

**UNIVERSIDADE FEDERAL DE UBERLÂNDIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE
FACULDADE DE MEDICINA**

**IMPACTO DOS EXERCÍCIOS FÍSICOS E SUPLEMENTAÇÃO DE
SUCO DE BETERRABA E DE ISOFLAVONAS NO ESTADO REDOX
DE MULHERES APÓS A MENOPAUSA**

ANA LUIZA AMARAL RIBEIRO

Doutorado

Uberlândia - 2023

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Faculdade de Medicina da Universidade Federal de Uberlândia, como requisito parcial para a obtenção do título de Doutora em Ciências da Saúde.

Área de concentração: Ciências da Saúde.

Orientador: Dr. Guilherme Morais Puga

Uberlândia

2023

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R484 2023	Ribeiro, Ana Luiza Amaral, 1995- Impacto dos exercícios físicos e suplementação de suco de beterraba e de isoflavonas no estado redox de mulheres após a menopausa [recurso eletrônico] / Ana Luiza Amaral Ribeiro. - 2023. Orientador: Guilherme Morais Puga. Tese (Doutorado) - Universidade Federal de Uberlândia, Pós-graduação em Ciências da Saúde. Modo de acesso: Internet. Disponível em: http://doi.org/10.14393/ufu.te.2023.606 Inclui bibliografia. Inclui ilustrações. 1. Ciências médicas. I. Puga, Guilherme Morais ,1982-, (Orient.). II. Universidade Federal de Uberlândia. Pós-graduação em Ciências da Saúde. III. Título. CDU: 61
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Título do trabalho: Impacto dos exercícios físicos e suplementação de suco de beterraba e de isoflavonas no estado redox de mulheres após a menopausa

Presidente da banca (orientador): Prof. Dr. Guilherme Morais Puga

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Agradecimentos

Ao Prof. Dr. Guilherme Morais Puga, pela orientação e apoio em qualquer circunstância durante estes anos.

Aos profissionais do Grupo de Pesquisa em Exercício, Saúde da Mulher e Cardiometabólica – GEPEMCardio da UFU pela disposição durante os dias de testes e coletas de dados.

A todos os meus colegas de laboratório, que acompanharam e auxiliaram de perto desde a concepção deste projeto.

Ao Igor que esteve ao meu lado em todos os momentos durante a pesquisa e fora dela.

A minha família, que sempre me apoiaram incondicionalmente.

Às voluntárias da pesquisa sem as quais este trabalho não existiria.

Às agências de fomento (Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, e Conselho Nacional de Desenvolvimento Científico e Tecnológico) que viabilizaram a execução deste projeto e a minha dedicação em tempo integral a este.

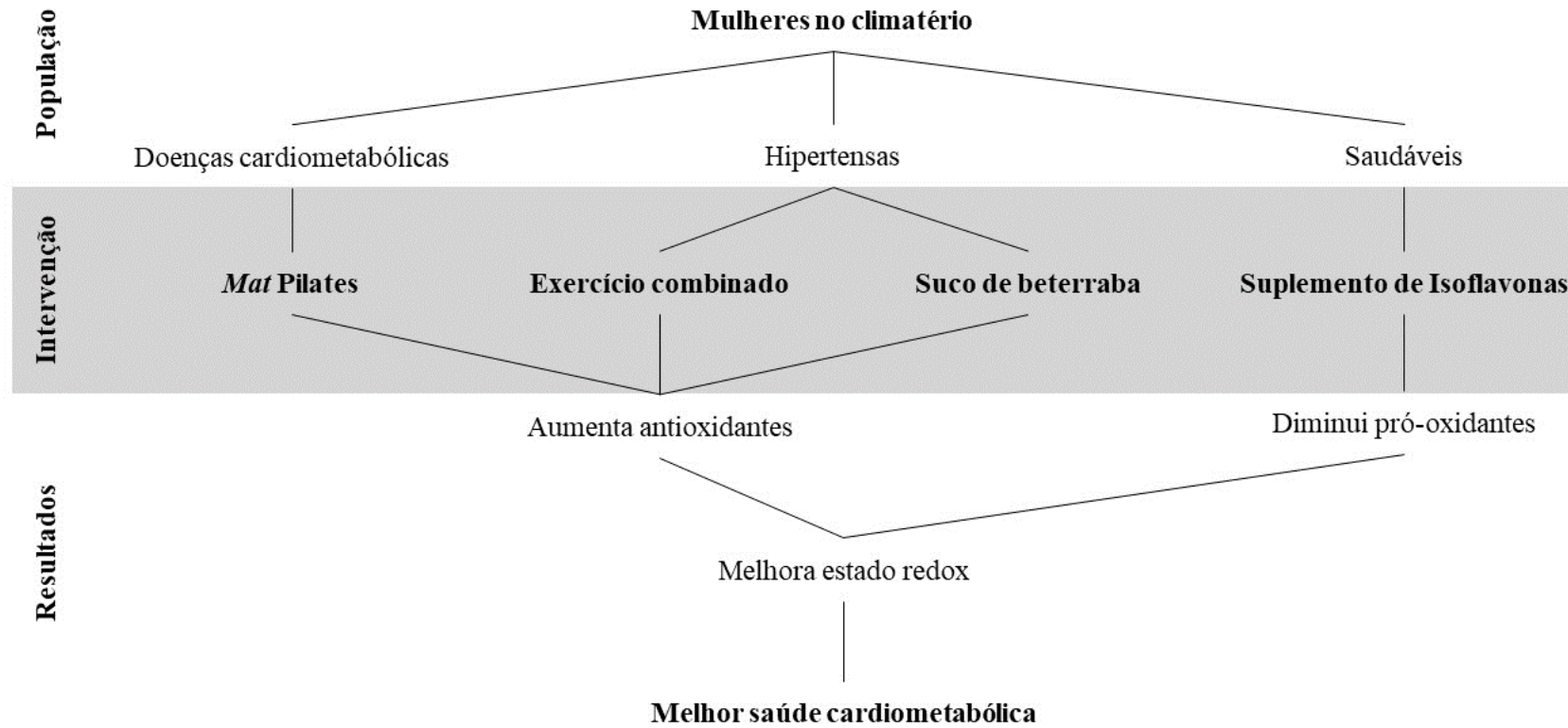
A todos que participaram e contribuíram de alguma maneira para a realização deste trabalho, meu sincero agradecimento.

Resumo

Introdução: Mulheres após a menopausa fazem parte dos grupos de risco de incidência de doenças cardiovasculares. Além disso, esta população apresenta aumento do estresse oxidativo. Duas possíveis estratégias para prevenir e tratar essas condições são o exercício físico e uma alimentação saudável. Contudo, não se sabe os efeitos de diferentes modalidades de exercício e suplementações no estado redox destas mulheres. **Objetivo:** Investigar o impacto do exercício físico e suplementação do suco de beterraba e de isoflavonas no estado redox de mulheres após a menopausa. **Métodos:** Capítulo 1) referencial teórico com embasamento para os artigos apresentados nos capítulos seguintes; Capítulo 2) discorre sobre os efeitos da ingestão de antioxidantes no sistema redox em mulheres após a menopausa; e Capítulo 3) discorre sobre os efeitos do exercício físico no sistema redox em mulheres após a menopausa. **Resultados:** Dentre os principais resultados destacamos: 1) a ingestão do suco de beterraba combinado ao exercício agudo aeróbio têm efeitos positivos em marcadores antioxidantes (catalase, $p < 0.001$; e GSH, $p < 0.001$), independente da concentração de nitrato no suco; 2) a combinação da suplementação de isoflavonas e treinamento físico promove efeitos antioxidantes, especialmente em TBARS ($p = 0.037$); 3) o estado redox de mulheres após a menopausa não apresentou mudanças mediadas pelo treinamento de *Mat Pilates* com uma ou mais doenças cardiometabólicas associadas, exceto por maiores reduções de FRAP no grupo sem multimorbidade ($p = 0.014$); e 4) o treinamento físico combinado não alterou os marcadores de estresse oxidativo salivar em mulheres após a menopausa normotensas ou hipertensas, embora, após o treinamento físico, os níveis de nitrito tenham aumentado em ambos os grupos ($p = 0.04$). **Conclusões:** O exercício físico e a suplementação do suco de beterraba e de isoflavonas podem melhorar o estado redox de mulheres após a menopausa, com ou sem doenças cardiometabólicas.

Palavras-chave: Hipertensão, Exercício, Menopausa, Multimorbidades, Pilates, Isoflavona, Suco de beterraba, Antioxidantes, Estresse Oxidativo.

Resumo Gráfico

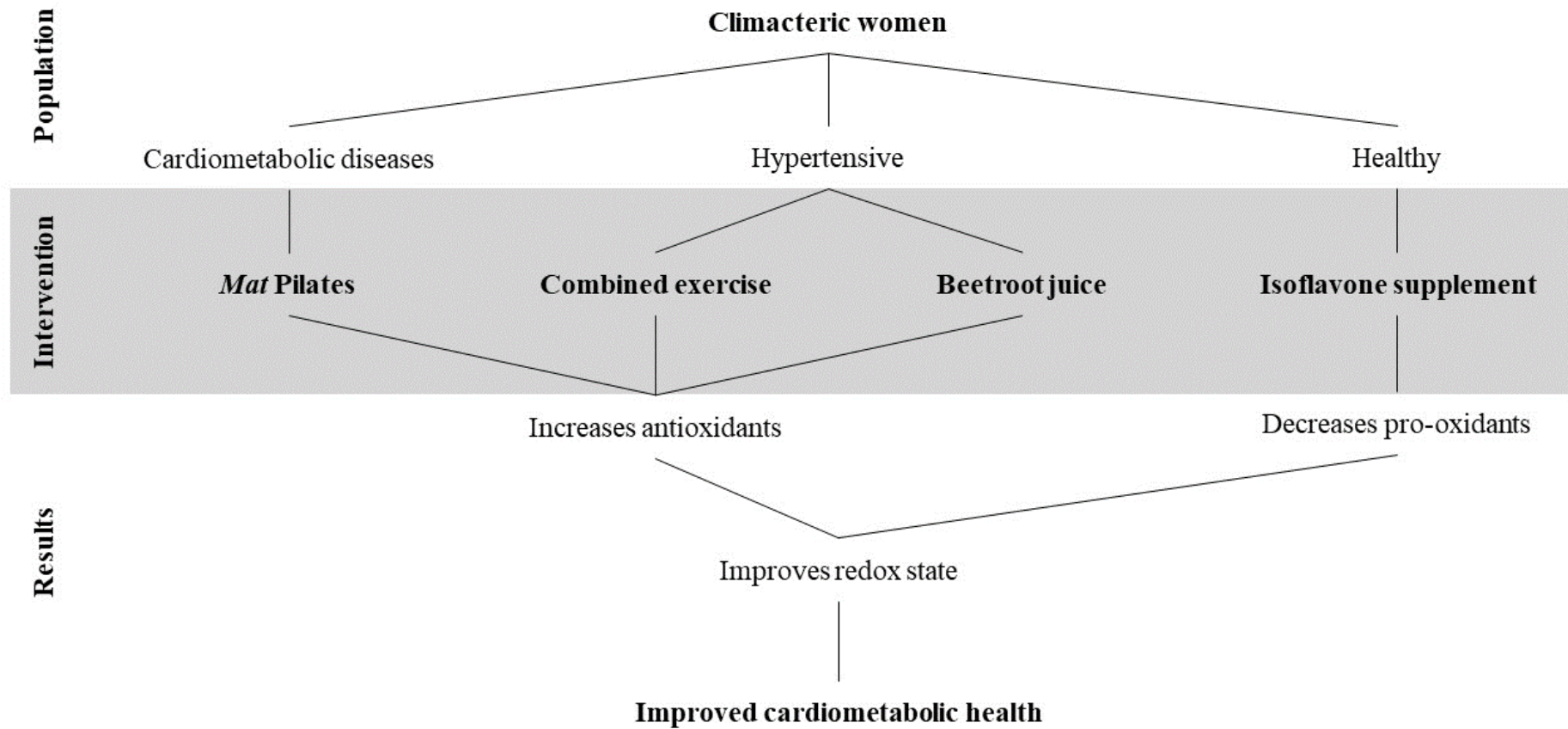


Abstract

Introduction: Postmenopausal women are considered a high-risk group for the development of cardiovascular diseases, often accompanied by increased oxidative stress. Strategies such as physical exercise and a healthy diet have been proposed to prevent and treat these conditions. However, the specific effects of different exercise modalities and antioxidant supplements on the redox status of postmenopausal women remain unclear. **Objective:** To investigate the impact of physical exercise and beetroot juice and isoflavone supplementation on the redox state of postmenopausal women. **Methods:** Chapter 1) theoretical framework - this chapter provides the theoretical foundation for the articles presented in subsequent chapters; Chapter 2) effects of antioxidant intake on the redox system in postmenopausal women - this chapter explores the effects of antioxidant supplementation on the redox system in postmenopausal women.; and Chapter 3) effects of physical exercise on the redox system in postmenopausal women - this chapter discusses the effects of different types of physical exercise on the redox system in postmenopausal women, considering factors such as cardiometabolic diseases. **Results:** Key findings from the study include: 1) beetroot juice consumption combined with acute aerobic exercise has positive effects on antioxidant markers (catalase, $p < 0.001$; and GSH, $p < 0.001$), regardless of the nitrate concentration in the juice; 2) a combination of isoflavone supplementation and physical training demonstrated potential antioxidant effects, specially evaluating by TBARS ($p = 0.037$); 3) no significant changes in the redox state of postmenopausal women following Mat Pilates training, with or without associated cardiometabolic diseases, except for greater reductions of FRAP in the group without multimorbidity ($p = 0.014$); and 4) combined physical training did not alter salivary oxidative stress markers in normotensive or hypertensive postmenopausal women, but an increase in nitrite levels was observed in both groups after physical training ($p = 0.04$). **Conclusions:** Physical exercise, as well as supplementation with isoflavones and beetroot juice, can improve the redox state of postmenopausal women, regardless of the presence of cardiometabolic diseases. These findings suggest the potential benefits of these interventions in managing oxidative stress in this population.

Keywords: Hypertension, Exercise, Menopause, Multimorbidities, Pilates, Isoflavone, Beetroot juice, Antioxidants, Oxidative Stress.

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Lista de abreviaturas e siglas

AFC	Folículos antrais
AMH	Hormônio anti mulleriano
CAT	Catalase
DCNTs	Doenças crônicas não transmissíveis
DCV	Doenças cardiovasculares
DM2	Diabetes melito tipo 2
eNOS	Óxido nítrico sintetase endotelial
ET1	Endotelina 1
FRAP	Capacidade de redução férrica do plasma
FSH	Hormônio folículo estimulante
GPx	Glutathione peroxidase
GSH	Glutathione reduzida
H ₂ O ₂	Peróxido de hidrogênio
HAS	Hipertensão arterial sistêmica
HDL	Lipoproteínas de Alta Densidade
IMC	Índice de massa corporal
LDL	Lipoproteínas de Baixa Densidade
NO	Óxido nítrico
NO ₃ ⁻	Nitrato inorgânico
O ₂ ^{-•}	Ânion superóxido
OH [•]	Radical hidroxila
ONOO ⁻	Peroxido nitrito
PA	Pressão Arterial
PA	Pressão arterial
PAD	PA diastólica
PAS	PA sistólica
RI	Resistência à insulina
ROS	Espécies reativas de oxigênio
SOD	Superóxido dismutase
TBARS	Ácido tiobarbitúrico
TG	Triglicérides

TGF- β	Fator de crescimento transformador beta
VFC	Variabilidade de Frequência cardíaca
VLDL	Lipoproteínas de Densidade Muito Baixa

LIST OF ABBREVIATIONS AND ACRONYMS

1RM	One-repetition maximum test
AC	Adjusted coefficients
ACEi or ACE	Angiotensin converting enzyme inhibitor
ANCOVA	Analysis of co-variance
ANOVA	Analysis of variance
ARB or AT1	Angiotensin receptor blocker
AUC	Area under the curve
BIA	Using electrical bioimpedance analysis
BJ	Beetroot juice
BMI	Body mass index
BP	Blood pressure
CAT	Catalase
DBP	Diastolic Blood pressure
EDTA	Ethylenediaminetetraacetic acid
FFM	Fat-free mass
FRAP	Iron-reducing antioxidant power
GEE	Generalized estimation equation
GPx	Glutathione peroxidase
GSH	Reduced glutathione
HDL	High density lipoprotein
High-NO ₃ ⁻	High Nitrate beetroot juice
HR	Heart rate
HRR	Heart rate reserve
HT	Hypertensive group
IPAQ	International physical activity questionnaire
ISO	Isoflavone supplementation
LDL	Low-density lipoproteins
Low-NO ₃ ⁻	Low Nitrate beetroot juice
MPA	Metaphosphoric acid
MULT	Women with multimorbidity
NO	Nitric oxide

NOS	Nitric oxide synthase
NT	Normotensive group
OFD	Non-Caloric orange flavored drink
PLA	Placebo supplementation
ROS	Reactive oxygen species
RPE	Rating perceived exertion scale
SBP	Systolic Blood pressure
SINGLE	Women with one cardiometabolic disease
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
TMP	Curve of 1,1,3,3-tetramethoxypropane
TP	Salivary total protein
USDA	United States Department of Agriculture
VCO ₂	Carbon dioxide output
VO ₂	Oxygen uptake
VT1	Ventilatory threshold 1
VT2	Ventilatory threshold 2
WHO	World health organization

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CAPÍTULO 1

Fundamentação Teórica

1.1. Introdução

O climatério representa a transição da fase reprodutiva para a não reprodutiva na vida da mulher, sendo a menopausa o marco dessa etapa (TAKAHASHI; JOHNSON, 2015). Com o envelhecimento populacional que ocorre devido ao aumento da expectativa de vida no Brasil (IBGE, 2020), observamos um aumento da quantidade de mulheres que vivem após a menopausa. Durante esse período, ocorrem alterações fisiológicas notáveis, como aparecimento de sintomas específicos do climatério, aumento significativo na incidência de doenças crônicas não transmissíveis (DCNTs), e aumento do estresse oxidativo. Por sua vez, estas condições impactam de maneira relevante a qualidade de vida e o processo de envelhecimento saudável das mulheres (HARLOW *et al.*, 2012).

As DCNTs estão relacionadas com altos níveis de mortalidade no Brasil, impulsionadas por fatores conhecidos como tabagismo, má alimentação, consumo excessivo de álcool e sedentarismo (WEHRMEISTER; WENDT; SARDINHA, 2022). Apesar da relação com a mortalidade, houve uma redução de 35% nas mortes por DCNTs de 1990 a 2017 (MALTA *et al.*, 2020; WEHRMEISTER; WENDT; SARDINHA, 2022). Dentre estas doenças, é possível observar aumento da prevalência de hipertensão arterial (HAS), obesidade, resistência insulínica (RI) e dislipidemia em mulheres após a menopausa (DI GIOSIA *et al.*, 2018; LOBO *et al.*, 2014; WALTON *et al.*, 1993; WHITCROFT; HERRIOT, 2011).

A HAS é um fator de risco para doenças cardiovasculares, renais e morte prematura. Afeta as mulheres após a menopausa, principalmente devido à redução da produção dos níveis de estrogênio desta população (WASSERTHEIL-SMOLLER *et al.*, 2000). Além disso, o acúmulo excessivo de gordura visceral, também está relacionado com a menopausa devido à redução dos níveis de estrogênio (LOBO *et al.*, 2014).

Neste sentido, as dislipidemias são caracterizadas pela desregulação das lipoproteínas. Esse desequilíbrio está associado com o aumento de mortalidade, principalmente relacionado a doenças cardiovasculares e com quadros agudos de doenças isquêmicas do miocárdio, por estímulo da produção da aterosclerose (FALUDI *et al.*, 2017; KOPIN; LOWENSTEIN, 2017). Concomitantemente, mulheres após a menopausa são mais vulneráveis para desenvolver RI, principalmente por modificações metabólicas relacionadas ao hipoestrogenismo, sobretudo relacionadas ao aumento da adiposidade na região central (WALTON *et al.*, 1993; WHITCROFT; HERRIOT, 2011).

Além disso, as doenças cardiometabólicas tem uma relação direta com o desbalanço do estado redox do indivíduo, tendendo para um aumento de pro-oxidantes (KATTOOR *et al.*, 2017). Por exemplo, as espécies reativas de oxigênio (ROS) tem um importante papel na patogênese da HAS. As ROS, também agem diretamente na produção de aterosclerose, estando assim associadas com eventos cardiovasculares (KATTOOR *et al.*, 2017). Além disso, o estrogênio está relacionado com a redução do estresse oxidativo vascular, logo, com a baixa da produção do estrogênio em mulheres após a menopausa, há um aumento do estresse oxidativo no organismo (SÁNCHEZ-RODRÍGUEZ *et al.*, 2012).

Há estratégias para o tratamento das DCNTs e para a regulação do estado redox no organismo de mulheres após a menopausa. Dentre estas estratégias, destacamos o exercício físico, que atua em diversos mecanismos do organismo, e é adjuvante no controle das DCNTs (CASONATTO; OLIVEIRA; GRANDOLFI, 2020; CELIK; YILDIZ, 2021; HE; YE, 2020; PAN *et al.*, 2018), no estresse oxidativo (LIN; LEE, 2018) e nos sintomas relacionados ao climatério (FLORES *et al.*, 2022; SIPILÄ *et al.*, 2020).

Além disso, a ingestão de compostos bioativos também atua como adjuvante nos tratamentos das DCNTs e no controle do estresse oxidativo. Por exemplo, as isoflavonas, por ter efeitos semelhantes ao estrogênio, são utilizadas para o alívio dos sintomas relacionados à menopausa (CHEN; KO; CHEN, 2019; KŘÍŽOVÁ *et al.*, 2019). Além disso, há indícios de que geram ações positivas na osteoporose e nas doenças cardiovasculares (KŘÍŽOVÁ *et al.*, 2019). Por fim, há indicativos de que a suplementação com isoflavonas também gera ação antioxidante e redução da peroxidação lipídica (DJURIC *et al.*, 2001).

Outra opção de tratamento com suplementação é o suco de beterraba, que é rico em compostos bioativos e em nitrato inorgânico (NO_3^-) e tem demonstrado eficácia na abordagem das DCNTs, principalmente na HAS (BORGHI; CICERO, 2017; JURIC *et al.*, 2001; REYNOLDS *et al.*, 2006; OCAMPO *et al.*, 2018). Além disso, há indícios que seus compostos podem atuar na melhora do estado redox através da ativação de vias metabólicas e ação antioxidante por eliminação de ROS (CLIFFORD *et al.*, 2015; LUNDBERG *et al.*, 2011; WINK *et al.*, 2001).

Frente a este cenário, percebemos a necessidade de estudar tratamentos relacionados ao estilo de vida de mulheres após a menopausa, com DCNTs, buscando respostas relacionadas ao exercício físico, ingestão de compostos bioativos e estado redox desta população.

1.2. Climatério, menopausa e pós-menopausa

O climatério consiste na transição entre o período reprodutivo e o não reprodutivo da vida da mulher. A menopausa é um marco desta fase, correspondendo à última menstruação, sendo reconhecida após 12 meses de amenorreia e ocorre próximo aos 50 anos de idade (TAKAHASHI; JOHNSON, 2015). Esta transição é marcada por um período de grandes mudanças fisiológicas. E de relevância clínica nesta transição, visto que elas podem desenvolver sintomas vasomotores, urogenitais, distúrbios de sono e depressão. Concomitante a estes sintomas, observamos aumento da prevalência de doenças cardiometabólicas nesta população, o que pode influenciar na qualidade de vida das mulheres e na probabilidade de envelhecimento saudável (HARLOW *et al.*, 2012).

Assim, a vida feminina adulta é dividida em três fases: reprodutiva, transição da menopausa e pós-menopausa (HARLOW *et al.*, 2012). A figura 1 apresenta didaticamente o sistema de estágios para o envelhecimento reprodutivo em mulheres. No final da fase reprodutiva, há declínio da fertilidade e neste momento a mulher pode começar a notar mudanças nos ciclos menstruais devido a parâmetros endócrinos. Neste contexto, não há modificações da concentração do FSH (hormônio folículo estimulante), entretanto as contagens do hormônio anti mulleriano (AMH) e dos folículos antrais (AFC) são baixas.

Com o passar do tempo, ocorrem modificações sutis no ciclo menstrual. Na fase folicular precoce (dias 2 a 5 do ciclo menstrual), o FSH eleva acima dos níveis normais e os níveis de AMH e AFC abaixam. Na transição da menopausa, ocorrem episódios de amenorreia acima de 60 dias. Neste estágio, os níveis de FSH às vezes estão elevados e as vezes estão dentro da faixa característica dos primeiros anos reprodutivos, principalmente associados a níveis elevados de estradiol e o hormônio anti mulleriano, por sua vez, reduz para níveis indetectáveis. Estima-se que esta fase dure de 1 a 3 anos, sendo que os sintomas costumam ser mais intensos nesta fase (HARLOW *et al.*, 2012).

A fase inicial da pós menopausa, consiste em até 8 anos após a última menstruação. Nos primeiros 2 anos após a menopausa, há rápidas mudanças nos níveis médios de FSH e estradiol e a mulher ainda apresenta sintomas, principalmente vasomotores. Nos próximos 3 anos, acontece a estabilização de altos níveis de FSH e baixos valores de estradiol e redução dos sintomas. Após este tempo, vem a fase tardia da pós menopausa, período em que as mudanças adicionais na função endócrina reprodutiva são mais limitadas e os processos de

envelhecimento tornam-se uma preocupação. Sintomas de secura vaginal e atrofia urogenital são cada vez mais prevalentes (HARLOW *et al.*, 2012).

Além disso, com a redução da produção de estrogênio, mulheres após a menopausa aumentam o risco de desenvolverem doenças cardiovasculares, já que esse hormônio têm um papel na função endotelial, tônus vascular e na função cardíaca (NEWSON, 2018). A prevalência de hipertensão arterial em mulheres na pós-menopausa é maior que em mulheres na pré-menopausa (MUIESAN *et al.*, 2016; STAESSEN *et al.*, 1989). Concomitante a isso, há um aumento da incidência de dislipidemia nessa população (FALUDI *et al.*, 2017). Além desses fatores, também observamos aumento da obesidade abdominal e da resistência à insulina nessas mulheres (STEFANSKA, *et al.*, 2015). Sendo assim, observamos a necessidade de se estudar doenças cardiometabólicas em mulheres após a menopausa, bem como o processo para a prevenção e tratamento dessas variáveis.

Menarca					Menopausa (0)						
Estágio	-5	-4	-3b	-3a	-2	-1	+1a	+1b	+1c	+2	
Terminologia	Reprodutivo				Transição		Pós menopausa				
					Perimenopausa						
Duração	Variável				Variável	1-3 anos	2 anos		3-6 anos	Tempo de vida restante	
Principal critério											
Ciclo menstrual	Variável a regular	Regular	Regular	Mudança súbita de fluxo/duração	Ao menos 7 dias de diferença na duração de ciclos consecutivos	Intervalo >= 60 dias	Inexistente				
Critério de suporte											
Endócrino											
FSH			Normal	Variável*	↑ Variável*	↑ >25IU/L	↑Variável*	Estabiliza			
AMH			Baixo	Baixo	Baixo	Baixo	Baixo	Muito baixo			
Inibina B			Baixo	Baixo	Baixo	Baixo	Baixo	Muito baixo			
Características descritivas											
Sintomas						Sintomas vasomotores	Sintomas vasomotores			Aumento dos sintomas de atrofia urogenitais	
						Provável	Mais provável				

Figura 1 –Estágios do envelhecimento reprodutivo em mulheres. Adaptado de (HARLOW *et al.*, 2012). * Coleta de sangue nos dias 2-5 do ciclo.

1.3. Doenças Crônicas não transmissíveis

O Brasil apresenta, atualmente, um cenário epidemiológico com predominância de DCNTs, que possuem fatores de risco bem conhecidos, como tabagismo, alimentação não saudável, consumo abusivo de bebidas alcoólicas, além de baixo nível de atividade física (WEHRMEISTER; WENDT; SARDINHA, 2022). Em 2017, cerca de três em cada quatro mortes foram atribuídas a DCNT no Brasil (MALTA *et al.*, 2020). Ainda que a mortalidade proporcional seja alta, de 1990 a 2017 houve uma redução de 35% nas mortes pelas DCNTs no país (MALTA *et al.*, 2020; WEHRMEISTER; WENDT; SARDINHA, 2022). Portanto, estudar DCNTs, bem como seus tratamentos, se mostra de suma importância, principalmente em mulheres após a menopausa que apresentam aumento da incidência dessas doenças (DI GIOSIA *et al.*, 2018; KO; KIM, 2020; LIVINGSTONE; COLLISON, 2002; LOBO *et al.*, 2014).

1.3.1. Hipertensão

A HAS é uma DCNT caracterizada pela elevação persistente da pressão arterial (PA), ou seja, PA sistólica (PAS) maior ou igual a 140 mmHg e/ou PA diastólica (PAD) maior ou igual a 90 mmHg, em que os benefícios do tratamento (não medicamentoso e/ou medicamentoso) superam os riscos. Trata-se de uma condição multifatorial, que depende de fatores genéticos/epigenéticos, ambientais e sociais (BARROSO *et al.*, 2020).

Por se tratar de condição frequentemente assintomática, a HAS costuma evoluir com alterações estruturais e/ou funcionais em órgãos-alvo, como coração, cérebro, rins e vasos. A hipertensão também é o principal fator de risco modificável para doenças cardiovasculares (DCV), doença renal crônica e morte prematura. Além disso, ela se associa a fatores de risco metabólicos para as doenças dos sistemas cardiocirculatório e renal, como dislipidemia, obesidade abdominal, intolerância à glicose, e diabetes melito tipo 2 (DM2) (BARROSO *et al.*, 2020).

Nas mulheres esta doença tem maior incidência a partir dos 50 anos de idade (DI GIOSIA *et al.*, 2018), podendo ser explicada pela deficiência de estrogênio causado pelo processo da menopausa. Este hormônio modula a PA principalmente através de ação endotelial (WASSTHEIL-SMOLLER *et al.*, 2000), elevando a produção de vasodilatadores como prostaciclina e óxido nítrico (CARDOSO JUNIOR *et al.*, 2007; ZANESCO; ZAROS, 2009).

O tratamento para controle da HAS é baseado na utilização de medicamentos anti-hipertensivos associados a tratamentos não medicamentosos relacionados a hábitos de vida, que incluem a prática regular de exercício físico, hábitos alimentares saudáveis e manejo do estresse

(BARROSO *et al.*, 2020; SOUSA *et al.*, 2017). Além disso, o controle da massa corporal se mostra uma importante intervenção, visto que a obesidade relacionada a adiposidade visceral tem relação com um pior prognóstico da HAS (SERAVALLE; GRASSI, 2017).

1.3.2. Obesidade

A obesidade é uma doença crônica, progressiva e recorrente, associada a diversas complicações. É definida como um acúmulo excessivo de gordura, que por sua vez causa e agrava outras doenças agudas e crônicas e reduzem a expectativa de vida (HALPERN *et al.*, 2022; WORLD HEALTH ORGANIZATION, 2000).

Para o diagnóstico da obesidade, são necessárias avaliações que se complementam. O Índice de massa corporal (IMC) é amplamente utilizado, sendo que o cálculo se baseia no peso e altura do indivíduo aplicado a seguinte fórmula: $(\text{peso(kg)} / \text{estatura(cm)}^2)$ (WORLD HEALTH ORGANIZATION, 2000). A classificação do IMC se encontra na tabela 2. Concomitante ao IMC, valores de circunferência da cintura também são utilizados, sendo medida no ponto médio entre a borda inferior da caixa torácica e a crista ilíaca, e com seu resultado é possível avaliar a adiposidade localizada na região central (WORLD HEALTH ORGANIZATION, 2000). Os valores de referência da circunferência da cintura se encontram na tabela 3. Outros meios disponíveis para avaliar a composição e distribuição corporal que podem ser úteis na prática clínica ou na pesquisa, são a análise de bioimpedância, absorciometria de raios X de dupla energia e tomografia computadorizada, porém essas ferramentas raramente são usadas para diagnóstico ou tratamento da obesidade (HALPERN *et al.*, 2022; LEMOS; GALLAGHER, 2017; WARD, 2019)

Tabela 1: Classificação do IMC para adultos.

Classificação	IMC	Risco de comorbidades
Baixo peso	<18,50	Pequeno (mas risco de desenvolve outros problemas clínicos)
Eutrofico	18,50-24,99	Médio
Sobrepeso	25,00-29,99	Aumentado
Obesidade I	30,00-34,99	Moderado
Obesidade II	35,00-39,99	Severo
Obesidade III	≥ 40	Muito Severo

Fonte: Adaptado de WHO (1998).

Tabela 2: Valores de referência para circunferência da cintura:

Circunferência da Cintura		
	Risco aumentado	Risco substancialmente aumentado
Masculino	≥ 94 cm	≥ 102 cm
Feminino	≥ 80 cm	≥ 88 cm

Fonte: Adaptado de WHO (2000).

Neste sentido, em mulheres após a menopausa a baixa disponibilidade de estrogênio no organismo está relacionada a modificação da distribuição da gordura corporal (LOBO *et al.*, 2014). O estrogênio ovariano é um dos responsáveis pelo acúmulo da gordura periférica, principalmente na região femoral e glútea. Este hormônio possui receptores expressos no tecido adiposo viscerais e subcutâneos, sendo que a ausência desses receptores causa hiperplasia e hipertrofia nas células adiposas, além de aumentar a resistência à insulina, hiperlipidemia e intolerância à glicose (KO; KIM, 2020). Sendo assim com a ausência da produção do estrogênio, a mulher aumenta o acúmulo de gordura na região central, aumentando por consequência a circunferência abdominal (KO; KIM, 2020).

Por fim, apesar das avaliações supracitadas serem amplamente utilizadas, o diagnóstico da obesidade tem sido cada vez mais complexo, já que nem sempre o tecido adiposo por si só é causador de doenças. Sendo assim, uma estratégia interessante para a melhor avaliação da saúde geral, é avaliar outros sinais e sintomas e relacioná-las com a obesidade, como por exemplo a resistência insulínica e a presença de gordura hepática (HALPERN *et al.*, 2022).

1.3.3. Dislipidemia

As dislipidemias consistem na desregulação das lipoproteínas, podendo ser classificadas em hiperlipidemias (níveis elevados de lipoproteínas) e hipolipidemias (níveis plasmáticos de lipoproteínas baixos) (FALUDI *et al.*, 2017). Os lipídeos são um dos principais compostos orgânicos naturais que constituem os seres vivos, sendo que os lipídeos biologicamente mais relevantes são os fosfolipídios, o colesterol, os ácidos graxos e os triglicérides (TG). Agregados moleculares de lipídeos e proteínas são chamados de lipoproteínas, que por sua vez transportam lipídeos em meio líquido. Esse transporte tem como destino os órgãos, que metabolizam esses lipídios nos tecidos periféricos e fígado (FALUDI *et al.*, 2017).

De forma mais específica, as lipoproteínas podem ser divididas em dois grupos: 1) ricas em TG, que são maiores e menos densas, representadas pelos quilomícrons, de origem intestinal, e pelas Lipoproteínas de Densidade Muito Baixa (VLDL, sigla do inglês *very low density lipoprotein*), de origem hepática; e 2) ricas em colesterol, que incluem as de Baixa Densidade (LDL, do inglês *low density lipoprotein*), popularmente conhecido por “colesterol ruim” e as de Alta Densidade (HDL, do inglês *high density lipoprotein*), conhecido popularmente por “colesterol bom” (FALUDI *et al.*, 2017).

Os desequilíbrios destas lipoproteínas estão associados com o aumento de mortalidade e morbidade, principalmente relacionado a doenças cardiovasculares e com quadros agudos de doenças isquêmicas do miocárdio. Esta resposta ocorre porque a dislipidemia estimula a formação de aterosclerose, uma doença crônica inflamatória de origem multifatorial, que ocorre em resposta à agressão endotelial, acometendo principalmente artérias de médio e grande calibre. A aterosclerose ocorre devido ao acúmulo de gorduras, colesterol e outras substâncias, formando placas de gorduras que causam obstrução do fluxo sanguíneo, sendo que esses depósitos dificultam a passagem de sangue dos vasos, podendo causar infartos, acidente vascular encefálico e até morte súbita (FALUDI *et al.*, 2017; KOPIN; LOWENSTEIN, 2017).

E como já discutido no tópico sobre obesidade, devido a progressiva redução até a cessação da produção de estrogênios pelos ovários, mulheres climatéricas possuem uma alteração no metabolismo lipídico, com aumento do acúmulo da gordura visceral. A gordura localizada na região central está associada com mudanças no metabolismo dos lipídios, pois ocorre aumento da lipólise, que pode gerar aumento do metabolismo de ácidos graxos (KO; KIM, 2020) e elevação do LDL, além de reduzir a relação entre o colesterol total e HDL (LOBO *et al.*, 2014). Além disso, o aumento da adiposidade visceral também está relacionado com a hiperinsulinemia, aumentando a resistência à insulina (KO; KIM, 2020). A deficiência do estrogênio também resulta na redução dos níveis de HDL plasmático e o aumento do LDL, colesterol total e dos triglicerídeos (KO; KIM, 2020). O HDL tem ação antioxidante, e evita a oxidação do LDL, sendo que este é um dos responsáveis pela aterosclerose. Além disso o HDL também colabora na prevenção da formação das placas de ateromas nos vasos sanguíneos (FALUDI *et al.*, 2017).

1.3.4. Resistência insulínica

A RI é uma condição em que há algum efeito biológico reduzido relacionado a qualquer concentração de insulina (WHITCROFT; HERRIOT, 2011). Pode ser definida usando uma

visão glucocêntrica, ou seja, quando um defeito na ação da insulina resulta em hiperinsulinemia de jejum para manter a euglicemia (ECKEL, 2007).

Com o metabolismo glicêmico prejudicado, é possível observar aumento da disfunção endotelial com comprometimento seletivo na produção de óxido nítrico (NO) mediada por insulina, resultando em vasodilatação desequilibrada, aumento da inflamação, aterogênese e HAS (SCHULMAN; ZHOU, 2009). Além disso, a RI também pode estar relacionada com a dislipidemia, sendo que nesse quadro há aumento do LDL e dos triglicerídeos, além da diminuição correspondente no colesterol HDL. Cronicamente, com a manutenção da RI, o indivíduo pode desenvolver a DM2, além de estar associado a outras DCV, a síndrome do ovário policístico, esteatose hepática não alcoólica e distúrbios de sono. Concomitante a este cenário, a RI é uma característica proeminente da síndrome metabólica (WHITCROFT; HERRIOT, 2011).

Por sua vez, mulheres após a menopausa são mais vulneráveis para desenvolver a RI principalmente por modificações metabólicas relacionadas ao hipoestrogenismo, sobretudo relacionadas ao aumento da adiposidade na região central (WALTON *et al.*, 1993; WHITCROFT; HERRIOT, 2011). A utilização de terapia hormonal a níveis fisiológicos de estrogênios gera uma diminuição no hiperandrogenismo e uma melhora na homeostase da glicose, portanto acredita-se que o hormônio estrogênio tenha um papel na manutenção da sensibilidade à insulina (LIVINGSTONE; COLLISON, 2002). Além disso, a deficiência de estrogênio está associada à deterioração da homeostase da glicose e da RI, sendo que mulheres após a menopausa aparentemente apresentam níveis semelhantes de glicose e insulina ao se comparar com mulheres na pré-menopausa. Porém, nas mulheres após a menopausa foi observado 50% a menos da produção de insulina, além disso sua eliminação ocorreu de forma mais lenta, apresentando um aspecto de compensação (GODSLAND, 2005; WALTON *et al.*, 1993). Concomitante a este cenário, a insulina se apresenta como um importante regulador da função ovariana, sendo que a RI interfere nesta regulação, portanto a insulina e o estrogênio parecem estar diretamente interligados.

Como elucidado, a HAS, obesidade, dislipidemia e RI, muitas vezes se encontram relacionadas. Atualmente, com o envelhecimento populacional, muitas mulheres passam um terço de suas vidas após a menopausa (LOBO *et al.*, 2014), e isso se relaciona diretamente com aumento da prevalência dessas condições crônicas. Sendo assim, esta população está vulnerável

a desenvolver essas doenças de forma associada, sendo importante conhecer esses efeitos para melhor manejo desta população.

1.3.5. Multimorbidades Cardiometabólicas

O termo multimorbidade consiste na coexistência de duas ou mais condições crônicas (BRICCA *et al.*, 2020), podendo englobar qualquer tipo de doença. Pessoas com multimorbidades tem um risco de mortalidade prematura maior ao se comparar com aquelas sem doenças (MENOTTI *et al.*, 2001). Condições acumuladas podem induzir ou exacerbar outras doenças por meio de fisiopatologia comum (por exemplo, vias inflamatórias crônicas de DCV aumentando os riscos de desenvolver DM2) (FORMAN *et al.*, 2018; FORTIN *et al.*, 2012). As sequelas comuns da multimorbidade incluem interações entre doenças, competição terapêutica quando um medicamento para uma doença inadvertidamente desestabiliza outra, além da eficácia do tratamento versus efeitos adversos (UHLIG *et al.*, 2014).

As doenças cardiometabólicas apresentadas neste capítulo, apesar da dificuldade na aderência aos tratamentos, já apresentam diretrizes de manejo bem definidas, porém quando essas doenças estão associadas, podem surgir dificuldades em unificar os tratamentos. Com o aumento do risco de desenvolvimento de DCV em mulheres após a menopausa, percebemos que esta é uma população suscetível ao desenvolvimento de multimorbidades, portanto é importante que tenhamos um olhar específico para elas.

1.3.6. Doenças cardiometabólicas e Estresse oxidativo

O acometimento das doenças cardiometabólicas tem uma relação direta com o desbalanço oxidativo do indivíduo, tendendo para um aumento de pro-oxidantes (KATTOOR *et al.*, 2017). As espécies reativas de oxigênio (ROS) tem um importante papel na patogênese destas doenças (SANTILLI; D'ARDES; DAVÌ, 2015). Na hipertensão, elas podem gerar disfunção do sistema renina-angiotensina (LANDMESSER; DREXLER, 2003; TANAKA *et al.*, 2005), e no diabetes através da desregulação do eixo produtos finais da glicação avançada (SANTILLI; D'ARDES; DAVÌ, 2015). Além disso, as ROS agem diretamente na produção de placas ateroscleróticas através da oxidação de LDL e colaboram com aumento da disfunção endotelial (KATTOOR *et al.*, 2017).

1.4. Estresse Oxidativo

Estresse oxidativo é "um desequilíbrio entre oxidantes e antioxidantes em favor dos oxidantes, levando a uma interrupção da sinalização e controle redox e/ou danos moleculares" (SIES, 2015). O estresse oxidativo constitui em um mecanismo que está relacionado a processos

de doenças, que ocorre quando há o desequilíbrio entre as ROS e os sistemas de defesa antioxidante no organismo.

1.4.1. Espécies Reativas de Oxigênio

As ROS são moléculas instáveis e extremamente reativas capazes de transformar outras moléculas com as quais reagem. Geralmente são geradas em grande quantidade durante o estresse oxidativo, condição em que são afetadas moléculas como proteínas, carboidratos, lipídeos e ácido nucleicos (YOUNG; WOODSIDE, 2001). Além disso, as ROS compreendem em muitas moléculas que possuem efeitos divergentes na função celular, sendo que o desequilíbrio produção/inativação tem papel fundamental na gênese das DCV. Os efeitos das ROS são mediados através da regulação sensível a redox de múltiplas moléculas de sinalização e segundos mensageiros (GONZÁLEZ, 2014).

As ROS mais conhecidas são: ânion superóxido ($O_2^{\cdot-}$), radical hidroxila ($OH^{\cdot}$), peróxido de hidrogênio (H_2O_2) e o peróxido nitrito ($ONOO^{\cdot-}$) (ZANESCO; PUGA, 2013). A função mais estudada do $O_2^{\cdot-}$ derivado de NOX é a inativação do NO para formar $ONOO^{\cdot-}$, levando à diminuição da vasodilatação dependente do endotélio e desacoplamento da óxido nítrico sintetase endotelial (eNOS) para produzir superóxido adicional (GONZÁLEZ, 2014), sendo que a principal função da eNOS é a produção de NO, que regula a vasodilatação.

1.4.2. Antioxidantes

Os antioxidantes são moléculas que podem doar elétrons aos oxidantes, interrompendo as reações em cadeia. Nosso organismo é dotado de um poderoso sistema de defesa antioxidante tem como função impedir as reações deletérias das ROS. Este sistema consiste em antioxidantes endógenos (albumina, bilirrubina, glutathione), antioxidantes exógenos (tocoferóis, carotenóides, vitamina C) e enzimas antioxidantes (superóxido dismutase – SOD; catalase – CAT; glutathione peroxidase - GPx) que convertem substratos ($O_2^{\cdot-}$ e H_2O_2) em formas menos reativas (WANG; CHUN; SONG, 2013).

Das enzimas antioxidantes, a SOD, do qual existem três isoformas, é particularmente importante, pois catalisa a dismutação de $O_2^{\cdot-}$ para H_2O_2 e localiza-se em compartimentos vasculares discretos: citosol para SOD1, mitocôndrias para SOD2 e matriz extracelular para SOD3. A SOD influencia a sinalização redox vascular de maneira regulada e compartimentada. Nas células endoteliais, a SOD dismuta o ânion superóxido antes que ele reaja com o NO, reduzindo assim a formação de $ONOO^{\cdot-}$ (MONTEZANO *et al.*, 2015).

As catalases são enzimas que desempenham papéis críticos na proteção das células contra os efeitos tóxicos do H_2O_2 . Esta enzima tem a função de proteger a hemoglobina, removendo mais da metade do H_2O_2 gerado em eritrócitos humanos normais, que são expostos a concentrações substanciais de oxigênio. Além disso, sugere-se que o papel da catalase na defesa antioxidante depende do tipo de tecido e do modelo de lesão tecidual mediada por oxidante (GOYAL; BASAK, 2010). Há indícios que a catalase está relacionada com a proteção contra H_2O_2 das células β pancreáticas (ELSNER; GEHRMANN; LENZEN, 2011; LUPPI *et al.*, 2020) e que pode proteger células contra a inflamação (GOYAL; BASAK, 2010).

Outra importante proteção antioxidante é a GPx, uma enzima intracelular que reduz o peróxido de hidrogênio a água para limitar seus efeitos nocivos, e em algumas condições fisiológicas pode ser considerado mais eficaz que a catalase na remoção de peróxidos intracelulares. A GPx também está relacionada com respostas patológicas adaptativas, como apoptose ou inflamação e processos de doença/lesão tecidual, tais como aqueles envolvidos na aterogênese, DM2, disfunção endotelial, toxicidade de drogas, e lesão de isquemia-reperfusão (LUBOS; LOSCALZO; HANDY, 2011).

1.4.3. Biomarcadores de Estresse Oxidativo

As ROS são instáveis e têm uma meia-vida muito curta, portanto, avaliar com precisão o $\text{O}_2^{\cdot -}$ e o H_2O_2 na clínica é um desafio. Para isso desenvolveu-se métodos para medir marcadores estáveis de ROS que refletem o status oxidativo, e marcadores de antioxidantes, que refletem a resposta antioxidante. Com isso, atualmente os biomarcadores do estresse oxidativo são utilizados para avaliar o estado redox em amostras humanas (MONTEZANO *et al.*, 2015).

1.4.4. Peroxidação lipídica

Os ácidos graxos poliinsaturados, incluindo fosfolipídios, glicolipídios e colesterol, são alvos vulneráveis da oxidação. Os níveis aumentados de ROS desencadeiam o processo de peroxidação lipídica. Os marcadores de peroxidação lipídica mais comumente estudados são o ácido tiobarbitúrico (TBARS) e o malondialdeído (MONTEZANO *et al.*, 2015; TSIKAS, 2017).

O malondialdeído é formado pela peroxidação de ácidos graxos poliinsaturados e pode interagir com proteínas. O malondialdeído pode ser detectado pelo ácido tiobarbitúrico, e os produtos medidos são chamados de espécies reativas de TBARS. O ensaio TBARS está entre

os mais amplamente empregados para avaliar a peroxidação lipídica em amostras humanas (MONTEZANO *et al.*, 2015).

1.4.5. Capacidade antioxidante total não enzimática

A capacidade antioxidante total é uma medida do efeito antioxidante combinado das defesas não enzimáticas nos fluidos biológicos e não leva em consideração os sistemas antioxidantes enzimáticos, como SOD, catalase e peroxidase (MONTEZANO *et al.*, 2015). São alguns exemplos, vitamina C, tióis, flavonóides, carotenóides, vitamina E, glutathione reduzida (GSH) e FRAP (capacidade de redução férrica do plasma).

A GSH é o antioxidante mais abundante nas células aeróbicas, presente nos fluidos corporais e nos tecidos e tem função fundamental para proteger o cérebro do estresse oxidativo, atuando como um eliminador de radicais livres e inibidor da peroxidação lipídica. Este antioxidante também participa da desintoxicação do peróxido de hidrogênio por várias glutathione peroxidases (OWEN; BUTTERFIELD, 2010). A FRAP, por sua vez, consiste em um teste que mede a capacidade de redução férrica do plasma e consiste em um método para avaliar o "poder antioxidante", através da redução de íon férrico em íon ferroso (BENZIE; STRAIN, 1996).

1.4.6. Antioxidantes enzimáticos

As células possuem um sistema antioxidante para combater o estresse oxidativo e remover as ROS para evitar danos nos tecidos. Além dos antioxidantes não enzimáticos supracitados, o sistema antioxidante enzimático é de suma importância neste processo (WANG; CHUN; SONG, 2013). Os biomarcadores do sistema antioxidante enzimático são a SOD, catalase, e GPx, que podem ser avaliados por diferentes fluidos, como a saliva e o plasma (CAIXETA *et al.*, 2018; JUSTINO *et al.*, 2017).

1.4.7. Estresse Oxidativo e Hipertensão Arterial

Há uma relação entre hipertensão arterial e valores altos de estresse oxidativo, sendo observado efeitos negativos das ROS no endotélio. Esta resposta é vista devido ao desbalanço causado pelo estresse oxidativo, que gera aumento da vasoconstrição derivado do estímulo de prostanóides e reduz a vasodilatação dependente do endotélio, derivado da diminuição do NO (MONTEZANO *et al.*, 2015). Além disso, indivíduos hipertensos, aparentemente, apresentam um produção aumentada de ROS, e níveis anormais de antioxidantes (GONZÁLEZ, 2014).

Neste sentido, nos indivíduos hipertensos, a PA parece se correlacionar positivamente com biomarcadores de estresse oxidativo e negativamente com níveis de antioxidantes (MONTEZANO *et al.*, 2015). Além disso, a disfunção endotelial, uma característica do fenótipo vascular na hipertensão, está associada ao aumento da produção de ROS vascular, estresse oxidativo e inflamação vascular (MONTEZANO *et al.*, 2015). Concomitantemente, parece haver redução na atividade enzimática antioxidante na mitocôndria nesses indivíduos (ZHOU *et al.*, 2006).

Além disso, os medicamentos utilizados no tratamento da HAS também apresentam relação com o estresse oxidativo. Agentes anti-hipertensivos clássicos, como bloqueadores β -adrenérgicos, inibidores da enzima de conversão da angiotensina, receptor da angiotensina antagonistas e bloqueadores dos canais de cálcio podem ser mediados, em parte, pela diminuição do estresse oxidativo vascular, através do aumento da atividade da NADPH oxidase, por exemplo (GHIADONI *et al.*, 2003; GONZÁLEZ, 2014; YOSHIDA *et al.*, 2004).

1.4.8. Estresse Oxidativo e Obesidade

O tecido adiposo visceral está relacionado com maiores níveis de estresse oxidativo. Os possíveis fatores que contribuem para este quadro são os níveis elevados de lipídios teciduais, a inflamação crônica, a disfunção endotelial, a hiperglicemia, a hiperleptinemia, a função mitocondrial prejudicada, o tipo de dieta, bem como deficiências de vitaminas e minerais (MANNA; JAIN, 2015).

Assim, a obesidade pode induzir o estresse oxidativo a nível sistêmico através de diferentes mecanismos, como a auto-oxidação de gliceraldeído, ativação de proteína quinase C e de vias de poliol, fosforilação oxidativa e geração de superóxido a partir de NADPH oxidases (SAVINI *et al.*, 2013). Outros fatores, como geração de ROS, baixa defesa antioxidante, inflamação crônica e hiperleptinemia também estão associados ao estresse oxidativo na obesidade (MANNA; JAIN, 2015).

Por fim, as ROS parecem estar envolvidas no controle do peso corporal, exercendo diferentes efeitos em neurônios hipotalâmicos, que controlam o comportamento da saciedade e da fome (HORVATH; ANDREWS; DIANO, 2009). Além disso, o tecido adiposo também desempenha um importante papel na regulação de mecanismos fisiopatológicos da obesidade e de suas comorbidades, sendo que o estresse oxidativo no indivíduo obeso pode causar o desenvolvimento de vários eventos patológicos, incluindo distúrbios do sono, complicações

cardiovasculares, RI e DM2, asma, problemas oncológicos, reprodutivos, reumatológicos e insuficiência hepática (JUNG, 1997; MANNA; JAIN, 2015; PI-SUNYER, 1991).

1.4.9. Estresse Oxidativo e Dislipidemia

As lipoproteínas desreguladas estão relacionadas com aumento do estresse oxidativo no organismo, associado com a elevação das concentrações de triglicerídeos e LDL plasmáticos e a diminuição das concentrações do HDL (RIZZO; BERNEIS, 2006). Concomitantemente, as principais características que definem a dislipidemia aterogênica são a hipertrigliceridemia, o baixo HDL e uma preponderância dessas partículas de LDL (RIZZO *et al.*, 2009).

Neste sentido, é possível observar que há associações entre os níveis plasmáticos de marcadores de estresse oxidativo e lipoproteínas aterogênicas em pacientes com DCV (RIZZO *et al.*, 2009). Sendo assim, a dislipidemia se mostra como um fator de risco para o desenvolvimento destas doenças, incluindo a aterosclerose, e está intimamente associada ao aumento da produção endotelial de ROS (OHARA; PETERSON; HARRISON, 1993).

Além disso, a hipercolesterolemia é capaz de induzir o estresse oxidativo nas células endoteliais através da peroxidação das membranas celulares e ácidos graxos livres (LEE; SONG, 2009). Os radicais livres são capazes de alterar a composição das lipoproteínas, produzindo LDL oxidado. Este, por sua vez, possui funções biológicas relacionadas ao aumento da expressão de citocinas pró-inflamatórias. Adicionalmente, as células espumosas são formadas pelo acúmulo de colesterol no interior dos macrófagos, favorecendo o desenvolvimento de placas ateroscleróticas (JABARPOUR *et al.*, 2019; LEE; SONG, 2009).

Por fim, o LDL oxidado aterogênico age perturbando a função de várias moléculas vasoativas, como o NO, endotelina 1 (ET1), angiotensina II e o fator de crescimento transformador beta (TGF- β), podendo causar outras alterações patológicas, principalmente relacionadas à DCV (JABARPOUR *et al.*, 2019). Sendo assim, tanto a dislipidemia quanto o estresse oxidativo são mecanismos-chave ligados ao desenvolvimento da aterosclerose e complicações associadas.

1.4.10. Estresse Oxidativo e Resistência Insulínica

O estresse oxidativo desempenha o papel principal na associação com a patogênese da RI pela interrupção dos sinais de insulina e desregulação das adipocitocinas (HOUSTIS; ROSEN; LANDER, 2006). Produtos de modificação oxidativa podem interromper o metabolismo e a sinalização celular, em particular aumentando a produção de citocinas pró-

inflamatórias, modificando a expressão gênica e promovendo a morte celular. Sob condições de RI, é possível perceber distúrbios em antioxidantes enzimáticos e não enzimáticos, bem como o aumento do conteúdo de produtos de modificação oxidativa, observados em fluidos e tecidos corporais (MACIEJCZYK; ŻEBROWSKA; CHABOWSKI, 2019).

Neste sentido, há uma relação entre indivíduos resistentes à insulina e níveis de estresse oxidativo alterados, sendo que pacientes com DM2 apresentam maior dano oxidativo induzido pela produção de ROS na circulação e mecanismos de defesas antioxidantes reduzidos (TANGVARASITTICHA, 2015). Neste cenário, observa-se que o H_2O_2 atua como oxidante e danifica as células β pancreáticas, interrompendo a via de sinalização da produção de insulina (NANDI *et al.*, 2019) e há indícios de que a catalase protege as células β pancreáticas dos danos causados pelo H_2O_2 (ELSNER; GEHRMANN; LENZEN, 2011; LUPPI *et al.*, 2020). Além disso, a catalase de eritrócitos também pode proteger as células contra os altos níveis de H_2O_2 exógeno, por exemplo, em áreas de inflamação (GOYAL; BASAK, 2010).

A hiperglicemia por sua vez, causa aumento do metabolismo da glicose e assim eleva a superprodução de NADH e $FADH_2$, que pode causar a maior produção SOD (TANGVARASITTICHA, 2015). Além disso, o estresse oxidativo aumentado na circulação de pacientes com DM2, pode ser a partir da via do poliol, sendo que na condição de superprodução de sorbitol, a disponibilidade de NADPH é reduzida, o que reflete na diminuição da regeneração da GPx e na atividade da NOS sintase, causando aumento do estresse oxidativo (BONNEFONT-ROUSSELOT, 2002).

Concomitante a essas vias, o indivíduo com RI apresenta inflamação, sendo que os mediadores inflamatórios, incluindo moléculas de adesão e interleucinas, induzem o estresse oxidativo. Além disso, a inflamação relacionada com a aterosclerose também está envolvida na resistência à insulina e na patogênese do DM2, sendo que o estado pró-inflamatório subclínico observado em muitas condições de patogênese, como aterosclerose e envelhecimento, é causado pela supergeração de ROS mitocondriais (TANGVARASITTICHA, 2015).

1.4.11. Estresse Oxidativo e Climatério

O estrogênio está relacionado com a redução do estresse oxidativo vascular, através da modulação da expressão e função da NADPH oxidase. Além disso, este hormônio também está associado com a produção de enzimas antioxidantes, como a SOD, GPx e catalase, demonstrando sua ação como efeito protetor contra o estresse oxidativo. Concomitantemente, há indícios de que este efeito antioxidante pode reduzir a oxidação do LDL (MILLER *et al.*,

2007; SÁNCHEZ-RODRÍGUEZ *et al.*, 2012), reduzindo estresse oxidativo celular e evitando aterogênese.

Com a baixa da produção do estrogênio em mulheres após a menopausa, há uma relação entre o aumento dos efeitos do estresse oxidativo através da NADPH, aumento de lipoperóxidos plasmáticos, bem como redução dos efeitos antioxidantes (SÁNCHEZ-RODRÍGUEZ *et al.*, 2012). Além disso, muitos sintomas da menopausa são considerados fatores pró-oxidantes, como insônia, depressão e ansiedade (D'ALMEIDA *et al.*, 2000; SÁNCHEZ-RODRÍGUEZ *et al.*, 2012; TSUBOI *et al.*, 2004). Assim sendo, há indícios de que os sintomas da pós-menopausa também são fatores pró-oxidantes e que a menopausa é um fator de risco para estresse oxidativo (SÁNCHEZ-RODRÍGUEZ *et al.*, 2012).

1.4.12. Estresse Oxidativo e Exercício físico

O exercício físico pode ser considerado como estímulo para o aumento da atividade inflamatória e do estresse oxidativo. Os radicais livres são produzidos durante o metabolismo celular aeróbico e têm papéis fundamentais como mediadores regulatórios nos processos de sinalização. A relação entre exercício físico e estresse oxidativo é extremamente complexa, dependendo da idade, sexo e nível de treinamento, bem como do modo, da intensidade e duração do exercício (PINGITORE *et al.*, 2015).

O treinamento moderado regular parece ser benéfico para o estresse oxidativo e a saúde. Por outro lado, o exercício agudo leva ao aumento do estresse oxidativo, embora esse mesmo estímulo seja necessário para permitir uma regulação positiva das defesas antioxidantes endógenas (PINGITORE *et al.*, 2015). As defesas endógenas podem ser complementadas com suplementação antioxidante oral adicional, o que pode representar uma ferramenta não invasiva adequada para prevenir ou reduzir o estresse oxidativo durante o treinamento. No entanto, o excesso de antioxidantes exógenos pode ter efeitos prejudiciais na saúde e no desempenho (PINGITORE *et al.*, 2015).

1.5. Exercício físico

A falta de atividade física é uma das principais causas de problemas de saúde e representa um considerável ônus econômico em escala global (STOJANOVSKA *et al.*, 2014). Os benefícios do exercício físico incluem: aumento da força, melhora na resistência, funcionamento cognitivo, metabolismo, energia e promove melhores padrões de sono (STOJANOVSKA *et al.*, 2014). Além disso, o exercício físico é um importante adjuvante no

tratamento das DCNTs, como a HAS (CASONATTO; OLIVEIRA; GRANDOLFI, 2020), DM2 (PAN *et al.*, 2018), dislipidemia (HE; YE, 2020) e obesidade (CELIK; YILDIZ, 2021).

Quanto a diferença entre as fases da vida, mulheres apresentam redução da massa muscular com a transição da menopausa (DOUCHI *et al.*, 1998; GREENDALE *et al.*, 2019; SIPILÄ *et al.*, 2020). Isso implica em aumento do risco cardiovascular, aumento do risco de sarcopenia, aumento do risco de osteopenia/osteoporose e aumento do risco de fraturas (CAPOZZA *et al.*, 2008; PEPPA *et al.*, 2014; SIPILÄ *et al.*, 2020). Desta forma, a manutenção da massa magra tem relevância clínica para saúde e longevidade desta população. Neste sentido, o exercício e o aumento da atividade física têm se mostrado eficazes na manutenção da massa magra em mulheres após a menopausa (FLORES *et al.*, 2022; SIPILÄ *et al.*, 2020).

Apesar de o exercício físico atuar em diversos mecanismos regulatórios do organismo, para cada modalidade há respostas específicas e que dependem da condição no indivíduo. Em uma única sessão de exercício físico, ocorrem milhares de mudanças moleculares e uma coreografia orquestrada de processos biológicos envolvendo metabolismo energético, inflamação, reparo tecidual, estresse oxidativo, resposta de fator de crescimento, bem como vias regulatórias (CONTREPOIS *et al.*, 2020).

Assim, a relação entre o exercício físico e o estresse oxidativo é extremamente complexa, dependendo do modo, intensidade e duração do exercício (PINGITORE *et al.*, 2015). O treinamento moderado parece ser benéfico para o estresse oxidativo e para a saúde. O exercício agudo, por sua vez, leva ao aumento da produção de radicais livres, embora esse mesmo estímulo seja necessário para permitir uma regulação positiva nas defesas antioxidantes endógenas (PINGITORE *et al.*, 2015).

Neste sentido, o aparecimento das DCNTs está diretamente relacionado ao desequilíbrio redox aumentando pró-oxidantes (KATTOOR *et al.*, 2017). Mulheres após a menopausa também apresentam aumento do EO (MONTROYA-ESTRADA *et al.*, 2020), associadas ou não às DCNTs. No entanto, o exercício físico nesta população tem se mostrado uma importante abordagem terapêutica no tratamento destas doenças (LIN; LEE, 2018), melhorando a densidade mineral óssea (DALY *et al.*, 2018) e manutenção/ganho de massa magra (NUNES *et al.*, 2016). O exercício aeróbico pode melhorar a saúde cardiovascular nessa população (LIN; LEE, 2018), reduzir o IMC e promover a regulação positiva de marcadores de status redox, como a atividade da SOD, sem alterar os níveis de cortisol/leptina (JARRETE *et al.*, 2014).

Já o treinamento de força é capaz de reduzir a hemoglobina glicada, a força muscular, a circunferência da cintura, o colesterol total e LDL, além de prevenir o aumento de marcadores pró-inflamatórios (NUNES *et al.*, 2016). A combinação desses dois tipos de treinamento, também pode levar a uma redução na rigidez arterial (MANOJLOVIĆ *et al.*, 2021; SON *et al.*, 2017), pressão arterial, endotelina-1, nitrito/nitrato sanguíneo, capacidade funcional e composição corporal nas mulheres na pós-menopausa (SON *et al.*, 2017).

Além disso, outras abordagens de exercício físico vêm se mostrando bastante prevalente na população de mulheres após a menopausa, como por exemplo o Pilates (FOURIE *et al.*, 2013). Este método consiste em um programa de condicionamento completo, que visa trabalhar a força, flexibilidade e equilíbrio (PILATES; MILLER, 1998). O *Mat Pilates* tem características de exercício resistido (com atributos semelhantes ao peso corporal e treinamento funcional), utilizando acessórios para aumentar a carga de exercício, mas sem usar o aparato clássico do método (BATISTA *et al.*, 2021). Porém, por ser uma prática mais recente, ainda há poucos estudos apresentando o comportamento dos marcadores de estresse oxidativo. Além disso, associado ao exercício, padrões dietéticos e alguns compostos bioativos também podem otimizar a resposta antioxidante (KŘÍŽOVÁ *et al.*, 2019; OCAMPO *et al.*, 2018).

1.6. Isoflavonas e suco de beterraba

Como visto anteriormente, alterações hormonais e metabólicas em mulheres na menopausa estão associadas ao aumento do peso corporal e acúmulo de gordura visceral, doenças cardiometabólicas e do estresse oxidativo. Como tratamento para todos estes parâmetros é indicada uma alimentação balanceada, rica em nutrientes que podem oferecer não só uma melhora da saúde geral, mas com a presença de compostos que podem agir de maneira específica, como as isoflavonas (KŘÍŽOVÁ *et al.*, 2019) e o suco de beterraba (OCAMPO *et al.*, 2018).

1.6.1. Isoflavona

As isoflavonas são compostos abundantes na soja, contendo daidzeína e genisteína (KŘÍŽOVÁ *et al.*, 2019) e há indícios de que seus efeitos são semelhantes ao estrogênio, por isso a relação com o alívio dos sintomas da menopausa. Há duas isoformas dos receptores de estrogênio: ER α , predominantemente presente nos testículos, útero, rins, hipófise e nas glândulas mamárias; e o ER β , predominante nos ossos, pulmões, trato urogenital, pele, cérebro

e no sistema cardiovascular, que por sua vez possuem uma melhor afinidade pelas isoflavonas (CHEN; KO; CHEN, 2019; KŘÍŽOVÁ *et al.*, 2019).

Algumas preocupações relacionadas à terapia hormonal, como o aumento do risco cardiovascular em mulheres (MARJORIBANKS *et al.*, 2012), trazem a suplementação com fitoestrogênios para o foco. As isoflavonas se tornaram populares por serem um tratamento alternativo para uma ampla gama de distúrbios hormonais, incluindo vários tipos de câncer, como câncer de mama e de próstata, osteoporose, doenças cardiovasculares e especialmente para sintomas da menopausa (KŘÍŽOVÁ *et al.*, 2019). Esse composto é capaz de reduzir as ondas de calor, atenuar a perda de densidade mineral óssea da coluna lombar e até apresenta efeitos benéficos na pressão arterial sistólica durante a menopausa precoce (CHEN; KO; CHEN, 2019). Mulheres após a menopausa apresentam, a princípio, bons resultados na suplementação de isoflavonas quanto a redução de sintomas relacionados a menopausa, embora seja dependente da intensidade dos sintomas (KŘÍŽOVÁ *et al.*, 2019; LETHABY *et al.*, 2007).

No sistema cardiovascular, há indícios de que a soja rica em isoflavonas age na redução do LDL (REYNOLDS *et al.*, 2006). Além disso, há indicativos de que a suplementação com isoflavonas melhora a resposta vasodilatadora em resposta ao NO e reduz a rigidez arterial, apesar de ainda não ser um consenso (KŘÍŽOVÁ *et al.*, 2019). Concomitantemente, há ação antioxidante através da ingestão de isoflavona, bem como redução de peroxidação lipídica (DJURIC *et al.*, 2001), porém as respostas de marcadores redox, como a catalase; SOD; e glutathiona peroxidase; ainda são inconclusivas (AMARAL *et al.*, 2020; KŘÍŽOVÁ *et al.*, 2019).

1.6.2. Suco de beterraba

A beterraba é uma hortaliça rica em compostos bioativos, como fibras, carboidratos, vitaminas do complexo B e minerais, como potássio, sódio, cobre e zinco. Além disso a beterraba é rica em NO_3^- sendo este um precursor de NO, com potente efeito vasodilatador (OCAMPO *et al.*, 2018). Grande parte dos estudos relacionando os efeitos ergogênicos da beterraba, a apresentam na forma de suco (BORGHI; CICERO, 2017; OCAMPO *et al.*, 2018), com o objetivo de aumentar a concentração dos seus nutrientes em um menor volume de alimento.

A coloração avermelhada da beterraba ocorre devido à presença de betalaínas, pigmentos nitrogenados que exibem atividades antioxidantes (ALVES *et al.*, 2008). É possível

observar redução da oxidação da membrana lipídica ativada por metomioglobina e ferro livre (KANNER; HAREL; GRANIT, 2001) através da atividade das betalainas. Além disso, o NO_3^- também é responsável por redução do estresse oxidativo, pela interação com a respiração mitocondrial, ativação de vias metabólicas e ação antioxidante por eliminação de ROS (por exemplo, superóxido e peróxido de hidrogênio) e menos formação de radicais livres (CLIFFORD *et al.*, 2015; LUNDBERG *et al.*, 2011; WINK *et al.*, 2001).

Alguns estudos (ASGARY *et al.*, 2016; BAHADORAN *et al.*, 2017; OCAMPO *et al.*, 2018) vem investigando os potenciais efeitos do suco de beterraba para colaborar com o tratamento para hipertensão arterial. Como discutido anteriormente, sabemos que a menopausa é fator de risco para hipertensão (MALACHIAS *et al.*, 2016) e observa-se aumento do estresse oxidativo (SÁNCHEZ-RODRÍGUEZ *et al.*, 2012) e alteração da biodisponibilidade do NO (WITKOWSKI; SERVIENTE, 2018), tanto relacionado com a menopausa, quanto a hipertensão. Sendo assim, a ingestão do suco de beterraba em mulheres após a menopausa hipertensas pode ser um aliado interessante no tratamento da saúde cardiovascular.

1.7. Considerações Gerais

O presente trabalho será apresentado em formato de quatro artigos científicos nos próximos capítulos. Os textos foram organizados em dois capítulos, com dois estudos clínicos em cada: **Capítulo 2)** discorre sobre os efeitos da ingestão de antioxidantes no sistema redox em mulheres após a menopausa; e **Capítulo 3)** discorre sobre os efeitos do exercício físico no sistema redox em mulheres após a menopausa.

O primeiro estudo do **capítulo 2** foi coletado entre 2015 e 2016, porém os dados relacionados ao estresse oxidativo ainda não tinham sido analisados, o que nos levantou ao questionamento sobre a suplementação de compostos antioxidantes no sistema redox. Já o segundo estudo do **capítulo 2** foi realizado durante 2018 e 2020, mas ainda não tínhamos analisado as respostas antioxidantes da suplementação do suco de beterraba. Os dois estudos do **capítulo 3** foram realizados em colaboração a outros projetos vigentes no programa de pós-graduação entre os anos de 2015-2019. Estes geraram dúvidas relacionadas ao treinamento com exercício físico no sistema redox de mulheres após a menopausa com doenças cardiometabólicas. As características gerais destes estudos estão apresentadas na tabela a seguir.

Tabela 3 – Características gerais dos estudos.

	Título adaptado ao português	Tipo de Estudo	Exercício	Status de Publicação	Revista
Capítulo 2	Efeitos da ingestão de antioxidantes no sistema redox em mulheres após a menopausa				
Estudo 1	Respostas antioxidantes em mulheres hipertensas na pós-menopausa após ingestão aguda de suco de beterraba e exercícios aeróbicos: ensaio duplo-cego e cruzado controlado por placebo	Cruzado, aleatorizado, placebo controlado e duplo-cego	Aeróbio agudo	Publicado.	Oxidative Medicine and Cellular Longevity
Estudo 2	Efeitos da suplementação de isoflavona e exercícios combinados sobre marcadores salivares de estresse oxidativo em mulheres após a menopausa	Aleatorizado, placebo controlado e duplo-cego	10 semanas de Treinamento combinado	Publicado.	Journal of Clinical Biochemistry and Nutrition
Capítulo 3	Efeitos do exercício físico no sistema redox em mulheres após a menopausa				
Estudo 3	Status redox de mulheres após a menopausa com uma ou múltiplas doenças cardiometabólicas tem respostas semelhante ao treinamento de Mat Pilates	Paralelo	12 semanas de Mat Pilates	Publicado.	Antioxidants
Estudo 4	Efeitos do treinamento físico combinado nos marcadores de estresse oxidativo salivar em mulheres hipertensas e normotensas após a menopausa	Paralelo	10 semanas de Treinamento combinado	Publicado.	Motriz

Frete ao que foi exposto, ao investigar as repostas redox ao exercício físico e à suplementação de antioxidantes em mulheres após a menopausa, produzimos informações ainda não descritas na literatura. Estas informações podem influenciar nas escolhas de diferentes características de exercício físico e da indicação de suplementação de forma individualizada.

1.8. Objetivo geral

O objetivo desta tese foi estudar o impacto do exercício físico e da suplementação de isoflavonas e suco de beterraba no estado redox de mulheres após a menopausa.

1.8.1. Objetivos específicos

- Investigar os efeitos de diferentes concentrações de nitrato presentes no suco de beterraba sobre marcadores antioxidantes salivares após exercício agudo em mulheres hipertensas após a menopausa.

- Investigar os efeitos da suplementação de isoflavonas associado a 10 semanas de treinamento combinado em marcadores de estresse oxidativo em mulheres não obesas após a menopausa.
- Investigar os efeitos de 12 semanas de *Mat* Pilates no estado redox em mulheres após a menopausa com uma ou mais doenças cardiometabólicas.
- Investigar os efeitos de 10 semanas de treinamento combinado nos marcadores de estresse oxidativo em mulheres hipertensas e normotensas após a menopausa.

CAPÍTULO 2

**Efeitos da ingestão de antioxidantes no sistema redox em
mulheres após a menopausa**

ESTUDO 01

ANTIOXIDANT RESPONSES IN HYPERTENSIVE POSTMENOPAUSAL WOMEN AFTER ACUTE BEETROOT JUICE INGESTION AND AEROBIC EXERCISE: DOUBLE BLIND AND PLACEBO-CONTROLLED CROSSOVER

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Status: publicado. Formato original disponível no Anexo 1.

Amaral AL, Mariano IM, Carrijo VHV, de Souza TCF, de Souza AV, Caixeta DC,

Teixeira, Teixeira RR, de Oliveira EP, Espindola FS, Puga GM. Antioxidant Responses in Hypertensive Postmenopausal Women after Acute Beetroot Juice Ingestion and Aerobic Exercise: Double Blind and Placebo-Controlled Crossover Trial. *Oxid. Med. and Cel. Longe.* 2021 Apr. doi: 10.1155/2021/5579864.

ABSTRACT:

This study aimed to analyze the effect of different nitrate concentrations [NO_3^-] present in beetroot juice (BJ) on salivary oxidative stress markers after acute exercise performance in hypertensive postmenopausal women. Thirteen hypertensive postmenopausal women participated in three experimental sessions, taking different beverages: Non-Caloric orange flavored drink (OFD); Low Nitrate (Low- NO_3^-) BJ; and High Nitrate (High- NO_3^-) BJ. The participants performed aerobic exercise on a treadmill, at 65–70% of heart rate reserve (HRR), for 40 min. Saliva samples were collected after an overnight fast, 10 minutes before BJ ingestion at 7:20 am (0'), 120 minutes after beverages ingestion (130'), immediately after exercise (170') and 90 min after exercise (260'). Salivary total protein (TP), catalase activity (CAT), reduced glutathione (GSH) and total antioxidant capacity by ferric-reducing antioxidant power (FRAP) concentrations were analyzed. No interaction (session*time) was found among three sessions over time. Catalase area under the curve (AUC) was lower after both Low- NO_3^- and High- NO_3^- consumption ($p < 0,001$); and GSH AUC was lower after High- NO_3^- ($p < 0,001$) compared with OFD. So, the acute intake of BJ with aerobic exercise seems to decrease catalase (in high- NO_3^- and low- NO_3^-) and GSH (in high- NO_3^-), besides not interfering with FRAP in hypertensive postmenopausal women.

Keywords: beetroot juice; antioxidants; hypertension; exercise; menopause.

INTRODUCTION

Postmenopausal hypertensive women have increased cardiovascular risk [1], which is due to and/or aggravated by excessive oxidative stress [2,3]. Oxidative stress is characterized by the imbalance between prooxidants and antioxidants factors in favor of prooxidants. It can lead to cell damage [4,5] due to excess of reactive oxygen species (ROS) [4,6]. ROS are involved in aging, inflammation and several cardiovascular diseases [3,4,6,7]. In postmenopausal women, hypertension is a prevalent risk factors for cardiovascular disease [8], and in these patients, there was an increased antioxidant enzymes production to combat the enhancement in ROS [9]. Besides that, postmenopausal estrogen cessation, alters the bioavailability of nitric oxide (NO), inflammation and oxidative stress [10] and in hypertensives, endothelium-derived relaxing factors (e.g. NO) was also altered [11,12].

An important precursor of NO is nitrate, available from two main sources: through diet and from oxidation by NO synthase [13]. Beetroot juice (BJ) is a common beverage that has a high nitrate concentration [14,15]. The ingestion of this beverage may generate vasodilation and blood pressure reduction in healthy [16] and hypertensive individuals [15,17]. Other nitrate effects include interaction with mitochondrial breathing, metabolic pathways activation and oxidative stress reduction [13]. In addition, BJ contains antioxidant compounds, like betalain, that reduce linoleate damage and lipid membrane oxidation activated by metmyoglobin and free iron [18]. Besides that, there is evidence of elimination of reactive oxygen and nitrogen species (e.g. superoxide and hydrogen peroxide) and less free radical formation by nitrite action, suggesting nitrate antioxidant effects [14,19,20].

As for nitrate concentration in BJ necessary to perform physiological effects, it is known that plasma, salivary and urinary nitrate/nitrite increases are dose dependents [21,22], with a peak approximately 2-3h after the ingestion. In addition, we observed in hypertensive postmenopausal women that there is a dose-dependent salivary nitrite increase for different nitrate concentrations in BJ [23]. However, different levels of nitrate in BJ have dose-dependent effects on exercise performance [21], but not in hypotensive responses [22,23], and it is not yet known whether the concentration of nitrate present in BJ alter oxidative stress.

On the other way, aerobic training is able to increase salivary nitrite, and these values were higher than that of plasma [24]. In a study with sodium nitrate supplementation, an increase in salivary nitrite was observed both 60 minutes after ingestion and after 5 days of supplementation [25]. Some studies have shown an improvement in antioxidant defense

[26,27], but acutely, the physical exercise increases pro-oxidants productions [28,29]. Thus, in hypertensives, exercises should be prescribed carefully. Therefore, the aim of this study was to analyze the effects of BJ intake with different concentrations of nitrate [NO_3^-] on the antioxidant response. Our initial hypothesis was that antioxidant biomarkers would increase acutely with high- NO_3^- and would not change with beverage without NO_3^- . For this, we evaluated the salivary levels of nitrite and oxidative stress markers after BJ ingestion and moderate aerobic exercise in hypertensive postmenopausal women.

MATERIAL AND METHODS

The study was carried out at the Federal University of Uberlândia, Uberlândia, MG, Brazil, between June and September 2018. It was registered at Clinicaltrials.gov (NCT03620227), approved by local Ethics Committee (70104717.0.0000.5152), has been conducted in accordance with the Helsinki Declaration, and all participants signed the consent form.

The sample calculation was performed using G.Power 3.1, using as main variable the variation of catalase after BJ ingestion and exercise (-33.18 ± 35 nmol/min/ml) [30]. Therefore, we found that the minimum sample should be 12 (power of 0.80; effect size of 0.474; alpha of 0.05; 3 groups and 4 measures).

The inclusion criteria were: hypertensive postmenopausal woman (amenorrhea for at least 12 months and $[\text{FSH}] > 40 \text{ mIU/mL}$); between 50 and 70 years old; without hormonal therapy; able to exercise on a treadmill; no history of food allergy, heart attack, stroke or Diabetes Mellitus; not being sensitive to nitrate; no β -blockers user; and not being smoker. The exclusion criteria were inability to carry out the protocol for exercise or juice intolerance, or even for not fulfilling/observing the fast.

The intervention began with anamnesis and questionnaire (short version IPAQ). Later anthropometric measures were taken, which included: body mass (electronic scale Filizola, São Paulo, Brazil); height (Sanny fixed stadiometer, São Bernardo do Campo, Brazil); abdominal circumference (Filizola inelastic tape, São Paulo, Brazil); and body composition (biobody impedance Inbody 230, Seoul, South Korea), as previously described [31]. For body composition, the volunteers were instructed not to consume alcohol or caffeine 72h before, and not to perform vigorous physical activities 24h before the exam.

STUDY DESIGN

The present study was a crossover, randomized, double blind, and placebo-controlled trial. The flow-diagram is shown in figure 1. The protocol extended for 3 visits over 10-21 days, with a wash-out interval of at least 5 days between sessions. On each visit, the volunteers consume one of the following beverages: BJ with high nitrate concentration (high- NO_3^-), BJ with low nitrate concentration (low- NO_3^-) and orange flavor non-caloric drink without nitrate (OFD). Study participants arrived at the laboratory at 7:00am and left at 11:40am. Figure 2 illustrates the experimental design.

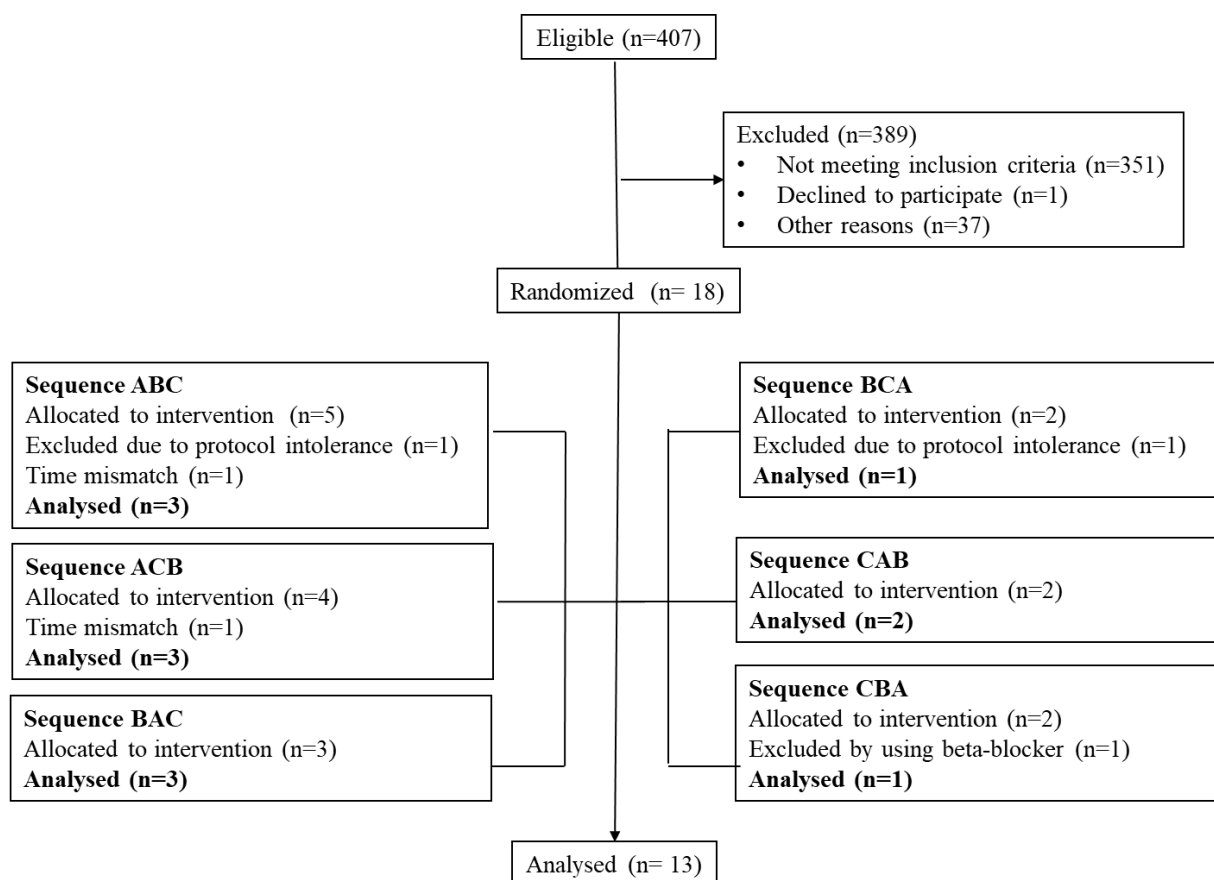


Figure 1: Flow Diagram. A: Beetroot juice with low nitrate (Low- NO_3^-); B: Beetroot juice with high nitrate (High- NO_3^-); C: Non-Caloric orange flavored drink without nitrate (OFD).

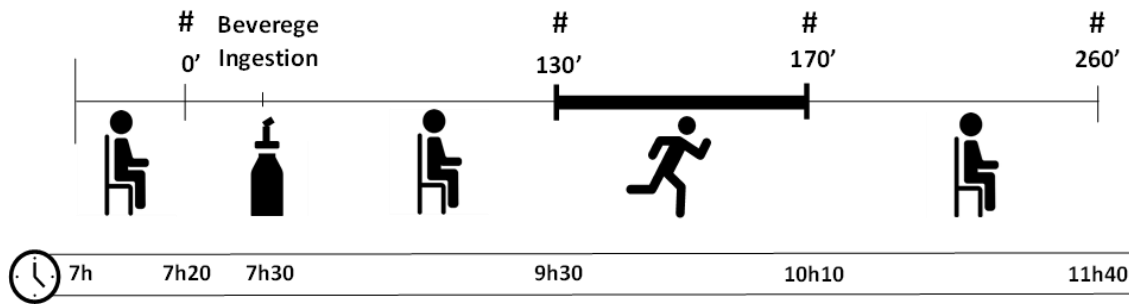


Figure 2: *Experimental design of the sessions. (#) Saliva sample collection.*

The volunteers arrived at the laboratory after 8h of overnight fasting. Samples of unstimulated saliva were collected after 20 minutes of rest (0'), immediately before exercise (130'), after exercise (170'), and 90 minutes after the exercise (260'). The beverage intake was performed after 10 minutes of rest, and participants had 15 minutes to consume it.

During the intervention, the volunteers were instructed not to ingest any type of food or liquid, except for water in small quantities. Before all sessions, volunteers went through an interview about their own food/drink consumption in the last 24h. As they could not consume food/drinks rich in nitrate in the last 24h, participants received a list of dietary restrictions, that includes: green vegetables (amaranth, lettuce, cabbage, spinach, broccoli, celery, cauliflower, Chinese radish), beetroot, sausage, salami, ham, turkey breast, coffee, energy drinks, soft drinks, alcoholic beverages, and to avoid mouthwashes.

EXERCISE PROTOCOL

The protocol consisted of 40 minutes of moderate continuous aerobic exercise on a treadmill. There were five minutes of warm up and two of cool down. The treadmill speed could reach 5.5km/h and the intensity was imposed by treadmill inclination until the volunteer reached the zone between 65% and 70% of HR (heart rate) reserve (HRR) [32]. To monitoring exercise intensity, a heart rate monitor and a Borg scale [33] were used. Whenever the volunteer's HR entered a non-stipulated HR, the exercise was readjusted.

BEVERAGE

The protocol was carried out with the ingestion of three different beverages: OFD; Low- NO_3^- ; and High- NO_3^- , with one beverage assigned to each session. The beverages intake order were randomly distributed to each volunteers using a randomization block design through the website (<https://www.random.org/lists/>). A researcher who did not participate in the data

collection was responsible for assigning the codes to each drink and blind them, adding the drink in a bottle with the volunteer's identification.

The High- NO_3^- had 20.78mmol/kg of nitrate and was prepared using 35mL of nitrate concentrated BJ (Beet-It Sport Shot, James White Drinks Ltd., Ipswich, UK), which was diluted in 315mL of distilled water with 6g of non-caloric orange juice flavored powder (Clight, Mondelez International, Inc.), totalizing 350mL of juice. The Low- NO_3^- had 3.86mmol/kg of nitrate and was prepared by filtering the High- NO_3^- on an ion exchange resin capable of depleting the nitrate (PA101 OH-, Permution®) [34], similarly to previously reported [22,23]. Lastly, OFD was prepared using 6g of orange juice powder diluted in 350mL of distilled water. High- NO_3^- and Low- NO_3^- were identical in taste, while OFD was slightly different. The beverages were delivered to volunteers in a black sealed bottle, with a lid and straw, with no possibility of viewing or even smelling the beverage.

SALIVARY SAMPLES

For the saliva samples, the spit method was used [35]. All samples were centrifuged at 3000rpm for 15 minutes, the supernatant was separated and stored at -80°C until analysis. From this, the following were analyzed: Total protein - was performed by the Bradford method, calculated using a bovine serum albumin curve [36]; Catalase activity - was based upon hydrogen peroxide decomposition by catalase present in the samples. Samples were mixed with 10mmol.L⁻¹ potassium phosphate buffer (pH 7.0) containing 0.2% hydrogen peroxide. The hydrogen peroxide decomposition was monitored at 240nm for 10 min [37]; Reduced Glutathione (GSH) sample was mixed with 185 μL of 100mM sodium phosphate buffer (pH 8.0) containing 5mM EDTA and 15 μL of ortho-phthaldialdehyde (1mg/mL in methanol). The solution was incubated in the dark at room temperature for 15 minutes. Fluorescence was read at 350nm (excitation) and 420nm (emission). Sample concentrations of GSH were calculated using a standard GSH curve (0.001-0.1mM) [38]; Total Antioxidant Capacity by Ferric-Reducing Antioxidant Power (FRAP): was determined by the capacity of the antioxidants present in saliva samples, to reduce Fe^{+3} to Fe^{+2} that is chelated by TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) and form the complex Fe^{+2} -TPTZ. This colored complex was read in the spectrophotometer at 593nm, and the antioxidant activity was determined using an analytical curve, constructed with trolox as standard [37].

STATISTICAL ANALYSIS

Results are presented as mean $\pm$ standard deviation and 95% confidence interval. The Shapiro-Wilk test was applied to verify data normality, and GEE (Generalized Estimation Equation) was used to analyze time points and interventions (OFD, Low- NO_3^- and High- NO_3^-) differences, using Bonferroni post hoc when necessary. The GEE model was set as Linear or Gamma with Log link based on normality and goodness of fit tests. The area under the curve (AUC) by the trapezoidal method was used to compare the interventions and a One-way ANOVA was used for AUC comparisons. Effect sizes were present as partial eta squared. Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS 20 and GraphPad Prism 6.

RESULTS

Two volunteers were excluded for inability to adapt to the protocol due to an abrupt decrease in blood pressure followed by syncope 15 minutes after exercise in the first collection session. One of these volunteers had consumed High- NO_3^- and the other Low- NO_3^- . Then, this study was completed by 13 medicated hypertensive postmenopausal women, with mean age of 58.1 ± 4.6 years and body mass index of $27.4 \pm 4.2 \text{ kg/m}^2$.

The IPAQ showed that 15% was very active, 62% active e 13% irregularly active, and there were no sedentary women. It is worth mentioning that blood pressure and nitrite concentration data from the same sample and intervention have already been published, just like the other general characteristics [23]. In this previous study, we emphasize that, as expected, salivary nitrite over time were different among the beverages (biggest increase in High- NO_3^- , smooth increase in Low- NO_3^- and no changes in OFD). In addition, we found an important post-exercise hypotension, but BJ intake was not able to potentiate it.

Among the antihypertensive drugs, 46% (6) used Angiotensin Receptor Blockers plus Diuretic polytherapy, 8% (1) Angiotensin Converting Enzyme Inhibitor plus Diuretic, 31% (4) Angiotensin Receptor Blockers, 8% (1) Diuretic, and 8% (1) Angiotensin Converting Enzyme Inhibitor. Besides that, 31% (4) used Levothyroxine and 23% (3) Statins.

Table I shows salivary oxidative stress markers and total protein. No interaction (session* time) was found in the salivary markers. It was possible to verify session effects on catalase (OFD did not change; Low- NO_3^- and High- NO_3^- increased) and GSH (High- NO_3^- increased). Regarding time effects, we found a significant difference in all variables being that:

in total protein, 170' was greater than all other time points; in catalase, 170' it was lower than baseline; in GSH, 130' and 170' were greater than baseline; and in FRAP 130' and 260' were lower than baseline, 170' was bigger than 130', and 260' was lower than 170'.

Table 1: Salivary oxidative stress markers and total protein, at baseline (0'), two hours after beetroot juice consume, and immediately before exercise (130'), immediately post-exercise (170'), and 90' post-exercise (260').

	0'	130'	170'	260'	P session	P time	P session*time	ES	95% CI
Total Protein (µg/µL)									
OFD	0.68 ± 0.23	0.74 ± 0.54	0.94 ± 0.64	0.74 ± 0.22					
Low-NO ₃ ⁻	0.74 ± 0.25	0.68 ± 0.26	1.24 ± 0.97	0.65 ± 0.18	0.166	< 0.001	0.353	0.102	0.65; 0.76
High-NO ₃ ⁻	0.74 ± 0.41	0.62 ± 0.21	0.98 ± 0.81	0.68 ± 0.21					
Catalase (U/µg prot)									
OFD	16.4 ± 10.3	17.6 ± 11.0	14.4 ± 7.2	17.8 ± 8.2					
Low-NO ₃ ⁻	17.5 ± 11.9	10.1 ± 6.8	7.5 ± 5.3	12.9 ± 6.8	< 0.001	< 0.001	0.412	0.092	10.71; 14.80
High-NO ₃ ⁻	16.1 ± 12.8	10.7 ± 7.7	7.4 ± 5.2	12.1 ± 9.0					
GSH (nmol/mg prot)									
OFD	31.6 ± 20.3	46.0 ± 29.6	42.7 ± 20.2	35.3 ± 11.6					
Low-NO ₃ ⁻	25.9 ± 9.3	40.9 ± 22.4	33.5 ± 13.7	41.5 ± 19.6	0.040	0.004	0.217	0.091	29.65; 36.47
High-NO ₃ ⁻	27.5 ± 13.3	38.5 ± 35.0	37.0 ± 26.4	29.9 ± 15.3					
FRAP (nmol Trolox)									
OFD	54.9 ± 30.2	44.5 ± 34.3	53.4 ± 20.4	46.4 ± 22.2					
Low-NO ₃ ⁻	61.5 ± 34.8	43.1 ± 20.8	63.6 ± 23.1	49.8 ± 30.7	0.098	< 0.001	0.680	0.055	43.28; 54.54
High-NO ₃ ⁻	59.0 ± 32.4	42.5 ± 29.7	52.3 ± 32.7	45.8 ± 29.3					

Mean ± standard deviation of salivary markers. ES: effect size (partial eta squared); CI: Confidence interval. GSH: Reduced Glutathione; FRAP: ferric reducing ability of plasma. OFD: Non-Caloric orange flavored drink without nitrate; Low- NO₃⁻: Beetroot juice with low nitrate; High- NO₃⁻: Beetroot juice with high nitrate; 0': Salivary sample at rest; 130': Salivary sample before aerobic exercise; 170': Salivary sample after aerobic exercise; 260': Salivary sample 90' after aerobic exercise.

Figure 3 shows the salivary oxidative stress markers over time and their respective AUC. The AUC of salivary catalase activity from both High- NO₃⁻ and Low- NO₃⁻ (figure 3B) were lower (p<0.01) than OFD session. Only salivary GSH AUC from High- NO₃⁻ was lower (p<0.01) than OFD session (Figure 2D). No difference was found in FRAP AUC among sessions.

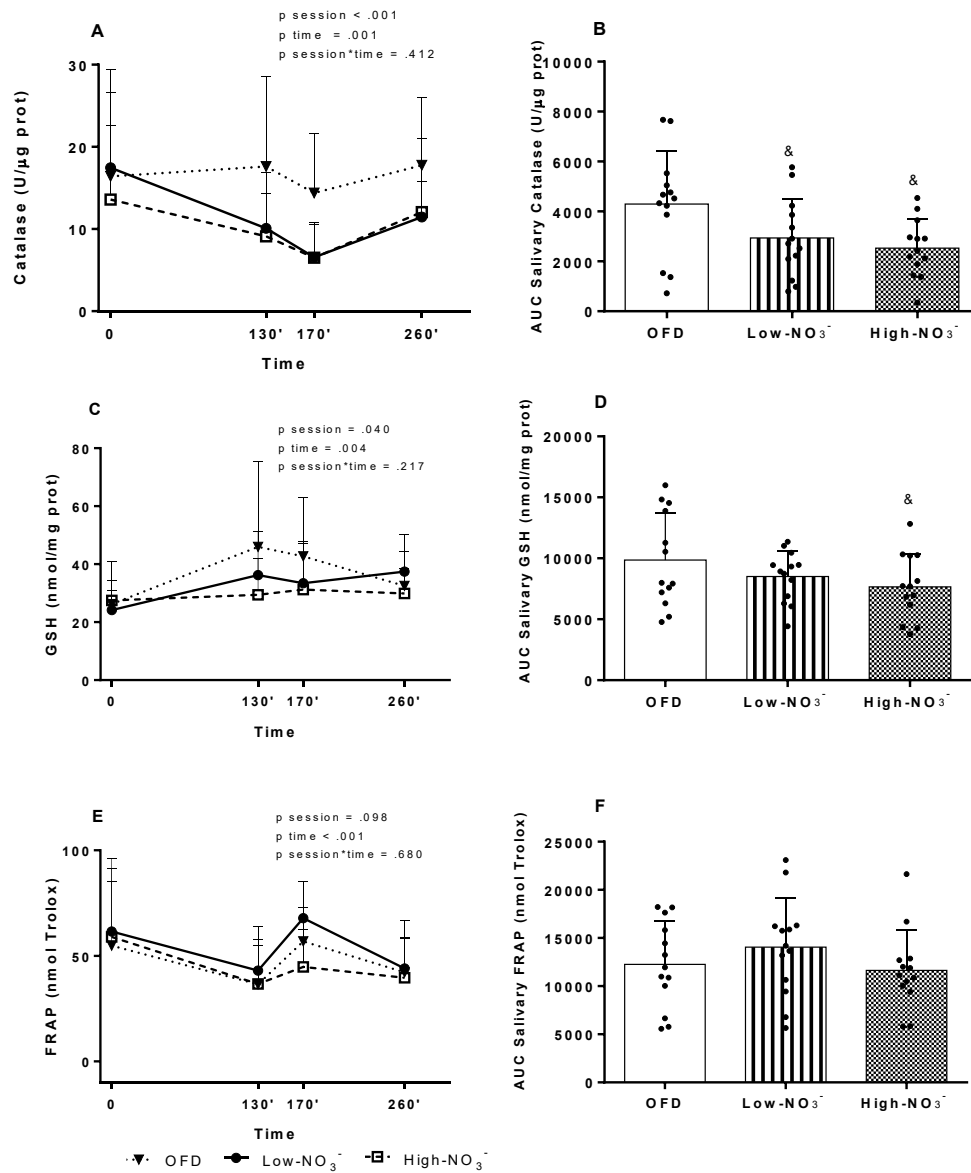


Figure 3: Salivary oxidative stress markers and AUC. Mean $\pm$ standard deviation of salivary oxidative stress markers. 0': Fasting rest collection; 130': Two hours after BJ consumption and immediately before aerobic exercise; 170': immediately after aerobic exercise; 260': 90 minutes after the aerobic exercise session. GSH: Reduced Glutathione; FRAP - Total Antioxidant Capacity by Ferric-Reducing Antioxidant Power. & p < 0.05 vs. OFD.

DISCUSSION

Our study investigated if acute BJ ingestion with different nitrate doses could change salivary oxidative stress markers after a moderate aerobic exercise in hypertensive postmenopausal women. The main results were that the antioxidant responses are better in high amounts of nitrate, although we also found a positive effect in catalase in both high- NO_3^- and low- NO_3^- . Catalase reduced with the intake of high- NO_3^- and low- NO_3^- , and post exercise a fall occurs. Besides that, we observed that GSH levels are maintained after high- NO_3^- , while OFD increases it. Furthermore, exercise does not alter GSH, since before and after exercise sessions were greater than the baseline. After interventions, FRAP reduces when compared to baseline, but high- NO_3^- or low- NO_3^- does not differ in their responses in relation to OFD.

Acute BJ ingestion with high doses of nitrate has been studied as potential antioxidant. These effects occur because nitrate is an important precursor of NO [21], and beetroot contains antioxidant nutrients, like polyphenoid compounds, and free radical scavengers [14,18,39]. In vitro [40] it was observed that a shot of BJ manages to supply a large number of bio-accessible antioxidants in gastric and duodenal cells, hypothesizing that this would be an interesting strategy to increase the body's antioxidant status. Some authors showed, in a study with diet control over four weeks of BJ [41], an increase in lipid peroxidation, indicating a change in oxidative behavior.

In this sense, in a previous study [23] with similar beverages and population, we found an increase in salivary nitrite with high- NO_3^- , but there was no potentiation of post-exercise hypotension. In addition, in a systematic review [42], it is commented that BJ is rich in antioxidants that suppress the accumulation of leukocytes after exercise, reducing muscle damage and that the betalain present in beetroot is able to attenuate muscle damage by analgesic and anti-inflammatory effects.

Our results showed that the catalase activity decreased after exercise in both high- NO_3^- and low- NO_3^- and this shows that possibly nitrate had no effect (or had a partial effect) on this antioxidant enzyme, but what may have reduced this enzyme are other antioxidant substances present in BJ. This enzyme acts as the first defense line against oxidative stress [43]. One of its functions is to catalyze the degradation/reduction of hydrogen peroxide [43]. Hydrogen peroxide in high concentrations is harmful to cells and is related to the pathophysiology of hypertension [44]. There is evidence that catalase production increases with the presence of ROS; however, with the presence of beet leaf extract its levels are restored to baseline [45]. It

was noticed that catalase responses by post intense exercise in amateur athletes there is an increase in its bioavailability for up to 24h post-exercise. In heart failure patients [46], post 30 minutes of moderate exercise, there is a catalase increase, but after low-intensity exercise with greater volume (45 minutes), there is a reduction in this enzyme. Which may indicate that the exercise intensity is more related to the oxidative stress than the volume. Regarding the ingestion of BJ, in athletes [30] the catalase activity increased after interval exercise, with no difference with placebo, distinguishing from the present study, in which the catalase activity reduced similarly between high- NO_3^- and low- NO_3^- .

Another important endogenous antioxidant is GSH, which is involved in cellular protection against excessive oxidative stress both directly and as an enzymatic cofactor of glutathione peroxidases [47]. This is an antioxidant molecule oxidized by glutathione peroxidase, an intracellular enzyme capable of reducing hydrogen peroxide with an important role in inhibiting the lipid peroxidation [43]. Our results showed that salivary GSH concentration decreases only after high- NO_3^- . In addition to betanine, beetroot is also rich in polyphenoid compounds, such as ascorbic acid, carotenoids, phenolic acids, flavonoids [48] and bioactive betalain [49], all of which are potential antioxidants [40]. This may indicate that the antioxidants present in beetroots increase GSH, although the difference with placebo was observed only with high- NO_3^- , which shows that nitrate also plays an important role. Unlike the present study, in a study with 7 days intake of 2 shots with 6.5-mmol of NO_3^- BJ per day with active and healthy young men [50], no changes were found in GSH, which may indicate that chronical beverage intake, with double nitrate concentration in two doses per day, does not modify these responses in a healthy population. When verifying these responses in intense exercise in amateur athletes [51], GSH did not undergo any changes within 72h after exercise. However, when we observe these responses post moderate resistance exercise with active young men [52], there is an increase in plasma GSH, but without differences in salivary analysis, which may indicate greater sensitivity of blood analysis. From our results, we realized that a high- NO_3^- reduces GSH, probably due to the presence of a high dose of nitrate related to the presence of antioxidants and other components, such as high levels of GSH and other antioxidant molecules not evaluated here.

The last variable analyzed was FRAP, which aims to analyze the antioxidant power [53]. In vitro [40], with the same BJ from the present study, an increase in FRAP was found after the gastric phase, with a marked reduction after the duodenal phase, which may be related to the

biotransformation of antioxidants caused by enzymes interactions. In a review study [14], it is argued that FRAP increases after BJ ingestion, contrary to our findings since in the present study there were no differences between drinks. It is worth noting that, although we found $p < 0.001$ in AUC, there was no significant value in the post hoc analysis. We have found no clinical study that analyzed FRAP associated with BJ intake, but in hypertensives, there is evidence of less production when compared with normotensive individuals [54,55]. In addition, negative correlations were found with systolic and diastolic blood pressures [55], suggesting a role of plasma antioxidant status in modulating blood pressure.

As for the present study, we found a reduction in FRAP after the beverages and 90 minutes post physical exercise. In trained healthy men [37], FRAP increased after continuous exercise and decreased 3h post exercise. These results corroborate our findings, considering that with the beverages intake there were reductions in FRAP, followed by increases caused by physical exercise, returning to the pre-exercise values 90 minutes after same. In addition, in physically active men [25], no FRAP modification was found associating sodium nitrate supplementation with 30 minutes of moderate aerobic exercise. This suggests that supplementations of dissociated nitrate from other compounds (such in BJ) may interfere with these responses.

In addition to the intervention characteristics, we must consider that the sample was composed by drug-controlled hypertensive women. We observed that 92% (12) of the volunteers used drugs that act on the renin-angiotensin system. In this sense, AT1 receptor antagonists or ACE inhibitors may have antioxidant actions by improving endothelial function [56] and reducing ROS production by reducing NADPH oxidase and regulating Cu/ZnSOD [57]. It is worth mentioning that since physical exercise was prescribed based on heart rate, we did not include β -blocking drug users. Another important point is the use of salivary evaluation as a way of analyzing the antioxidant profile. The antioxidant profile of saliva was similar to that of plasma [37], suggesting that as an alternative and non-invasive tool. So, saliva may be an interesting method for studying antioxidants in sports medicine.

Among the limitations of our study, we have the use of antihypertensive drugs, since these drugs can exercise specific actions not analyzed. To minimize these effects, we indicated that the volunteers marked the time of day that they used the medication. In addition, with this study, we assessed only the acute effects of BJ intake, and we did not assess its isolated effects (without exercise). We also did not carry out the dietary control of these volunteers, but as a

way of minimizing this, they should follow a restriction list. In addition, we have not evaluated several other antioxidant enzymes, which could give a better oxidant profile. These results can be applied specifically to hypertensive postmenopausal women and cannot be stratified for other populations.

As a clinical application, we realized the use of BJ as a treatment for hypertension has been studied in recent years [58]. Since there are discussions about the increase in hypertension incidence, especially low- and middle-income countries [59]. We believe that this study can contribute to finding food benefits that are already present in the population's diet and can be used to add knowledge to alternative treatments of hypertension.

CONCLUSION

We conclude that the acute intake of BJ associated with moderate-intensity aerobic exercise seems to decrease catalase activity (in high- NO_3^- and low- NO_3^-) and GSH (in high- NO_3^-), besides not interfering with FRAP in hypertensive postmenopausal women. Thus, BJ may have positive effects in oxidative stress markers independent of nitrate concentration, although high- NO_3^- can have smooth better results.

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ESTUDO 02

THE EFFECTS OF ISOFLAVONE SUPPLEMENTATION PLUS COMBINED EXERCISE ON SALIVARY MARKERS OF OXIDATIVE STRESS IN POSTMENOPAUSAL WOMEN

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Status: publicado. Formato original disponível no Anexo 2.

Amaral AL, Mendonça AM, Giolo JS, Costa JG, Mariano IM, de Souza TCF, Batista JP, Rodrigues ML, de Souza AV, Caixeta DC, Peixoto LG, de Oliveira EP, Espindola FS, Puga GM. The effects of isoflavone supplementation plus combined exercise on salivary markers of oxidative stress in postmenopausal women. J Clin Biochem Nutr. 2020 Jan;66(1):43-48. doi: 10.3164/jcbn.19-44. Epub 2019 Oct 31. PMID: 32001955; PMCID: PMC6983439.

ABSTRACT:

This study tested the effect of isoflavone supplementation in addition to combined exercise training in salivary oxidative stress markers in non-obese postmenopausal women. Thirty-two postmenopausal women without hormone therapy were randomly assigned to exercise + placebo (n=15) or exercise + isoflavone supplementation (n=17) groups. They performed 30 sessions of combined exercises (aerobic plus resistance) over ten weeks and consumed 100 mg of isoflavone supplementation or placebo. Saliva samples were collected after an overnight fast. Superoxide dismutase, total antioxidant capacity, thiobarbituric acid reactive substances, catalase, total protein and nitrite were determined before and after ten weeks of the intervention. The ANOVA two-way Analysis of Variance was applied with α of 5%. Both groups increase ($p<0.05$) superoxide dismutase activity and decrease Catalase levels. There was interaction (time*group) in both Nitrite and thiobarbituric acid reactive substances results, with increase ($p<0.05$) in placebo group and decrease ($p<0.05$) in isoflavone group. No difference was found for total antioxidant capacity or total protein. The combination of isoflavone supplementation and exercise training can promote an antioxidant effect through reduction of lipid peroxidation and concentrations of salivary nitrite.

Keywords: aerobic and resistance exercises; soy protein; menopause; anti-oxidant system.

INTRODUCTION

Women in post menopause are predisposed to the development of cardiovascular diseases [1], and one possible reason to that is an increase in oxidative stress during this life period [2], leading to endothelial cells dysfunction [3]. Endothelial cells are responsible for biological functions, mainly in cardiovascular and endocrine systems. Further, a prooxidant-antioxidant balance is necessary in order to have these cells functioning properly [3,4]. Oxidative stress is an imbalance of this system to prooxidant molecules, thus, generating more reactive oxygen species (ROS), which are involved in several cardiovascular diseases, inflammation, cancer and aging [3–5]. Clinically, there are methods to evaluate oxidative stress markers, such as the enzymes catalase (CAT) and superoxide dismutase (SOD) [6], and also nitrite [7], an important nitric oxide (NO) precursor and a key to endothelium vasoprotection [8].

Saliva is an important factor in oral health maintenance of individuals [9]. Salivary analysis for oxidative stress markers is an effective and non-invasive method [10], however research about salivary oxidative markers are still lacking. An imbalance of ROS and consequent changes in oxidative stress salivary markers are associated with not only oral diseases, such as dental caries [9,10], and periodontal diseases [10,11], but also with other systemic diseases, such as type II diabetes [11,12], and obesity [13]. Importantly, studies have shown that salivary free radical-scavenging activity was affected by physical and mental activities [14]. Furthermore, some studies suggest that saliva may reflect the current physiological condition of the body [15,16].

The maintenance of a prooxidant-antioxidant balance is important to keep healthy conditions to physiological activities, and there are reports that physical exercise leads to positive responses to this balance [7,17,18]. Regular exercise training has been demonstrated to improve antioxidant defense [17,18], and to increase nitrite concentrations [19]. Acute exercise results in an increased production of ROS [20], however, chronic exercise promotes an adaptation to the antioxidant defense system, leading to adequate responses to the higher ROS generation [18,21]. Moderate intensity aerobic training promotes mechanisms for this adaptation, such as an increased production of endothelium vasoactive substances [22]. Further, the combination of aerobic with resistance exercise has been demonstrated to effectively diminish ROS production, and enhance the enzymatic antioxidant defense [23].

Associated with physical exercise, postmenopausal women often use alternative treatments to reduce climacteric symptoms, mainly supplements structurally similar to estrogen, such as isoflavones, a phytoestrogen derived from soy [24] which can change the oxidative balance. Studies have controversial data regarding oxidative stress markers and isoflavone supplementation. In some studies, there was a decrease in ROS production, and an increase in SOD in postmenopausal women [25,26], other showed that genistein, a soy isoflavone, could decrease oxidative stress via lowering lipid peroxidation, and improving antioxidant enzymes [27]. Besides, other studies find that a high isoflavone consumption, as well as exercise, is effective in increasing nitrite concentrations and improving endothelial function [28,29]. However, there are also studies that did not report any effect in oxidative markers with isoflavone supplementation [30].

Therefore, the objective of this study was to verify if there is an additional effect of isoflavone supplementation associated with combined aerobic and resistance exercise training, in salivary oxidative stress markers in postmenopausal women. Our hypothesis was that isoflavone supplementation together with combined exercise performance would improve oxidative stress markers.

MATERIALS AND METHODS

This study was a randomized, double-blind, parallel prospective clinical trial, carried out at the Laboratory of Cardiorespiratory and Metabolic Physiology of the Physical Education Department at the Federal University of Uberlândia, Uberlândia MG, Brazil. The study was approved by the local Ethics Committee for human studies (CAAE: 40622414.9.0000.5152). All participants agreed and signed an informed consent form prior to admission to the study, and experiments were performed in accordance to the World Medical Association Declaration of Helsinki.

PARTICIPANTS

Postmenopausal women, aged between 50 and 70 years old, were recruited by traditional media (TV, radio and posters). The inclusion criteria were: a body mass index ≤ 30 kg/m², amenorrhea for at least 12 months, ability to participate in treadmill and resistance exercises, no hormone therapy or supplementation of soy derived products, no use of drugs that alter lipid profile, and no history of high blood pressure, diabetes or cardiovascular disease. Figure 1 presents the flowchart with the distribution of the participants in the study.

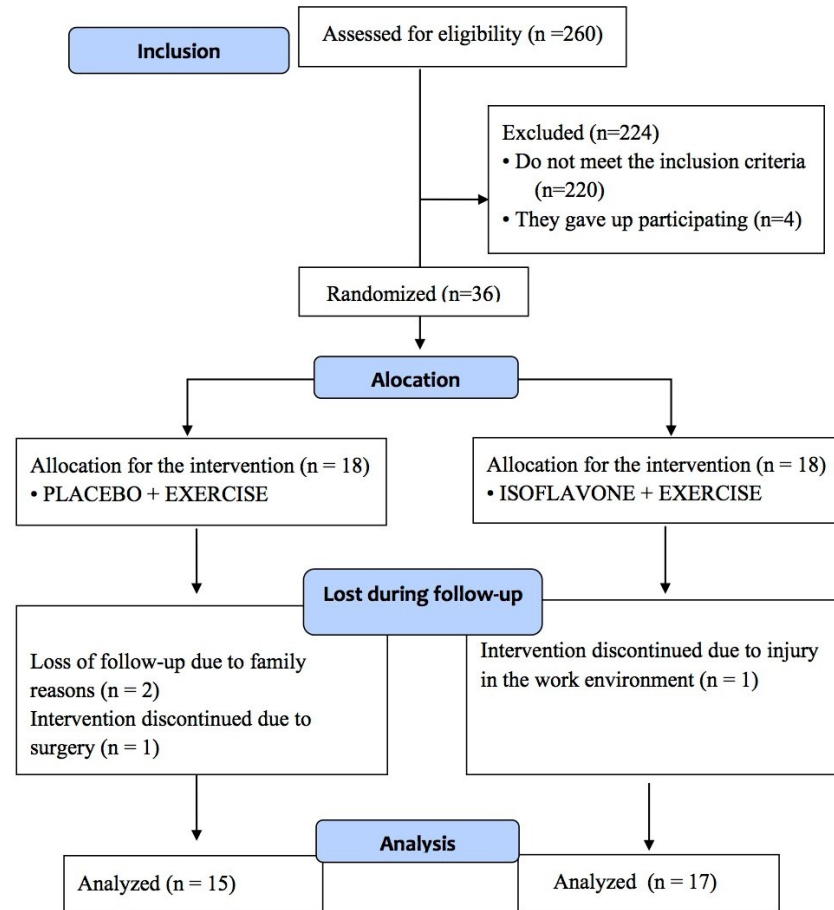


Figure 1: Follow-up flowchart.

All participants who met the inclusion criteria were randomly allocated, through electronic lottery, to two different groups: exercise training and isoflavone supplementation (ISO) or exercise training and placebo supplementation (PLA). Saliva samples of all participants were collected before and after 10 weeks of placebo or isoflavone supplementation, and combined aerobic and resistance exercise training. Participants also went through anthropometric measurements, a test for aerobic capacity and maximum strength (1RM) and were familiarized with the ergometers and exercises of the trial prior to start.

SUPPLEMENTATION

ISO group received one capsule per day of 100mg of isoflavones containing 3.3 mg of genistein, 93.5 mg of dadzein and 3.2 mg of glycitein derived from soybean (Xi'An Green-Life

Natural Products, Xian, Shaanxi, China). These amounts correspond to approximately 37.58g of soy [31]. PLA group received one capsule per day containing 100mg of cornstarch. The capsules for both groups were identical in appearance, smell, and flavor.

DIETARY ASSESSMENT

Food intake was assessed by trained nutritionists that instructed all participants to maintain their regular diet throughout the study. Three dietary recalls (two weekdays and one weekend day) were gathered on non-consecutive days at baseline and at the end of study. Dietary data was analyzed by the United States Department of Agriculture (USDA) food composition table and by the software by Dietpro® 5.7i (Minas Gerais, MG, Brazil) and there was no difference in dietary pattern between groups during the intervention (data not shown).

BODY COMPOSITION

Height and weight were measured using a standard stadiometer Sanny (São Paulo, SP, Brazil) and an electronic scale Micheletti (São Paulo, SP, Brazil), respectively. Body mass index (BMI) was calculated and rated according to the World Health Organization (WHO) [32]. To assess total fat mass and fat-free mass participants went through a bioimpedance test (Biodynamics model 450c—Biodynamics, Shoreline, WA, USA). The test was performed in the morning after at least eight hours of fasting, and hydration was controlled as previously reported [5].

EXERCISE TRAINING

Exercise training was performed as previously described [30]. Briefly, the exercise training consisted of 30 sessions of combined aerobic and resistance exercises during the same session. Exercise sessions took place three times per week on non-consecutive days, over 10 weeks. All sessions included a warm-up on a treadmill for 5 min, aerobic exercise on a treadmill for 20 min, resistance exercises for 20 min, and cool down exercises for 5 min, with a total of 50 minutes for each session. Aerobic and resistance exercise order was changed in every training session. All women went through a familiarization session with the treadmill and resistance exercises before the intervention start.

SALIVA SAMPLES COLLECTION AND ANALYSIS

Saliva samples were collected after an overnight fast, five days before, and 72 h after, the last exercise training session to eliminate possible acute effects of exercise. All volunteers

were instructed not to consume alcoholic beverages or caffeine, and not to practice intense physical activities 24 hours prior to sample collection. After a 30 seconds mouthwash with distilled water, unstimulated saliva was collected into graduated Falcon tubes by the spitting method for 2 minutes [33]. Samples were centrifuged at 3500rpm and 4°C for 15 minutes, transferred to microtubes of 1.5ml and stored at -80°C. All analyses were performed using an automated system (Cobas Mira, Roche Instruments Inc. Bellport, NY, USA), using commercial kits (Labtest, Minas Gerais, Brazil).

The total antioxidant capacity in saliva was evaluated by the ferric reducing ability potential (FRAP) methodology and calculated from the standard trolox curve, as described by Justino et al. [34]. The activity of the superoxide dismutase enzyme (SOD) was determined based on the auto-oxidation capacity of pyrogallol [34]. Lipid peroxidation levels were analyzed by the TBARS (thiobarbituric acid reactive substances) method, using a curve of 1,1,3,3-tetramethoxypropane (TMP) as the standard [34].

STATISTICS

Results are reported as means $\pm$ standard errors. Data distribution was analyzed using the Shapiro-Wilk test. Sample size was calculated using data from GPower 3.0.10, using an α value of 0.05, a β value of 0.15 and a power analysis of 85% with a final number of volunteers of 26 women. The two-way Analysis of Variance (ANOVA) for repeated measures was used to analyze the time (pre and post), group (PLA and ISO) and group versus time interactions with a Bonferroni post hoc test, when appropriate. A p-value of < 0.05 was set for statistical significance, and all statistical analyses were performed using SPSS software version 20.0 (IBM, NewYork, NY, USA).

RESULTS

ISO group had mean age of 56 ± 5.40 years, body mass of 65.35 ± 8.73 kg, height of 1.57 ± 0.04 m, BMI 26.29 ± 3.38 kg / m², and abdominal circumference of 93.15 ± 9.72 cm. The PLA group had a mean age of 52.67 ± 4.90 years, body mass of 64.67 ± 8.60 kg, height of 1.56 ± 0.06 m, BMI of 26.52 ± 3.14 kg / m², and abdominal circumference of 92.80 ± 7.57 cm.

Oxidative stress markers in saliva are displaced in Figure 2 and Table 1. There were significant time effects in catalase (decreases in both groups) and SOD (increase in both groups) concentrations, whereas no interaction, time or group effect was found for FRAP or TP. There was an interaction for nitrite concentrations in saliva ($p=0.007$), in which the concentrations for

PLA group increased and ISO group decreased after the intervention (Figure 2). There was also an interaction for TBARS concentrations ($p=0.035$) in which this marker was slightly lower in ISO group and slightly increased in PLA group after intervention (Table 1). Catalase concentration was slightly lower after intervention in both groups with a significant effect of time ($p=0.035$). SOD concentration was increased in both groups after the exercise protocol with a significant effect of time ($p=0.001$).

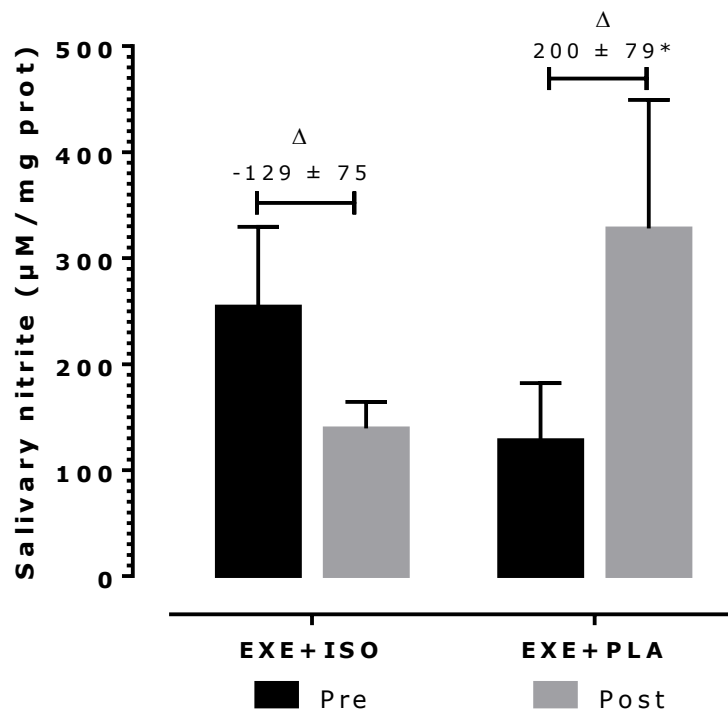


Figure 2. Salivary nitrite of postmenopausal women in placebo and ISO groups, in pre- and post- intervention moments.

Table 1: Oxidative stress markers in saliva of postmenopausal women.

	Pre	Post		<i>p</i>	<i>p</i>	<i>p</i>	Effect Size
	Mean ± SE	Mean ± SE	Δ	(Groups)	(Moments)	(Groups * Moments)	
CATALASE U/mg prot)							
ISO	21.32 ± 2.30	16.02 ± 2.04	-5.29 ± 1.48	0.46	0.035	0.708	0.008
PLA	17.59 ± 2.45	16.28 ± 2.37	-1.25 ± 1.46				
FRAP (umol/ L eq. Trolox)							
ISO	42.28 ± 3.56	38.49 ± 4.80	-3.57 ± 3.90	0.97	0.064	0.12	0.291
PLA	50.29 ± 8.01	59.09 ± 11.61	8.04 ± 9.72				
SOD (SOD/mg prot)							
ISO	5.36 ± 0.63	8.66 ± 0.66	2.91 ± 0.73	0.669	0.001	0.79	0.154
PLA	3.91 ± 0.71	11.16 ± 2.22	6.74 ± 2.07				
TBARS Umol/L							
ISO	4.93 ± 0.24	4.52 ± 0.18	-0.41 ± 0.17	0.698	0.735	0.037	0.21
PLA	5.02 ± 0.29	5.57 ± 0.44	0.26 ± 0.67				
TP (µg/µl)							
ISO	0.64 ± 0.05	0.65 ± 0.04	0.01 ± 0.03	0.999	0.133	0.221	0.078
PLA	0.69 ± 0.04	0.76 ± 0.06	0.07 ± 0.04				

Interaction: groups vs time interaction; ISO, isoflavone supplementation; FRAP, plasma ferric reduction capacity; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TP, total protein.

DISCUSSION

To the best of our knowledge this is the first-time salivary oxidative markers of postmenopausal women have been evaluated before and after 10 weeks of isoflavone supplementation with combined aerobic and resistance exercise. This study demonstrates an effect of the association of isoflavone with exercise for nitrite and TBARS concentrations in saliva, indicating lower production of nitrate with isoflavone supplementation, and a slightly lower production of lipid peroxides by the TBARS evaluation. Combined exercise had a positive effect on SOD, which had its concentration increased, and a negative effect on catalase, that was slightly lower after the exercise protocol in both groups.

Besides that, during physical exercise, the high shear stress caused by blood in vessels can lead to an increase in the release of a potent vasodilator named nitric oxide (NO), that improves endothelial function [19]. However, NO can react rapidly with the anion superoxide resulting in peroxynitrite, a potent oxidant related to mitochondrial damage and increase of oxidative stress [8]. In this study, there was a chronic increase in salivary nitrite concentrations (i.e. NO precursor) in the PLA group, which can be explained by the mechanism of shear stress

stimulated by exercise. Previous studies have shown an increase of salivary nitrite in active man in up to one hour after aerobic exercise [35], and after combined exercise in patients with peripheral arterial disease [36]. Besides, the nitrite/nitrate concentrations were also higher after three months of an exercise protocol in healthy postmenopausal women [37]. Interestingly, in ISO group nitrite concentrations were lower after intervention. Isoflavone supplementation can also increase serum nitrite/nitrate, and improve endothelial function, as well as exercise, in postmenopausal women [28,29]. Although some studies show that both isoflavone consumption, and exercise alone can increase nitrite concentrations, our results indicate that when associated, isoflavone does not seem to have any additional benefits to this variable.

Therefore, there are other mechanisms that may explain nitrite reduction in ISO group. A study [38] showed that genistein and dadzein can promote an antioxidant mechanism through NO or peroxynitrite clearance consequently reducing DNA damage [38]. There was also a study showing that isoflavones can attenuate excessive NO generation in vitro [39][39]. In our study, it is possible that isoflavone supplementation blocked the increase in salivary nitrite induced by exercise training and lowered its concentration in order to prevent peroxynitrite production and an increase in oxidative stress in postmenopausal women.

Besides decreasing nitrite, our study shows that isoflavone supplementation also slightly decreased TBARS production in postmenopausal women engaged in a combined exercise program. One of the theories for this event is the increase in peroxynitrite, a free radical that can induce the oxidation of low density lipoprotein (LDL) and increase the risk of developing atherosclerosis [40]. Accordingly, studies have shown decreased TBARS concentrations with isoflavone supplementation in postmenopausal women [41] and in animal models [42]. Isoflavones, including dadzein and genistein, are able to inhibit TBARS formation, demonstrating an antioxidant effect [43,44]. The efficiency of TBARS inhibition may depend on the individual isoflavones concentration in the extract, with some compounds having low or moderate efficiency to inhibit TBARS [44], which may explain the slight interaction observed in this study ($p=0.037$), and also why other studies found no changes in lipid peroxidation markers with isoflavone supplementation [30,31].

One of the main benefits from exercise training is related to improvements in antioxidant defense. In this way, we found an increase in SOD in both groups after combined exercise training indicating that this type of exercise training, independently of isoflavone supplementation, can improve the antioxidant activity of this enzyme in saliva. This data was

already expected, since exercise training leads to an adaptation of the antioxidant system [20] by increasing prooxidants cells in each exercise session [45]. Therefore, in the long-term, exercise training stimulates the antioxidant defense system to attenuate the acute effect generated by exercise[22,45].

The limitations of this study must be taken into consideration. The study was conducted with healthy, non-obese women; therefore, the results might not be applicable to groups receiving higher potency medical treatment, or for longer than 10 weeks. It is important to note that this result is applicable only for isoflavone supplementation and may not be extrapolated to isoflavone consumption from natural and regular food and nor in different amounts of isoflavone and concentrations of its compounds. Other limitations were the absence of an evaluation of isoflavone levels in the urine to detect how much soy protein was actually absorbed in the study population. More studies are needed with larger numbers of participants and with patients with dyslipidemia, obesity or with high oxidative stress levels to investigate the effects of these interventions.

Isoflavone supplementation associated with a combined exercise program can promote an antioxidant effect through reduction of lipid peroxidation and concentrations of salivary nitrite. Furthermore, combined exercise training alone can increase the antioxidant activity of the enzyme SOD after 10 weeks of intervention. Differences in salivary FRAP, catalase and TP were not detected after the intervention period.

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CAPÍTULO 3

**Efeitos do exercício físico no sistema redox em mulheres após a
menopausa**

ESTUDO 03

REDOX STATUS OF POSTMENOPAUSAL WOMEN WITH SINGLE OR MULTIPLE CARDIOMETABOLIC DISEASES HAS A SIMILAR RESPONSE TO MAT PILATES TRAINING

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Status: publicado. Formato original disponível no Anexo 3.

Amaral AL, Batista JP, Mariano IM, Gonçalves LF, Tavares JB, de Souza AV, Caixeta DC, Teixeira RR, de Oliveira EP, Espindola FS, Puga GM. Redox Status of Postmenopausal Women with Single or Multiple Cardiometabolic Diseases Has a Similar Response to Mat Pilates Training. *Antioxidants (Basel)*. 2022 Jul 26;11(8):1445. doi: 10.3390/antiox11081445. PMID: 35892647; PMCID: PMC9331979.

ABSTRACT

Postmenopausal women have a high prevalence of cardiometabolic diseases and that may associate with higher oxidative stress. Exercise can contribute to the treatment of such diseases, but some modalities, such as Mat Pilates, need to be further studied in terms of their physiological responses. Our aim was to investigate the effects of 12 weeks of Mat Pilates on redox status in postmenopausal women with one or more than two comorbidities of cardiometabolic diseases. Forty-four postmenopausal women were divided into two groups: SINGLE, composed of women with one cardiometabolic disease (n=20) and MULT, with multimorbidity (n=24). Mat Pilates training was conducted for 3 times a week for 12 weeks, and each session lasted 50 minutes. Plasma samples were collected before and after training to analyze the following redox markers: superoxide dismutase, catalase, glutathione peroxidase, total antioxidant capacity due to iron-reducing antioxidant power (FRAP), reduced glutathione (GSH), uric acid, and carbonyl protein. ANCOVA showed interaction effects in FRAP ($p=0.014$). Both groups had reduced levels of catalase ($p=0.240$) and GSH ($p=0.309$), and increased levels of carbonyl protein ($p=0.053$) after intervention. In conclusion the redox status of postmenopausal women shows no changes mediated by Mat Pilates training between SINGLE and MULT, except for greater reductions of FRAP in SINGLE.

Keywords: climacteric; exercise; endogenous antioxidants; Pilates training; oxidative stress balance.

INTRODUCTION

Multimorbidity is the coexistence of two or more chronic health complications [1], and may include cardiometabolic multimorbidity disease. People with multimorbidity have a higher risk of premature mortality compared to those without disease [2]. In addition, there is an increased incidence of diseases with aging [2], especially cardiometabolic diseases, which are the leading cause of death, hospitalization, and outpatient care worldwide, including in developing countries such as Brazil [3]. Obesity [4], arterial hypertension [3] and dysfunction lipid [5] and glycemic profiles [6] are the most common chronic health complications. On their own, these conditions already have well-defined treatment guidelines, but when associated with other conditions, their potential interactions are not yet well defined [7]. The emergence of these diseases is directly related to the individual's redox imbalance, trending towards an increase in pro-oxidants [8]. Reactive oxygen species (ROS) play an important role in the pathogenesis of these diseases [9]. In hypertension, they can generate ren-in-angiotensin system dysfunction [10–12], and in diabetes they cause dysregulation of the end products axis of advanced glycation [9]. Furthermore, ROS act directly in the production of atherosclerotic plaques through the oxidation of low-density lipoproteins (LDL) and increase endothelial dysfunction [8].

Postmenopausal women are predisposed to the development of cardiometabolic diseases, mainly due to the cessation of estrogen production, an increase in the concentration of visceral adipose tissue, inflammation, sympathetic activity, production of vasoconstrictors, renal vascular resistance, and increased oxidative stress [13]. However, physical exercise in postmenopausal women has been shown to be an important therapeutic approach treating for cardiometabolic diseases [14], improving bone mineral density [15] and maintenance/gain of lean mass [16]. Aerobic exercise can improve cardio-vascular health in this population [14], reduce body mass index and promote upregulation of redox status markers such as superoxide dismutase (SOD) activity, without altering cortisol/leptin levels [17]. Meanwhile, resistance training is capable of improving glycated hemoglobin, muscle strength, waist circumference, and total and LDL cholesterol, as well as preventing the increase in pro-inflammatory markers [16]. Combining these two types of training can lead to an improvement in arterial stiffness [18,19], blood pressure, endothelin-1, blood nitrite/nitrate, functional capacity, and body composition in postmenopausal women [19]. In addition, other physical exercise practices, such as yoga, may improve the level of adiponectin, serum lipids and metabolic syndrome risk

factors in obese postmenopausal women [20]. However, the Pilates training remains unclear.

Mat Pilates has resistance exercise characteristics (with features similar to bodyweight and functional fitness training), using accessories to increase the exercise load, but without using the classic apparatus of the method [21]. While Pilates does not appear on the Fitness Trends list, related terms are found in the global Top 20, such as resistance training, bodyweight training, and functional fitness training [22]. In addition, it is a method with high adherence in the postmenopausal population [23]. Physical training, in turn, is a strategy for the treatment of multimorbidity, as it generates benefits [1] such as psychosocial effects, increased strength, blood pressure regulation and improved insulin sensitivity [24], and has anti-inflammatory effects [25]. However, while exercise can be safely practiced by patients with multimorbidities, there are still no specific recommendations for exercise therapy for this population [26]. In this sense, the effects of physical training in populations with different types of isolated cardiometabolic diseases are relatively well explored, however, the evidence in populations with multimorbidities, especially in populations with an increased risk of these diseases such as in women after menopause, are still scarce. Furthermore, there are indications that health interventions in individuals with multimorbidities may have minor or insignificant effects [27], and the effects of different kinds of exercise training on anti-inflammatory and antioxidant systems in patients with multimorbidities remain unclear in the literature. Given this gap, understanding the effects of physical training in postmenopausal women with multimorbidities in relation to those with only one cardiometabolic disease is important in the management of these patients with their idiosyncrasies.

Since exercise training is a strategy for the treatment of multimorbidity [1] and can also generate redox adaptations [28], Mat Pilates therapy could be beneficial. However, its effects on populations with cardiometabolic multimorbidity and increased oxidative stress have been poorly explored. Although the consequences of exercise-induced oxidative stress remain a controversial, a moderate level of ROS production during exercise can promote adaptation through mitochondrial biogenesis, antioxidant enzymes, and stress protein synthesis. On the other hand, high levels of ROS production result in damage to macromolecular structures, such as DNA, proteins, and lipids [29]. Thus, our aim was to investigate whether the effects of 12 weeks of Mat Pilates on redox status differ between postmenopausal women with single or multiple cardiometabolic diseases. We hypothesized that exercise training with Mat Pilates could lead to improvements in the redox status in postmenopausal women, regardless of the

number of cardiometabolic diseases. This would be less evident in women with multimorbidity due to the association of these diseases with a high level of ROS production.

MATERIALS AND METHODS

PARTICIPANTS

This was a parallel clinical trial. All steps of this study were carried out at the Laboratory of Cardiorespiratory and Metabolic Physiology at the Federal University of Uberlândia between August 2017 and March 2019. Data collection started after August 2017. All participants signed the Consent Form and were allocated to groups for convenience in a non-probabilistic way. The present study followed the ethical principles of the Declaration of Helsinki. In addition, the study was approved by the local Ethics Committee of the Federal University of Uberlândia (68408116.9.0000.5152) and was registered on “clinicaltrials.gov” (NCT03626792). This study was part of a larger research project in which the effects of Mat Pilates exercise training on climacteric symptoms, ambulatory blood pressure responses, lipid, and glucose profile and pro- and anti-inflammatory and antioxidant markers in postmenopausal normotensive and hypertensive women, were investigated.

We included in the study i) post-menopausal women, ii) aged between 50 and 70 years, iii) with a low level of physical activity, iv) who did not present physical limitations that prevented them from performing physical exercise; v) without hormone therapy; vi) and who did not smoke. We excluded those women who missed two consecutive training sessions and/or did not complete 80% of the training schedule; who changed their medication or the dose of their medication during the study; and performed physical exercise in parallel with this intervention.

Initially, 806 women were registered following wide dissemination of information (TV, radio, and posters), of which 758 were excluded for not meeting the inclusion criteria. Therefore, 48 women were properly allocated to the two groups, and 44 participants completed the entire study (Figure 1).

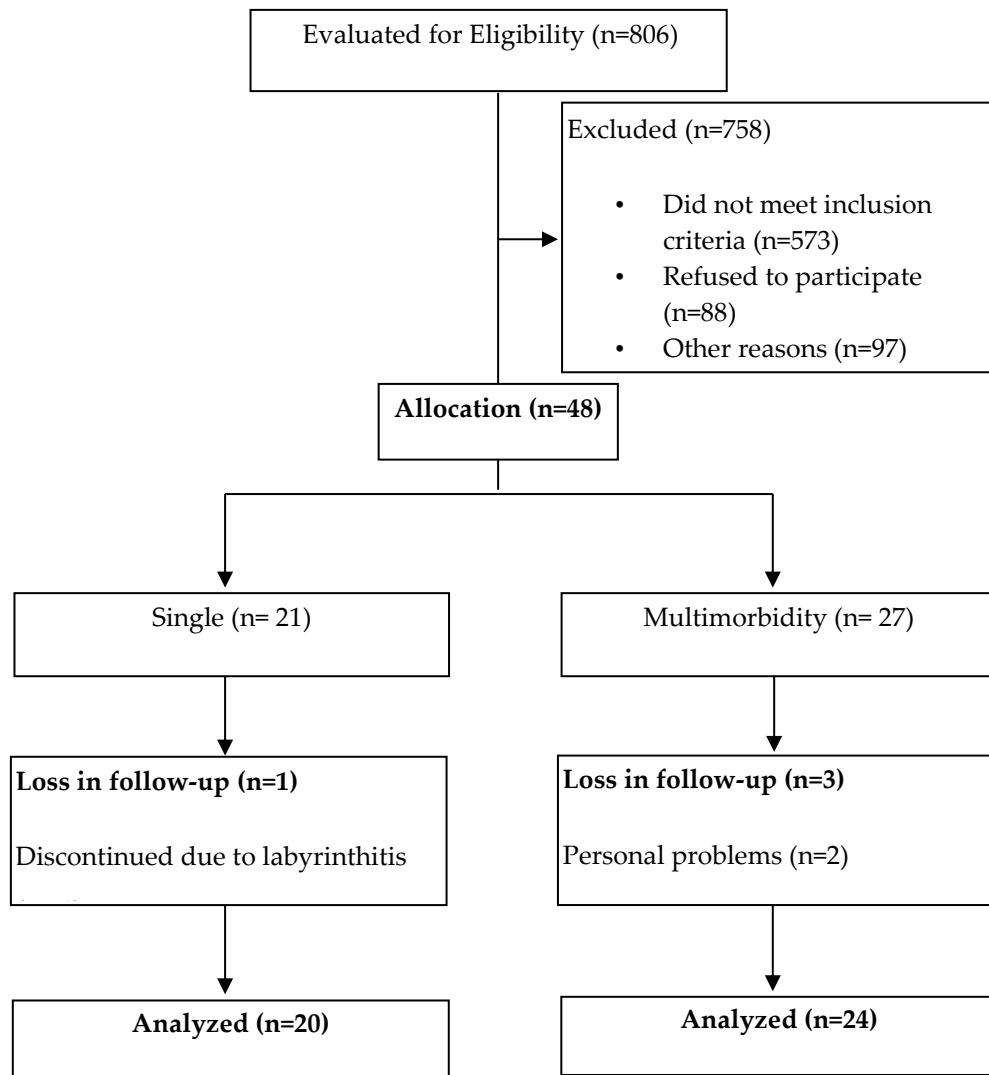


Figure 1: Follow-up flowchart.

PROCEDURES

The Pilates exercise program was conducted three times a week for 12 weeks. First, we performed familiarization sessions, explaining the Pilates exercises, its principles, and Borg's Rating Perceived Exertion 6 –20 Scale (RPE; 6 being no exertion and 20 being maximum exertion) [30].

Prior to and 72 hours after execution of the training protocol, we performed anthropometric assessments, blood pressure measurements at rest, food recalls, and blood collection. There was no blinding of the participants or evaluators, but the evaluations were always carried out by the same researchers.

After the initial assessment, the volunteers were divided into two groups according to their number of cardiometabolic diseases: SINGLE – women with one cardiometabolic disease; and MULT – women with two or more cardiometabolic diseases (multimorbidity). The criteria for the multimorbidity of cardiometabolic diseases was the coexistence of two or more of the following chronic diseases [31]: blood glucose > 126 mg/dL [6]; obesity (BMI ≥ 30 kg/m² [32]); dyslipidemia (LDL ≥ 160 mg/dL and/or triglycerides ≥ 150 mg/dL and/or total cholesterol ≥ 190 mg/dL and/or HDL ≤ 50 mg/dL) [5]; hyper-tension (SBP > 139 mmHg and DBP > 89 mmHg) [3]; and/or use of medication to control any of these diseases. Therefore, while participating in the study, volunteers with cardiometabolic diseases were being treated with medication.

EXERCISE TRAINING PROGRAM

Classical Pilates exercises were chosen for the 12-week exercise training program [33]. Two types of sessions (A and B) were performed alternately by all volunteers. These sessions consisted of three warm-up exercises, eight exercises in the main part of the session, and three exercises in the cool down in accordance with our previous study [34] (detailed information about exercises and sessions can be found in Supplementary Table 1). Mat Pilates sessions were conducted in the morning, with a maximum of 10 participants and a duration of 50 minutes. The first 5 minutes were dedicated to warming up, and the final 5 minutes to cooling down. The rest period between exercises was 45 seconds. The volunteers were instructed to maintain the principles of the method, especially breathing, and to maintain the correct posture during each exercise. All sessions were supervised by certified Pilates exercise specialists. The exercise load progression is presented in Table 1.

Table 1: Exercise load progression.

Weeks of training	Exercise load progression
1-3rd	10 repetitions, body weight
4-6th	12 repetitions, body weight
7-9th	12 repetitions, shin guards and free weight
10-12th	15 repetitions, shin guards and free weight

MEASUREMENT OF GENERAL CHARACTERISTICS

Initially, an anamnesis was taken by asking general questions. Stature was measured using a fixed stadiometer (Sanny, São Bernardo do Campo, Brazil) and body mass, and its distribution, were measured by bioimpedance using In Body 230 (Seoul, South Korea). The body mass index (BMI) was calculated using the value of body mass and height, according to the formula: $BMI = \text{body mass (kg)} / \text{height}^2 \text{ (cm)}$. Waist circumference was measured above the umbilical scar using an inelastic flexible measuring tape (Filizola, São Paulo, Brazil) without tissue compression. Blood pressure and resting heart rate were measured before and after training using an OMRON HEM-7113 automatic monitor, properly validated and calibrated. Three measurements of blood pressure and heart rate per day were taken on 3 non-consecutive days, and the means were used for analysis. These assessments always took place in the morning, in a sitting position after resting for 20 minutes in a silent environment, with restricted conversations and with relaxed arms and legs.

DIETARY INTAKE

Participants answered the 24-hour dietary recall administered by trained nutritionists. The volunteers were instructed to remember the foods and beverages they had consumed the day before. To calculate food consumption, 3 dietary records were collected, on 2 days during the week and one day at the weekend, on nonconsecutive days, and the mean recalls were calculated. Dietary data were evaluated at the beginning of the study and after 12 weeks of intervention. The total intake of energy, protein, fat, carbohydrates, fiber, and micronutrients was quantified using the Dietpro® software (version 5.7i, Agromídia Software®, Minas Gerais, Brazil) and the US Department of Agriculture food composition table [35,36] and manufacturers' nutrition labels.

BLOOD COLLECTION AND ANALYSIS

For the blood samples, 15 mL of blood were collected after an overnight fast, 5 days before and 72 h after the last exercise session. The samples were placed in tubes with EDTA (ethylenediamine tetraacetic acid) or separating gel and centrifuged at 3000 rpm for 15 minutes, and the supernatant (plasma) was aliquoted. All samples were kept frozen at -80°C until analysis and biochemical determinations were made in duplicate. The analyses performed were:

- Total protein: performed using the Bradford method and a bovine serum albumin curve [37].
- Enzymatic antioxidants: the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) was evaluated. SOD activity was evaluated by inhibiting pyrogallol auto-oxidation. Samples were mixed with 50 mmol L⁻¹ Tris-HCl buffer (pH 8.2) containing 1 mmol.L⁻¹ EDTA to deactivate metal-dependent enzymes, 80 U.mL⁻¹ catalase and 24 mmol.L⁻¹ of pyrogallol, and the kinetic assay was monitored for 10 minutes at 420 nm using an analytical curve constructed with SOD as the standard [38]. CAT activity was based on the decomposition of hydrogen peroxide. The samples were mixed with a 10 mmol.L⁻¹ potassium phosphate buffer (pH 7.0 containing 0.2% hydrogen peroxide. The decomposition of hydrogen peroxide was monitored at 240 nm for 10 min [38]. To measure GPx activity, the plasma was incubated with GPx buffer (100 mM potassium phosphate containing 1 mM EDTA, pH 7.7), sodium azide (40 mM), GSH (diluted in 5% metaphosphoric acid), GR (diluted in GPx buffer), NADPH (diluted with 5% sodium bicarbonate) and tert-butyl hydroperoxide (0.5 mM). The reduction in NADPH concentration was evaluated for 10 minutes in a spectrophotometer at 340 nm [39].
- Total antioxidant capacity due to ferric-reducing antioxidant power (FRAP) was determined by the ability of antioxidants present in blood samples to reduce Fe⁺³ to Fe⁺² which is chelated by TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) and forms the Fe⁺² TPTZ complex. This complex was quantified in a spectrophotometer at 593 nm, and antioxidant activity was determined by means of an analytical curve, constructed with trolox as the standard [40].
- Reduced glutathione (GSH): Plasma proteins were precipitated with metaphosphoric acid (MPA) and centrifuged at 7000xg for 10 minutes. The supernatant was collected and mixed with 100 mM sodium phosphate buffer (pH 8.0) containing 5 mM EDTA and 15 µL of orthophthaldialdehyde (1 mg/mL in methanol, for HPLC, ≥ 99.9%). The solution was incubated in the dark at room temperature for 15 minutes. Fluorescence was read at 350 nm (excitation) and 420 nm (emission). Sample GSH concentrations were calculated using a standard curve of GSH (0.001– 0.1 mM) [40].
- Uric acid: analysis performed by the automated uricase-PAP method.
- Carbonylated protein: Protein carbonyls were identified by 2,4-dinitrophenylhydrazine (DNPH). Samples were incubated with 10 mM DNPH (diluted in 2.5 N HCl) for 1 h

and then precipitated with 20% trichloroacetic acid (TCA). After centrifugation (9000 x g for 5 min), the pellet was washed with ethanol-ethyl acetate and resuspended in guanidine hydrochloride 6 mol.L⁻¹ (diluted in 2.5 N HCl). A blank was performed with the sample incubated with 2.5 N HCl (without DNPH) and followed the same procedures, in order to reduce the contribution of the color to the absorbance. Absorbance values were recorded at 370 nm (Molecular Devices, Menlo Park, CA, 213 USA) [38].

STATISTICAL ANALYSIS

We were unable to find any studies that assessed postmenopausal women with enough similarities multimorbidities undergoing exercise training to justify their use for a priori sample calculation, and therefore, we chose to present the power analysis values found a posteriori by G*power 3.1.9.7 in the results section ($\alpha = 0.05$; 2 groups, $n = 47$, effect sizes based on groups delta). The results are presented as mean $\pm$ standard deviation. To verify the normality of the results, we applied the Shapiro-Wilk test, and for the homogeneity of variance, we used the Levene test. Baseline characteristics of the groups were compared using an unpaired t-test. Variables that did not meet the assumptions of normal distribution and homogeneity of variance were evaluated using the Mann-Whitney test. Analysis of co-variance (ANCOVA) was used to compare the redox markers and food consumption between groups. This comparison was adjusted according to baseline values due the high individual variance of redox markers values. Data are presented in both pre- and post-adjusted. Variation between pre-and post-training and confidence intervals were calculated from the unadjusted post-training data. Effect sizes were calculated using Cohen's d, from the variation between pre-and post-training groups. All analyzes were performed using SPSS software version 21.0. The level of significance adopted was $p < 0,05$.

RESULTS

Of the 48 volunteers who started the study, a total of 44 volunteers completed it, 20 participants in SINGLE and 24 in MULT. One volunteer from the SINGLE group discontinued the study due to labyrinthitis, and 3 from the MULT group discontinued due to personal problems or medication modifications. None of the participants had training related injuries, and they had performed at least 33 of the 36 sessions. The general characteristics of the sample are shown in Table 2. We found that MULT had lower triglycerides ($p = 0.050$) and a higher

BMI ($p = 0.003$) and waist circumference [$p = 0.0002$; MULT had a substantially increased cardiovascular risk (≥ 88 cm) and SINGLE had a normal to increased risk (≥ 80 cm) [41]], and there were no obese women in SINGLE. At rest, the values of blood pressure, blood glucose, and lipid profile were not different between groups. The mean value and percentage of drugs used that control cardiometabolic diseases are shown in Table 2. We analyzed dietary recall data from SINGLE ($n = 13$) and MULT ($n = 20$) and found no significant differences in baseline or training effects for macronutrients, saturated and unsaturated lipids, cholesterol, fiber, zinc, vitamin A and vitamin C (Supplementary Table 2). Data are presented in pre-training and post training adjusted by ANCOVA for the baseline value.

Table 2: Baseline general characteristics, drugs, and comorbidities.

	SINGLE n = 20	MULT n = 24	TOTAL n = 44
Characteristics			
Age (years)	57 ± 4	58 ± 6	58 ± 5
Postmenopausal time (years)	10 ± 6	9 ± 7	9 ± 7
Waist circumference (cm)	79 ± 7	96 ± 9*	84 ± 8
BMI (kg/m ²)	25 ± 3	29 ± 4*	27 ± 4
Obesity (n(%))	0 (0)	11 (46)	11 (25)
Rest SBP (mmHg)	121 ± 8	119 ± 10	120 ± 9
Rest DBP (mmHg)	77 ± 8	76 ± 8	77 ± 8
Hypertension (n(%))	3 (15)	20 (83)	23 (52)
Fasting glucose (mg/dL)	97 ± 10	93 ± 8	95 ± 9
Diabetes Mellitus (n(%))	0 (0)	0 (0)	0 (0)
Total cholesterol (mg/dL)	231 ± 40	218 ± 35	224 ± 37
HDL (mg/dL)	60 ± 11	61 ± 11	61 ± 11
LDL (mg/dL)	142 ± 39	135 ± 33	138 ± 36
Triglycerides (mg/dL)	162 ± 89	113 ± 44*	135 ± 72
Dyslipidemia (n(%))	17 (85)	22 (92)	39 (89)
Drugs (n (%))			
Statins	1 (5)	1 (4)	2 (5)
ARB	1 (5)	6 (5)	7 (16)
ARB + Diuretic	2 (10)	7 (29)	9 (20)
Diuretic	0 (0)	2 (9)	2 (5)
ACEi	0 (0)	4 (17)	4 (9)
ACEi + Diuretic	0 (0)	1 (4)	1 (2)
Levothyroxine	3 (15)	3 (13)	6 (14)
Number of comorbidities (n (%))			
None	0 (0)	0 (0)	0 (0)
One	20 (100)	0 (0)	20 (45)
Two	0 (0)	20 (83)	20 (43)
Three	0 (0)	4 (17)	4 (9)

Data are presented as mean ± standard deviation or n (%). SINGLE: women with 1 disease; MULT: women with ≥ 2 comorbidities; BMI: body mass index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; HDL: high density lipoprotein; LDL: low density lipoprotein; ARB: angiotensin receptor blocker; ACEi: Angiotensin Converting Enzyme Inhibitor. * $p < 0.05$, significant difference between groups. Obesity was considered with BMI ≥ 30 (kg/m²). Diabetes Mellitus was considered with fasting glucose > 126 mg/dL.

Table 3 presents the redox data measured in plasma and the data are presented as pre-training and post-training adjusted by ANCOVA for the baseline value. This analysis showed interaction effects only in FRAP ($\Delta\%$ = 12 in SINGLE, and 8 in MULT; ES = 0.908; p = 0.014). Additionally, there was no significant difference at baseline between groups in any of the analyzed variables (SOD, p = 0.28; CAT, p = 0.46; GPx, p = 0.90; FRAP p = 0.90; GSH p = 0.44; carbonylated protein p = 0.88). Although the ANCOVA did not show isolated exercise training effects, the results can be explored using 95% confidence intervals. These showed a reduction in both groups in catalase [Δ = -577.34 (-702.27 to -452.41) for SINGLE; Δ = -540.87 (-733.64 to -348.1) for MULT], and in GSH [Δ = -1.93 (-2.34 to -1.52) for SINGLE; Δ = -2.75 (-3.32 to -2.18) for MULT] and an increase in carbonylated protein [Δ = 1.31 (0.95 to 1.67) for SINGLE; Δ = 1.68 (1.27 to 2.09) for MULT]. Table 4 shows the achieved power analysis for the redox data.

Table 3: Plasma redox status in SINGLE (n=20) and MULT (n=24) before and after 12 weeks of Mat Pilates training.

	PRE Mean ± SD	POST adjusted Mean ± SD	Mean difference (95%CI)	ANCOVA p
SOD (U/mL)				
SINGLE	0.93 ± 0.04	0.82 ± 0.16	-0.11 (-0.16 to -0.06)	0.972
MULT	0.88 ± 0.17	0.82 ± 1.48	-0.06 (-0.48 to 0.36)	
Catalase (U/μg prot)				
SINGLE	1674.48 ± 186.16	1097.14 ± 357.57	-577.34 (-702.27 to -452.41)	0.240
MULT	1508.66 ± 598.25	967.79 ± 326.21	-540.87 (-733.64 to -348.1)	
GPx (U/mg prot)				
SINGLE	1.20 ± 0.07	1.11 ± 0.41	-0.09 (-0.22 to 0.04)	0.102
MULT	1.23 ± 0.29	0.90 ± 0.38	-0.33 (-0.47 to -0.19)	
FRAP (nmol Trolox)				
SINGLE	178.89 ± 5.32	157.12 ± 29.70	-21.77 (-31.12 to -12.42)	0.014
MULT	195.58 ± 83.75	180.38 ± 27.09	-15.2 (-40.1 to 9.7)	
GSH (nmol/mg prot)				
SINGLE	5.96 ± 0.45	4.03 ± 1.24	-1.93 (-2.34 to -1.52)	0.309
MULT	6.39 ± 1.68	3.64 ± 1.13	-2.75 (-3.32 to -2.18)	
Uric acid (mg/dL)				
SINGLE	4.05 ± 0.25	3.81 ± 0.76	-0.24 (-0.49 to 0.01)	0.418
MULT	4.22 ± 1.14	4.10 ± 0.70	-0.12 (-0.5 to 0.26)	
Carbonylated protein (nmol/mg of protein)				
SINGLE	3.05 ± 0.16	4.36 ± 1.16	1.31 (0.95 to 1.67)	0.053
MULT	3.14 ± 0.99	4.82 ± 1.06	1.68 (1.27 to 2.09)	

Data are presented as mean $\pm$ standard deviation. SINGLE: women with 1 disease; MULT: women with ≥ 2 comorbidities. Data presented in pre- and post-adjusted. Mean difference from pre- to post- intervention values were calculated from the unadjusted post. SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; FRAP: Total antioxidant capacity due to iron-reducing antioxidant power; GSH: reduced glutathione.

Table 4: Statistical power of analysis.

	Power
SOD (U/mL)	0.050
Catalase (U/ μ g prot)	0.999
GPx (U/mg prot)	0.167
FRAP (nmol Trolox)	0.908
GSH (nmol/mg prot)	0.999
Uric acid (mg/dL)	0.277
Carbonylated Protein (nmol/mg of prot)	0.999

SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; FRAP: Total antioxidant capacity due to iron-reducing antioxidant power; GSH: reduced glutathione.

DISCUSSION

Our study investigated whether 12 weeks of Mat Pilates exercise training could affect the redox status in postmenopausal women with single or multiple cardiometabolic diseases. Our main result shows no significant baseline group differences in the redox status. We also found no significant changes in most of the markers of redox status after the exercise training program in either group. Only FRAP differed significantly after training with Mat Pilates, with confidence intervals showing a greater reduction in the SINGLE group. Moreover, the baseline values of blood pressure, blood glucose, and lipid profile did not differ between groups due to the use of drugs to control cardiometabolic diseases; however, there were differences in for BMI and waist circumference, which were higher in women with multimorbidity as expected.

In previous studies, postmenopausal women [42] and hypertensive individuals [43] had lower FRAP values, demonstrating the presence of oxidative damage. The ferric-reducing ability of plasma – FRAP, has been used as an indirect measurement to determine the total antioxidant activity, and some studies have shown high values of this marker in patients with one or more morbidity such as hypertension, obesity, diabetes, and metabolic syndrome [44]. However, the correlation between FRAP and other enzymatic antioxidant markers such as GSH and uric acid measured in human blood remains controversial [44–47]. The lack of correlation among redox biomarkers could be due to the inactivation of these enzymes under conditions of high oxidative stress [45,46].

In the present study, the SINGLE group showed greater reductions in FRAP values when compared to MULT, which is consistent with Smith et al. [27], who found that individuals with multimorbidities may have experience only minor or negligible effects of physical training. In postmenopausal women, the reduction in 17 β -estradiol directly impacts aspects of mitochondrial function, including the production of reactive oxygen species and ATP, and the membrane potential [48]. Considering that cardiometabolic diseases and the climacteric are associated with oxidative stress, these women probably started the exercise protocol under higher oxidative stress [8,42,49–52]. There is a lack of studies investigating the effects of the Pilates method on redox status, so given that this method has some characteristics similar to resistance and/or isometric training method, to compared our results with studies using those types of exercise. However, we know that even though they share similar characteristics, it would be better to compare our results with other studies using the Pilates method. Although

training with Mat Pilates is an interesting proposal for adherence for this group moderate-intensity aerobic exercise has better effects on oxidative stress in hypertensive postmenopausal women [14] and also in type 2 diabetic individuals [53].

A study with elderly women undergoing 12 weeks of resistance training showed that there was a reduction in uric acid and an increase in catalase and SOD [54], contradicting our findings. In older adults with and without metabolic syndrome undergoing 6 months of aerobic training, there were no significant differences in most of the oxidative stress variables, including protein carbonylation, antioxidant enzymes, and uric acid [55]. In addition, women with metabolic syndrome had significantly higher FRAP levels compared to women without metabolic syndrome [55], showing an increase in antioxidant capacity probably generated by increased production of pro-oxidants. Therefore, although physical training is considered the cornerstone of non-pharmacological therapy for postmenopausal hypertension [14], the oxidative profile responses in populations of postmenopausal women and those associated with cardiometabolic disease still need to be further elucidated.

Carbonyl protein can be used as a marker of protein damage given that postmenopausal women, showed an increase in its expression when compared to pre-menopausal women [13]. In patients with chronic obstructive pulmonary disease, lower values of antioxidant enzymes and higher values of protein carbonylation were found after training, probably due to disturbances in the mitochondrial respiratory chain [56].

We found no difference in the baseline of the redox status between variables between the SINGLE versus MULT groups. This may be due to the following possibilities: unlike SINGLE, half of the MULT sample had a BMI classifying them as obese 1 [41], in addition to a higher waist circumference. In this sense, MULT presents a substantially increased cardiovascular risk while SINGLE had a normal to increased risk [41]. However, the lipid profile data did not differ significantly, and the total and LDL cholesterol values for both groups were above the recommended level (< 190 mg/dL for total cholesterol; < 130 for LDL) [5] which could be problematic as oxidized LDL stimulates inflammation and the accumulation of reactive oxygen species generates damage [8]. Besides, the fact that MULT were well treated by drugs may have led to similar responses to the SINGLE group.

When we analyzed the preexisting cardiometabolic diseases in MULT, we found that 46% of the volunteers were obese and 83% hypertensive. Obesity is related to oxidative stress

without a well-defined causal direction. Oxidative stress can stimulate white adipose tissue deposition and increase preadipocyte proliferation, adipocyte differentiation, and mature adipocyte size and obesity can elevate chronic inflammation with increased oxidative stress [57]. Increased oxidative stress and redox imbalance damage cell structures, generate underproduction of antioxidant mechanisms, and modify mitochondrial activity, leading to the development of obesity-related complications [49,57]. This pro-oxidant state could explain why we did not find changes in plasma oxidative markers after 12 weeks of Mat Pilates training. Hypertension, on the other hand, can intensify oxidative stress through increased lipid peroxidation, endothelial dysfunction, and inflammation caused by the sustained increase in blood pressure values [50]. However, the present study found similar results in both groups, since both groups had the same types of morbidities (differentiated by the amount), and despite having isolated effects on the redox status, an additional effect of these results has not yet been demonstrated.

Lastly, despite the presence of hypertension, all study participants were taking drugs to control blood pressure. However, the use of antihypertensive drugs could also explain the absence of differences in redox biomarkers found between the SINGLE and MULT groups. The antihypertensive drugs used by volunteers act mainly on the renin-angiotensin system. Both angiotensin converting enzyme inhibitors and angiotensin receptor antagonists inhibit vascular remodeling and reduce ROS [58] through the reduction of NADPH oxidase and upregulation of Cu/ZnSOD [11,12]. These drugs can also improve endothelial function [58] and appear to be mediated by decreased lipoxygenase enzyme expression [59]. In addition, by decreasing Angiotensin II, angiotensin-converting enzyme inhibition, limits the stimulation of vascular NAD(P)H oxidase, preventing the increase in superoxide flow associated with activation of the renin-angiotensin system. Finally, angiotensin-converting enzyme inhibitors also limit the formation of hydrogen peroxide [59,60] and lipid peroxidation by the reduction of peroxynitrite formation [60]. As for thiazide diuretics, there is scant evidence of effects on oxidative stress [61,62]; however, it may be that they act on the antioxidant protection expressed by higher levels of FRAP [62].

For dietary intake, there are indications that the high intake of macronutrients, especially saturated fats and refined carbohydrates consumed in excess, can promote oxidative stress and, consequently, contribute to inflammation [63,64]. On the other hand, fiber, vitamin C, and some unsaturated lipids can act as antioxidants, contributing to the reduction of oxidative stress [64].

Although consumption of these nutrients is related to the increase in cardiometabolic diseases and oxidative stress [64], we did not find changes in intake during the training protocol. Therefore, the results found in this study are probably not related to food consumption by the sample.

Regarding the potential clinical application of this study, Mat Pilates is a type of exercise with high adherence in the postmenopausal population [23]. Furthermore, despite not having found significant differences in the redox parameters, the Pilates method has relevance for other clinical parameters, such as the improvement of sexual function and the quality of sexual life of climacteric women [65] and physical and motor benefits in the elderly [66]. Therefore, understanding the physiological and clinical responses of the Pilates method, associated or not with cardiometabolic multimorbidity, is important when thinking about the best training approach for the climacteric population, given the scarcity and/or low methodological quality of previous studies [66].

This study had some limitations. Some redox markers presented a low or moderate power in the statistical analysis, and this could be the reason for the lack of results in some variables. Having a control group without training could help us understand the effects of Mat Pilates, not just differences in responses between populations; however, we did not have this group, as our aim was to compare the exercise-mediated responses in women with single or multiple cardiovascular disease. In addition, the volunteers were medicated, which may make it difficult to generalize these results to populations with non-existent or less effective treatments. Another limitation we had was that not all volunteers answered the dietary recall, and dietary patterns were not controlled for all participants. In addition, there was an imbalance between the number of pro-oxidant and antioxidant markers analyzed in the present study, which limits our interpretation of the results in relation to the global redox status but prioritizes the expected antioxidant results of physical training. In this sense, there are other markers that we did not analyze that could increase our understanding of the redox status of these women, such as the metabolism of lipid peroxidation and other non-enzymatic and exogenous antioxidants. Therefore, future studies should include participants with more severe conditions of cardiometabolic multimorbidity and a protocol that uses a control group that does not practice Mat Pilates.

CONCLUSIONS

We conclude that there were no changes in redox status mediated by 12 weeks of Mat Pilates exercise training between SINGLE and MULT, except that FRAP in SINGLE was lower. This study points to new perspectives to investigate in patients with multimorbidities the impacts of this physical training program on changes in the systemic redox state.

Supplementary Materials:

Supplementary Table 1: Mat Pilates exercise program.

Exercise	Description
Warm up	
<i>Roll up</i>	Dorsal decubitus, arms going toward the shoulder line, and from there the head starts to rise and the trunk curls towards the feet.
<i>Knee folds</i>	Lie on your back with your pelvis in neutral position, with your knees bent and feet flat. Exhale and tighten your abs to lift your right leg straight up to 90 degrees. Inhale, then exhale and lift your left leg straight up to 90 degrees. Lower your legs to the floor.
<i>Knee sways</i>	Lying in a dorsal decubitus with a bend in your knees and your feet relaxed flat on the surface. Keep your knees and feet lightly touching and your shoulders flat against the surface as you slowly sway your knees together from side to side.
Training A	
<i>The one leg stretch</i>	Neutral position lying on back, slowly raise one leg and bend it, forming a 90 ° angle, without leaving the neutral position. Extend the leg forward taking care not to arch your back and raise the other leg extending to which it was folded but without lowering the leg.
<i>One leg circle (right / left)</i>	Dorsal decubitus, straight legs and circular movements with both legs, one at a time clockwise.
<i>One leg circle (right / left)</i>	Dorsal decubitus, straight legs and circular movements with both legs, one at a time in the counterclockwise direction.
<i>The shoulder bridge in the ball</i>	Lying face up, bend the knees and place the feet on top of a stability ball. Rest on the shoulders and the upper back while keeping the body in a straight line from the knees to the head. Slowly lower the hips back down to the floor, keeping the ball still.
<i>The Swan dive</i>	Ventral decubitus, palms resting on the floor, elbows extended. Keep the spine extension, triggered abs, buttocks and contractors and shoulders away from the ears as the body swings upward and returns toward the floor.
<i>Abdominal with the ball</i>	Contraction of the abdominal muscle, leave the hip on the floor and support both legs on top of the ball, with the hands resting on the chest, remove the shoulder blades from the floor and return to the starting position.

The Hundred with flex Lying with the backrest on the mat, head and torso slightly raised, legs raised and arms stretched to the side of the hip without touching the floor. Inhale to raise the head and trunk until reaching the base of the scapula, maintaining control of the body and returning to the initial position. The flex ring should be held between the ankles.

Abdominal with the ball (alternating legs) Abdominal muscle contraction, leave the hips on the floor and support both legs at the top of the ball, with the hands resting behind the neck, withdraw the shoulder blades from the floor going with the right elbow towards the left knee that will leave the ball support and return to the starting position to repeat the exercise with the left elbow toward the right knee.

Training B

The double leg stretch Neutral position lying on your back, slowly lift your legs and bend them at a 90° angle, without leaving the neutral position. Extend your legs forward taking care not to arch but without letting your legs touch the floor.

The Shoulder Bridge Lying face up, bend the knees and place the feet on the floor. Rest on the shoulders and the upper back while keeping the body in a straight line from the knees to the head. Slowly raise and lower hips back to the floor.

The Shoulder Bridge with one leg (right / left) and flex Lying straight up, bend your knees and extend one leg toward the ceiling. Rest on the shoulders and upper back, keeping the body straight from the knees to the head. Slowly raise and lower your hip back to the floor. Repeat this movement with the other leg. Hold the flex ring at chest height with your elbows bent at shoulder height and tighten every time your hips are raised.

The shoulder bridge with flex ring Lying face up, bend the knees and place the feet on the floor. Rest on the shoulders and the upper back while keeping the body in a straight line from the knees to the head. Slowly lower the hips back down to the floor. Position the flex ring between the inner thigh and press every time you lift your hip from the floor.

Swimming Neutral position facing down, raise arms and place forward for a V with shoulders. The lumbar area should be stable, without letting the belly yield towards the floor, but keeping the abdomen contracted. Lift both the right arm and the left leg simultaneously at the same height. Lower your right arm and left leg at the same time. Repeat the exercise using the left arm and right leg this time.

The Swan dive in the ball Ventral decubitus on top of the ball, palms resting on the floor, elbows extended. Hold the extension of the spine, trigger abs, buttocks, contractors and shoulders away from the ears as the body moves up and returns to the ball.

The Hundred Lying with the backrest on the mat, head and torso slightly raised, legs raised and arms stretched to the side of the hip without touching the floor. Inhale to raise the head and trunk until reaching the base of the scapula, maintaining control of the body and returning to the initial position.

Board Lean forward, lean on the elbows in the floor aligned with the shoulder. Then raise the hip, keeping only supported by the tip of the feet and the elbows. Exercise has no movement, characterizing isometry.

Cooling down

Spine Stretch	Sitting with the spine straight, legs stretched and open slightly beyond the width of the hips. The arms remain stretched forward, always maintaining the alignment of the shoulder blades. It takes the chin to the chest and rolls the column forward, forming a "C". The chest should pass over the thigh and the inner part of the thighs should be lengthening. Return the movement to the starting position.
Cat Stretch	In four supports, with hands under the shoulders, the knees under the hips and the legs apart in the distance of the hips. Pelvis and spine in the neutral position. The head should follow the alignment of the thoracic spine. The movement is to articulate the spine, without misaligning the hands and legs. Tilt posteriorly to the pelvis and round the spine, sequentially joining the coccyx to the head. Maintain the position with the abs contracted and supporting the head with relaxed shoulders. We rejoined the spine sequentially to an extension of the thoracic.
Roll down	Stand your feet shoulder-width apart. The column must be in a neutral position. Lower the body, rolling it forward, keeping the muscles of the neck and arms totally relaxed. Try touching the shins or feet with your hands. Slowly return to the starting position.

Supplementary Table 2: Analysis of food consumption pattern in SINGLE (n=13) and MULT (n=20) before and after 12 weeks of Mat Pilates training.

	PRE Mean ± SD	POST adjusted Mean ± SD	Difference in means (CI 95%)	ANCOVA p
Energy (kcal)				
SINGLE	1608.19 ± 499.62	1597.94 ± 355.35	-10.25 (-706.92 to 686.42)	0.847
MULT	1649.27 ± 603.15	1622.59 ± 440.76	-26.68 (-214.02 to 160.66)	
Protein (g)				
SINGLE	61.37 ± 13.88	59.55 ± 19.22	-1.82 (-10.93 to 7.29)	0.472
MULT	74.36 ± 36.08	64.58 ± 19.12	-9.78 (-22.43 to 2.87)	
Carbohydrate (g)				
SINGLE	186.37 ± 54.06	188.11 ± 48.29	1.74 (-26.12 to 29.6)	0.790
MULT	187.57 ± 50.74	192.73 ± 48.29	5.16 (-16.55 to 26.87)	
Lipids (g)				
SINGLE	69.51 ± 29.14	68.86 ± 21.33	-0.65 (-14.53 to 13.23)	0.661
MULT	76.41 ± 38.06	65.48 ± 21.31	-10.93 (-24.45 to 2.59)	
Saturated (g)				
SINGLE	22.74 ± 11.98	19.05 ± 7.02	-3.69 (-9.03 to 1.65)	0.980
MULT	21.13 ± 8.71	18.99 ± 7.02	-2.14 (-5.61 to 1.33)	
Monounsaturated (g)				
SINGLE	19.26 ± 8.32	17.47 ± 6.73	-1.79 (-5.9 to 2.32)	0.599
MULT	20.71 ± 9.78	18.74 ± 6.73	-1.97 (-5.65 to 1.71)	
Polyunsaturated (g)				
SINGLE	18.11 ± 7.27	19.27 ± 6.55	1.16 (-2.6 to 4.92)	0.538
MULT	19.78 ± 7.73	17.81 ± 6.55	-1.97 (-5.11 to 1.17)	

Cholesterol (mg)				
SINGLE	279.27 ± 149.16	220.92 ± 84.95	-58.35 (-124.33 to 7.63)	0.800
MULT	243.65 ± 118.05	213.14 ± 84.78	-30.51 (-75.55 to 14.53)	
Fiber (g)				
SINGLE	15.69 ± 4.36	15.64 ± 4.26	-0.05 (-2.39 to 2.29)	0.967
MULT	15.36 ± 5.03	15.58 ± 4.26	0.22 (-1.82 to 2.26)	
Zinc (mg)				
SINGLE	6.57 ± 2.20	6.96 ± 3.16	0.39 (-1.09 to 1.87)	0.533
MULT	8.72 ± 5.70	7.68 ± 3.14	-1.04 (-3.06 to 0.98)	
Vitamin A (µg)				
SINGLE	115.7 ± 76.05	116.13 ± 41.97	0.43 (-32.96 to 33.82)	0.007
MULT	94.66 ± 60.47	72.72 ± 41.86	-21.94 (-44.73 to 0.85)	
Vitamin C (mg)				
SINGLE	176.24 ± 96.71	113.31 ± 86.78	-62.93 (-112.88 to -12.98)	0.510
MULT	125.74 ± 95.48	134.20 ± 107.64	8.46 (-21.22 to 38.14)	

Data presented as mean ± standard deviation. SINGLE: women with 1 disease; MULT:

women with ≥ 2 comorbidities. Data presented in pre- and post-adjusted. Variation values and confidence intervals were calculated from the unadjusted post.

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**EFFECTS OF COMBINED EXERCISE ON SALIVARY
OXIDATIVE STRESS IN HYPERTENSIVE AND
NORMOTENSIVE POSTMENOPAUSAL WOMEN**

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Status: publicado. Formato original disponível no Anexo 4.

Amaral AL, Mariano IM, Giolo JS, Dechichi JGC, de Souza AV, Batista JP, de Souza TCF, Caixeta DC, Peixoto LG, Teixeira RR, Espindola FS, Puga GM. Effects of combined exercise on salivary oxidative stress in hypertensive and normotensive postmenopausal women. Motriz. 2022; 28, Especial 1. doi: 10.1590/S1980-657420220012321.

Abstract

Aim: This study evaluated the effects of 10 weeks of combined exercise training on the salivary oxidative profile of hypertensive and normotensive postmenopausal women. **Methods:** Twenty-six non-obese postmenopausal women were divided into two groups: the hypertensive group (HT; $n = 13$; 58.9 ± 3.9 years; and BMI of 27.7 ± 4.6 kg/m²) or the normotensive group (NT; $n = 13$; 52.7 ± 5.2 years; and BMI of 26.9 ± 2.9 kg/m²). They performed 30 sessions of combined exercises over 10 weeks: 45 min per session, three times a week. Resting saliva samples were collected after an overnight fast to evaluate salivary nitrite levels and oxidative stress markers before and after training. **Results:** Two-way ANOVA showed that there was no difference in the responses over time between the hypertensive and normotensive groups in catalase, superoxide dismutase salivary activity, total antioxidant capacity, or lipid peroxidation. However, superoxide dismutase activity (Δ HT -0.87 ± 14.53 SOD / mg protein; Δ NT: 7.13 ± 9.39 SOD / mg protein; $p < 0.01$) and nitrite levels (Δ HT 10.32 ± 60.83 mM; Δ NT 101.92 ± 149.57 mM; $p = 0.03$) were higher overall in the hypertensive group compared to the normotensive group. Moreover, salivary nitrite levels increased over time ($p = 0.04$) in both groups. **Conclusion:** 10 weeks of combined exercise training did not change salivary oxidative stress markers in either normotensive or hypertensive postmenopausal women, although, after exercise training, nitrite levels increased in both groups, even with higher baseline salivary nitrite levels in hypertensive women. Thus, recurrent exercise seems to be a safe strategy after menopause from the standpoint of oxidative stress, regardless of the presence of hypertension.

Keywords: superoxide dismutase; nitrite; saliva; climacteric; combined exercise.

INTRODUCTION

Menopause is characterized by the cessation of estrogen production by the ovaries, resulting in permanent amenorrhea [1]. Additionally, estrogen is a cardioprotective hormone [2] that plays a role in the modulation of endothelial function [3]. So, the lack of this hormone can lead to endothelial dysfunction and increase oxidative stress [4] which may trigger cardiovascular diseases such as hypertension [5]. In this way, the imbalance between pro-oxidant and antioxidant factors leads to oxidative stress, causing cell damage [6] due to excess reactive oxygen species (ROS) production [6]. Furthermore, oxidative stress may be accentuated by hypertension [7] and cause deregulation of vascular tone control through changes in the bioavailability of nitric oxide (NO) [7], an endothelium-derived relaxing factor that promotes vasodilation [7].

NO metabolism produces inorganic anions such as nitrate and nitrite, which are commonly evaluated in biological samples [8]. The main form of NO production is the L-arginine pathway, which occurs from NO synthase (NOS) isoforms, mainly by endothelial NOS, which can be produced by the vascular endothelium and is directly linked to the regulation of vascular tone and maintenance of endothelial integrity [9]. Oxidative stress caused by diseases associated with endothelium dysfunction can increase the activation of antioxidant enzymes, such as catalase and superoxide dismutase (SOD), in an attempt to maintain body homeostasis [10]. In this sense, assessment of antioxidant enzymes, total antioxidant capacity, and oxidative damage in saliva could provide alternative ways to find information about oxidative balance through less invasive methods than venipuncture.

Endothelial dysfunction and the lack of NO production that can be observed in postmenopausal women may lead to increased peripheral vascular resistance [11], which facilitates the onset of hypertension. Non-pharmacological treatments such as physical exercise practice can have positive responses, improving both endothelial function [12] and the antioxidant system [13]. In this sense, hypertension guidelines [14,15] recommend the practice of moderate aerobic training and resistance exercise as support, since they can reduce resting blood pressure (BP) [16] and improve oxidative balance [17,18]. However, only a few studies have investigated the effects of combined aerobic and resistance exercise training on oxidative stress, especially in saliva or in postmenopausal women. Furthermore, considering that these women have a higher prevalence of hypertension [5], it is still unknown whether the presence of this disease generates any different response in oxidative stress markers compared to women

without the disease when submitted to combined training. Therefore, this study aimed to assess the effects of 10 weeks of combined aerobic and resistance training in salivary oxidative stress markers in non-obese hypertensive and normotensive postmenopausal women. We hypothesized that hypertensive women would have worse oxidative balance and that combined exercise training would be able to improve both nitrite and the antioxidant system in both groups.

MATERIAL AND METHODS

PARTICIPANTS

This was a parallel clinical trial study developed in two stages: before and after 10 weeks of combined exercise. Participants were divided into two groups: HT hypertensive (n = 13) and NT normotensive (n=13). A total of 260 women, aged 50-70 years, were recruited from traditional media (TV, radio, and posters) and registered from January 2015 to December 2017 at the Laboratory of Cardiorespiratory and Metabolic Physiology of the Federal University of Uberlândia, Uberlândia, MG, Brazil; women were recruited at the same time as sample collection occurred. The inclusion criteria for the study were as follows: amenorrhea for at least 12 months, body mass index ≤ 32 kg/m², ability to engage in treadmill and resistance exercise training, without diabetes or cancer treatment, not undergoing dental treatment, no use of beta-blockers, no hormone therapy, non-smokers, and not using drugs that alter the lipid profile. To be included in the hypertension group, women had to self-report being hypertensive and using antihypertensive medication. The study design is presented in Figure 1. The Human Research Ethics Committee of the Federal University of Uberlandia approved this study (CAAE: 40622414.9.0000.5152). All the volunteers signed a consent form. The experiments were carried out in accordance with the principles set out in the World Medical Association Declaration of Helsinki. This study was registered at Clinicaltrials.gov (number: NCT03008785).

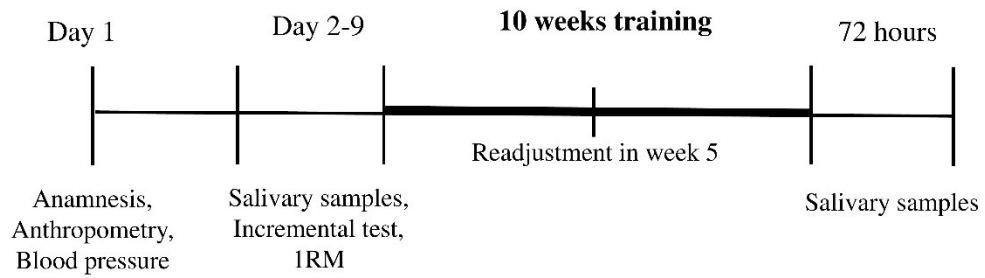


Figure 1. Study design. 1RM: one maximum repetition test.

ANTHROPOMETRY EVALUATION

The volunteers were instructed to maintain their regular diet. Their body mass was measured using an electronic scale (Micheletti, São Paulo, SP, Brazil), height was measured using a stadiometer (Sanny, São Paulo, SP, Brazil) and abdominal circumference was measured with an inelastic tape (Sanny, São Paulo, SP, Brazil) measuring 0.5 cm wide. A tetrapolar bioelectrical impedance analysis (Biodynamics model 450c, Biodynamics, Shoreline, WA, United States) was used to evaluate the total lean mass, fat mass, and percentage of total body fat. It was performed in the morning after at least eight hours of fasting, and hydration was controlled.

PHYSICAL FITNESS ASSESSMENT

A short version of the International Physical Activity Questionnaire (IPAQ) [19] was used to evaluate the initial physical activity level of the volunteers, classifying them as sedentary, irregularly active, active, or very active. The aerobic capacity assessment was determined through an incremental treadmill test adapted from previous study [20]. Briefly, all volunteers performed a submaximal incremental test on a treadmill at 5.5 km/h and the intensity was increased using only treadmill inclination (1% every two minutes) until the volunteers reached 85% of their predicted maximum heart rate (HR) or their rate of perceived exertion (RPE) reached 18 using the Borg scale. Oxygen uptake (VO_2) and carbon dioxide output (VCO_2) were recorded during all tests using a Cosmed Quark CPET gas analyzer (Rome, Italy). The goal of this test was to identify ventilatory thresholds (VT1 and VT2) based on ventilatory equivalents for oxygen (VE/VO_2 ratio) and carbon dioxide (VE/VCO_2 ratio) [21]. The intensity of resistance exercise was evaluated and prescribed based on a one-repetition maximum test

(1RM) [22], which happened once before and once in the fifth week of training in the following exercises: leg press 45°, seated low row, vertical chest press machine, pec deck, and wide grip lat pull-down. This test consisted of the workload performed with no more than one repetition in five tries with three minutes of rest between tries [22].

EXERCISE PROGRAM

The exercise program consisted of 30 sessions of combined aerobic and resistance exercise training during 10 consecutive weeks (three sessions per week). Absences were made up on the nearest day when there were no sessions scheduled. Each session lasted 45 min and consisted of five min warm-up, 20 min of resistance exercise, and 20 min of aerobic exercise, with the order of the exercises alternated between sessions. After five weeks of training, the 1RM test was performed again to readjust the resistance training load. The aerobic intensity was readjusted with a 20% increase in inclination the same week. Resistance training was performed in two sets of 15 repetitions in seven weight training exercises (from a new 1RM test; we readjusted 60% of 1RM in the fifth week) for large muscle groups: leg press 45°, seated low row, vertical chest press machine, pec deck, wide grip lat pull-down, Swiss ball squat and abdominal crunch. The aerobic exercise was performed on a treadmill, at a speed of 5.5 km/h and intensity (imposed by treadmill inclination and heart rate) between VT1 and VT2.

SALIVARY ANALYSES

Saliva samples were collected after 12 h of fasting before the first and 72 h after the last exercise training session. All samples were kept frozen at -80 °C until analysis and biochemical determinations were made in duplicate. Total antioxidant capacity was evaluated using the FRAP method and calculated from the standard Trolox curve [23]. The activity of SOD was determined based on the auto-oxidation capacity of pyrogallol and catalase by monitoring the consumption of hydrogen peroxide at 240 nm [23]. Lipid peroxidation levels were determined by the TBARS method, using as the standard a curve of 1,1,3,3-tetramethoxypropane [24]. The amounts of NO were estimated by the determination of total nitrite by the Griess colorimetric method [25].

STATISTICAL ANALYSIS

The data are presented as mean $\pm$ standard deviation. The data distribution was analyzed using the Shapiro-Wilk test, and non-parametric data were transformed until a normal distribution was achieved (log or z-score). The sample size was calculated by GPower 3.1.9.2,

considering variations of 4 ± 4 mM in nitrite as acceptable for this population after a training program [26], with an α value of 0.05 and power analysis of 85%, resulting in 26 volunteers. An unpaired Student's t-test was applied to compare baseline groups and two-way analysis of variance (ANOVA) for repeated measures was used to analyze time (pre and post) and group (NT and HT) effects with a Bonferroni post hoc test, when appropriate. For outlier identification, the extreme studentized deviate method was used. Effect sizes (Hedges' g) are presented. A p-value of < 0.05 was considered statistically significant, and all statistical analyses were performed using SPSS software version 20.0 (IBM, New York, NY, USA).

RESULTS

Forty volunteers who fulfilled the inclusion criteria were recruited, and 14 volunteers were excluded: one modified their antihypertensive medication during the protocol, one sample analysis failed, two left due to health problems not related to the study, four did not complete for personal reasons, and six due to incompatible frequency or scheduling. So, 26 completed the 10 weeks of training and performed the post-tests. Figure 2 shows the flowchart.

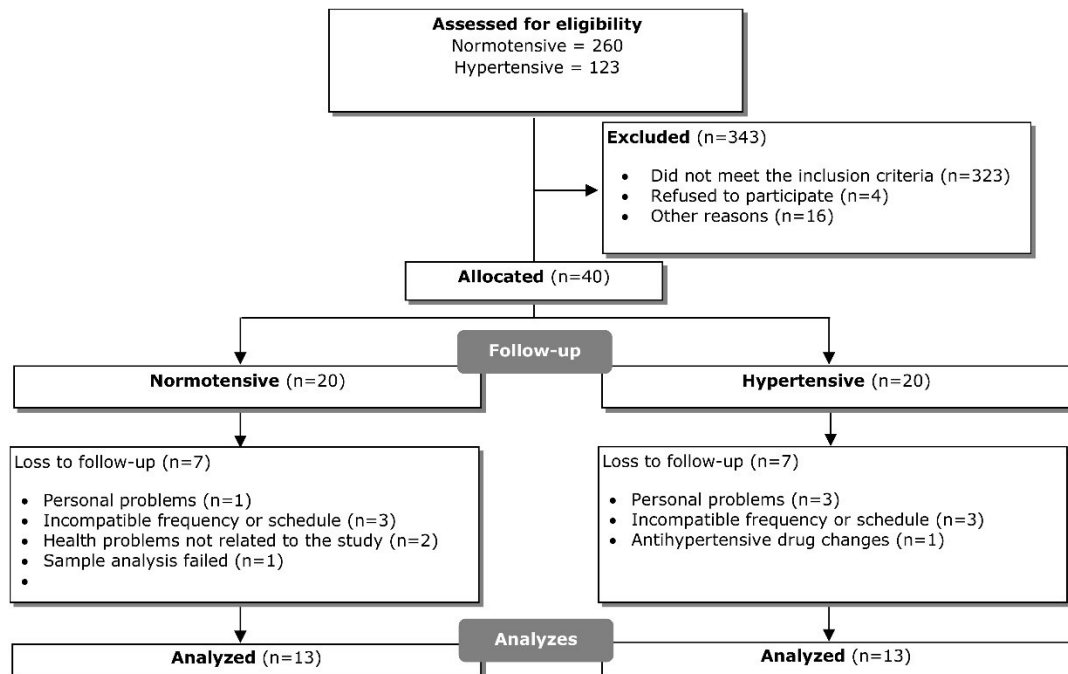


Figure 2: Flowchart.

Table 1 shows the anthropometric and general characteristics of all participants from both groups and the antihypertensive drugs used by the HT participants. HT subjects were older

($p = 0.01$) when compared to NT (58.9 ± 3.9 years for HT and 52.7 ± 5.2 years for NT). There were no significant differences in other variables between groups.

Table 1 - Volunteers characteristics

	NT (n=13)	HT (n=13)	p
Age (years)	52.7 ± 5.2	58.9 ± 3.9	0.01
Body Mass (kg)	64.8 ± 9.0	68.5 ± 8.3	0.29
BMI (kg/m ²)	26.9 ± 2.9	27.7 ± 4.6	0.60
Abdominal Circumference (cm)	92.9 ± 7.9	93.6 ± 9.2	0.84
Body Fat (%)	35.4 ± 3.7	38.4 ± 7.0	0.18
Fat Mass (kg)	23.0 ± 4.6	26.5 ± 6.9	0.14
Lean Mass (kg)	41.6 ± 4.0	39.2 ± 4.0	0.14
Resting Systolic Blood Pressure (mmHg)	129.1 ± 17.4	121.9 ± 13.6	0.25
Resting Diastolic Blood Pressure (mmHg)	84.3 ± 12.2	76.1 ± 8.1	0.06
Antihypertensive drugs – (n (%))			
ACE	-	2 (15.4)	
AT ₁	-	4 (30.8)	
Thiazide Diuretics	-	2 (15.4)	
ACE + Thiazide Diuretics	-	1 (7.7)	
AT ₁ + Thiazide Diuretics	-	4 (30.8)	

Data are present in mean $\pm$ standard deviation. NT: normotensive group; HT: hypertensive group; BMI: body mass index. ACE: angiotensin-converting-enzyme inhibitors; AT₁: angiotensin II type 1 receptor blocker.

Table 2 shows the salivary oxidative stress marker levels, analyzed (ANOVA two-way) before and after the 10-week intervention in both groups. There were no interaction effects (group * time) in nitrite, catalase, FRAP, SOD, and TBARS. SOD levels were lower ($p < 0.01$) in NT at both the pre- and post-time points. Two-way ANOVA showed that nitrite values increased (pre vs. post) ($p < 0.01$) in both hypertensive (pre: 87.6 ± 44.0 ; post 98.0 ± 47.2 mM) and normotensive (pre: 50.2 ± 28.6 ; post 152.2 ± 146.5 mM) groups with no difference between groups ($p = 0.16$).

Table 2 - Salivary oxidative stress markers in HT and NT before and after intervention.

	Pre	Post	Δ	<i>ANOVA</i>	<i>ANOVA</i>	<i>ANOVA</i>	Effect	<i>t test p</i>
	Mean $\pm$ SD	Mean $\pm$ SD		p group	p time	p interaction	size	Baseline
Nitrite (mM)								
NT	50.25 $\pm$ 28.62	152.16 $\pm$ 146.51	101.92 $\pm$ 149.57	0.16	0.04	0.07	-0.97	0.03
HT	87.64 $\pm$ 43.98	97.96 $\pm$ 47.24	10.32 $\pm$ 60.83				-0.23	
Catalase (U/mg prot)								
NT	15.51 $\pm$ 7.23	15.15 $\pm$ 7.38	-0.36 $\pm$ 5.72	0.20	0.74	0.25	0.05	0.32
HT	20.38 $\pm$ 15.20	17.31 $\pm$ 11.16	-3.07 $\pm$ 15.11				0.23	
FRAP (umol/L eq. Trolox)								
NT	59.62 $\pm$ 43.82	57.09 $\pm$ 44.97	-2.34 $\pm$ 55.68	0.09	0.81	0.93	0.06	0.66
HT	53.28 $\pm$ 22.97	52.24 $\pm$ 25.65	-1.04 $\pm$ 27.32				0.04	
SOD (SOD/mg prot)								
NT	3.30 $\pm$ 0.82	11.85 $\pm$ 10.04	7.13 $\pm$ 9.39	< 0.001	0.38	0.36	-1.20	< 0.001
HT	23.23 $\pm$ 12.37	22.36 $\pm$ 17.78	-0.87 $\pm$ 14.53				0.06	
TBARS (umol/L)								
NT	5.12 $\pm$ 1.17	5.80 $\pm$ 1.69	0.69 $\pm$ 1.65	0.67	0.13	0.12	-0.47	0.26
HT	4.58 $\pm$ 1.19	4.90 $\pm$ 1.09	0.32 $\pm$ 1.51				-0.28	

Data are present in mean $\pm$ standard deviation. NT: normotensive group; HT: hypertension group; FRAP: ferric reducing ability of plasma; SOD: superoxide dismutase; TBARS: thiobarbituric acid reactive substances; Δ : Values of salivary variation (post – pre values).

DISCUSSION

The present study investigated if 10 weeks of combined exercise could provide different responses in salivary oxidative stress between hypertensive and normotensive non-obese postmenopausal women. First, we found that women in the HT group were a little older than NT subjects, but they were all in the same age group as postmenopausal women and not yet elderly. In the oxidative stress variables, our results showed no differences in these markers between groups, although SOD activity was higher at baseline for the HT group. Moreover, salivary nitrite levels increased in both the HT and NT groups after exercise training, even with higher baseline levels in the HT group.

The hypertensive population had greater cardiovascular risk and worse vascular health [27,28], mainly due to endothelial dysfunction [11] involved in the production and action of substances related to BP control, such as relaxation factors (e.g. NO) [9]. The oxidative stress caused by this high BP can chronically intensify endothelial dysfunction [7], lipid peroxidation, and inflammation [7]. Furthermore, oxidative stress may compromise other organs and systems that play a key role in the regulation of BP, such as the kidneys [14,29], where activation of the

renin-angiotensin-aldosterone system causes oxidative imbalance via NADPH-oxidase and decreased availability of NO and antioxidant enzymes [29].

Some studies have found lower oxidative stress in healthy compared with hypertensive patients; while ROS production plays an important role in the development of hypertension, chronic moderate-intensity exercise training has been documented to ameliorate these ROS levels [18]. Unlike these results, we did not find improvements in salivary oxidative stress markers after exercise training in these women, but we believe that some factors were important in determining our results, such as BP levels and exercise training duration. In a previous study, we found that there were no significant differences in 24-h ambulatory blood pressure values between groups [30]. These data also showed no undiagnosed hypertensive women from the outpatient examination. Furthermore, the hypertensive women in our study had been under antihypertensive drug treatment for more than three years, so they were treated, well-controlled hypertensive patients. Blood pressure levels are positively correlated with markers of oxidative stress and negatively correlated with antioxidant capacity [18], so we believe that good regulation of BP levels in hypertensive women may attenuate oxidative stress markers over time. Another important factor was that we used a combined aerobic and resistance exercise training intervention for 10 weeks; this may have been too short a period to see differences in salivary oxidative stress markers in our volunteers.

The effects of physical training on oxidative balance in patients with hypertension are not unequivocal. Dantas et al. [31] found improvements in plasma oxidative balance and nitrite levels in patients similar to those taking part in the present study (elderly hypertensive women) after 10 weeks of resistance training. A further study in hypertensive patients suggested that isometric handgrip training protected against plasma oxidative stress at rest and after exercise [32]. However, as in the present results, no improvements in nitrite or lipid peroxidation were found. Lastly, Fearheller et al. [33] demonstrated that six months of moderate-intensity aerobic exercises with 94 hypertensive and prehypertensive adults was sufficient to improve the plasma and urinary oxidative profiles, independent of the analyzed polymorphisms.

Similar to our results, some studies have also found no changes in oxidative stress markers after exercise training. Jarrete et al. [34] showed higher SOD activity but did not find changes in the plasma levels of catalase or TBARS in hypertensive postmenopausal women after eight weeks of aerobic exercise training. Plasma oxidative stress markers (ex. SOD, catalase, glutathione peroxidase, and total antioxidant status) were also not different after 12

weeks nor 10 weeks of combined exercise training in women of the same age [35,36]. So, although our hypothesis was that exercise training could improve oxidative stress markers, and, in accordance with the literature, exercise training tends to improve antioxidant markers and decrease pro-oxidant markers, the intensity, volume, type of exercise, and type of population may directly influence these results [37]. It is worth mentioning that most studies use plasma analysis, and although saliva analysis is advantageous due to its ease of collection, there are still few studies that use it for their main analysis.

Another important point was that 84.7% of the HT group in our sample used drugs that act on the renin-angiotensin system. It has been reported that AT1 receptor antagonists or angiotensin-converting enzyme inhibitors inhibit vascular remodeling and reduce ROS, not only by the reduction of NADPH oxidase but also by the positive regulation of Cu/ZnSOD [38]. In addition, these drugs also partially improve endothelial function regardless of BP reduction [39]. These antioxidant actions are important because oxidative stress and NO elimination due to excess ROS are a major cause of reduced NO bioavailability [39]. Therefore, this result leads us to hypothesize that endothelial dysfunction, vascular remodeling, and increased lipid peroxidation in women after menopause [40] reduces the bioavailability of NO and generates the need for increased SOD activity in both groups. Nevertheless, within the HT group in the present study, this was corrected by antihypertensive drugs.

Regarding the increase in NO expression after training, we know that during exercise there is an overproduction of ROS due to increased endothelial shear stress and mitochondrial respiratory chain inefficiency [18]. However, this acute momentary increase in oxidative stress possibly signals positive chronic antioxidant upregulation, providing long-term antioxidant capacity [18]. Despite some inconsistent results, some literature reviews [13,17,18] seem to indicate positive oxidative balance effects of regular exercise. However, in relation to these parameters, it is worth mentioning that: 1) few studies have evaluated the chronic effects of exercise; 2) fewer studies are completed with hypertensive subjects; 3) almost all studies use aerobic exercise and 4) the influence of exercise load variables (i.e. intensity, volume, frequency) is still not very clear.

In this way, a clinical study with hypertensive women after menopause who underwent aerobic training for eight weeks (three days/week) showed higher plasma NO levels after training [34]. A meta-analysis [41] on the effects of high-intensity interval training in patients with lifestyle-induced cardiovascular problems found a greater availability of NO and less

oxidative stress when compared to lower intensity training. However, in general terms, moderate-intensity aerobic exercise is recommended, especially in populations more vulnerable to mechanical injuries, such as middle-aged and elderly people [17].

To our knowledge, this is the first study to evaluate the relationship between combined training and salivary markers of oxidative stress in humans, especially in hypertensive patients. However, the present study has some limitations, such as the duration of the exercise training and the fact that BP was well-controlled by antihypertensive drugs in the HT group. Additionally, analyses comparing the classes of antihypertensive agents and the various characteristics of training load control (such as volume, frequency, and intensity) should be completed with this population on salivary oxidative markers in future studies. Thus, the results suggest that combined exercise at moderate intensity may be a good strategy to maintain vascular health and to improve NO release from the endothelium in both hypertensive and normotensive postmenopausal women, but that exercise training is not effective in improving oxidative stress markers.

CONCLUSION

Ten weeks of combined aerobic and resistance exercise training did not change salivary oxidative stress markers in either normotensive or hypertensive non-obese postmenopausal women but was able to increase salivary nitrite in both groups. Thus, recurrent exercise seems to be a safe strategy after menopause from the standpoint of oxidative stress, regardless of the presence of hypertension.

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CONCLUSÕES GERAIS DA TESE

A partir dos estudos expostos, chegamos as seguintes conclusões: **1)** a ingestão aguda do suco de beterraba associada ao exercício aeróbio moderado parece reduzir a atividade da catalase (em High- NO_3^- e Low- NO_3^-) e GSH (em High- NO_3^-); **2)** Isoflavona e exercício físico combinado pode promover redução da peroxidação lipídica e de concentrações salivares de nitrito; **3)** não houve mudanças no estado redox mediado por 12 semanas de treinamento de *Mat* Pilates entre mulheres com uma doença e multimorbidades, exceto que FRAP em SINGLE (grupo com apenas uma doença cardiometabólica) foi menor; E **4)** 10 semanas de treinamento físico combinado não alteraram os marcadores de estresse oxidativo salivar em mulheres após a menopausa normotensas ou hipertensas, embora, após o treinamento físico, os níveis de nitrito tenham aumentado em ambos os grupos, mesmo com níveis basais de nitrito salivar mais elevados em mulheres hipertensas.

Assim, como aplicação clínica temos que o exercício físico auxilia na regulação do estado redox independentemente da quantidade de doenças associadas; e a suplementação com isoflavonas ou suco de beterraba se mostram como importantes aliados no controle do estado redox em mulheres após a menopausa.

Como perspectivas futuras, sugerimos o estudo da manipulação de doses destas intervenções, seja nas suplementações, como em diferentes frequências de consumo de suco de beterraba ou isoflavonas, diferentes composições de isoflavonas ou até diferentes concentrações de nitrato no suco de beterraba; ou com relação aos exercícios como diferentes intensidades, volumes ou frequências de treino nessa população (mulheres climatéricas, multimórbidas, etc), especialmente em modalidades menos estudadas como no Pilates.

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ANEXO 1 - COMPROVANTE DE PUBLICAÇÃO DO ESTUDO 1

Hindawi
Oxidative Medicine and Cellular Longevity
Volume 2021, Article ID 5579864, 9 pages
<https://doi.org/10.1155/2021/5579864>



Research Article

Antioxidant Responses in Hypertensive Postmenopausal Women after Acute Beetroot Juice Ingestion and Aerobic Exercise: Double Blind and Placebo-Controlled Crossover Trial

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Received 5 February 2021; Revised 25 March 2021; Accepted 2 April 2021; Published 14 April 2021

Academic Editor: Ana Lloret

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This study is aimed to analyze the effect of different nitrate concentrations [NO₃⁻] present in beetroot juice (BJ) on salivary oxidative stress markers after acute exercise performance in hypertensive postmenopausal women. Thirteen hypertensive postmenopausal women participated in three experimental sessions, taking different beverages: noncaloric orange flavored drink (OFD), low nitrate (low-NO₃⁻) BJ, and high nitrate (high-NO₃⁻) BJ. The participants performed aerobic exercise on a treadmill, at 65–70% of heart rate reserve (HRR), for 40 min. Saliva samples were collected after an overnight fast, 10 minutes before BJ ingestion at 7:20 am (0[']), 120 minutes after beverages ingestion (130[']), immediately after exercise (170[']), and 90 min after exercise (260[']). Salivary total protein (TP), catalase activity (CAT), reduced glutathione (GSH), and total antioxidant capacity by ferric-reducing antioxidant power (FRAP) concentrations were analyzed. No interaction (session*time) was found among three sessions over time. Catalase area under the curve (AUC) was lower after both low-NO₃⁻ and high-NO₃⁻ consumption ($p < 0.001$), and GSH AUC was lower after high-NO₃⁻ ($p < 0.001$) compared with OFD. So, the acute intake of BJ with aerobic exercise seems to decrease catalase (in high-NO₃⁻ and low-NO₃⁻) and GSH (in high-NO₃⁻), besides not interfering with FRAP in hypertensive postmenopausal women.

ANEXO 2 - COMPROVANTE DE PUBLICAÇÃO DO ESTUDO 2

Original Article

The effects of isoflavone supplementation plus combined exercise on salivary markers of oxidative stress in postmenopausal women

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(Received 24 May, 2019; Accepted 17 July, 2019; Published online 31 October, 2019)

This study tested the effect of isoflavone supplementation in addition to combined exercise training in salivary oxidative stress markers in non-obese postmenopausal women. Thirty-two postmenopausal women without hormone therapy were randomly assigned to exercise + placebo ($n = 15$) or exercise + isoflavone supplementation ($n = 17$) groups. They performed 30 sessions of combined exercises (aerobic plus resistance) over ten weeks and consumed 100 mg of isoflavone supplementation or placebo. Saliva samples were collected after an overnight fast. Superoxide dismutase, total antioxidant capacity, thiobarbituric acid reactive substances, catalase, total protein and nitrite were determined before and after ten weeks of the intervention. The ANOVA two-way analysis of variance was applied with α of 5%. Both groups increase ($p < 0.05$) superoxide dismutase activity and decrease catalase levels. There was interaction (time $\times$ group) in both nitrite and thiobarbituric acid reactive substances results, with increase ($p < 0.05$) in placebo group and decrease ($p < 0.05$) in isoflavone group. No difference was found for total antioxidant capacity or total protein. The combination of isoflavone supplementation and exercise training can promote an antioxidant effect through reduction of lipid peroxidation and concentrations of salivary nitrite.

Key Words: aerobic and resistance exercises, soy protein, menopause, anti-oxidant system

Women in post menopause are predisposed to the development of cardiovascular diseases,⁽¹⁾ and one possible reason to that is an increase in oxidative stress during this life period,⁽²⁾ leading to endothelial cells dysfunction.⁽³⁾ Endothelial cells are responsible for biological functions, mainly in cardiovascular and endocrine systems. Further, a prooxidant-antioxidant balance is necessary in order to have these cells functioning properly.^(4,5) Oxidative stress is an imbalance of this system to prooxidant molecules, thus, generating more reactive oxygen species (ROS), which are involved in several cardiovascular diseases, inflammation, cancer and aging.⁽²⁻⁵⁾ Clinically, there are methods to evaluate oxidative stress markers, such as the enzymes catalase and superoxide dismutase (SOD),⁽⁶⁾ and also nitrite,⁽⁷⁾ an important nitric oxide (NO) precursor and a key to endothelium vasoprotection.⁽⁸⁾

Saliva is an important factor in oral health maintenance of individuals.⁽⁹⁾ Salivary analysis for oxidative stress markers is an effective and non-invasive method,⁽¹⁰⁾ however research about salivary oxidative markers are still lacking. An imbalance of ROS

and consequent changes in oxidative stress salivary markers are associated with not only oral diseases, such as dental caries,^(9,10) and periodontal diseases,^(10,11) but also with other systemic diseases, such as type II diabetes,^(11,12) and obesity and it also increases with aging process.^(13,14) Importantly, studies have shown that salivary free radical-scavenging activity was affected by physical and mental activities.⁽¹⁵⁾ Furthermore, some studies suggest that saliva may reflect the current physiological condition of the body.^(16,17)

The maintenance of a prooxidant-antioxidant balance is important to keep healthy conditions to physiological activities, and there are reports that physical exercise leads to positive responses to this balance.^(7,18,19) Regular exercise training has been demonstrated to improve antioxidant defense,^(18,19) and to increase nitrite concentrations.⁽²⁰⁾ Acute exercise results in an increased production of ROS,⁽²¹⁾ however, chronic exercise promotes an adaptation to the antioxidant defense system, leading to adequate responses to the higher ROS generation.^(19,22) Moderate intensity aerobic training promotes mechanisms for this adaptation, such as an increased production of endothelium vasoactive substances.⁽²³⁾ Further, the combination of aerobic with resistance exercise has been demonstrated to effectively diminish ROS production, and enhance the enzymatic antioxidant defense.⁽²⁴⁾

Associated with physical exercise, postmenopausal women often use alternative treatments to reduce climacteric symptoms, mainly supplements structurally similar to estrogen, such as isoflavones, a phytoestrogen derived from soy which can change the oxidative balance.⁽²⁵⁾ Studies have controversial data regarding oxidative stress markers and isoflavone supplementation (ISO). In some studies, there was a decrease in ROS production, and an increase in SOD in postmenopausal women,^(26,27) other showed that genistein, a soy isoflavone, or the soy isoflavone from hypocotyl could decrease oxidative stress via lowering lipid peroxidation, and improving antioxidant enzymes.^(28,29) Besides, other studies find that a high isoflavone consumption, as well as exercise, is effective in increasing nitrite concentrations and improving endothelial function.^(30,31) However, there are also studies that did not report any effect in oxidative markers with ISO.⁽³²⁾

Therefore, the objective of this study was to verify if there is an additional effect of ISO associated with combined aerobic and resistance exercise training, in salivary oxidative stress markers in postmenopausal women. Our hypothesis was that ISO together

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ANEXO 3 - COMPROVANTE DE PUBLICAÇÃO DO ESTUDO 3



antioxidants



Article

Redox Status of Postmenopausal Women with Single or Multiple Cardiometabolic Diseases Has a Similar Response to Mat Pilates Training

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Citation: Amaral, A.L.; Batista, J.P.; Mariano, I.M.; Gonçalves, L.F.; Tavares, J.B.; de Souza, A.V.; Caixeta, D.C.; Teixeira, R.R.; de Oliveira, E.P.; Espindola, F.S.; et al. Redox Status of Postmenopausal Women with Single or Multiple Cardiometabolic Diseases Has a Similar Response to Mat Pilates Training. *Antioxidants* **2022**, *11*, 1445. <https://doi.org/10.3390/antiox11081445>

Academic Editors: Tinna Traustadóttir and David Marcinek

Received: 12 May 2022

Accepted: 12 July 2022

Published: 26 July 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Postmenopausal women have a high prevalence of cardiometabolic diseases and that may associate with higher oxidative stress. Exercise can contribute to the treatment of such diseases, but some modalities, such as Mat Pilates, need to be further studied in terms of their physiological responses. Our aim was to investigate the effects of 12 weeks of Mat Pilates on redox status in postmenopausal women with one or multiple comorbidities of cardiometabolic diseases. Forty-four postmenopausal women were divided into two groups: SINGLE, composed of women with one cardiometabolic disease ($n = 20$) and MULT, with multimorbidity ($n = 24$). Mat Pilates training was conducted three times a week for 12 weeks, and each session lasted 50 min. Plasma samples were collected before and after training to analyze the following redox markers: superoxide dismutase, catalase, glutathione peroxidase, total antioxidant capacity due to ferric-reducing antioxidant power (FRAP), reduced glutathione (GSH), uric acid, and carbonyl protein. ANCOVA showed interaction effects in FRAP ($p = 0.014$). Both groups had reduced levels of catalase ($p = 0.240$) and GSH ($p = 0.309$), and increased levels of carbonyl protein ($p = 0.053$) after intervention. In conclusion, the redox status of postmenopausal women shows no changes mediated by Mat Pilates training between SINGLE and MULT, except for greater reductions of FRAP in SINGLE.

Keywords: climacteric; exercise; endogenous antioxidants; Pilates training; oxidative stress balance

ANEXO 4 - COMPROVANTE DE PUBLICAÇÃO DO ESTUDO 4

Motriz, Rio Claro, v. 28, n. Especial 1, 2022, e10220012321

DOI: <http://dx.doi.org/10.1590/S1980-657420220012321>

Exercise Physiology

Effects of combined exercise on salivary oxidative stress in hypertensive and normotensive postmenopausal women

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Abstract - Aim: This study evaluated the effects of 10 weeks of combined exercise training on the salivary oxidative profile of hypertensive and normotensive postmenopausal women. **Methods:** Twenty-six non-obese postmenopausal women were divided into two groups: the hypertensive group (HT; n = 13; 58.9 ± 3.9 years; and BMI of 27.7 ± 4.6 kg/m²) or the normotensive group (NT; n = 13; 52.7 ± 5.2 years; and BMI of 26.9 ± 2.9 kg/m²). They performed 30 sessions of combined exercises over 10 weeks: 45 min per session, three times a week. Resting saliva samples were collected after an overnight fast to evaluate salivary nitrite levels and oxidative stress markers before and after training. **Results:** Two-way ANOVA showed that there was no difference in the responses over time between the hypertensive and normotensive groups in catalase, superoxide dismutase salivary activity, total antioxidant capacity, or lipid peroxidation. However, superoxide dismutase activity (Δ HT -0.87 ± 14.53 SOD/mg protein; Δ NT: 7.13 ± 9.39 SOD/mg protein; p < 0.01) and nitrite levels (Δ HT 10.32 ± 60.83 mM; Δ NT 101.92 ± 149.57 mM; p = 0.03) were higher overall in the hypertensive group compared to the normotensive group. Moreover, salivary nitrite levels increased over time (p = 0.04) in both groups. **Conclusion:** 10 weeks of combined exercise training did not change salivary oxidative stress markers in either normotensive or hypertensive postmenopausal women, although, after exercise training, nitrite levels increased in both groups, even with higher baseline salivary nitrite levels in hypertensive women. Thus, recurrent exercise seems to be a safe strategy after menopause from the standpoint of oxidative stress, regardless of the presence of hypertension.

Keywords: superoxide dismutase, nitrite, saliva, climacteric, combined exercise.

ANEXO 5 - QUESTIONÁRIOS UTILIZADOS

International Physical Activity Questionnaire (IPAQ)

ID: _____

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

- Atividades **VIGOROSAS**: precisam de grande esforço e fazem respirar MUITO mais forte que o normal.
- Atividades **MODERADAS**: precisam de algum esforço e fazem respirar UM POUCO mais forte que o normal.

1A) Em quantos dias da última semana você caminhou por pelo menos 10 minutos contínuos em casa ou no trabalho, como forma de transporte para ir de um lugar para outro, por lazer, por prazer ou como forma de exercício? Dias _____ por **SEMANA** () Nenhum

1B) Nos dias em que você caminhou por pelo menos 10 minutos contínuos quanto tempo no total você gastou caminhando **por dia**? Horas: _____ Minutos: _____

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

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

Horas: _____ Minutos: _____

3A) Em quantos dias da última semana, você realizou atividades **VIGOROSAS** por pelo menos 10 minutos contínuos, como por exemplo correr, fazer ginástica aeróbica, jogar futebol, pedalar rápido na bicicleta, jogar basquete, fazer serviços domésticos pesados em casa, no quintal ou cavoucar no jardim, carregar pesos elevados ou qualquer atividade que fez aumentar **MUITO** sua respiração ou batimentos do coração. Dias _____ por **SEMANA** () Nenhum

3B) Nos dias em que você fez essas atividades vigorosas por pelo menos 10 minutos contínuos quanto tempo no total você gastou fazendo essas atividades por dia?

Horas: _____ Minutos: _____

As últimas questões são sobre o tempo que você permanece sentado no trabalho, na escola ou faculdade, em casa e durante seu tempo livre. Isto inclui o tempo sentado estudando, sentado enquanto descansa, visitando um amigo, lendo, sentado ou deitado assistindo TV. **Não inclua o tempo gasto sentando durante o transporte** em ônibus, trem, metrô ou carro.

4A) Quanto tempo no total você gasta sentado em um dia de semana? _____ horas ____ minutos

4B) Quanto tempo no total você gasta sentado em um dia de fim de semana?

_____ horas ____ minutos

Avaliador: _____

Data: ____ / ____ / ____

Anamnese e Antropometria

ID: _____

Data de nascimento: ____/____/____

Idade: _____

Hipertensão: Sim () Não()

Fumante: Sim() Não()

Menopausa: Sim() Não()

Anos na menopausa: _____

Uso de TH: Sim () Não()

Anos de TH: _____

Nível de Escolaridade: ()sem estudos ()primário ()secundário ()universitário

Patologias: _____

Alergias ou intolerância alimentar: _____

Medicamentos: _____

Horário que toma medicamento: _____

Suplementos alimentares: _____

Patologias gastrointestinais: _____

Histórico de DCV: _____

Possuí acompanhamento médico: Sim () Não() _____

Avaliação da saúde bucal: _____

Exercício regular: Sim () Não() Qual: _____

MC (Kg): _____ Estatura (m): _____ IMC (Kg/m²): _____ C. Abd (cm): _____

OBS.:

Avaliador: _____ Data: ____/____/____