± 0.4	47.9 ± 1.0	1.1 ± 0.4*	0.699	**0.023**
Total fat free mass (kg)	44.8 ± 1.8	-1.2 ± 0.2*	45.4 ±1.3	-0.6 ± 0.2	0.813	0.142
Total fat free mass (%)	51.9 ± 1.3	-0.14 ± 0.3	51.2 ± 1.0	1.2 ± 0.4*	0.701	**0.031**

Values are mean ± SEM. Δ = final – baseline assessment. * p ≤ 0.05 or **p ≤ 0.001 are significant differences within-group (paired t-test or Wilcoxon test). Baseline p-values and Δ p-values refer to the comparison between groups (independent-samples t-test or Mann-Whitney U test). BMI, body mass index; WHtR, Waist-to-height ratio; WHR, Waist-to-hip ratio. [§] Subsample analyze (control n= 10; mixed nut n= 11).

Table 5.2.2. Effect of 8-week intervention on cardiometabolic risk markers and endothelial function according to diet groups.

	Control (n= 15)		BN-group (n= 14)		Baseline p-values	Δ p-values
	Baseline	Δ	baseline	Δ		
Cardiometabolic risk markers						
Total cholesterol (mg/dL)	173.9 ± 8.8	**-7.4 ± 3.03***	172.8 ± 7.3	**-8.3 ± 2.6***	0.927	0.828
Triglycerides	128.5 ± 26.8	4.1 ± 10.0	109.2 ± 14,4	-4.64 ± 14.1	0.621	0.377
LDL-c (mg/dL)	89.4 ± 6.08	**-4.8 ± 1.6***	87.8 ± 6.3	**-5.5 ± 2.3***	0.862	0.787
HDL-c (mg/dL)	49.6 ± 3.2	-1.4 ± 2.3	55.7 ± 3.8	-3.7 ± 2.5	0.236	0.524
VLDL-c (mg/dL)	25.7 ± 5.3	0.8 ± 2.01	21.8 ± 2.8	-0.9 ± 2.8	0.621	0.377
Non-HDL-c	124.3 ± 6.81	**-6.0 ± 2.8***	117.1 ± 8.6	-4.6 ± 3.4	0.515	0.914
Total cholesterol:HDL-c	3.6 ± 1.6	-0.02 ± 0.1	3.2 ± 0.2	0.01 ± 0.1	0.305	0.826
LDL-c:HDL-c	1.8 ± 0.1	-0.03 ± 0.08	1.7 ± 01	-0.01 ± 0.06	0.499	0.870
Fasting glucose (mg/dL)	97.7 ± 2.5	0.2 ± 2.3	94.0 ± 2.4	0.3 ± 2.5	0.477	0.979
Insulin (µUI/mL)	11.3 ± 1.2	-0.7 ± 1.2	14.4 ± 2.8	-0.16 ± 1.6	0.847	0.621
TyG index	8.5 ±0.1	-0.009 ± 0.09	8.4 ± 0.1	-0.04 ± 0.1	0.437	0.822
Apo AI (mg/dL)	128.5 ± 7.2	-2.4 ± 5.2	131.2 ± 7.09	-2.7 ± 3.2	0.794	0.960
Apo B (mg/dL)	82.0 ± 3.9	-2.4 ± 1.2	79.6 ± 4.2	-4.1 ± 2.06	0.686	0.492
Apo E (mg/L)	42.1 ± 4.9	-0.9 ± 2.0	38.5 ± 3.9	0.22 ± 2.8	0.578	0.743
ApoB/ApoA	0.6 ± 0.03	0.009 ± 0.02	0.6 ± 0.05	-0.02 ± 0.02	0.767	0.477
hs-PCR (mg/dL)	5.1 ± 0.8	0.1 ± 0.8	4.4 ± 0.7	-0.6 ± 0.4	0.591	0.447
Selenium status marker						
Se (µg/L)	57.4 ± 3.8	8.9 ± 7.3	57.6 ± 4.1	35.4 ± 7.2**	0.979	**0.010**
Endothelial function markers						
SBP (mmHg)	80.3 ± 1.4	**-4,2 ± 1,4***	80.2 ± 1.7	**-4.0 ±1.3***	0.959	0.920
DBP (mmHg)	117.2 ± 2.2	-2,4 ± 2,1	119.7 ± 2.7	-3.8 ± 2.7	0.480	0.688
ABI	1.1 ± 0.03	0,02 ± 0,03	1.07 ± 0.04	-0.02 ± 0.02	0.556	0.374
NO (µM/mL)	27.7 ± 15.9	1,3 ± 2,05	15.0 ± 1.8	0.9 ± 2.9	0.652	0.913
ICAM-1 (pg/mL)	3,538.7 ± 207.1	35.3 ± 366.2	3,106.6 ± 311.2	79.5 ± 449.7	0.252	0.939
VCAM-1 (pg/mL)	85.0 ± 7.5	24.3 ± 14.6	92.6 ± 8.4	**-25.8 ± 10.4***	0.451	**0.010**

Values are mean ± SEM. Δ = final – baseline assessment * p ≤ 0.05 or **p ≤ 0.001 are significant differences within-group (paired t-test or Wilcoxon test). Baseline p-values and Δ p-values refer to the comparison between groups (independent-samples t-test or Mann-Whitney U test). ABI, ankle brachial index; Apo A, Apolipoprotein A; Apo B, Apolipoprotein B; Apo E, Apolipoprotein E; hs-PCR, high sensitivity protein C reactive; HDL, high-density lipoprotein; ICAM-1, intercellular adhesion molecule-1; LDL, low-density lipoprotein; VLDL-c, very low-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; VCAM-1, Vascular cell adhesion molecule-1.

Table 5.2.3 Dietary compliance of women who completed the study according to diet groups.

Daily intake	Control group (n= 15)		BN-group (n= 14)		Difference between	
	Baseline	Δ	Baseline	Δ	Baseline p-values	Δ p-values
Total energy intake (kcal)	1,747.6 ± 183.3	-201.9 ±	1,810.3 ± 109.6	-287.7 ± 107.3*	0.316	0.339
Carbohydrate (g)	230.3 ± 22.1	-44.0 ± 23.9	224.0 ± 20.3	-51.9 ± 17.0*	0.839	0.794
Protein (g)	78.9 ± 8.8	-5.6 ± 10.0	78.6 ± 6.0	-3.0 ± 7.8	0.650	1.000
Total fat (g)	58.2 ± 7.8	0.6 ± 9.6	68.2 ± 5.0	-6.4 ± 5.8	0065	0.551
MUFA (g)	19.04 ± 3.1	-1.0 ± 3.9	23.1 ± 1.9	1.9 ± 2.1*	**0.008**	**0.009**
PUFA (g)	10.7 ± 1.8	6.6 ± 1.8*	13.2 ± 1.3	-1.1 ± 1.4	**0.041**	**<0.001**
SFA (g)	20.8 ± 2.5	-4.0 ± 3.5	23.6 ± 1.7	-4.9 ± 2.0*	0.170	0.843
Cholesterol (mg)	322.4 ± 40.7	-96.8 ± 37.3*	370.4 ± 35.0	-113.7 ± 29.3*	0.388	0.713
Fiber (g)	20.7 ± 2.9	1.5 ± 3.5	16.4 ± 1.9	2.9 ± 1.8	0.294	0.786

Values are mean ± SEM. Δ = final – baseline assessment *p ≤ 0.05 significant differences within-group (paired t-test or Wilcoxon test). Baseline p-values and Δ p-values refer to the comparison between groups (independent-samples t-test or Mann-Whitney U test). † One-way ANCOVA adjusted by baseline value. MUFA, monounsaturated fatty acid; and PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid

Suplemmental table 5.2-1. Fatty acid profile of the Brazil nuts and Cashew nuts (% of total fatty acids).

Fatty Acids	Brazil nut	Cashew nut
Palmitic acid (C16:0)	14.0	8.19
Estearic acid (C18:0)	10.0	7.23
Oleic acid (C18:1n9)	36.3	67.0
Linoleic acid (C18:2n6)	39.5	17.5
Alpha-linolenic acid (C18:3n3)	0.1	0.07
Total SFA	24.0	15.4
Total MUFA	36.3	67.0
Total PUFA	39.6	17.6

Values are mean of triplicate

Suplemmental table 5.2-2. Effect of 8-week intervention on body composition according to diet groups.

	Control (n= 15)		BN-group (n= 14)			
	Baseline	Δ	Baseline	Δ	Baseline p-value	Δ p-value
Body composition (DEXA) §						
Gynoid lean mass (kg)	6.3 ± 0.3	-0.17 ± 0.06*	6.6 ± 0.2	-0.2 ± 0.1	0.387	0.561
Gynoid lean mass (%)	44.2 ± 1.4	-0.3 ± 0.4	45.03 ± 1.2	0.6 ± 0.6	0.555	0.203
Gynoid fat mass (kg)	7.9 ± 0.5	-0.2 ± 0.1	8.03 ± 0.5	-0.3 ± 0.1*	0.871	0.543
Gynoid fat mass percentage (%)	55.8 ± 1.2	-0.8 ± 1.4	56.3 ± 1.1	-5.4 ± 1.7*	0.856	0.057
Gynoid fat free mass (kg)	6.6 ± 0.3	-0.1 ± 0.06*	6.9 ± 0.2	-0.2 ± 0.1	0.440	0.508
Gynoid fat free mass (%)	46.1 ± 1.4	-0.2 ± 0.4	47.0 ± 1.2	0.7 ± 0.6	0.586	0.216
Android lean mass (kg)	3.01 ± 0.1	-0.2 ± 0.04*	3.01±0.1	-0.1 ± 0.05	0.995	0.206
Android lean mass (%)	43.2 ± 1.2	0.2 ± 0.6	42.6 ± 1.1	1.2 ± 0.5*	0.757	0.263
Android fat mass (kg)	3.9 ± 0.2	-0.2 ± 0.06*	4.07 ± 0.3	-0.3 ± 0.1*	0.703	0.794
Android fat mass (%)	49.5 ± 1.7	4.1 ± 1.4*	48.3 ± 1.6	8.5± 1.5*	0.612	**0.045**
Android fat free mass (kg)	3.0 ± 0.1	-0.2 ± 0.04*	3.08 ± 1.5	-0.1± 0.05	0.979	0.262
Android fat free mass (%)	44.1 ± 1.2	0.4 ± 0.6	43.6 ± 1.1	1.2 ± 0.5	0.783	0.382
Truncal lean mass (kg)	20.0 ± 0.9	-1.2 ± 0.3*	19.8± 0.7	0.08 ± 0.2	0.869	**0.004**
Truncal lean mass (%)	47.2± 1.4	-1.0 ± 0.7	45.2 ± 1.1	1.9 ± 0.7*	0.307	**0.010**
Truncal fat mass (kg)	21.6 ± 1.2	-0.7 ± 0.4	23.2 ± 1.4	-1.5 ± 0.4*	0.399	0.218
Truncal fat mass e (%)	42.6 ± 1.8	9.8 ± 2.0*	44.1 ± 2.0	6.5 ± 2.0*	0.593	0.272
Truncal fat free mass (kg)	20.9 ± 0.9	-1.2 ± 0.3**	20.8 ± 0.7	0.03 ± 0.2**	0.919	**0.003**
Truncal fat free mass (%)	49.4 ± 1.4	-0.7 ± 0.6	47.6 ± 1.1	1.8 ± 0.6*	0.330	**0.013**

Values are mean ± SEM. Δ = final – baseline assessment. Significant differences within-group are expressed by * p ≤ 0.05 or **p ≤ 0.001 (paired t-test or Wilcoxon test). Baseline p-values and Δ p-values refer to the comparison between groups (independent-samples t-test or Mann-Whitney U test). BMI, body mass index; WHtR, Waist-to-height ratio; WHR, Waist-to-hip ratio. § Subsample analyze (control n= 10; mixed nut n= 11).

5.3 Artigo 03: Effect of Brazilian nuts intake on liver function and oxidative status markers of cardiometabolic risk women: Brazilian Nuts Study

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ABASTRACT

Objective: To evaluate the effect of a Brazilian mixed nuts associated with energy-restricted diet on oxidative status and liver function markers in cardiometabolic risk women.

Methods: This is a randomized controlled parallel 8-week clinical trial. Forty women were randomly allocated to 1) Control group: energy-restricted diet without mixed nuts or 2) Brazilian nut group (BN-group): energy-restricted diet containing daily 45 g of Brazilian nuts (15 g of Brazil nuts + 30 g of cashew nuts). The Brazilian nuts provided 66 µg of Selenium/day. Antioxidant status (superoxide dismutase, glutathione peroxidase, malondialdehyde, oxidized low-density lipoprotein, and Ferric Reducing Antioxidant Power) and liver function (aspartate aminotransferase, alanine aminotransferase, γ- glutamyltransferase, and alkaline phosphatase) markers were evaluated at baseline and final intervention period.

Results: After 8-week, plasma selenium concentration was significantly improved in BN-group (Δ = + 31.5 ± 7.8 µg/L; p= 0.001). After the intervention, no significant effect of Brazilian nuts intake was observed on liver function markers. Similarly, antioxidant status markers remained unchanged after Brazilian nuts intake.

Conclusion: According to the present data, the addition of Brazilian mixed nuts to an energy-restricted seems not to affect the liver function or antioxidant status in women at cardiometabolic risk.

Trial registration: Brazilian Registry of Clinical Trials: U1111-1236-1647.

Keywords: Brazil nut; cashew nut; obesity; Selenium. oxidative stress.

INTRODUCTION

Cardiovascular diseases (CVDs) remain a leading cause of death worldwide. Besides abdominal obesity, insulin resistance, inflammation, and lifestyle, the emergent cardiovascular risk factors also include oxidative stress, which is hallmarked by an imbalance between pro-oxidants and antioxidants (1). To protect the body's cells and organ systems against reactive oxygen species (ROS), humans have a sophisticated and complex antioxidant protection system. It involves several components, both endogenous (e.g.. bilirubin, uric acid, SOD, CAT, GPx) and exogenous antioxidants (e.g.. vitamin C, E, and bioactive compounds), that act synergistically to neutralize free radicals and maintain oxidative status balance (2). When established, the oxidative stress induces insulin resistance within adipose and peripheral tissues leading to alterations in metabolic homeostasis (3). Also, obesity and insulin resistance cause liver metabolic disruptions, and consequently impairment on lipid and glucose metabolism (4). Although commonly observed in overweight and obese subjects, liver disruptions have been ignored as one potential cause of CVD (5). Serum aminotransferases such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), combined with alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT), are commonly measured sensitive markers of liver function (6). Subtle abnormalities on these markers are frequently observed in patients at cardiometabolic risk.

Currently. nuts have been disseminated as one of the main sources of vitamins, minerals, and bioactive compounds with antioxidant functions (7). Previously, these foods' antioxidant capacity was widely discussed, highlighting the bioactive potential of nut phenolic compounds (8). Despite that, our recent review about the antioxidant effect of nuts emphasized inconsistent results and the need for more studies to confirm and comprehend the involved mechanisms in the antioxidant effect of nuts. Regarding the impact of nuts intake on liver function, the available clinical studies are controversial. However, observational studies suggest that a higher intake of nuts can be associated with a decreased risk of non-alcoholic fatty liver disease (9).

Therefore. the present randomized clinical trial 8-week parallel-arm aimed to evaluate the effect of Brazilian nuts intake associated with an energy-restricted diet on the liver function and antioxidant status markers in cardiometabolic risk women.

MATERIALS AND METHODS

Forty women at cardiometabolic risk were selected from applicants satisfying the following eligibility criteria: adult women 20-55 years old, with overweight (BMI ≥ 27 kg/m^2 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/m2), regardless of the presence of metabolic complications. Ineligibility included 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; usual consumption of nuts greater than 30 g/day; alcohol consumption higher than 21 units (168g) per week; dental problems that interfere with chewing; use of vitamin. mineral and omega 3 supplements

The study was a randomized controlled parallel 8-week design with 2 intervention groups: control and Brazilian nuts group (BN-group), carried out in a free-living condition. Treatment was randomly allocated by the researchers using minimization method, adopting age, BMI, and body fat percentage as prognostic factors. The randomization was performed in the WinPepi software. version 11.65 (Copyright J.H. Abransom. Aug 23. 2016). The Brazilian Nuts study was approved by the institutional review board of the Universidade Federal de Viçosa (registration number: CAAE: 92004818.0.0000.5153; N: 2.832.601/ 2018) and registered on the Brazilian Registers of Clinical Trials – REBEC (protocol: RBR-3ntxrm). The trial was run in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice.

The intervention trial included an energy-restricted diet (-500 kcal) absent in any type of nuts (control group), or an energy-restricted diet (-500 kcal) containing 45

g (30 g of cashew plus 15g of Brazil nut) of Brazilian nuts daily (BN-group). At beginning and final intervention period, we collected fasting blood sample and evaluated anthropometric variables for all participants. For 48-hour before the procedures, all women were advised to avoid caffeine and alcohol and to maintain their habitual physical activity levels. For each woman was prescribed an eating plan with five nutritionally-balanced menus. The total energy intake was estimated using the Estimated Energy Requirement (EER) for adult women with overweight or obesity (10); then, 500 kcal/day were deducted for the dietary prescription. The average distribution of macronutrients was 22.0%. 32.6%. and 45.4% of daily energy from proteins, lipids, and carbohydrates, respectively. For the intervention group, the diets were calculated including the energy provided by the daily portion of 45 g of mixed nuts. Due to the high-fat content of the mixed nuts, the control group was asked to consume two tablespoons of a salad dressing based on soy oil and lemon (2:1 ratio. respectively), which was provided by the researchers. The Brazilian nuts were donated by Embrapa Agroindustria Tropical - Fortaleza - Ceará (cashew nuts) and by the company Inovam Brasil® (Brazil nuts). After received, all nuts were manually selected and portioned in laminated packages, vacuum sealed (Selovac Sealer model 200 B), and stored in a freezer at -20ºC until distribution to the volunteers. The quantity of Brazilian nuts on the mixed nuts was defined based on the selenium content of Brazil nut, to meet daily selenium recommendation. Each portion of 15g of Brazil nut provide approximately 51µ of Selenium; the 30g of cashew nut is supported by previously published studies evaluating tree nuts cardiovascular benefits (11–13).

All dietary advice was individualized and provided by dietitians every two weeks. On these occasions, body weight, 24-h dietary recalls, and questions about physical activity practice were taken to monitoring study compliance. The mixed nuts consumption over the study was checked by the return of not consumed nut packages. Besides, at the beginning and the end of the study, the plasma selenium concentration was assessed. All women were asked to maintain their lifestyle during the study and inform any change in the type or dosage of medication of continuous use.

RESULTS

Forty women were randomized. After 8-week intervention period, a total of twenty-nine concluded the study, fifteen in the control and fourteen in the mixed nut

group; all in accordance with the study protocol. The basal mean of age was 31.4 ± 1.6 years and the BMI was 33.4 ± 0.7 kg/m². At beginning, there was no significant difference between groups for plasma selenium concentration, anthropometric characteristics, liver function, and antioxidant status markers. Regarding food consumption, MUFA and PUFA intake was higher in the BN-group them in the control group. Energy intake, total fat, protein, carbohydrate, cholesterol, and fiber intake were similar between groups at baseline (**Supplemental table 5.3-1**).

Over time, both groups had a significant reduction in body weight, BMI, and waist circumference. However, these variables remain similar in between groups comparison after the intervention (**Table 5.3.1**). The liver function enzymes remained unchanged throughout the intervention. On the other side, Fatty liver index showed a significant reduction after mixed nuts intake, but no difference in comparison to the control group was observed. Regarding oxidative status markers, SOD, MDA, MDA/SOD ratio, FRAP, and GPx remained unchanged after intervention. However, oxLDL concentration was significantly reduced in the control group in comparison to the mixed nuts group.

DISCUSSION

This study aimed to evaluate the effect of an energy-restricted diet associated with Brazilian nuts intake compared to the control on the liver function and antioxidant status markers. After the intervention, liver function markers remain unchanged. Also, contrary to our expectations, we did not verify any change in the oxidant status markers evaluated after Brazilian nuts intake.

The liver performs a broad array of biochemical functions necessary for whole-body metabolic homeostasis. Harm to liver function is commonly observed in overweight and obese conditions hallmarked by an increase in liver enzymes, which increases progressively with BMI (14). In the present study, no evaluated liver function marker showed significant change after the Brazilian nuts intake. It is important to note that all investigated liver enzymes had values at the normal range at baseline, explaining the lack of impact of Brazilian nuts on these markers. Additionally, since weight can be considered one of the major determining factors for liver enzyme values, the similar weight loss between groups supports the no effect of the Brazilian mixed nuts intake on liver enzymes. The evidence about the nuts effect on liver enzymes is

currently limited, especially in the context of weight loss. Dikariyanto et al. (2020) and Bowen et al. (2018) observed no effect on GGT, ALT, and AST after supplementation with almonds for 6 and 8 weeks. respectively (15,16). On the other hand, contrary to our results, the association between almond supplementation (50 g) and a hypocaloric diet for three months lead to a significant reduction of ALT, AST, GGT in women with overweight (6). Moreover, no previous chronic study investigated Brazil or cashew nuts effect on liver function makers.

Oxidative stress emerges as a key characteristic at high cardiometabolic risk conditions. Dyslipidemia, hyperglycemia, insulin resistance, excessive weight, endothelial dysfunction, and others are some possible contributors to oxidative stress inducement (17). Glutathione peroxidase (GPx) and superoxide dismutase (SOD) are two majors endogenous cytoprotective antioxidant enzymes present in humans and considered essential indicators of oxidative stress (18). In our investigation, both enzymes kept statistically similar in within and between groups comparison. Despite the improvement in plasma Se over the intervention, no impact on GPx activity was verified. To ensure the optimal activity of selenium-dependent proteins, serum selenium values should be between 100 and 130 µg/L (19). In the present study, despite significant selenium improvement, the optimal plasma selenium level was not achieved, which can justify the no impact on GPx activity. Previous studies with acute (20) and chronic (21–27) designs, that evaluated GPx activity after Brazil nut intake observed opposite results. However, these studies provided at last three times more Se than in the present investigation.

In addition, ferric reducing antioxidant power (FRAP) has been used as comprehensive measures of radical quenching capacity by antioxidants in plasma (17). Unlike other markers, FRAP is directly influenced by diet quality. Nevertheless, despite the mineral, vitamin, and bioactive compounds provided by the daily mixed nuts intake, no effect on FRAP values were verified. Malondialdehyde (MDA), a lipid peroxidation product by ROS and a recognized biomarker of oxidative damage, also remains unchanged after the intervention. To our knowledge, no previous studies evaluated the effect of cashew nut on MDA. Only one study evaluated MDA after six months of Brazil nut intake (1 unit). Similar to our investigation, no impact was observed in MDA plasma concentration, even with GPx activity improvement, which protects against lipid peroxidation (23).

oxLDL is a critical oxidative signal in the onset and progression of atherosclerosis (28). A systematic review recently published by our group about the effect of chronic nuts intake on oxidative stress highlighted that nuts supplementation is potentially effective on oxLDL reduction (29). Contrary to the evidence that an intake of MUFA-rich diet and bioactive compounds decreases the susceptibility to LDL oxidation, in our study, there was no significant change of oxLDL concentration in the BN-group; surprisingly, a significant reduction of this marker was observed in the control group. The reason for this result is unclear. Compared to the baseline, both groups showed a similar decrease in total LDL concentration (data not shown). Also, the fat intake profile seems to be more unfavorable in the control group due to reduction in MUFA and increased in PUFA intake, which acts as protective and risk factors for oxLDL formation, respectively. Additionally, GPx and SOD, two antioxidant enzymes with a protective effect on lipid peroxidation, remain unchanged after the intervention period (30). At last, although Brazil nut has the highest SFA content compared to other nuts, the SFA from Brazil nut provided in the BN-group is not enough to promote any pro-oxidative effect (29). Taken together, these results appear not to support the differential reduction of oxLDL on the control group when compared with the BN-group.

The current study had some limitations that should be mentioned. The short sample investigated limits our possibilities for statistical analysis and conclusions. Also, all participants were females; thus, the findings may not be generalized to males. On the other hand, the study strengths may be represented by the close control of lifestyle throughout the intervention period, forward to minimize its influence on the outcomes. Furthermore, this clinical trial provides the first scientific evidence about the effects of Brazilian nuts intake on liver function and antioxidant status in women at cardiometabolic risk, besides be the first study that evaluates Brazilian nuts combined with an energy-restricted diet.

CONCLUSION

According to the present study, the daily intake of a realistic quantity of Brazilian nuts associated with an energy-restricted diet did not affect the liver enzymes. Furthermore, despite the exogenous antioxidant content provided by nuts, the

antioxidant status improvement hypothesis was not confirmed. Our results interpretations require caution and should be validated on a larger number of subjects of both sexes and by other prospective long-term controlled trials.

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Table 5.3.1 Effect of mixed nuts consumption on anthropometric, liver function, antioxidant markers, and selenium concentration.

	Control (n= 14)		Mixed nuts (n= 15)			
Parameters	**Baseline**	**Δ**	**Baseline**	**Δ**	**Basal p value**	**Δ p value**
Age (years)	31.6 ± 2.1	-	31.2 ± 2.7	-	0.972	
Body weight (kg)	87.9 ± 3.9	-1.8 ± 0.6*	90.5 ± 3.8	-3.5 ± 0.6**	0.644	0.073
BMI (kg/m²)	33.0 ± 1.0	-0.6 ± 0.2*	33.8 ± 1.2	-1.3 ± 0.2**	0.596	0.071
Waist (cm)	107.7 ± 2.6	-2.9 ± 0.6**	107.7 ± 2.8	-5.5 ± 1.1**	0.997	0.104
AST (mg/dL)	23.0 ± 1.5	-0.6 ± 1.3	24.3 ± 2.3	-2.4 ± 2.2	0.598	0.742
ALT (mg/dL)	15.4 ± 1.4	0.1 ± 1.8	15.5 ± 1.9	2.0 ± 2.8	0.944	0.578
GGT (mg/dL)	22.6 ± 2.4	-0.4 ± 1.7	22.5 ± 3.5	-0.3 ± 2.4	0.970	0.496
Alkaline phosphatase (U/L)	73.7 ± 5.8	-3.1 ± 1.8	78.5 ± 9.7	2.2 ± 2.3	0.668	0.082
FLI	72.8 ± 4.8	-5.3 ± 3.8	69.4 ± 6.6	**-9.8 ± 3.1***	0.679	0.368
SOD (U/mL)	243.2 ± 35.6	-11.7 ± 13.0	209 ± 16.1	-22.6 ± 17.7	0.616	0.622
MDA (µM/mL)	14.7 ± 0.4	0.5 ± 0.6	16.04 ± 0.7	-0.3 ± 0.6	0.445	0.366
FRAP (µM/mL)	47161.0 ± 2965.1	1325.3 ± 1987.6	44646.3 ± 1966.2	-1560.9 ± 1618.7	0.492	0.274
MDA/SOD	0.06 ± 0.04	0.005 ± 0.004	0.08 ± 0.004	0.006 ± 0.007	0.073	0.920
oxLDL (ng/mL)	130.2 ± 11.1	**-25.9 ± 7.0***	113.1 ± 10.6	13.3 ±5.8	0.277	**>0.001**
GPx (U/L)	179.0 ± 15.3	26.0 ± 21.0	206.4 ± 16.2	23.8 ± 31.5	0.231	0.955
Se (µg/L)	57.4 ± 3.8	8.9 ± 7.3	57.6 ± 4.1	35.4 ± 7.2**	0.979	**0.010**

Values are mean ± SEM. *Significant difference within-group ($p < 0.05$*; $p<0.01$**; paired t-test or Wilcoxon test). p values columns refer to the comparison between groups for baseline and change values (independent-samples t-test or Mann-Whitney U test). Δ = final assessment – baseline assessment. AST. aspartate aminotransferase; ALT. alanine aminotransferase; GGT. gamma glutamyl transferase; FLI. Fatty liver index; SOD. superoxide dismutase; MDA. malondialdehyde; FRAP. ferric reducing antioxidant power; MDA/SOD. ratio malondialdehyde/superoxide dismutase; GPx. Glutathione peroxidase; oxLDL. Oxidized low-density lipoprotein; Se. selenium.

SUPPLEMENTAL MATERIAL

Blood sampling e biochemical analyses

Venous blood samples from the antebrachial vein were collected after overnight fasting (12 h), by a registered nursing using vacuum tubes precoated with EDTA or heparin as an anticoagulant. Following, blood samples were centrifugated (1500rpm. 15 min. 4 Cº). aliquoted. and stored at -80 Cº until analysis.

Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), γ-glutamyltransferase (GGT), and Alkaline phosphatase were carried out in the Laboratory of Clinical Analysis of the Department of Nutrition of Universidade Federal de Viçosa by colorimetric methods using Mindray BS-200 Chemistry Analyzer. Fatty Liver Index (FLI) was calculated for fatty liver estimation according to the previous studies (31.32). $FLI = (e^{[0.953 \times \ln(TG) + 0.139 \times BMI + 0.718 \times \ln(GGT) + 0.053 \times WC - 15.745]} / (1 + e^{[0.953 \times \ln(TG) + 0.139 \times BMI + 0.718 \times \ln(GGT) + 0.053 \times WC - 15.745]}) \times 100$. Oxidative status markers were measured in plasma samples by colorimetric methods. Malondialdehyde (MDA) was determined in triplicate by the measurement of thiobarbituric acid reactive substances (TBARS), described by Buege and Aust (1975) (33). The total activity of superoxide dismutase (SOD) enzyme was determined in triplicate following the method described by Marklund and Marklund (1974) and your activity was expressed as U of SOD/L (34). The total antioxidant capacity of plasma was assessed by the iron reduction antioxidant test Ferric Reducing Antioxidant Power (FRAP) as proposed by Benzie and Strain (1996). For oxidized low density lipoprotein (oxLDL) and glutathione peroxidase (GPx) there was used Human Oxidized Low-Density Lipoprotein (OxLDL) ELISA Kit (EZ assay®) and EnzymChrom™ glutathione peroxidase assay kit, respectively, according to recommendations.

Dietary assessment

All women completed one 3-day food records (two nonconsecutive weekdays and one weekend day), before the study baseline assessments. During the follow-up, every fifteen days a 24-hour dietary recall was applied to monitoring food intake compliance. Food records were analyzed using REC24h-ERICA software adapted for the Brazilian population.

Supplemental table 5.3-1. Dietary compliance (by self-reported average of three 24-h dietary recalls) of women who completed the study. according to the experimental group.

	Control	Mixed nut	Basal p value	Δ p value
Total energy intake				
Baseline	1747.6 ±	1810.3 ±	0.316	0.339
Final	1545.68 ±	1492.01 ±		
Δ	-201.9 ± 206.6	-287.7 ±		
Carbohydrate (g)				
Baseline	230.3 ± 22.1	224.0 ± 20.3	0.839	0.794
Final	186.3 ± 11.2	168.6 ± 11.0		
Δ	-44.0 ± 23.9	-51.9 ± 17.0*		
Protein (g)				
Baseline	78.9 ± 8.8	78.6 ± 6.0	0.650	1.000
Final	73.3 ± 5.0	74.8 ± 6.1		
Δ	-5.6 ± 10.0	-3.0 ± 7.8		
Total fat (g)				
Baseline	58.2 ± 7.8	68.2 ± 5.0	0065	0.551
Final	58.8 ± 5.4	60.2 ± 3.2		
Δ	0.6 ± 9.6	-6.4 ± 5.8		
MUFA (g)				
Baseline	19.04 ± 3.1	23.1 ± 1.9	**0.008**	**0.009**
Final	18.08 ± 1.9	24.4 ± 1.3		
Δ	-1.0 ± 3.9	1.9 ± 2.1*		
PUFA (g)				
Baseline	10.7 ± 1.8	13.2 ± 1.3	**0.041**	**<0.001**
Final	17.4 ± 1.0	11.8 ± 0.8		
Δ	6.6 ± 1.8*	-1.1 ± 1.4		
SFA (g)				
Baseline	20.8 ± 2.5	23.6 ± 1.7	0.170	0.843
Final	16.7 ± 1.8	18.2 ± 1.1		
Δ	-4.0 ± 3.5	-4.9 ± 2.0*		
Cholesterol (mg)				
Baseline	322.4 ± 40.7	370.4 ± 35.0	0.388	0.713
Final	225.6 ± 27.1	246.1 ± 25.0		
Δ	-96.8 ± 37.3*	-113.7 ± 29.3*		
Fiber (g)				
Baseline	20.7 ± 2.9	16.4 ± 1.9	0.294	0.786
Final	22.3 ± 2.2	19.3 ± 1.3		
Δ	1.5 ± 3.5	2.9 ± 1.8		

Values are mean ± SEM. *Significant difference within-group ($p < 0.05$; paired t-test or Wilcoxon test). p values columns refer to the comparison between groups for baseline and change values (independent-samples t-test or Mann-Whitney U test). † One-way ANCOVA adjusted by baseline value. MUFA. monounsaturated fatty acid; and PUFA. polyunsaturated fatty acid; SFA. saturated fatty acid.

After intervention, there were no differences in total energy, protein, carbohydrate, total fat, cholesterol, or fiber intake at baseline or final intervention (**Supplemental table 5.3-1**). Regarding fatty acids, MUFA and PUFA intake was significantly higher in the intervention group at baseline. As expected, after 8-weeks there was a higher intake of PUFA in the control group and higher MUFA intake in the mixed nuts group. The caloric restriction was similar in the control and mixed nuts group (-201.9 ± 206.6 kcal/day vs. -287.7 ± 107.3 kcal/day, respectively), while the planned did was -500 kcal/day were not achieved. Both treatments showed a significant but similar reduction in cholesterol intake compared to baseline.

6 CONCLUSÃO

Em suma, nossos resultados demonstram que o consumo de uma mistura de amêndoas de castanhas brasileiras associado a uma dieta restrita em calorias promoveu maior redução do percentual de gordura corporal e menor perda de massa magra em comparação ao grupo controle, demonstrando o impacto positivo do consumo desses alimentos na promoção de uma composição corporal mais saudável ao longo do processo de perda de peso. Além disso, houve redução da molécula de adesão VCAM-1, sugerindo redução da inflamação endotelial. O consumo das amêndoas brasileiras também levou ao aumento do selênio plasmático, porém sem impacto sobre o status antioxidante e função hepática das voluntárias. Assim, a associação do consumo das amêndoas de castanhas brasileiras à abordagem nutricional tradicional direcionada às mulheres com excesso de peso pode auxiliar na redução do risco cardiovascular.

APÊNDICE A - Dietary fatty acids as nutritional modulators of sirtuins: a systematic review

Emerging Science

Dietary fatty acids as nutritional modulators of sirtuins: a systematic review

Ana Paula S. Caldas, Daniela Mayumi U. P. Rocha, Josefina Bressan, and Helen Hermana M. Hermsdorff

Context: *The sirtuins (SIRT1 to SIRT7) constitute a family of highly conserved nicotinamide adenine dinucleotide–dependent proteins. When activated, sirtuins control essential cellular processes to maintain metabolic homeostasis, while lack of expression of sirtuins has been related to chronic disease.* ***Objective:*** *The aim of this systematic review is to analyze the role of fat consumption as a modulator of human sirtuins.* ***Data Sources:*** *This review was conducted according to PRISMA guidelines. Studies were identified by searches of the electronic databases PubMed/MEDLINE, Scopus, and Web of Science.* ***Study Selection:*** *Randomized clinical trials assessing the effect of fatty acid consumption on sirtuin mRNA expression, sirtuin protein expression, or sirtuin protein activity were eligible for inclusion.* ***Data Extraction:*** *Two authors screened and determined the quality of the studies; disagreements were resolved by the third author. All authors compared the compiled data.* ***Results:*** *Seven clinical studies with 3 different types of interventions involving healthy and nonhealthy participants were selected. Only SIRT1 and SIRT3 were evaluated. Overall, the evidence from clinical studies to date is insufficient to understand how lipid consumption modulates sirtuins in humans. The best-characterized mechanism highlights oleic acid as a natural activator of SIRT1.* ***Conclusion:*** *These results draw attention to a new field of interest in nutrition science. The possible activation of sirtuins by dietary fat manipulation may represent an important nutritional strategy for management of chronic and metabolic disease.* ***Systematic Review Registration:*** *PROSPERO registration number CRD42018114456.*

INTRODUCTION

Sirtuins are nicotinamide adenine dinucleotide $(NAD)^+$–dependent protein deacetylases and mono-adenosine diphosphate (mono-ADP) ribosyltransferase enzymes that regulate diverse biological processes, including energy metabolism, stress responses, DNA regulation, and longevity.[1–3] The sirtuin family comprises 7 members (SIRT1–SIRT7) that possess conserved NAD^+-binding and catalytic domains. The flanking N- and C-termini of the different sirtuins are distinct from one another, which contributes to differences between sirtuins in subcellular localization, enzymatic activity, and substrate specificity.[4] Besides exhibiting deacetylase activity, some sirtuins also exhibit weak adenosine diphosphate (ADP)-ribosyltransferase activity.[5] The process of ADP-ribosylation is a reversible post-translational modification of proteins catalyzed by ADP-ribosyltransferases and some sirtuins (SIRT4–SIRT7).[6] Proteins with ADP-ribosyltransferase activity catalyze the transfer of adenosine diphosphate ribose (ADP-ribose) from NAD^+ onto specific target proteins.[7]

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Sirtuins are also classified as class III histone deacetylases, and, according to phylogenetic analysis, are grouped into 4 main classes (I–IV) of protein deacetylases, which include the human isotypes as well as at least 1 more class containing enzymes found in archaea and bacteria (class U).[8] In this classification, SIRT1–SIRT3 belong to class I, SIRT4 to class II, SIRT5 to class III, and SIRT6 and SIRT7 to class IV.[1] Different sirtuins also have different subcellular localizations: SIRT1, SIRT6, and SIRT7 have nuclear localization, SIRT2 has mainly cytosolic localization but can also shuttle to the nucleus during mitosis, while SIRT3, SIRT4, and SIRT5 have mitochondrial localization. In addition, some isoforms of SIRT1 and SIRT5 have been found in the cytoplasm.[1]

All sirtuins play fundamental roles in health maintenance. Sirtuins regulate several metabolic pathways and improve cellular and systemic adaptive responses to stress by modulating the activity of proteins.[9] Of the 7 human sirtuins, SIRT1 is studied most widely. This sirtuin deacetylates target proteins, such as transcription factors and histones,[8] mainly those involved in the regulation of energy metabolism, stress, and inflammatory responses.[10] For example, activated SIRT1 upregulates peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) by deacetylation, leading to an increase in mitochondrial biogenesis and, consequently, the expression of PGC-1α target genes linked to fatty acid oxidation pathways.[11,12] Currently, the SIRT1/PGC-1α pathway is described as a major regulator of metabolic processes in various tissues, such as liver, skeletal muscle, and white adipose tissue.[13] The main activity of SIRT2 is related to cell cycle control.[14] This sirtuin deacetylates forkhead box protein O transcription factors in response to caloric restriction and oxidative stress. SIRT3 improves mitochondrial activity by deacetylating and activating acetyl coenzyme A synthetase and several components of the electron transport chain complexes I and II. It also increases respiration in mitochondria by stimulating cyclic adenosine monophosphate (cAMP)[15] and protects cells from reactive oxygen species.[16] The mitochondrial SIRT4 regulates the pyruvate dehydrogenase complex via enzymatic hydrolysis of the lipoamide cofactor, thus modulating acetyl coenzyme A production, Krebs cycle activity, and generation of reactive oxygen species.[17] SIRT6, which is widely distributed in biological tissues, modulates telomeric chromatin and plays a role in DNA repair, human aging, glucose homeostasis, and genomic stability.[18–20] This sirtuin was also recently reported to preferentially hydrolyze long-chain fatty acyl groups over acetyl groups.[21] Lastly, SIRT7 is an activator of RNA transcription and may regulate cellular growth, metabolism, and survival.[10] As for catalytic activities, SIRT1, SIRT2, and SIRT3 have potent deacetylase and long-chain deacylase activities. SIRT4 exhibits deacylase, ADP-ribosyltransferase, and substrate-specific deacetylase and lipoamidase activities. SIRT5 displays unique affinity for negatively charged acyl modifications to lysine and is involved in protein desuccinylation, demalonylation, and deglutarylation reactions.[22] SIRT6 exhibits deacetylase, ADP-ribosyltransferase, and long-chain deacylase activities, and SIRT7 mediates deacetylation, histone desuccinylation, and long-chain deacylation reactions and is involved in shutting down rDNA transcription.[20,22]

Since the discovery of the sirtuins and their functions, different modulators of sirtuin enzymatic activity have been investigated, and several studies have found a vast array of synthetic and natural compounds with the ability to modulate sirtuin function.[1,23,24] Recently, a wide spectrum of studies proposed an association between lipids and sirtuins, since weight loss and fat mass loss by caloric restriction interventions have been associated with an increase in *SIRT1*, *SIRT3*, and *SIRT6* mRNA concentrations in the subcutaneous tissue of severely obese individuals.[25] Feldman et al[21] demonstrated that low intrinsic activity of SIRT6 might be activated in vivo in response to elevated levels of fatty acids, such as oleic acid and linoleic acid. In addition, SIRT1 appears to promote greater rates of lipolysis and a reduction in inflammatory processes; hence, the reduction in *SIRT1* mRNA levels observed in the adipose tissue of obese individuals might be associated with excessive fat accumulation and development of obesity-related comorbidities.[26] In animal studies, a high-fat diet (≈ 42%–60% fat) was shown to reduce both the expression of *SIRT1* mRNA and SIRT1 protein levels in liver,[27,28] adipose,[29,30] and cardiac tissue.[31,32]

Despite the above findings, the mechanisms through which sirtuins modulate fatty acids remain unknown. Here, evidence from interventions investigating the effect of high-fat diets or supplementation with specific fatty acids on human sirtuins is reviewed.

METHODS

Protocol and registration

The present review was conducted according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) statement[33] and has been registered with PROSPERO (https://www.crd.york.ac.uk/prospero/), registration number CRD42018114456. The PRISMA checklist is provided as Appendix 1 in the Supporting Information online.

Table 1 **PICOS criteria for inclusion of studies**

Parameter	Inclusion criteria
Participants	Adults
Intervention	Dietary intervention with high-fat diet or supplementation with specific fatty acids
Comparison	Placebo diet; supplementation with different types or the quantity of fatty acids; or before and after the intervention period, in studies with a single group
Outcomes	Change in mRNA expression or activity of sirtuin proteins after the intervention period (with fatty acid intake)
Study design	Clinical trials

Literature search

Studies were identified by searching the following electronic databases: MEDLINE/PubMed, Scopus, and Web of Science. Table 1 lists the PICOS (Participants, Intervention, Comparison, Outcomes, and Study design) criteria adopted for this review. The following search terms were used to search titles and abstracts in the databases: (sirtuin OR SIRT OR SIRT1) AND (fatty acid OR fatty acids OR polyunsaturated fatty acid OR polyunsaturated fatty acids OR PUFA OR omega 3 OR n3 OR n-3 OR w3 OR w-3 OR monosaturated fatty acid OR eicosapentaenoic acid OR EPA OR docosahexaenoic acid OR DHA OR monosaturated fatty acids OR MUFA OR saturated fatty acid OR saturated fatty acids OR SFA). The search was conducted from database inception to November 11, 2018.

Eligibility criteria

The following criteria were applied for inclusion of studies: (1) original, randomized controlled trials; (2) fatty acid consumption used as an intervention; (3) expression or activity of sirtuins or expression of sirtuin mRNA as the outcome of interest. If data were duplicated in more than 1 study, the most complete and detailed study was included. The following exclusion criteria were applied: (1) publications that did not report original studies, such as letters, comments, or reviews; (2) comparisons not based on fatty acid interventions; (3) interventions in which fatty acids were consumed together mineral/vitamin supplements, other nutrient supplements, or alcohol; and (4) interventions that included behavior modifications, such as physical activity.

Study selection and data collection process

Study selection was performed independently in an unblinded standardized manner by 2 authors. Disagreements were resolved by consensus or by consulting a third author. The following information was extracted from each study: authors; year of publication; characteristics of study participants; intervention design; study duration; and results. This information was summarized in a standard data extraction template.

Risk-of-bias assessment

Risk of bias in the included studies was assessed using the Cochrane risk of bias tool.[34] Independently, 2 authors (A.P.S.C. and D.M.U.P.R.) assessed the 7 included studies to determine whether there was low, high, or unclear risk of bias in the following domains: (1) selection bias, (2) performance bias, (3) detection bias, (4) attrition bias, and (5) reporting bias. Disagreements were resolved by an independent evaluation of a third author. The outcomes reported in published reports were compared with the outcomes described in the study protocol, when available. The evaluation of risk of bias was recorded in Review Manager (RevMan) software, Version 5.3.[35] Studies with a low risk of bias for at least 3 items were considered to be of good quality; studies with a low risk of bias for 2 items were considered to be of fair quality, and studies with a low risk of bias for only 1 item or no items were considered to be of poor quality.[34]

RESULTS

Study selection

A total of 1646 records were identified through searches of the MEDLINE/PubMed, Scopus, and Web of Science databases. Duplicate records were removed using Mendeley reference manager software, resulting in 1005 records. During screening of titles and abstracts, 979 records were removed because they did not meet the inclusion criteria: 125 were not original articles, 392 were not relevant to the topic, and 462 reported in vitro or animal studies. The remaining 26 records were retrieved and reviewed for further assessment. After reviewing the full-text articles for eligibility, 7 studies were included (Figure 1).

Study characteristics

Study design. Only SIRT1[36–41] and SIRT3[13] were evaluated in the 7 selected studies. Initially, all selected

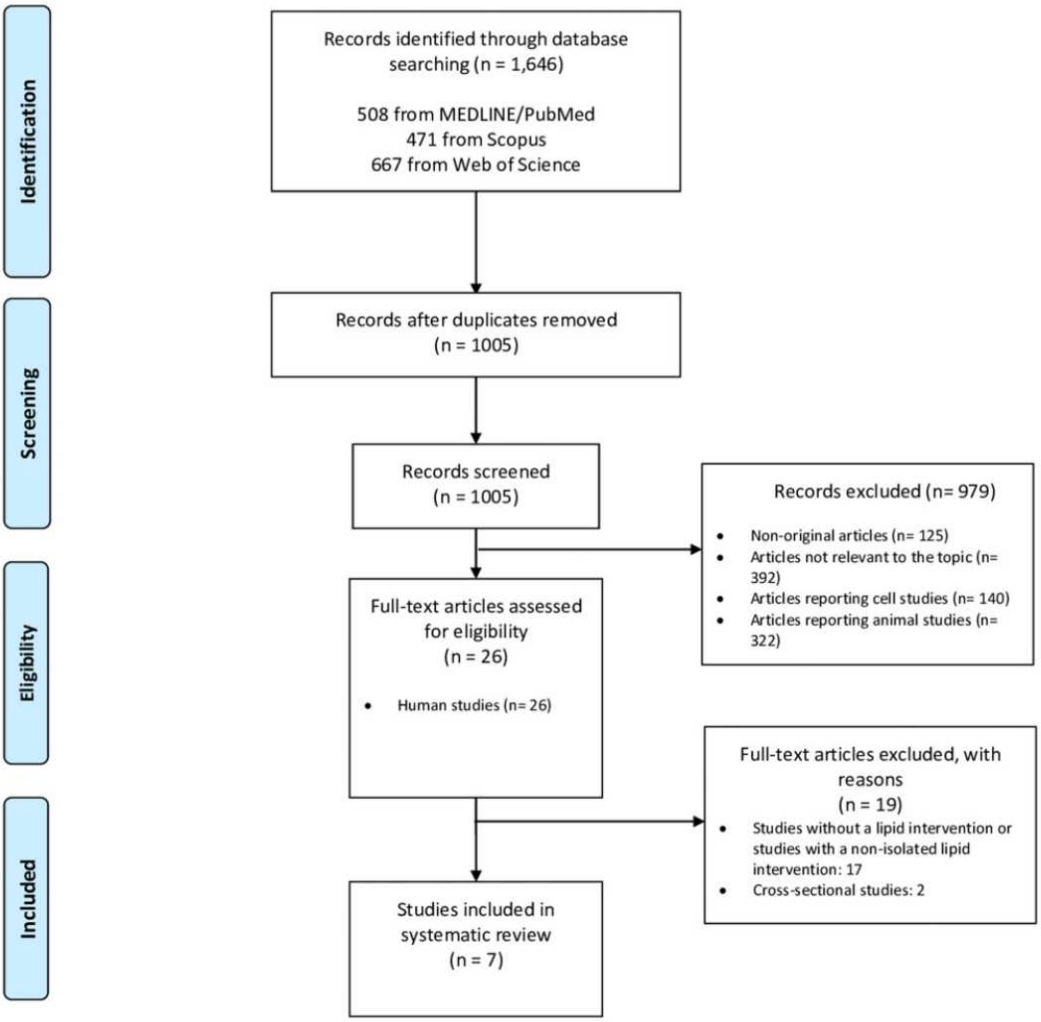

Figure 1 **Flow diagram of the literature search process.**

studies had to be randomized clinical trials, but non-randomized clinical trials also were considered, given their importance to ensuring the objective of the review was met. Four studies were conducted with subgroups of previous studies.[13,36,37,39] Two of the included studies had a single-arm design,[13,39] 2 had a crossover design,[36,38] and 3 had a parallel-arm design.[37,40,41] Two are short-term studies (2 days of intervention)[36,38] and 5 are intervention studies (8–12 weeks).[13,37,39–41] Two studies evaluated overfeeding,[13,39] 3 assessed dietary interventions,[36–38] and 2 examined the effects of supplementation with fatty acids on the expression or activity of sirtuins.[40,41]

Study participants. Eligible trials included healthy participants (n = 118),[13,36,38,39] patients with coronary artery disease (n = 60),[40,41] patients with type 2 diabetes mellitus (n = 85), and patients with metabolic syndrome (n = 75).[37] Three studies involved both men and women.[36,39,40] Three studies were conducted with men only,[13,38,41] and 1 study did not describe the sample.[37] The mean age of the participants ranged from 25 ± 5 to 56 ± 7 years. The mean body mass index at baseline ranged from 25.3 ± 3.1 to 35.1 ± 3.1 kg/m^2.

Type of intervention Three different types of interventions were used: hyperlipidic overfeeding,[13,39] dietary

interventions,[36–38] and supplementation with fatty acids[40,41] (Table 2[13,36–41]). One hyperlipidic overfeeding intervention was based on the daily addition of 70 g of lipids (100 g of cheese, 20 g of butter, and 40 g of almonds, consisting of 46.3% saturated fatty acids [SFAs], 44.7% monounsaturated fatty acids [MUFAs], and 9% polyunsaturated fatty acids [PUFAs]), which represented 760 kcal/d.[13] The other hyperlipidic overfeeding intervention provided a diet containing 1.4 times the baseline energy requirements, composed of 41% carbohydrate, 15% protein, and 44% fat (40% of which comprised saturated fatty acids).[39] In both supplementation studies, omega-3 (n-3) fatty acids were used.[40,41] The dietary interventions used a high-fat diet with or without the addition of specific fatty acids.[36–38] The controls in the interventions varied: low-fat diets,[36,37] high-fat meals,[38] and placebo, which was either paraffin[40] or unspecified.[41] Two studies used the baseline values of interest variables.[13,39]

Outcomes evaluated All of the included studies assessed SIRT1.[13,36–41] *SIRT1* expression was verified in all 7 included studies, while the SIRT1 protein level was assessed only in 1 study.[36] Although SIRT1 activity was not evaluated directly in any study, Bergouignan et al[36] and Seyssel et al[13] measured AMP-activated kinase (AMPK), acetylation of PGC-1α, and NAD^+ concentrations as markers of SIRT1 activity. One study evaluated *SIRT3* expression.[13] No study evaluated SIRT2, SIRT4, SIRT5, SIRT6, or SIRT7.

Risk of bias within studies

Figure 2[13,37–41] and Figure 3 summarize the results of the risk of bias assessment. Most studies did not provide sufficient information to be graded as having a low risk of bias and were classified as having an unclear risk of bias for most of the domains evaluated. No study was classified as "high risk of bias" for any domain evaluated. Only the study by Mazaherioun et al[40] was classified as having a low risk of bias in 6 of the 7 domains evaluated. The study by Bergouignan et al[36] was classified as having an unclear risk of bias in all 7 domains.

Short-term response studies

The effect of fat on SIRT1 activity was evaluated in 2 short-term studies.[36,38] In a randomized crossover study (with a 2-week washout period) in healthy adults, postprandial expression of *SIRT1* in peripheral blood mononuclear cells (PBMCs) was unchanged after 2 days of a high-fat meal supplemented with 15 mL of sacha inchi oil, a vegetable oil containing high levels of alpha-linolenic acid and linoleic acid (65.0% energy from fat: 19.4 percent energy (E%) from SFAs, 20.0 E% from MUFAs, 14.9 E% from PUFAs) in comparison with *SIRT1* expression after a high-fat meal without sacha inchi oil (59% energy from fat: 18.8 E% from SFAs, 13.5 E% from MUFAs, 2.3 E% from PUFAs).[38] Similarly, a high-fat diet (50.0% energy from fat: 16.0 E% from SFAs, 20.0–30.0 E% from MUFAs, 8–12 E% from PUFAs) for 2 days compared with a low-fat control diet (20.0% energy from fat: 6.0 E% from SFAs, 8.0–12.0 E% from MUFAs, 3.0–5.0 E% from PUFAs) did not modify *SIRT1* mRNA expression or SIRT1 protein levels in skeletal muscle of healthy adults.[36] Acetylation of PGC-1α was significantly reduced but total PGC-1α protein was unchanged, suggesting that the deacetylating activity of SIRT1 was markedly enhanced after the high-fat diet.

Intervention studies

Overfeeding interventions Two studies evaluated *SIRT1* expression after chronic overfeeding (8–12 weeks).[13,39] Toledo et al[39] reported that *SIRT1* expression in the skeletal muscle of healthy volunteers increased in comparison with baseline values after 8 weeks of overfeeding (diet providing 1.4 times the baseline energy requirement, containing 44% fat, of which 44% was saturated fat).

Similarly, Seyssel et al[13] reported *SIRT1* and *SIRT3* expression to be significantly increased after 56 days of hyperlipidic overfeeding (usual diet plus 760 kcal from fat per day).[13] However, they suggest that SIRT1 activity decreased, given that NAD^+ concentrations were reduced and PGC-1α acetylation was increased after overfeeding. SIRT1 is activated by NAD^+ bioavailability, and when activated, it is responsible for PGC-1α deacetylation.[13]

High-fat dietary interventions

In the study by Lopez-Moreno et al,[37] conducted in adults with metabolic syndrome, the intervention groups that received a high-MUFA diet (38% energy from fat; 20 E% from MUFAs) or a low-fat, high-complex-carbohydrate diet supplemented with long-chain n-3 PUFAs (28.0% energy from fat, supplemented with 1.24 g of n-3 PUFAs daily) showed increased expression of *SIRT1* in PBMCs when compared with baseline after 12 weeks of intervention. In addition, after long-term adaptation (12 weeks) to the corresponding intervention diet, study participants were subjected to a fat challenge consisting of a fat overload (breakfast containing 65% fat) in order to assess the postprandial response of *SIRT1* mRNA. After 4 hours, when compared with fasting values at week 12, *SIRT1* expression was increased in the high-MUFA group and decreased in the group that received

Table 2 **Summary of human studies investigating the role of lipid consumption on SIRT1 expression**

Reference	Study design	Characteristics of participants	Characteristics of intervention			Sample analyzed	Results[a]
			Type	Diet	Duration		
Seyssel et al (2014)[13]	Single-arm clinical trial[b]	n: 39 Sex: 0 F; 39 M Mean age: 34 ± 4 y Mean BMI: 25.3 ± 3.1 kg/m^2 Healthy individuals	Overfeeding	Hyperlipidic overfeeding: supplementation with 70 g of lipids (46.3% SFAs and 44.7% MUFAs)	8 wk	Skeletal muscle (20 individuals)	↑ *SIRT3* mRNA ↑ *SIRT1* mRNA
Toledo et al (2018)[39]	Single-arm clinical trial[b]	n: 26 Sex: 5 F; 21 M Mean age: 25 ± 5 y Mean BMI: 25.5 ± 2.4 kg/m^2 Healthy individuals	Overfeeding	Hyperlipidic overfeeding (40% of baseline energy requirements): 44% energy from fat (17.6 E% SFAs, 16.3 E% MUFAs, 10.1 E% PUFAs)	8 wk	Skeletal muscle	↑ *SIRT1* mRNA
Bergouignan et al (2012)[36]	Crossover clinical trial[b]	n: 11 Sex: 5 F; 6 M Mean age: 35 ± 9 y BMI: 29.6 ± 7.2 kg/m^2 Healthy individuals	Dietary interventions	Dietary interventions: Low-fat meal: 20% energy from fat (6 E% SFAs, 8–12 E% MUFAs, 3–5 E% PUFAs) High-fat meal: 50% energy from fat (16 E% SFAs, 20–30 E% MUFAs, 8–12 E% PUFAs)	2 d	Skeletal muscle	↔ *SIRT1* mRNA (both interventions) ↔ SIRT protein (both interventions)
Lopez-Moreno et al (2017)[37]	Randomized clinical trial[b]	n: 75 Sex: NA Mean age: 56 ± 7 y Mean BMI: 35.1 ± 3.1 kg/m^2 Patients with MetS	Dietary interventions	Dietary interventions: High-fat diet: 38% energy from fat (16 E% SFAs) High MUFA diet: 38% energy from fat (20 E% MUFAs) LFHCC n-3 PUFA diet: 28% energy from fat (supplemented with n-3 PUFAs, 1.24 g/d) LFHCC diet: 28% energy from fat	12 wk	PBMCs	Long-term interventions: ↑ *SIRT1* mRNA for high MUFA and LFHCC n-3 ↔ *SIRT1* mRNA for LFHCC and high SFA Postprandial status: ↑ *SIRT1* mRNA for high MUFA ↓ *SIRT1* mRNA for LFHCC

(continued)

Table 2 **Continued**

Reference	Study design	Characteristics of participants	Characteristics of intervention			Sample analyzed	Results[a]
			Type	Diet	Duration		
				(supplemented with sunflower oil as placebo, 1.0 g/d)			↔ *SIRT1* mRNA for LFHCC n-3 and high SFA
Alayón et al (2018)[38]	Crossover randomized clinical trial	n: 42 Sex: 0 F; 42 M Mean age: 40 ± 8.9 y Mean BMI: 26.6 ± 4.8 kg/m^2 Healthy individuals	Dietary interventions	Dietary interventions: High-fat meal: 59% energy from fat (18.8 E% SFAs, 13.5 E% MUFAs, 2.3 E% PUFAs) High-fat meal plus 15 mL of SIO: 65% energy from fat (19.4 E% SFAs, 20 E% MUFAs, 14.9 E% PUFAs)	2 d	PBMCs	↔ *SIRT1* mRNA (both interventions)
Saboori et al (2016)[41]	Double-blind, parallel, randomized, placebo-controlled clinical trial	n: 60 Sex: 0 F; 60 M Mean age: NA Mean BMI: 27.3 ± 0.8 kg/m^2 Patients with CAD	Supplementation	Supplementation: n-3 PUFAs (0.72 g EPA and 0.48 g DHA) Placebo: NA	8 wk	PBMCs	↔ *SIRT1* mRNA
Mazaherioun et al (2017)[40]	Double-blind, parallel, randomized, placebo-controlled clinical trial	n: 85 Sex: 32 F; 53 M Mean age: 51 ± 7 y Mean BMI: 29.2 ± 3.2 kg/m^2 Patients with T2DM	Supplementation	Supplementation: n-3 PUFAs (1.8 g EPA and 0.9 g DHA) Placebo: paraffin (2.7 g)	10 wk	PBMCs	↔ *SIRT1* mRNA

Abbreviations and symbols: BMI, body mass index; CAD, coronary artery disease; DHA, docosahexaenoic acid; E%, proportion of total energy intake; EPA, eicosapentaenoic acid; LFHCC, low-fat, high-complex-carbohydrate; MetS, metabolic syndrome; mRNA, messenger RNA; MUFA, monounsaturated fatty acid; n, number of participants; NA, not available; PBMC, peripheral blood mononuclear cell; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SIRT 1, sirtuin 1; SIO, sacha inchi oil; T2DM, type 2 diabetes; ↑, increased; ↓, decreased; ↔, unchanged.

[a]Comparison with control or baseline (in single-arm clinical trials).

[b]Studies conducted with subgroups of previous studies.

the low-fat high-complex-carbohydrate diet supplemented with n-3 PUFAs. Interestingly, the group that received the high-SFA diet had lower *SIRT1* expression in comparison with the other groups after both the long-term and the postprandial interventions.

Supplementation interventions

Neither daily supplementation with n-3 PUFAs (0.72 g of EPA and 0.48 g of DHA for 8 weeks) in patients with coronary artery disease[41] nor supplementation with n-3 PUFAs (1.8 g of EPA and 0.9 g of DHA for 10 weeks) in adults with type 2 diabetes mellitus[40] was able to modify *SIRT1* or *PGC1α* expression in PBMCs.

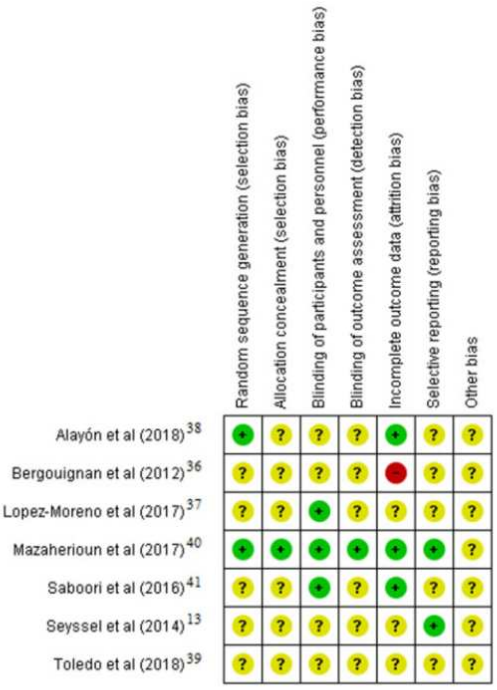

Figure 2 **Summary of risk of bias of each study included in the systematic review.**

DISCUSSION

To the best of knowledge, the present study is the first systematic review to analyze the effect of lipid consumption on human sirtuins. Currently, there is considerable interest in the exploration of dietary modulators of sirtuins, given the importance of sirtuins to health maintenance. This systematic review included 7 studies involving 338 individuals who were healthy or who had metabolic syndrome, type 2 diabetes mellitus, or coronary artery disease. These studies comprised 2 short-term studies and 5 long-term studies that used different types of interventions (supplementation, dietary intervention, and overfeeding) to examine the effects of different types of dietary fat (SFAs, MUFAs, and PUFAs) on sirtuins 1 and 3. However, there is limited evidence to establish how fatty acid intake affects the activity of human sirtuins.

Of the 7 members of the sirtuin family, SIRT1 has been studied most extensively and was evaluated in all studies included in this review, while SIRT3 was evaluated in only 1 study. SIRT1 is involved in the regulation of multiple essential cellular functions, such as mitochondrial biogenesis, glucose and lipid metabolism, DNA repair, apoptosis, stress resistance, and inflammation.[8,42–45] Consequently, alterations in SIRT1 expression or function have been associated with several chronic conditions, including cancer, diabetes, cardiovascular disease, insulin resistance, and metabolic syndrome.[26,46–48] SIRT3 is a major regulator of mitochondrial function.[49] Given its role in the control of inflammation, oxidative stress, and cellular metabolism, SIRT3 can be involved in several pathological processes. Accumulating evidence suggests that SIRT3 has a critical role in cardiovascular diseases such as hypertrophic cardiomyopathy, myocardial infarction,

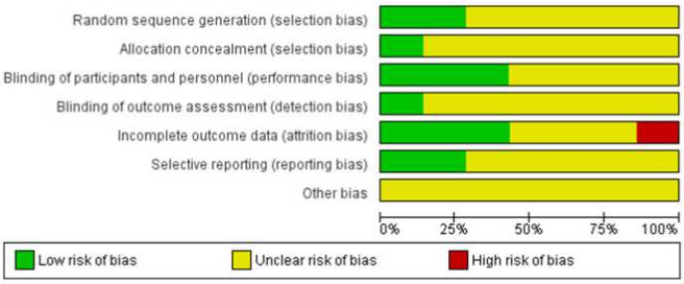

Figure 3 **Risk of bias of each item assessed, presented as percentages across all studies included in the systematic review.**

ischemia-reperfusion injury, hypertension, atherosclerosis, and heart failure.[50–53] In addition, SIRT3 might regulate systemic inflammation by improving metabolic balance.[54]

The studies of short-term lipid intake included in this review demonstrated that 2 days of a high-SFA meal or high-fat diet does not promote any modification of *SITR1* mRNA or SIRT1 protein levels in the skeletal muscle or PBMCs of healthy individuals.[36,38] However, Bergouignan et al[36] demonstrated that the amount of acetylated PGC-1α in muscle decreased without a change in total PGC-1α content, indicating an improvement in SIRT1 activity after a high-fat diet vs an isocaloric low-fat diet. Thus, in healthy individuals, an increase in dietary fat intake from 20% to 50% (of which 20% to 30% were MUFAs) of total calories for 2 days might lead to an increase in skeletal muscle oxidative capacity, since PGC-1α is a transcription factor involved in the control of fat oxidation pathways.[39,55] This suggests an acute physiological adaptive response to excessive fat intake, possibly linked to increased activity of SIRT1.

In fact, high energy status, including high-fat diet feeding, decreases cellular NAD^+ levels, which reduces SIRT1 activity.[44] Moreover, fatty acids have been highlighted as activators or inhibitors of SIRT1. In vitro studies showed that the addition of PUFAs to cell cultures was able to increase *SIRT1* mRNA expression and SIRT1 protein levels as well as SIRT1 activity.[56–58] Similarly, mice fed a diet supplemented with PUFAs also showed an increase in *SIRT1* mRNA expression and SIRT1 protein levels.[59] However, in humans, Alayón et al[38] showed that the addition of 15 mL of sacha inchi oil, a vegetable oil containing high amounts of alpha-linolenic acid and linoleic acid, to a high-SFA meal was not able to modify *SIRT1* expression in PBMCs when compared with a high-SFA meal only. In that study, the experimental meals (100 g of buttered bread and sweetened coffee with or without 15 mL of sacha inchi oil) were similar in organoleptic characteristics but contained different amounts of energy (874 kcal vs 998 kcal), which is a key factor in modulation of sirtuin function. The high-SFA meal with sacha inchi oil had more calories than the control meal. Thus, the higher amount of energy available may have prevented the PUFAs in sacha inchi oil from modulating *SIRT1* expression.[38]

Caloric restriction, which increases NAD^+ levels, was the first method proven to increase SIRT1 activity.[60] NAD^+ acts as a positive modulator of SIRT1, whereas the accumulation of nicotinamide and nicotinamide adenine dinucleotide (NADH), the reduced form of NAD^+, leads to inhibition of SIRT1.[24] NAD^+ is synthesized via 2 major pathways, the NAD^+ de novo and the salvage pathways, both of which converge at nicotinic acid mononucleotide. In the de novo pathway, the nicotinic acid moiety of NAD^+ is synthesized from tryptophan via the kynurenine pathway.[61] In the NAD^+ salvage pathway, NAD^+ is generated through the recycling of its degradation products, such as nicotinamide. During caloric restriction, there is a decline in glycolytic rates in favor of respiratory metabolism as the main energy source. These alterations change the equilibrium of the reduced/oxidized forms of NAD towards NAD^+, thus increasing SIRT1 activity.[60] Unexpectedly, the 2 included studies that used long-term hyperlipidic overfeeding and conditions of excess nutrient intake showed an increase in expression of *SIRT1* and *SIRT3* mRNA in muscle.[13,39] Because the proportions of PUFAs, MUFAs, and SFAs provided in these overfeeding studies were similar, it is difficult to attribute any observed effect to the type of fatty acid used. Furthermore, the increase in *SIRT1* expression was not necessarily associated with improved SIRT1 activity. For example, Seyssel et al[13] showed PGC-1α hyperacetylation, decreased lipid oxidation rates, and reduced NAD^+ levels after 56 days of overfeeding, suggesting a lower deacetylase activity of SIRT1. Toledo et al[39] suggested an adaptive metabolic process in the face of an increase in available energy to explain the increase in *SIRT1* expression. Thus, an adaptative metabolic process might lead to an increase in the expression of *SIRT1* and *SIRT3* toward metabolic homeostasis to compensate for a possible reduction in sirtuin activity caused by chronic consumption of a hyperlipidic diet.

Currently, the best-characterized mechanism for sirtuin modulation by fatty acids shows a MUFA as a SIRT1 modulator. Lim et al[62] demonstrated that oleic acid stimulates the cAMP/protein kinase A (cAMP/PKA) signaling pathway and activates the SIRT1/PGC-1α transcriptional complex to modulate rates of fatty acid oxidation. This new lipid signaling/transcriptional cellular route is dependent on the activation of the cAMP/PKA signaling pathway that phosphorylates SIRT1 at Ser-434 and increases its deacetylase activity. Once activated, SIRT1 deacetylates PGC-1α, which increases the expression of fatty acid oxidation genes, leading to increases in complete oxidation of fatty acids (Figure 4[27–32,56–58,62–68]). These findings were supported by Lopez-Moreno et al,[37] who conducted a study in patients with metabolic syndrome. After long-term dietary intervention with a high-MUFA diet but not a high-SFA diet, patients showed an increase in *SIRT1* expression in PBMCs.[37] Similarly, increased levels of SIRT1 protein and increased expression of *SIRT1* mRNA after stimulation of hepatic cells with oleic acid (200μM and 500μM) were observed in vitro.[62,69,70]

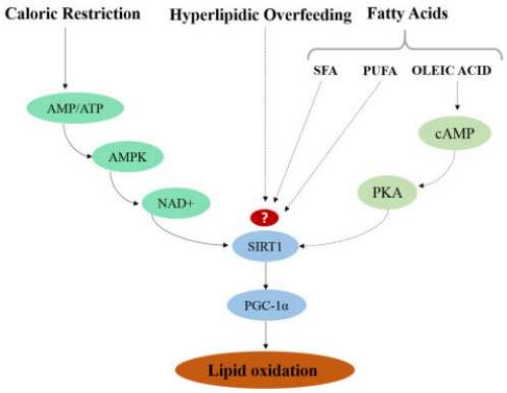

Figure 4 **Classical sirtuin modulators (solid lines) and new potential dietary sirtuin modulators (dotted lines) of SIRT1.** Under fasting conditions, SIRT1 is activated by NAD^+ and promotes PGC-1α activation through deacetylation. Once activated, PGC-1α increases the expression of target genes linked to the fatty acid oxidation pathway, thereby increasing lipid oxidation.[62,63] Current evidence suggests that sirtuins in humans also appear to be responsive to specific nutrient intake.[64] Lipids have been suggested as potential modulators of sirtuins in experimental and in vitro studies. Suppression of SFAs and improvement in SIRT1 function, both related to PUFAs, have been observed, but no mechanisms have been proposed yet.[27–32,56–58,65–68] On the other hand, MUFAs might activate SIRT1 via the cAMP/PKA pathway, which consequently promotes PGC-1α activation and, finally, improvement in lipid oxidation.[62] *Abbreviations*: cAMP, cyclic adenosine monophosphate; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; MUFA, monosaturated fatty acid; NAD^+, nicotinamide adenine dinucleotide; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PKA, protein kinase A; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SIRT1, sirtuin 1.

In 2 randomized controlled trials of n-3 PUFA supplementation (1.2–2.7 g of EPA + DHA) for 8 to 10 weeks, no changes in *SIRT1* expression in PBMCs was observed in patients with cardiovascular disease or type 2 diabetes mellitus.[40,41] Similarly, no changes in *SIRT1* expression were observed in patients with metabolic syndrome who consumed a low-fat diet. However, when those patients were supplemented with n-3 PUFAs, *SIRT1* expression increased after 12 weeks of intervention.[37] Although in vitro and animal studies have demonstrated the ability of PUFAs to enhance *SIRT1* expression, no mechanism for modulation of sirtuins by PUFAs has yet been proposed. Furthermore, both cardiovascular disease and type 2 diabetes are associated with a severe decline in sirtuin function, especially SIRT1 function.[47,71–73] Levels of SIRT1 activity are lower in diabetic patients than in healthy individuals.[74] Insulin resistance and type 2 diabetes are both associated with inflammation, oxidative stress, and mitochondrial dysfunction. Inflammation and oxidative stress also promote pancreatic β-cell dysfunction, which contributes to the progression of type 2 diabetes.[75,76] SIRT1 may reduce inflammation and oxidative stress, thereby improving mitochondrial function and resulting in the protection of pancreatic β-cells and a reduction in insulin resistance.[76] Furthermore, SIRT1 was recently reported to have a protective effect against the pathogenesis of diabetic nephropathy by modulating metabolic inflammation, autophagy, apoptosis, and oxidative stress and suppressing inflammation through deacetylation of transcription factors.[75] Nissoli et al[77] provided the first evidence linking expression of SIRT1 to increased production of endothelial nitric oxide synthase. SIRT1expression is lower in patients with heart disease than in healthy individuals.[78,79] Moreover, sirtuins exhibit atheroprotective, antithrombotic, anti-inflammatory, and antioxidant effects.[46] Thus, it is plausible to suggest that the reduced sirtuin activity observed in chronic disease may decrease the modulatory effect of fatty acids such as PUFAs.

The studies included in this review exhibited substantial heterogeneity in trial design, characteristics of study participants, type of interventions, outcomes investigated, and duration of follow-up, which prevents definitive conclusions from being reached. Nevertheless, despite the limited evidence available, this review summarizes information about the relation between sirtuins and fatty acids in humans and represents an important step toward better understanding of these mechanisms. Because sirtuins play a role in various metabolic processes and, thus, may influence the development of chronic disease, the ability of dietary components, such as fatty acids, to modulate sirtuins warrants further investigation.

CONCLUSION

This review summarizes the most recent information about the relation between consumption of fatty acids and the activity of sirtuins. Currently, the ability of fatty acids to modulate sirtuin activity is best documented in preclinical studies. It is not yet possible to establish a specific effect of PUFA, MUFA, or SFA consumption on sirtuin function in humans. Thus far, the best-described mechanism highlights the ability of oleic acid to improve SIRT1 function and, consequently, to increase fatty acid oxidation by the SIRT1/PGC-1α pathway. Additional randomized clinical trials with homogeneous populations and better controls are needed to demonstrate whether fatty acid consumption may favorably modulate the activity of sirtuins. An improvement in sirtuin activity through dietary intake of fatty acids might not only protect against obesity by

increasing fatty acid oxidation but also prevent the development of chronic disease, given the role of sirtuins in metabolic homeostasis.

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Declaration of interest. The authors have no relevant interests to declare.

Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website.

Appendix S1 **PRISMA 2009 checklist**

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APÊNDICE B - Características clínicas dos estudos conduzidos com amêndoa da castanha-do-Pará.

Autor (data)	Tipo de estudo (nº registro)	Indivíduos	Intervenção	Duração	Intervenção vs. Controle	Resultados Inicial vs. Final
1. Strunz et al. (2008)	Estudo clínico não randomizado. não controlado (N.D)	n:15 (H/M) Idade: 27.3 ± 3.9 anos IMC: 23.8 ± 2.8 kg/m² Indivíduos saudáveis	**Intervenção:** 45g/dia de amêndoa de castanha-do-Pará (~1 unid.) †862.65 µg de Se/dia	15 dias	-	↑ Selênio plasmático ↔ CT. LDL e HDL-c ↔ Apo AI e Apo B ↔ Atividade da PON1 ↑ Na captação de ésteres de colesterol pela partícula de HDL-c ↔ Na captação de fosfolipídios. colesterol livre e TGL pela partícula de HDL-c
2. Stockler-Pinto et al. (2010)	Estudo clínico não randomizado. não controlado (N.D)	n:81 (H/M) Idade: 52.0 ± 15.2 anos IMC: 24.9 ± 4.4 kg/m² Indivíduos em hemodiálise	**Intervenção:** 5g/dia de amêndoa de castanha-do-Pará (~1 unid.) †290.5 µg de Se/dia	3 meses	-	↑ Selênio plasmático ↑ Selênio eritrocitário ↑ Atividade da GPx
3. Thomson et al. (2008)	Estudo clínico randomizado controlado (N.D)	n: 59 (H/M) Idade: Controle: 42.5 ± 9.9 anos Intervenção 1: 45.6 ± 11.0 anos Intervenção 2: 49.5 ± 8.6 anos IMC: Controle: 25.9 ± 4.2 kg/m² Intervenção 1: 26.0 ± 3.6 kg/m² Intervenção 2: 28.2 ± 5.3 kg/m² Indivíduos saudáveis	**Controle:** placebo **Intervenção 1:** 1 comprimido/ dia contendo 100 µg/dia de selenometionina **Intervenção 2:** 2 unid. /dia de amêndoa de castanha-do-Pará contendo 53 µg de Se /dia	12 semanas	↑ Selênio plasmático ↑ atividade da GPx plasmática ↑ atividade da GPx no sangue total	↑ Selênio plasmático ↑ atividade da GPx plasmática ↑ atividade da GPx no sangue total

(Continua)

(Continuação)

APÊNDICE A - Características clínicas dos estudos conduzidos com amêndoa da castanha-do-Pará.

Autor (data)	Tipo de estudo (nº registro)	Indivíduos	Intervenção	Duração	Resultados	
					Intervenção vs. Controle	Inicial vs. Final
4. Cominetti et al. (2011)	Estudo clínico não randomizado. não controlado (N.D)	n:37 (M) Idade: Pro/Pro. 32.4 ± 6.5 anos Pro/Leu. 36.1 ± 7.0 anos Leu/Leu. 36.8 ± 6.8 anos IMC: Pro/Pro. 44.5 ± 3.9 kg/m2 Pro/Leu. 45.6 ± 4..7 kg/m2 Leu/Leu. 46.8 ± 3.9 kg/m2 Mulheres obesas	**Intervenção:** 5g/dia de amêndoa de castanha-do-Pará (~1 unid.) 290 µg de Se /d ia	8 semanas	-	Pro/Pro: ↑ Selênio plasmático ↑ Selênio eritrocitário ↑ Atividade da GPx ↓ Danos ao DNA Pro/Leu: ↑ Selênio plasmático ↑ Selênio eritrocitário ↑ Atividade da GPx ↔ Danos ao DNA Leu/Leu: ↑ Selênio plasmático ↑ Selênio eritrocitário ↑ Atividade da GPx ↑ Danos ao DNA
5. Stockler-Pinto et al. (2012)	Estudo clínico não randomizado. não controlado (N.D)	n:21 (H/M) Idade: 54.2 ± 15.2anos IMC: 24.4 ± 3.8 kg/m2 Indivíduos em hemodiálise	**Intervenção:** 5g/dia de amêndoa de castanha-do-Pará (~1 unid.) †290.5 µg de Se / dia	3 meses	-	3 meses após a intervenção: ↑ Nitrogênio da ureia plasmático ↔ Creatinina plasmática ↔ Cálcio plasmática ↔ Fósforo plasmático ↔ Potássio plasmático ↔ Ktv ↑ Selênio plasmático

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(Continuação)

APÊNDICE A - Características clínicas dos estudos conduzidos com amêndoa da castanha-do-Pará.

Autor (data)	Tipo de estudo (nº registro)	Indivíduos	Intervenção	Duração	Resultados	
					Intervenção vs. Controle	Inicial vs. Final
6. Maranhão et al. (2011)	Estudo clínico randomizado controlado por placebo (NCT00937599)	n:17 (M) Idade: 15.4 ± 2.0 anos IMC: 35.6 ± 3.3 kg/m2 Adolescentes obesas	**Control:** 1 capsula/dia contendo lactose **Intervenção:** 15 – 25g/dia (3 a 5 unids.) de amêndoa da castanha-do-Pará 108.5 ± 27 µg de Se/dia	16 semanas	↔ Peso corporal ↔ IMC ↔ Perímetro da cintura ↔ Insulina ↔ Glicose de jejum ↔ HOMA-IR ↔ hs-PCR ↓ CT ↔ HDL-c ↓ LDL-c ↔ TGL ↔ 8-epi-PGF2a ↓ LDL oxidada ↔ GPx3 ↔ Selênio plasmático ↔ Densidade capilar funcional ↔ Diâmetro aferente ↔ Diâmetro apical ↑ RBCV ↔ $RBCV_{max}$ ↔ $TRBCV_{max}$	↔ Peso corporal ↔ IMC ↔ Perímetro da cintura ↔ Insulina ↔ Glicose de jejum ↔ HOMA-IR ↔ hs-PCR ↓ CT ↔ HDL-c ↓ LDL-c ↔ TGL ↔ 8-epi-PGF2a ↔ LDL oxidada ↔ GPx3 ↑ Selênio plasmático ↔ Densidade capilar funcional ↔ Diâmetro aferente ↔ Diâmetro apical ↑ RBCV ↑ $RBCV_{max}$ ↔ $TRBCV_{max}$

(Continua)

(Continuação)

APÊNDICE A - Características clínicas dos estudos conduzidos com amêndoa da castanha-do-Pará.

Autor (data)	Tipo de estudo (nº registro)	Indivíduos	Intervenção	Duração	Intervenção vs. Controle	Resultados Inicial vs. Final
7. Colpo et al. (2014)	Estudo clínico agudo randomizado cruzado (N.D)	n:10 (H/M) Idade: 24.7 ± 3.4 anos IMC: 24.4 ± 3.8 kg/m2 Indivíduos saudáveis	**Intervenção:** 3 doses diferentes de amêndoa de castanha-do-Pará. 5g. 20g e 50g Avaliações nos tempos 0. 1h. 3h. 6h. 9h. 24h. 48h. 5d e 50d 31.25 ± 18.7 µg de Se/g de amêndoa de castanha-do-Pará	Agudo	-	↔ Leucócitos ↔ Eritrócitos ↔ Hemoglobina ↔ Hematócrito ↔ Plaqueta ↔ Glicose ↔ AST. ALT. GT e Fosfatase alcalina ↔ Proteína plasmática ↔ Albumina ↔ Ureia ↔ Creatinina ↔ hs-PCR ↔ Atividade eritrocitária da GSH-Px ↔ Danos ao DNA ↓ IL-1 nos tempos 24h. 48h. 5d e 30d após consumo de 20g e 50g. ↓ IL-6 nos tempos 5d e 30d após consumo de 20g e 24h. 48h. 5d e 30d após consumo de 50g. ↓ TNF-α nos tempos 24h. 48h. 5d e 30d após consumo de 20g e 50g. ↓ IFN-γ nos tempos 24h. 48h. 5d e 30d após consumo de 20g e 50g. ↑ IL-10 nos tempos 9h. 24h. 48h. 5d e 30d após consumo de 20g e 50g.

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(Continuação)

APÊNDICE A - Características clínicas dos estudos conduzidos com amêndoa da castanha-do-Pará.

Autor (data)	Tipo de estudo (nº registro)	Indivíduos	Intervenção	Duração	Resultados	
					Intervenção vs. Controle	Inicial vs. Final
8. Rita Cardoso et al. (2016)	Estudo clínico randomizado controlado (NCT02121457)	n: 31 (H/M) Idade: 77.7±5.3 anos IMC: N.D Idosos com comprometimento cognitivo	**Controle:** sem intervenção **Intervenção:** 1 unid/dia de amêndoa de castanha-do-Pará 288.75 µg de Se/dia	6 meses	↑ Se eritrocitário ↑ Se plasmático ↑ Atividade da GPx ↔ ORAC ↔ MDA ↔ Score CERAD ↔ Fluência verbal ↔ Teste de nomeação de Boston ↔ Práxis construtiva ↔ Word list learning test ↔ Word list recall	↑ Se eritrocitário ↑ Se plasmático ↔ Atividade da GPx ↔ ORAC ↔ MDA ↔ Score CERAD ↑ Fluência verbal ↔ Teste de nomeação de Boston ↑ Práxis construtiva ↔ Word list learning test ↔ Word list recall
9. Stockler-Pinto et al. (2015)	Estudo clínico não randomizado. não controlado (N.D)	n: 21 (H/M) Idade: 51.0 ± 3.3 anos IMC: 23.6 kg/m^2 (17.7–40.3) Indivíduos em hemodiálise	**Intervenção:** 5g/dia de amêndoa de castanha-do-Pará (~1 unid.) 290 µg de Se/dia	3 meses	-	Após intervenção: ↑ Se plasmático e atividade da GPx ↓ 8-Isoprostan e 8-OHdG ↓ IL-6 ↔ CT. LDL e TGL ↑ HDL 12 meses após a intervenção: ↓ Se plasmático e atividade da GPx ↑ 8-Isoprostan ↓ 8-OHdG ↑ IL-6 ↔ CT. LDL-c. HDL-c e TGL

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APÊNDICE A - Características clínicas dos estudos conduzidos com amêndoa da castanha-do-Pará.

Autor (data)	Tipo de estudo (nº registro)	Indivíduos	Intervenção	Duração	Resultados	
					Intervenção vs. Controle	Inicial vs. Final
10. Stockler-Pinto et al. (2014)	Estudo clínico não randomizado. não controlado (N.D)	n: 40 (H/M) Idade: 53.3 ± 16.1 anos IMC: N.D Indivíduos em hemodiálise	**Intervenção:** 5g/dia de amêndoa de castanha-do-Pará (~1 unid.) 290 µg de Se/dia	3 meses		↑ Se plasmático ↑ atividade da GPx ↑ HDL-c ↓ LDL-c ↓ 8-Isoprostan ↓ 8-OHdG ↓ IL-6 ↓ CT/HDL-c ↓ LDL-C/HDL-c
11. Carvalho et al. (2015)	Estudo clínico randomizado duplo cego controlado por placebo (NCT01990391)	n: 89 (H/M) Idade: controle. 60.4 ± 9.9 anos; intervenção. 59.6 ± 10.8 anos IMC: controle. 29.3 ± 4.8 kg/m^2. intervenção 29.9 ± 6.5 kg/m^2 Indivíduos hipertensos e com dislipidemia	**Controle:** dieta seguindo diretrizes para dislipidemia e hipertensão contendo 10g/dia de farinha de mandioca como placebo **Intervenção:** dieta seguindo diretrizes para dislipidemia e hipertensão contendo 13g/dia de farinha de amêndoa de castanha-do-Pará parcialmente desengordurada 227.5 µg de Se/dia	3 meses	↑ Se plasmático ↔ IMC ↔ Perímetro da cintura ↔ T3. T4 e TSH ↔ PAS e PAD ↓ CT e Colesterol não HDL-c ↔ LDL. HDL e TGL ↓ Apo AI ↔ ApoB e Apo B/Apo A ↔ Lipoproteína A	↑ Se plasmático ↔ IMC ↔ Perímetro da cintura ↔ T3. T4 e TSH ↔ PAS ePAD ↔ CT. LDL. HDL e TGL ↔ Colesterol não DHL-c ↔ Apo AI. ApoB e Apo B/Apo A ↔ Lipoproteína A

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APÊNDICE A - Características clínicas dos estudos conduzidos com amêndoa da castanha-do-Pará.

Autor (data)	Tipo de estudo (nº registro)	Indivíduos	Intervenção	Duração	Resultados	
					Intervenção vs. Controle	Inicial vs. Final
12. Huguenin et al. (2015)	Estudo clínico randomizado cruzado duplo cego controlado por placebo (NCT01990391)	n: 91 (H/M) Idade: 62.1 ± 9.3 anos IMC: N.D Indivíduos hipertensos e com dislipidemia	**Controle:** dieta seguindo diretrizes para dislipidemia e hipertensão contendo 10g/dia de farinha de mandioca como placebo **Intervenção:** dieta seguindo diretrizes para dislipidemia e hipertensão contendo 13g/dia de farinha de amêndoa de castanha-do-Pará parcialmente desengordurada 227.5 µg de Se / dia	3 meses 1 mês de washout	-	↑ Se plasmático ↔ Peso. IMC e Perímetro da cintura ↔ PAS e PAD ↔ Glicose de jejum ↑ Nitrito e nitrato ↑ HDL-c ↔ CT . LDL e TGL ↔ Densidade microvascular da Pele ↔ Recrutamento Capilar ↔ Reatividade microvascular da pele
13. Grazielle V.B. Huguenin et al. (2015)	Estudo clínico randomizado cruzado duplo cego controlado por placebo (NCT01990391)	n: 91 (H/M) Idade: 62.1 ± 9.3 anos IMC: N.D Indivíduos hipertensos e com dislipidemia	**Controle:** dieta seguindo diretrizes para dislipidemia e hipertensão contendo 10g/dia de farinha de mandioca como placebo **Intervenção:** dieta seguindo diretrizes para dislipidemia e hipertensão contendo 13g/dia de farinha de amêndoa de castanha-do-Pará parcialmente desengordurada 227.5 µg de Se / dia		↑ Se plasmático ↑ Atividade da GPx3 ↔ LDLoxidada ↑ Capacidade antioxidante total ↔ 8-epi PGF2α ↔ LDL oxidada/LDL-c	↑ Se plasmático ↑ Atividade da GPx3 ↓ LDLoxidada ↔ Capacidade antioxidante total ↔ 8-epi PGF2α ↔ LDL oxidada/LDL-c

(Continua)

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APÊNDICE A - Características clínicas dos estudos conduzidos com amêndoa da castanha-do-Pará.

Autor (data)	Tipo de estudo (nº registro)	Indivíduos	Intervenção	Duração	Resultados	
					Intervenção vs. Controle	Inicial vs. Final
14. Stockler-Pinto et al. (2015)	Estudo clínico não randomizado. não controlado (N.D)	n: 40 (H/M) Idade: 53.3 ± 16.1anos IMC: 23.0 ± 5.1 kg/m^2 Indivíduos em hemodiálise	**Intervenção:** 5g/dia de amêndoa de castanha-do-Pará (~1 unid.) 290 µg de Se / dia	3 meses	-	↑ Se plasmático ↑ Atividade da GPx ↑ T3 ↑ FT4 ↓ FT4/T3
15. Duarte et al. (2019).	Ensaio clínico randomizado. controlado	n: 55 (m) Idade: controle. 39.4 ± 9.5 anos; intervenção. 40.4 ± 9 anos. IMC: controle. 34.8 (33.1-40.2) kg/m²; intervenção 34.6 (30.8-37.4) kg/m²; Mulheres com obesidade	**Controle:** dieta habitual **Intervenção:** 1 unid. de castanha-do-Pará ~1261 µg de Se / dia	2 meses	↑ Se plasmático e eritrocitário ↑ atividade da GPx1 ↑ Selenoproteína P ↔ PCR. MCP-1. IL-6. IL-10. IL-1β. TNF-α. IFN-γ e fibrinogênio ↑ expressão gênica de selenoproteína P. TNF-α. IL-6. IL-10. TLR2. TLR4 ↓ expressão gênica de GPx1	-

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APÊNDICE A - Características clínicas dos estudos conduzidos com amêndoa da castanha-do-Pará.

Autor (data)	Tipo de estudo (nº registro)	Indivíduos	Intervenção	Duração	Resultados	
					Intervenção vs. Controle	Inicial vs. Final
16. Reis et al. (2019)	Ensaio clínico randomizado. controlado	n: 54 (m) Idade: controle. 39.4 ± 9.5 anos; intervenção. 40.4 ± 9 anos IMC: controle. 36.6 ± 6.5 kg/m²; intervenção. 34.9 ± 4.7 kg/m² mulheres com obesidade	**Controle:** dieta habitual **Intervenção:** 1 unid. de castanha-do-Pará ~1261 µg de Se / dia	2 meses	↑ Se plasma e eritrocitário ↑ expressão de miR-454-3p e miR-584-5p	-

↑ . aumentou; ↓. reduziu; ↔. sem efeito; AST. aspartato aminotransferase; ALT. alanina aminotransferase; Apo A. apolipoproteína A; Apo B. apolipoproteína B; CT. colesterol total; FT4. torixina livre; GPx. glutationa peroxidase; H. homem; HDL-c. lipoproteína de alta densidade; HOMA-IR. homeostasis model assessment; IMC. índice de massa corporal; IL-6. interleucina; IL-1. interleucina 1; IL-10. interleucina 10; IFN-γ. interferon γ; LDL-c. lipoproteína de baixa densidade; M. mulher; MDA. malondialdeído; ORAC. oxygen radical absorbance capacity; PAS. pressão arterial sistólica; PAD. pressão arterial diastólica; PON1. antioxidante enzyme paraoxonase 1; ; RBCV. red blood cell velocity; $RBCV_{max.}$ red blood cell velocity after 1 min arterial occlusion; Se. selênio; T3. triiodotironina; T4. tiroxina; TNF-α. fator de necrose tumoral α; ; TSH. hormônio tireoestimulante; TGL. triglicerídeos; $TRBCV_{max.}$ time taken to reach $RBCV_{max}$; 8-epi PGF2α. 8-epi prostaglandine F2α; 8-OHdG. 8-hidróxi-2'-deoxiguanosina.

† Quantidade estimada com base em tabelas de referência.

APÊNDICE C - Características clínicas dos estudos conduzidos com amêndoa de castanha de caju.

Autor (data)	Tipo de estudo (nº registro)	Indivíduos	Intervenção	Duração	Resultados	
					Intervenção vs. Controle	Inicial vs. Final
1. Pieters et al. (2005)	Estudo clínico. randomizado. controlado. paralelo (N.D)	n: 68 (H/M) Idade: controle 45.1 anos (IC: 40.8-49.3); Intervenção 45.7 anos (IC: 40.7-50.7) IMC: controle 35.1 kg/m² (C: 32.8-37.4) intervenção 45.7 kg/m² (IC: 40.7-50.7) Adultos com SM	**Controle**: dieta controle (16.4% ptn; 51.3 % de cho; 32.8% de lip) **Intervenção:** dieta contendo 20%E de amêndoa de castanha de caju sem sal (63-108g/dia; 16.2% ptn; 46.8% cho; 37.1% lip) * Dietas normocalórica. isocalóricas.	8 semanas	↔ Insulina ↔ HOMA-IR ↔ Fator de von Willebrand ↔ Fibrinogênio ↔ Atividade coagulante do fator VII ↔ Atividade do ativador do plasminogênio tecidual ↔ Atividade do inibidor de ativador do plasminogênio 1 ↔ Inibidor de fibrinólise ativável por trombina	↔ Insulina e HOMA-IR ↔ Fator de von Willebrand ↔ Fibrinogênio ↔ Atividade coagulante do fator VII ↔ Atividade do ativador do plasminogênio tecidual ↔ Atividade do inibidor de ativador do plasminogênio 1 ↔ Inibidor de fibrinólise ativável por trombina
2. Schutte et al. (2006)	Estudo clínico. randomizado. controlado. paralelo (N.D)	n: 62 (H/M) Idade: controle 44.4 (IC: 40.2-48.6); intervenção 45.7 (IC: 40.7-50.7) IMC: controle 35.5 kg/m² (IC: 33.1-37.8); intervenção 34.7 kg/m² (32.2-36.6) Adultos com SM	**Controle**: dieta controle (16.2% ptn; 46.8% cho; 37.1% lip) **Intervenção:** dieta contendo 20%E de amêndoa de castanha de caju sem sal (63-108g/dia; 16.4% ptn; 51.3 % de cho; 32.8% de lip) * Dietas normocalórica. isocalóricas.	8 semanas	↑ Sensibilidade barorreflexa ↑ Glicemia de jejum ↔ Perímetro da cintura ↔ IMC ↔ PAS ↔ PAD ↔ hs-PCR ↔ TGL ↔ HDL-c	↑ Sensibilidade barorreflexa ↑ Glicemia de jejum ↔ Perímetro da cintura ↔ IMC ↔ PAS ↔ PAD ↔ hs-PCR ↔ TGL e HDL-c

(Continua)

(Continuação)

Apêndice B - Características clínicas dos estudos conduzidos com amêndoa de castanha de caju.

Autor (data)	Tipo de estudo (nº registro)	Indivíduos	Intervenção	Duração	Resultados	
					Intervenção vs. Controle	Inicial vs. Final
3. Mukuddem-Petersen et al. (2007)	Estudo clínico. randomizado. controlado. paralelo (N.D)	n: 64 (H/M) Idade: 45 ± 10 anos IMC: controle. 35.1 (IC:32.8-37.4); intervenção. 34·4 (IC: 32.2-36.6) Adultos com SM	**Controle**: dieta controle (16.4% ptn; 51.3 % de cho; 32.8% de lip) **Intervenção:** dieta contendo 20%E de amêndoa de castanha de caju sem sal (63-108g/dia; 16.2% ptn; 46.8% cho; 37.1% lip) * Dietas normocalóricas. isocalóricas.	8 semanas	↔ Peso corporal ↔ LDL ↔ CT ↔ TGL ↔ HDL ↔ Frutosaminas ↑ Glicose de jejum ↔ Teste de tolerância oral a glicose ↔ PAS ↔ PAD ↔ Ácido úrico ↔ hs-CRP	↔ Peso corporal ↔ LDL ↔ CT ↔ TGL ↔ HDL ↑ Glicose de jejum ↔ Frutosaminas ↔ Teste de tolerância oral a glicose ↔ PAS ↔ PAD ↔ Ácido úrico ↔ hs-CRP
4. Davis et al. (2007)	Estudo clínico. randomizado. controlado. paralelo (N.D)	n: 64 (H/M) Idade: 45 (IC: 40.4-50.2); 46 (IC: 40.7- 50.7) IMC: controle 35.1(32.8-37.4); intervenção 34.4 (IC: 32.2-36.6) Adultos com SM	**Controle**: dieta controle (16.4% ptn; 51.3 % de cho; 32.8% de lip) **Intervenção:** dieta contendo 20%E de amêndoa de castanha de caju sem sal (63-108g/dia; 16.2% ptn; 46.8% cho; 37.1% lip) * Dietas normocalórica. isocalóricas.	8 semanas	↔ GSSG. ↔ dRom ↔ GSH ↔ GSH/GSSG ↔ ORAC	↓ GSSG. ↔ dRom ↔ GSH ↑ GSH/GSSG ↔ ORAC

(Continua)

(Continuação)

Apêndice B - Características clínicas dos estudos conduzidos com amêndoa de castanha de caju.

Autor (data)	Tipo de estudo (nº registro)	Indivíduos	Intervenção	Duração	Resultados	
					Intervenção vs. Controle	Inicial vs. Final
5. Mah et al. (2017)	Estudo clínico crossover. randomizado. controlado (NCT02769741)	n: 51 (H/M) Idade: 55.7 ± 1.42 anos IMC: 26.9 ± 0.39 kg/m² Adultos com dislipidemia ou em risco de dislipidemia	**Controle:** dieta contendo ~11% E (32–64 g/d) de chips de batata (15.7% ptn; 56.9% cho; 28.4% lip) **Intervenção**: dieta contendo ~11% E (28-64g/dia) de amêndoa de castanha d caju assada e sagada (16.3% ptn; 51.6 cho; 33.2% lip) * Dietas normocalóricas. isocalóricas.	28 dias 2 Semanas de "*washout"*	↓ LDL ↓ CT ↓ HDL-não colesterol ↓ CT: HDL ↔ HDL ↔ TGL ↑ % AGMI plasmático ↓ % AGS plasmático	↔ LDL ↔ CT: HDL ↔ HDL ↔ TGL ↔ Colesterol total ↓ HDL-não colesterol ↔ % AGMI plasmático ↔ % AGS plasmático
6. Mohan et al. (2018)	Estudo clínico. randomizado. controlado. paralelo (CTRI/2017/07/009022)	n: 300 (H/M) Idade:50.8 ± 9.5 anos IMC: 26.0 ± 3.4 kg/m² Adultos com DM2	**Controle:** dieta indiana para DM2[§] **Intervenção:** dieta indiana para DM2[§] + 30g/d (~182 kcal) de amêndoa de castanha de caju quebrada. crua e sem sal	12 semanas	↑ [ ] plasmática de ácido graxo oleico plasmático ↑ HDL-C ↓ PAS ↔ Glicemia de jejum ↔ Insulina ↔ HbA1c ↔ HOMA-IR ↔ Peso. IMC e CC ↔ LDL ↔ CT ↔ TGL ↔ VLDL ↔ CT/HDL-C	N.D

(Continua)

(Continuação)

Apêndice B - Características clínicas dos estudos conduzidos com amêndoa de castanha de caju.

Autor (data)	Tipo de estudo (nº registro)	Indivíduos	Intervenção	Duração	Resultados	
					Intervenção vs. Controle	Inicial vs. Final
7. Baer and Novotny (2019)	Estudo clínico crossover. randomizado. controlado (NCT02628171)	n: 40 (H/M) Idade: 56.8 ± 1.7 anos IMC: 29.0 ± 0.7 kg/m² Adultos saudáveis	**Intervenção**: dieta controle (33% lip; 15% ptn; 52% cho) + 42 g/dia de amêndoa de castanha de caju **Controle**: dieta controle (33% lip; 15% ptn; 52% cho) * Dietas normocalóricas. isocalóricas.	8 semanas Washout (N.D)	↓ PCSK9 ↑ TNF-a ↔ CT. LDL. HDL e TGL ↔ Apo AI. Apo AII e Apo B ↔ Glicose ↔ Endotelina ↔ Fibrinogênio ↔ Fator VII ↔ hs-PCR e IL-6 ↔ Sérum amiloide A ↔ ICAM-1 e VCAM-1 ↔ PAS e PAD ↔ Pressão arterial central ↔ Velocidade da onda de pulso	↔ PCSK9 ↔ CT. LDL. HDL e TGL ↔ Apo AI. Apo AII e Apo B ↔ Glicose ↔ Endotelina ↔ Fibrinogênio ↔ Fator VII ↔ hs-PCR. TNF-a e IL-6 ↔ Sérum amiloide A. ↔ ICAM-1 e VCAM-1 ↔ PAS e PAD ↔ Pressão arterial central ↔ Velocidade da onda de pulso

↑ . aumentou; ↓. reduziu; ↔. sem efeito; AGS. ácido graxo saturado; AGM. ácido graxo monoinsaturado; Apo AI. apolipoproteína A1; Apo AII. apolipoproteína A II; Apo B. apolipoproteína B; CT. colesterol total; DCV. doenças cardiovasculares; dRom. do inglês: "Diacron reactive metabolites"; GSH. glutationa; GSSH. do ingês: *"oxidized glutathione"*; H. homem; HDL. lipoproteína de alta densidade; hs-PCR. proteína C reativa ultra sensível; HOMA-IR. homeostatic model assessment (HOMA) index; ICAM-1. intercellular adhesion molecule 1; IMC. índice de massa corporal; M. mulher; N.D. não disponível; PAD. pressão arterial diastólica; PAS. pressão arterial sistólica; PCSK9. proprotein convertase subtilisin/kexin type 9; SM. síndrome metabólica; TGL. triglicerídeos; TNF-α. Fator de necrose tumoral α; ORAC. do inglês: "Oxygen radical absorbance capacity". VCAM-1. vascular cell adhesion protein 1 .

§ Dieta Indiana para indivíduos com DM2: (1400–1600 kcal/dia; 60–65% CHO; 15–25% LIP e completar as calorias com PTN).

APÊNDICE D – Termo de consentimento livre e esclarecido

ESTUDO
castanhas brasileiras

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (Folha 1/2)

Você está sendo convidada com voluntária a participar da pesquisa "Efeito do Consumo de um mix de Castanhas sobre a redução do Peso e Composição Corporal, Apetite, Ingestão Alimentar, Reguladores Metabólicos e Marcadores Genéticos". Todas as informações necessárias sobre a pesquisa encontram-se descritas abaixo e caso existam dúvidas, favor esclarecê-las antes da assinatura do presente termo.

Título do projeto de pesquisa: Efeito do Consumo de um mix de Castanhas sobre a redução do Peso e Composição Corporal, Apetite, Ingestão Alimentar, Reguladores Metabólicos e Marcadores Genéticos.

Pesquisadores responsáveis: Professores Josefina Bressan (coordenadora) e Helen Hermana Miranda Hermsdorff

1. Objetivos do estudo

Avaliar o efeito de um acompanhamento nutricional com dieta restritiva, associada ou não a uma suplementação de castanhas (castanha de caju e/ou castanha-do-Brasil), sobre a perda de peso, ingestão alimentar, marcadores bioquímicos e parâmetros comportamentais em mulheres adultas com excesso de peso.

2. Dos procedimentos para a coleta de dados:

Local de execução: A pesquisa será desenvolvida no Laboratório de Metabolismo Energético e Composição Corporal (LAMECC) do Departamento de Nutrição e Saúde da Universidade Federal de Viçosa.

Voluntários: Mulheres adultas (20 a 55 anos) com excesso de peso (≥ 27 kg/m^2, ≥ 32% gordura corporal e perímetro da cintura ≥ 80 cm), que estão a procura de acompanhamento nutricional para emagrecimento e que apresentem alteração na pressão arterial (≥130/≥85 mmHg ou anti-hipertensivo), na glicemia (≥ 100 mg/dL ou medicação hipoglicemiante) ou triglicerídeos (≥ 150 mg/dL ou medicação).

Coleta de dados: Todos os voluntários do estudo responderão a questionários estruturados contendo perguntas sobre história clínica e socioeconômica, hábitos alimentares, nível de atividade física, comportamental frente ao alimento, e qualidade do sono. O acompanhamento terá duração de 8 semanas e a coleta dos dados será realizado no início, durante, e ao final do estudo. Durante esse período o voluntário seguirá uma dieta individualizada com ou sem redução de 500 calorias, a qual poderá ou não ser acompanhada da ingestão de 45g de uma mistura de castanhas fornecida semanalmente pelos pesquisadores. Os parâmetros avaliados serão: peso, altura, gordura corporal, gasto de energia, análises sanguíneas completas e atividade de genes relacionados com inflamação, estresse oxidativo e metabolismo. Para a avaliação dos dados, será necessário coletar amostras de sangue e urina, no início e ao final do estudo. Durante esse período de acompanhamento nutricional, os voluntários serão orientados a manter o nível de atividade física habitual e a não ingerir bebida alcoólica.

Da utilização e armazenamento dos dados: Os dados coletados serão arquivados no laboratório de realização da pesquisa assegurando-se a privacidade dos participantes e ficarão à disposição da equipe envolvida no projeto. Estes dados poderão ser utilizados para a publicação de trabalhos científicos e outros materiais, sendo assegurado o sigilo dos voluntários. Além disso, os mesmos serão divulgados apenas de forma agregada na forma de gráficos e/ou tabelas em veículos acadêmicos.

3. Dos benefícios para os indivíduos

Todos participantes incluídos na pesquisa terão seu estado nutricional avaliado e receberão acompanhamento nutricional por 8 semanas, exceto em caso de desistência, visando a perda de peso e a promoção da sua saúde. Além disso, os participantes do estudo terão acesso aos seus dados de avaliação antropométrica, composição corporal e bioquímicos.

4. Dos potenciais riscos para os indivíduos

Não existem riscos para a saúde dos participantes, pois os procedimentos invasivos serão realizados por pessoas treinadas, minimizando ao máximo eventuais 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. No caso de eventuais complicações no momento da punção venosa, serão prestados os primeiros socorros no local pelo técnico em enfermagem e, caso aja necessidade, o voluntário será encaminhado para a Divisão de Saúde e receberá os cuidados necessários. Ainda, os alimentos fornecidos no estudo terão boa procedência e qualidade e serão bem acondicionados visando manutenção da qualidade nutricional e microbiológica.

A equipe de trabalho não se responsabiliza por informações não prestadas pelo avaliado, que possam interferir na sua saúde. O voluntário também terá direito à indenização caso ocorram danos não previstos na pesquisa.

5. Da assistência

Durante a pesquisa, os voluntários receberão avaliações nutricionais completas de maneira individualizada, como avaliações da perda de peso e composição corporal.

APÊNDICE D – Termo de consentimento livre e esclarecido

ESTUDO
castanhas brasileiras

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (Folha 2/2)

6. Das despesas

A participação como voluntário na presente pesquisa não resultará em qualquer ônus. Além disso, não haverá nenhuma forma de contrato de trabalho ou remuneração para com as voluntárias.

7. Da garantia de recusar, desistir ou revogar o consentimento

A participação no estudo é voluntária e você pode recusar-se a participar ou pode se retirar do estudo a qualquer momento sem justificativa ou penalização desde que formalizem por escrito.

Eu, _____,

fui informada dos objetivos da pesquisa "Efeito do Consumo de um mix de Castanhas sobre a redução do Peso e Composição Corporal, Apetite, Ingestão Alimentar, Reguladores Metabólicos e Marcadores Genéticos", de maneira clara e detalhada, e esclareci as minhas dúvidas. Sei que a qualquer momento poderei solicitar novas informações e modificar minha decisão de participar se assim o desejar. Declaro que concordo em participar desse estudo. Recebi uma cópia do Termo de Consentimento Livre e Esclarecido e me foi dada a oportunidade de ler e esclarecer minhas dúvidas.

Nome do pesquisador Responsável: _____

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, 6° andar. Campus Universitário. Viçosa/MG.

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Em caso de discordância ou irregularidades sob o aspecto ético desta pesquisa, você 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)3899-2492
Email: cep@ufv.br
www.cep.ufv.br

Viçosa, _____ de _____ de 20_____

_____
Assinatura do Participante

_____
Assinatura do Pesquisador

ANEXO 1 - Parecer consubstanciado do cep

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,

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inflamação, estresse oxidativo, micro organismos intestinais e expressão de genes relacionados à regulação metabólica, inflamação e estresse oxidativo. Dessa forma, poderemos compreender melhor os mecanismos pelos quais o consumo de amêndoas promove benefícios à saúde de mulheres com alterações metabólicas.

Objetivo da Pesquisa:
De acordo com os pesquisadores,
Objetivo Primário: Avaliar a perda de peso
Objetivos secundários: Avaliar a redução de gordura corporal, melhora do risco cardiometabólico, inflamação, estresse oxidativo e metabolismo energético

Avaliação dos Riscos e Benefícios:
Os pesquisadores apresentam no formulário online da Plataforma os seguintes Riscos: Não existem riscos para a saúde dos participantes, pois os procedimentos invasivos serão realizados por pessoas treinadas. 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. Este técnico será orientado a ser o mais preciso possível para evitar estes incômodos aos participantes do estudo. O uso da bioimpedância elétrica para avaliação da composição corporal e do Deltatrac para avaliação de gasto energético não envolvem riscos. 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. As amostras biológicas de fezes e urina serão coletadas e entregues em frascos adequados, fornecidos pelos pesquisadores, e sem identificação nominal para evitar eventuais constrangimentos.

E os seguintes Benefícios: Todas as voluntárias incluídas no estudo terão seu estado nutricional avaliado e receberão um plano alimentar individualizado, visando à redução de peso. Além disso, tanto o consumo de amêndoas quanto a dieta restrita em calorias estão associados a benefícios sobre o perfil lipídico. Ainda, as voluntárias do estudo terão acesso aos seus dados de avaliação antropométrica, composição corporal e bioquímicos. Ao final do estudo, todas as voluntárias receberão um novo plano alimentar individualizado, visando à redução de peso e adequação dos dados bioquímicos que se apresentarem fora dos níveis de normalidade.

Avaliação:

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Riscos e Benefícios adequadamente escritos

Comentários e Considerações sobre a Pesquisa:

Para tanto, propõe-se realizar um estudo clínico simples-cego, prospectivo, controlado, com 8 semanas de duração envolvendo 78 voluntárias distribuídos 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).

Os voluntários dos grupos testem 1 e 2 irão ingerir diariamente, por 8 semanas consecutivas, 45g de um mix de nuts, enquanto que os voluntários do grupo controle terão alimentação isenta deste alimento. A todas as voluntárias será prescrita uma dieta hipocalórica (-500 kcal) equilibrada em macronutrientes e livre de qualquer outra oleaginosa. Dados de antropometria, composição corporal, gasto energético, ingestão alimentar, além de amostras de sangue, fezes e urina, serão coletados ao início e ao final da intervenção. Adicionalmente, o peso corporal será avaliado ao final da quarta semana e a ingestão alimentar será avaliada em outros quatro momentos, ao final das semanas 2, 4, 6 e 8.

Considerações sobre os Termos de apresentação obrigatória:

Considerações sobre os documentos apresentados pelo pesquisador:

1. Formulário online - Plataforma Brasil (PB)
2. Projeto:
3. TCLE: Adequado
4. Cronograma: Adequado
5. Folha de rosto:
6. Autorização:
7. Ficha de avaliação:

Recomendações:

Quando da coleta de dados, o TCLE deve ser elaborado em duas vias, rubricado em todas as suas páginas e assinado, ao seu término, pelo convidado a participar da pesquisa ou responsável legal, bem como pelo pesquisador responsável, ou pessoa(s) por ele delegada(s), devendo todas as assinaturas constar na mesma folha.

Não é necessário apresentar os TCLEs assinados ao CEP/UFV. Uma via deve ser mantida em

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arquivo pelo pesquisador e a outra é do participante da pesquisa.

Conclusões ou Pendências e Lista de Inadequações:

Aprovado

Considerações Finais a critério do CEP:

Ao término da pesquisa é necessário apresentar, via notificação, o Relatório Final (modelo disponível no site www.cep.ufv.br). Após ser emitido o Parecer Consubstanciado de aprovação do Relatório Final, deve ser encaminhado, via notificação, o Comunicado de Término dos Estudos para encerramento de todo o protocolo na Plataforma Brasil.

Projeto aprovado autorizando o início da coleta de dados com os seres humanos a partir da data de emissão deste parecer.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1163118.pdf	03/08/2018 09:48:33		Aceito
Outros	Carta_Resposta_Estudo_castanhas_brasileiras.odt	03/08/2018 09:48:01	Josefina Bressan	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_DE_PESQUISA_modificado.docx	03/08/2018 09:46:10	Josefina Bressan	Aceito
Cronograma	CRONOGRAMA_modificado.docx	03/08/2018 09:45:33	Josefina Bressan	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_CEP_modificado.docx	03/08/2018 09:44:41	Josefina Bressan	Aceito
Outros	Questionarios_pesquisa.pdf	21/06/2018 10:52:04	Josefina Bressan	Aceito
Folha de Rosto	folha_de_rosto.pdf	20/06/2018 17:47:45	Josefina Bressan	Aceito
Cronograma	CRONOGRAMA.docx	20/06/2018 17:17:05	Josefina Bressan	Aceito
Orçamento	ORCAMENTO.docx	20/06/2018 17:16:58	Josefina Bressan	Aceito
Outros	Altorizacoes.pdf	20/06/2018 17:14:51	Josefina Bressan	Aceito
Projeto Detalhado / Brochura	PROJETO_DE_PESQUISA.docx	20/06/2018 17:12:47	Josefina Bressan	Aceito

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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)