PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
DOUTORADO EM FISIOPATOLOGIA E SAÚDE ANIMAL

LETICIA ESTEVAM ENGEL

EFEITOS DE DIFERENTES MODALIDADES DE EXERCÍCIOS E INTERVENÇÃO NUTRICIONAL SOBRE BIOMARCADORES DE ESTRESSE OXIDATIVO E ASPECTOS HISTOPATOLÓGICOS MUSCULARES: EVIDÊNCIAS DE ESTUDOS PRÉ-CLÍNICOS

Presidente Prudente – SP
2025

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Prof.ª Drª. Francis Lopes Pacagnelli

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AUTOR(A): LETICIA ESTEVAM ENGEL

ORIENTADOR(A): Profa. Dra. FRANCIS LOPES PACAGNELLI

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Área de Concentração FISIOPATOLOGIA ANIMAL, pela Comissão Examinadora:

Profa. Dra. FRANCIS LOPES PACAGNELLI
UNOESTE - Universidade do Oeste Paulista / Presidente Prudente (SP)

Profa. Dra. SABRINA ALVES LENQUISTE
UNOESTE - Universidade do Oeste Paulista / Presidente Prudente (SP)

Profa. Dra. INES CRISTINA GIOMETTI CEDA
UNOESTE - Universidade do Oeste Paulista / Presidente Prudente (SP)

Profa. Dra. MARIANA JANINI GOMES
Universidade de Texas A&M University

Prof. Dr. ROBSON CHACON CASTOLDI
UNOPAR - Universidade Norte do Paraná / Londrina (PR)

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SABRINA ALVES LENQUISTE (PROFESSOR)
sabrina.alves@unoeste.br
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FRANCIS LOPES PACAGNELLI (PROFESSOR)
francispacagnelli@unoeste.br
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Assinatura Interna UNOESTE
Usando endereço IP: 177.131.39.1
ID da assinatura: 4749431

INES CRISTINA GIOMETTI CEDA (PROFESSOR)
inesgiometti@unoeste.br
Recebido em 25/04/2025 14:47
Assinado em 28/04/2025 08:21
Assinatura Interna UNOESTE
Usando endereço IP: 177.131.39.1
ID da assinatura: 4749433

ROBSON CHACON CASTOLDI (SIGNATÁRIO EXTERNO)
castoldi_rc@yahoo.com.br
Recebido em 25/04/2025 14:47
Assinado em 25/04/2025 17:51
Assinatura Interna UNOESTE
Usando endereço IP: 2804:14d:5283:8e6c:cca6:c1ed:8363:2339
ID da assinatura: 4749435

MARIANA JANINI GOMES (SIGNATÁRIO EXTERNO)
m.janinigomes@tamu.edu
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Assinado em 29/04/2025 11:03
Assinatura Interna UNOESTE
Usando endereço IP: 47.218.34.150
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DEDICATÓRIA

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O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca e vence obstáculos, no mínimo fará coisas admiráveis." (José de Alencar)

RESUMO

Efeitos de diferentes modalidades de exercícios e intervenção nutricional sobre biomarcadores de estresse oxidativo e aspectos histopatológicos musculares: evidências de estudos pré-clínicos na obesidade e isquemia e reperfusão

Dietas ricas em gordura (HFD) e estilos de vida sedentários estão diretamente associados à obesidade que leva a disfunção do músculo esquelético e ao aumento do risco de doenças cardiovasculares. Por outro lado, a inclusão de alimentos funcionais, como o óleo de chia, reconhecido por suas propriedades anti-inflamatórias e antioxidantes, pode atenuar esses efeitos negativos. Paralelamente, a prática regular de exercício físico exerce influência significativa sobre os níveis de estresse oxidativo, promovendo benefícios musculares e efeitos cardioprotetores. O objetivo dessa tese foi desenvolver duas pesquisas 1)avaliar os efeitos do exercício físico aeróbico isoladamente ou em combinação com a suplementação de óleo de chia, na modulação do estresse oxidativo e parâmetros histopatológicos no músculo esquelético em modelos experimentais de obesidade e 2) performar uma revisão sistemática com metanálise para avaliar os efeitos do exercício intervalado de alta intensidade (HIIT) em animais com lesão por isquemia-reperfusão em relação ao estresse oxidativo cardíaco. Na primeira pesquisa, 35 ratos Wistar machos de 60 dias foram aleatoriamente divididos em cinco grupos: (1) controle (C), alimentados com uma dieta padrão de ração; (2) HFD, alimentado com uma dieta rica em gordura ad libitum; (3) HFD+Ex, alimentado com HFD e em natação, três vezes por semana; 30minutos (4) HFD+CO, recebendo HFD com suplementação de óleo de chia via gavagem na dose de 1 mL/dia, três vezes por semana e (5) HFD+Ex+CO, alimentados com HFD, em fase de natação e com suplementação de óleo de chia. Após 8 semanas, os ratos foram eutanasiados e os músculos sóleos foram coletados, pesados e processados para análises histopatológicas, morfométricas e de estresse oxidativo. A suplementação com óleo de chia reduziu o acúmulo de colágeno e carbonilação de proteínas, enquanto o exercício aeróbico sozinho melhorou a deposição de colágeno, mas aumentou a carbonilação de proteínas. A

combinação de ambas as intervenções reduziu efetivamente o ganho de peso e reservas adiposas. Além disso, no segundo artigo, uma meta-análise examinou os efeitos do HIIT nos marcadores de estresse oxidativo na lesão de isquemia-reperfusão (IR), uma condição caracterizada pela interrupção transitória do fluxo sanguíneo seguida de reperfusão, levando a danos celulares. Seis estudos com 88 ratos foram analisados, revelando que o HIIT aumentou significativamente os níveis de enzimas antioxidantes, como superóxido dismutase e catalase. No entanto, não foram observadas diferenças significativas nos níveis de glutationa peroxidase, glutationa reduzida, malondialdeído e mieloperoxidase. Embora o HIIT pareça benéfico no aumento das defesas antioxidantes, mais estudos de alta qualidade são necessários para confirmar esses achados. Em conclusão, tanto o treinamento aeróbico combinado com óleo de chia quanto o HIIT demonstram benefícios potenciais na melhora dos parâmetros musculares e cardíacos relacionados ao estresse oxidativo. Esses achados destacam a importância da suplementação nutrição funcional e programas de exercícios estruturados para combater distúrbios metabólicos e disfunção muscular esquelética e cardíaca.

Palavras-chave: Dieta hiperlipídica, estresse oxidativo, alimentação funcional, exercício aeróbico, HIIT, lesão de isquemia-reperfusão.

ABSTRACT

Effects of different exercise modalities and nutritional intervention on oxidative stress biomarkers and muscle histopathological aspects: evidence from preclinical studies in obesity and ischemia and reperfusion

High-fat diets (HFD) and sedentary lifestyles are directly associated with obesity, which leads to skeletal muscle dysfunction and increased risk of cardiovascular disease. On the other hand, the inclusion of functional foods, such as chia oil, recognized for its anti-inflammatory and antioxidant properties, can mitigate these negative effects. In parallel, regular physical exercise significantly influences oxidative stress levels, promoting muscle benefits and cardioprotective effects. The objective of this thesis was to develop two studies: 1) to evaluate the effects of aerobic physical exercise alone or in combination with chia oil supplementation on the modulation of oxidative stress and histopathological parameters in skeletal muscle in experimental models of obesity; and 2) to perform a systematic review with meta-analysis to evaluate the effects of high-intensity interval training (HIIT) in animals with ischemia-reperfusion injury in relation to cardiac oxidative stress. In the first study, 35 60-day-old male Wistar rats were randomly divided into five groups: (1) control (C), fed a standard chow diet; (2) HFD, fed a high-fat diet ad libitum; (3) HFD+Ex, fed HFD and swimming three times a week; (4) HFD+CO, receiving HFD with chia oil supplementation via gavage at a dose of 1 mL/day, three times a week; and (5) HFD+Ex+CO, fed HFD, swimming and supplemented with chia oil. After 8 weeks, the rats were euthanized and the soleus muscles were collected, weighed and processed for histopathological, morphometric and oxidative stress analyses. Chia oil supplementation reduced collagen accumulation and protein carbonylation, while aerobic exercise alone improved collagen deposition but increased protein carbonylation. The combination of both interventions effectively reduced weight gain and adipose stores. Furthermore, in the second article, a meta-analysis examined the effects of HIIT on markers of oxidative stress in ischemia-reperfusion (IR) injury, a condition characterized by transient interruption of blood flow followed by reperfusion, leading to cellular damage. Six studies with 88 rats were analyzed, revealing that HIIT significantly

increased the levels of antioxidant enzymes, such as superoxide dismutase and catalase. However, no significant differences were observed in the levels of glutathione peroxidase, reduced glutathione, malondialdehyde, and myeloperoxidase. Although HIIT appears beneficial in increasing antioxidant defenses, more high-quality studies are needed to confirm these findings. In conclusion, both aerobic training combined with chia oil and HIIT demonstrate potential benefits in improving muscle and cardiac parameters related to oxidative stress. These findings highlight the importance of functional nutrition supplementation and structured exercise programs to combat metabolic disorders and skeletal and cardiac muscle dysfunction.

Keywords: High-fat diet, oxidative stress, functional feeding, aerobic exercise, HIIT, ischemia-reperfusion injury.

LISTA DE ABREVIATURAS

µm²	- Square micrometer
ANOVA	- Analysis of variance
AU-	arbitrary unit
LVDD	- Diastolic diameter of the left venticulus
LVSD	- Left ventricular systolic diameter
g	- Grass
h	- Time
mg/kg	- Milligram per kilogram
min	- Minute
mmol/L	- Millimol per liter
ms	- Millisecond
VO2max	- Maximum body oxygen consumption
WKY	- Ratos Wistar-Kyoto
PUFAs	- supplementation of polyunsaturated fatty acids
HFD	- high-fat diet
CT	- control group
Ex	- physical exercise
CO	- chia oil
MDA	- malondialdehyde
HE	- Hematoxylin-Eosin
VVG	- Verhoeff Van Gienson
ECM	- extracellular matrix
PSR	- Picrosirius Red
GPx	- glutathione peroxidase
SOD	- superoxide dismutase
Nrf2	- nuclear factor 2 erythroid 2
MI	- myocardial infarction
CVD	- Cardiovascular diseases
ALA	- alpha-linolenic acid
TBA	- Thiobarbituric acid
ROS	- reactive oxygen species
HSPs	- heat shock proteins

SD	- standard deviation
IR	- ischemia-reperfusion
HIIT	- High Intensity Interval Training
HIIT+IR	- HIIT and were exposed to ischemia-reperfusion
CTRL+IR	- ischemia-reperfusion from sedentary rats
LAD	- Left anterior descending coronary artery
LDH	- lactate dehydrogenase
CK-MB	- creatine kinase-MB
SV	- stroke volume
EF	- ejection fraction
SVI	- Stroke Volume Index
CDI	- Cardiac output index
LVDD	- left ventricular diastolic diameter
LVSD	- left ventricular systolic diameter
HR	- heart rate
FC	- coronary flow
PVLS	- l eft ventricular systolic pressure
FS	- fractional shortening
EF	- left ventricular ejection fraction
CO	- cardiac output
H_2O_2	- hydrogen peroxide
NO−2NO2	- nitrite
NO	- nitric oxide
O−2O2	- superoxide anion radical
dp/dt max	- Maximum rate of pressure development in the left ventricle
dp/dt min	- Minimum rate of pressure development in the left ventricle

SUMÁRIO

1 ARTIGO ORIGINAL 1

CHIA OIL SUPPLEMENTATION ENHANCES AEROBIC EXERCISE REDOX BALANCE BENEFITS ON SKELETAL MUSCLE IN DIET- INDUCED OBESE RAT

Chia and exercise in skeletal muscle of obese rats

Letícia Estevam Engel[1], Camila Renata Correa[2], Natália Cervantes Uzeloto Guazi[1], Tiago Lyria da Silva Pazinato[1], Valentina Trombini da Silva[1], Giovana Rampazzo Teixeira[3], Sabrina Alves Lanquiste[1], Rayana Loch Gomes[4], Luiz Carlos Marques Vanderlei[5], Ivan J Vechetti[6], Francis Lopes Pacagnelli[1]

[1] University of Western São Paulo, UNOESTE, Presidente Prudente, Brazil.

[2]Medical School, São Paulo State University (UNESP), Botucatu, Brazil.

[3]Department of Physical Education, School of Technology and Sciences, São Paulo State University (UNESP), Presidente Prudente, Brazil.

[4]Facult of Health Sciences, Federal University of Grande Dourados, MS, Brazil.

[5]Department of Physiotherapy, School of Technology and Sciences, São Paulo State University (UNESP), Presidente Prudente, Brazil.

[6] University of Nebraska-Lincoln, Department of Nutrition and Health Sciences, Lincoln, NE 68583, USA

Corresponding author:

Francis Lopes Pacagnelli, Universidade do Oeste Paulista -Unoeste, Rodovia Raposo Tavares, km 572, Bairro Limoeiro, Presidente Prudente – SP - Brazil. CEP: 19.067-175.

Phone: 55 (18) 3229-2000. e-mail: francispacagnelli@unoeste.br

Summary

The high-fat diet (HFD) and sedentary lifestyle contribute to increased lipid accumulation and can negatively impact skeletal muscle function. Chia seed oil, known for its high nutritional value, anti-inflammatory, and antioxidant properties, has the potential to alleviate metabolic disorders. The combination of aerobic physical exercise promotes benefits such as improved MEC remodeling, and chia oil supplementation can be more effective and reduce the skeletal muscle dysfunction. The purpose of this study was to examine the effects of chia oil supplementation and aerobic training on skeletal muscle of obese rats those fed with HFD. Thirty-five 60 days male Wistar rats were randomly assigned to five groups: (1) control (C), fed a standard chow diet; (2) HFD, fed an ad libitum high-fat diet; (3) HFD+Ex, fed a HFD and undergoing swimming, three times a week; 30minutos (4) HFD+CO, receiving HFD with chia oil supplementation via gavage at a dose of 1 mL/day, three times per week and (5) HFD+Ex+CO, fed an HFD, undergoing swimming and with chia oil supplementation. Following 8 weeks, rats were euthanized and the soleus muscles were collected, weighed, and processed for histopathology, morphometric, and oxidative stress analyses. Our results demonstrated that HFD increased final body weight, adipose reserves, collagen deposition, and carbonyl in soleus muscle. Chia oil supplementation reduced collagen accumulation and carbonyl, while exercise alone decreased collagen accumulation and increased carbonyl. In conclusion, both interventions improved collagen deposition, with chia oil showing additional benefits in mitigating carbonyl. The combination of aerobic exercise and chia oil supplementation significantly reduced adipose reserves. The combination of interventions produces clear synergistic effects body weight and adipose tissue deposits epididymal, mesenteric and retroperitoneal, both strategies have significant benefits when isolated and associated with.

Keywords: Physical exercise, diet, functional food, obesity

1| INTRODUCTION

Obesity is currently one of the world's biggest public health crises, with epidemiological data pointing to a growing prevalence in all age groups, driven by high-fat diets and low levels of physical activity[1,2] This condition is directly associated with the emergence of metabolic diseases such as type 2 diabetes, cardiovascular diseases, and muscle dysfunctions, which impact quality of life and increase mortality. [3-6] Additionally, previous studies demonstrated that HFD and obesity promote adaptations in skeletal muscle with dysfunction of extracellular matrix remodeling (ECM)[7-11]. Adaptations of the ECM regarding modifications of collagen types I and III that are implicated in the decrease in muscle strength, transmission of contraction force, which is directly related to functional and quality of life worsening, and is also a predictor of mortality[11-14]. These changes are accompanied by an increase in reactive oxygen species (ROS) and an impairment on redox state balance[15].

ROS play a fundamental role in muscle redox signaling and are essential for the preservation of cellular functions[16]. However, when present at high levels, they can cause cell damage by alter the protective antioxidant[16]. Therefore, maintaining redox homeostasis is crucial to ensure that ROS-mediated signaling occurs without compromising cellular integrity. To achieve this redox state balance, cells carefully regulate both the generation and removal of these ROS[16]. Metabolic conditions such as obesity cause dysregulation of the redox state and impacts on skeletal muscle[17-18]. These impacts included diminished muscular function, quality, and mass and increases the incidence risk of several metabolic comorbidities[17-18].

Aerobic exercise has been long recommended to obesity by promotes muscle benefits as improved MEC remodeling, metabolic profiles and attenuating oxidative stress[19-25]. In addition to exercise, the use of natural compounds present in foods has been widely investigated as a therapeutic strategy for the treatment of skeletal muscle dysfunction associated with HFD consumption. These nutraceuticals can provide a practical approach for the prevention and treatment of muscular dysfunction[26].

Chia seed oil (Salvia hispanica L.) has attracted a great deal of scientific attention due to its unique nutritional composition, being one of the main plant sources of polyunsaturated fatty acids, especially α-linolenic acid, a precursor to omega-3 fatty

acids. [25,26] In addition, chia contains fiber, proteins of high biological value, vitamins, minerals, and bioactive compounds, such as polyphenols and flavonoids, which confer antioxidant and anti-inflammatory properties.[26,27] Evidence suggests that these components may contribute to the improvement of the lipid profile, enhancing blood glucose, modulation of inflammation, and the regulation of energy metabolism, playing a relevant role in the prevention and management of obesity. [28-34]

Despite the various existing therapeutic strategies, there is still an important gap in the understanding of combined approaches that associate specific nutritional interventions with physical exercise programs to restore redox homeostasis and structural integrity of skeletal muscle in obese individuals. Therefore, the present research proposes to investigate the effects of supplementation with chia oil, rich in antioxidant compounds, associated with aerobic exercise on redox balance and muscle histomorphology in a preclinical model of diet-induced obesity. This innovative approach seeks to offer new insights into affordable and sustainable alternatives to mitigate the deleterious effects of obesity, contributing not only to the improvement of muscle function, but also to the advancement of strategies to promote public health and reduce the socioeconomic burden related to obesity.

The study contributes to the advancement of knowledge regarding nutritional and exercise strategies to mitigate the metabolic effects of obesity and is in line with United Nations Sustainable Development Goals (SDGs): SDG 3 (Good Health and Well-Being): the study seeks alternatives to improve metabolic health and reduce the impact of obesity, contributing to overall well-being; SDG 2 (Zero Hunger and Sustainable Agriculture): the research highlights the role of chia as a functional food, promoting a more balanced and sustainable diet, and SDG 9 (Industry, Innovation, and Infrastructure): the research fosters innovation in the field of nutrition and health by exploring new strategies to combat metabolic diseases. Thus, the research aimed to evaluate whether the combination of aerobic exercise and chia oil could reduce the negative impacts of obesity on skeletal muscle. The analysis included assessments of muscle histopathology, morphometric, and oxidative stress analyses. By addressing these parameters, the research introduces a novel approach to mitigate the detrimental effects of obesity on skeletal muscle.

2 | MATERIAL AND METHODS

2.1 | Ethical approval

All experimental procedures involving animals were conducted in accordance with the ethical principles for animal research adopted by the Brazilian College of Animal Experimentation (COBEA). The study protocol was approved by the Ethics Committee on Animal Use (CEUA) of the University of Western São Paulo (Unoeste), Presidente Prudente (protocol number 3962).

2.2 | Experimental design

Thirty-five adults male Wistar rats (60 days old) were individually housed and maintained at a temperature of 22 ±1°C, 60–70% humidity. The chia oil and exercise groups began oil supplementation and the exercise protocol lasted eight weeks (Fig. 1). All procedures involving animals were performed between 19:00 and 23:00.

The animals were randomized into five groups (n=7). (1) control (C), fed a standard chow diet; (2) HFD, fed an ad libitum high-fat diet; (3) HFD+Ex, fed a HFD and undergoing swimming, three times a week; 30minutos (4) HFD+CO, receiving HFD with chia oil supplementation via gavage at a dose of 1 mL/day, three times per week and (5) HFD+Ex+CO, fed an HFD, undergoing swimming and with chia oil supplementation.

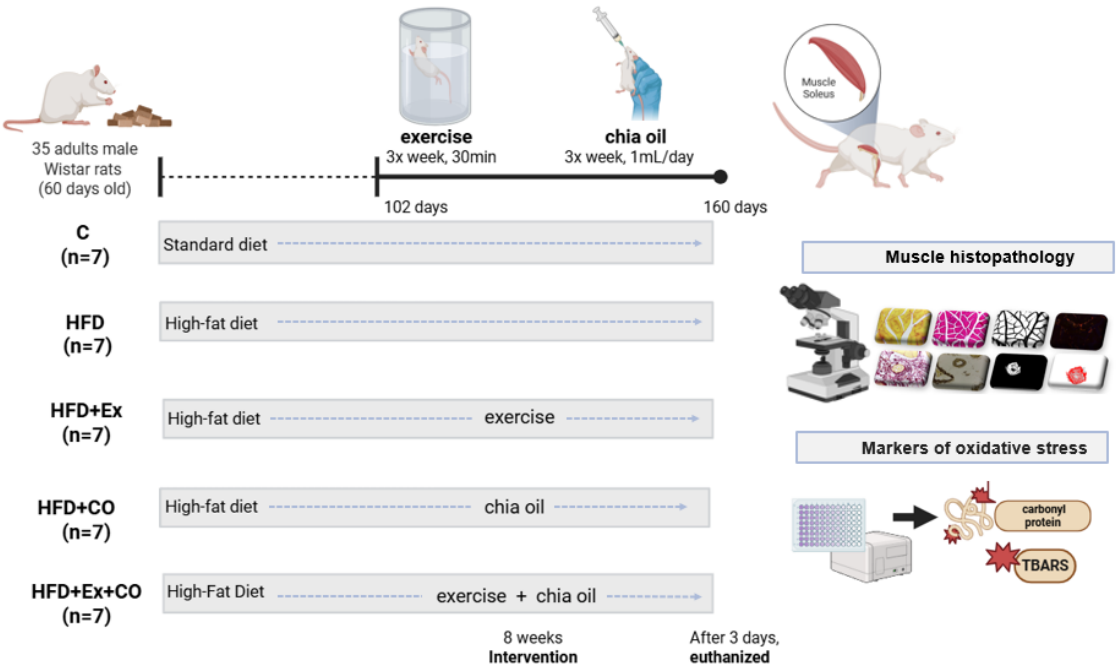

FIGURE 1. Schematic figure summarizing the experimental design. C: control group, HFD: high-fat diet group, HFD+Ex: high-fat diet and aerobic exercise group, HFD+CO: high-fat diet and chia oil supplementation group, HFD+CO+Ex: high-fat diet, chia oil, and exercise group.

2.3 | High-fat diet

At 60 days old, the rats were maintained on a standard diet (commercial Supralab) or started the induction period with a high-fat diet (HFD). The HFD was prepared weekly and care with storage was essential such as, dark packaging, used in this study consisted of a hypercaloric mixture (normoproteic and HFD) containing ground and mixed commercial Supralab feed, roasted peanuts, milk chocolate, and biscuit (sweet without filling) in a 3:2:2:2 ratio[21,31]. The HFD was composed of 59% lipids, 28% carbohydrates, and 13% proteins. The centesimal composition of the experimental diets was evaluated according to analytical methods recommended by the Association of Official Analytical Chemists. The commercial diet consisted of 24.11% proteins, 4.27% lipids, and 52.20% carbohydrates, providing 3.4 kcal/g, while the high-fat diet consisted of 18.84% proteins, 23.80% lipids, and 50.4% carbohydrates, providing 4.9 kcal/g. [31]

2.4 | Exercise protocol

The groups (HFD+Ex) and (HFD+Ex+CO) underwent aerobic exercise through swimming in a 25 cm diameter polyvinyl chloride (PVC) tube specifically designed for this type of exercise protocol. The tube contained 38 cm of heated water (30°C), allowing each animal to train individually.

The exercise sessions were conducted three times per week (Mondays, Wednesdays, and Fridays) for 30 minutes. The intensity used was 70% of the threshold, determined by the maximum lactate steady state in rats, which corresponds to 5% of the animal's body weight.[32] Adjustments were made after the first 4 weeks of training to avoid adaptation. During the exercise, a custom-made pouch containing a weight equivalent to 70% of the threshold was attached to the posterior thoracic region of the animals.[33,34] After 3 days, with 48 hours of rest + 12 hours of fasting, at the end of the exercise protocol, the rats were euthanized, and soleus muscle was collected for analysis.

2.5 | Chia Oil Supplementation

The animals were supplemented with chia oil from RSBLUMOS (RSBLUMOS Comercial Produtos Alimentícios LTDA) via gavage at a dose of 1 mL/day, based on previous investigations by Marinelli et al. (2015).[27] This supplementation was conducted three times per week for 8 weeks. Animals in other groups received daily gavage with water as a placebo to ensure all animals experienced the same stress. The chia oil was composed of 0.11 g/day of saturated fatty acids, 0.7 g/day of monounsaturated fatty acids, and 58.4 mg/day of linoleic acid and 170.84 mg/day of linolenic acid from polyunsaturated fatty acids, with a caloric value of 9 kcal/g.[21]

2.6 | Material Collection

At the end of the experimental period, the animals were anesthetized with intraperitoneal ketamine (60 mg/kg) and xylazine hydrochloride (1 mg/kg) and euthanized after 3 days of the last aerobic exercise session, by exsanguination. Adipose tissue deposits were dissected: epididymal: located in the inguinal region, adjacent to the testicles; mesenteric: located in the mesentery, which connects the small intestine to the abdominal wall; and retroperitoneal: located in the retroperitoneal region, adjacent to the kidneys; The soleus muscles were dissected, weighed, with the upper portion preserved in buffered formalin for subsequent histological analysis, and the lower portion frozen in liquid nitrogen. The tibia was removed and measured as a normalization parameter.

2.7 | Body weight and nutritional analyses

Body weight was also measured; to evaluate weight gain, we calculated body mass gain (Δ = final weight—starting weight). Relative fat (%) was calculated for each adipose tissue deposit using the following formula: Relative Fat (%) = (Fat Storage Weight (g) / Total Body Weight (g)) x 100. During the experimental period, weekly consumption of food (g/day) and changes in rat body mass were monitored. [21]

2.8 | Histology Muscle

2.8.1 | Muscle transverse sectional areas

Samples of the soleus muscles were fixed in 10% buffered formalin for 48 hours. After fixation, the tissue was embedded in paraffin blocks to obtain two 4-micrometer coronal histological sections per animal. The histological sections were stained with Hematoxylin-Eosin (HE) for evaluation of the transverse sectional areas using a LEICA microscope (model DM750, Germany) equipped with a video camera that transmitted digital images to a computer with the Image Pro-Plus software (Media Cybernetics, Silver Spring, Maryland, USA). All images were captured at analyzed using a 40X objective with 400x magnification. Image selection for capture and digitization was performed visually. The mean sectional areas obtained for each group were used as an indicator of cell size.[35-37]

2.8.2 | Collagen Analysis

Initially, a small number of slides were tested due to differences in staining time among different fixatives and tissues. Before staining, the sections were deparaffinized using xylene, alcohol, and distilled water. The slides were stained with Picrosirius Red (PSR) for 30 minutes, rinsed with alcohol, and dehydrated. Images were obtained using a Leica DMLB optical microscope (Germany) equipped with a camera (Leica DFC300 FX, Germany) and the Leica Qwin Plus-Leica Qwin Colour software (GB). Three fields were selected, and images were captured in a standardized manner at 400x magnification. Collagen quantification was performed using the same software, which generated a histogram of color intensity frequencies (red for type I collagen and green for type III collagen) and detected only the regions stained with vibrant red. Three fields of each slide were analyzed. The results were expressed as percentages, representing the proportion of collagen fibers relative to the respective field. [38-41]

2.8.3 | Fractal Dimension

For fractal dimension analysis, slides stained with HE and PSR were binarized for reading, and the fractal dimension was estimated using the box-counting method with ImageJ software (National Institutes of Health, USA). The software considers two-dimensional box-counting, allowing quantification of pixel distribution in this space, without accounting for image texture. Thus, fractal dimension analysis is based on the relationship between resolution and the evaluated scale, quantitatively expressed as the fractal dimension of the object: $DF = (\text{Log } Nr/\text{Log } r^{-1})$, where Nr is the number of identical elements needed to fill the original object, and r is the applied scale. With this

method, fractal dimension values calculated using ImageJ always range between 0 and 2, without distinguishing different textures. [35]

2.9 | Histology Muscle Arterioles

2.9.1| Arteriolar Wall Thickness

Histological sections were stained with Verhoeff Van Gieson (VVG) solution, and muscle arterioles from each animal were analyzed using a 40X objective with 400x magnification using a LEICA DM750 optical microscope (Leica Microsystems, Germany) equipped with a video camera, which transmitted digital images to a computer with Image Pro-Plus software (Media Cybernetics, Silver Spring, Maryland, USA). Four measurements of each arteriole's thickness were taken, and the mean value was calculated using ImageJ software [National Institutes of Health (NIH), USA].[42]

2.9.2| Collagen in Arterioles

Initially, a small number of slides were tested due to differences in staining time among different fixatives and tissues. Before staining, sections were deparaffinized using xylene, alcohol, and distilled water. Slides were stained with Picrosirius Red (PSR) for 30 minutes, rinsed with alcohol, and dehydrated. Images were captured using a Leica DMLB optical microscope (Germany) with a camera (Leica DFC300 FX, Germany) and Leica Qwin Plus-Leica Qwin Colour software (GB). Three fields were selected, and images were captured in a standardized manner at 400x magnification. Collagen quantification was performed using the same software, which generated a histogram of color intensity frequencies (red for type I collagen and green for type III collagen) and detected only the regions stained with vibrant red. Results were expressed as percentages, representing the proportion of collagen fibers related to the field. Analysis of collagen types was performed using polarized light microscopy. [38-41]

2.10| Markers of oxidative stress

2.10.1| Thiobarbituric acid (TBARS)

Malondialdehyde (MDA) levels were used to evaluate lipid peroxidation. Briefly, 250 µL of supernatant from hepatic and epididymal adipose tissues were used and added to 750 µL of 10% trichloroacetic acid for protein precipitation. The samples

were centrifuged (3000 rpm for 5 minutes; Eppendorf® Centrifuge 5804-R, Hamburg, Germany), and the supernatant was collected. Thiobarbituric acid (TBA) at a concentration of 0.67% (1:1) was added, and the samples were heated for 15 minutes at 100°C. After cooling, absorbance was read at 535 nm using the Spectra Max 190 microplate reader (Molecular Devices®, Sunnyvale, CA, USA). The MDA concentration was calculated using the molar extinction coefficient (1.56 x 10^5 $M^{-1}cm^{-1}$), and the results were expressed in nmol/g of protein.[43]

2.10.2| Carbonyl

For the quantification of protein carbonylation, 100 µL of tissue homogenate was mixed with 100 µL of 2,4-dinitrophenylhydrazine (DNPH, 10 mM in 2 M HCl). The samples were incubated for 10 minutes at room temperature, followed by the addition of 50 µL of sodium hydroxide (6 M NaOH), and incubated again for 10 minutes at room temperature. Absorbance was measured at 450 nm using the Spectramax 190 microplate reader (Molecular Devices®, Sunnyvale, CA, USA). Results were obtained using the molar extinction coefficient of DNPH (22,000 $M^{-1}cm^{-1}$) and expressed in nmol/g of protein. Protein carbonylation levels were reported in nmol of DNPH/mg of protein.[44]

2.11| Statistics Analysis

All results were analyzed for normality using the Shapiro-Wilk test. Variables with parametric distribution were subjected to analysis of variance (ANOVA) to compare the means of the four groups, followed by Dunn's post hoc test. Non-parametric variables were analyzed using the Kruskal-Wallis's test to compare means among groups. A significant level of 5% was adopted for all comparisons.

3 | RESULTS

3.1 | Body weight and nutritional analyses

The high-fat diet induced weight gain, increased total, relative fat and adipose reserves when compared to group C. Chia oil supplementation and exercise decreased weight gain. Associated HFD+Ex+CO interventions decreased final weight, weight gain, total fat, and adipose reserves compared to the HFD group (Table 1). Retroperitoneal adipose tissue, also decreased with HFD+Ex (Table 1). As expected, food consumption was lower in the groups that consumed HFD than in the control

group. The absolute weight of the soleus and the ratio of soleus to tibial length did not show significant differences between the groups (Table 1).

TABLE 1. Animal morphometric

Variables	Groups				
	C (n=7)	**HFD (n=7)**	**HFD+Ex (n=7)**	**HFD+CO (n=7)**	**HFD+Ex+CO (n=7)**
Initial body weight (g)	208.6 ±6.23	199.30 ±9.56	193.40 ±8.31	194.70 ± 6.39	198.3 ± 5.86
Final body weight (g)	408.1 ±17.90	458.1±38.88	393.8±23.40	375.8±33.48 [b]	371.7±27.40 [b]
Weight gain (g)delta	199.5±28.14	258.8±45.04 [a]	200.4±11.82[b]	181.1±28.20 [b]	173.5±32.44[b]
Total fat (g)	17.87 ±0.82	28.41± 1.74[a]	22.26± 1.94	21.90± 2.15	19.40 ± 1.31[b]
Relative fat (%)	4.36 ± 0.18	6.24± 0.43[a]	5.64± 0.49	5.72 ± 0.43	5.23± 0.43
Epididimal adipose tissue (g)	5.88± 0.14	9.29± 0.75 [a]	7.45± 0.80	6.97±0.96	5.94± 0.47[b]
Mesenteric adipose tissue (g)	5.08± 0.33	7.38±0.41[a]	6.38± 0.46	6.08±0.46	5.30±0.28[b]
Retroperitoneal adipose tissue (g)	6.89± 0.62	11.73± 0.85[a]	8.42 ± 0.78 [b]	8.62± 0.81	8.16± 0.69[b]
Food consumption (g/day)	29.04±0.70	17.07±0.3[a]	17.09±0.57 [a]	14.87±0.41[abc]	15.06±0.30[abc]
Soleus weight (g)	0.17 ± 0.005	0.17 ± 0.010	0.15 ± 0.008	0.16 ± 0.007	0.15 ± 0.002
Soleus/tibia length	4.32 ± 0.12	4.03 ± 0.31	3.53 ± 0.20	3.92± 0.22	3.86 ± 0.31

Data on initial and final body weight, weight gain, absolute and relative fats and soleus weight, relationship of soleus to tibia length chia oil intake, fed to high-fat diet for 14 weeks. Data are presented as the mean ± SEM (n= 7). The significance of $p< 0.05$ is indicated by lowercase letters indicating a difference between the groups. a Referring to the control group. b Referring to the high-fat diet group. c Referring to the high-fat diet group chia. C: control group, HFD: high-fat diet group, HFD+Ex: high-fat diet and aerobic exercise group, HFD+CO: high-fat diet and chia oil supplementation group, HFD+CO+Ex: high-fat diet, chia oil, and exercise group. The Two-Way ANOVA test was used to compare the means with the Tukey post-test

3.2 | Muscle transverse sectional areas

The mean transverse sectional areas obtained for each group were used as an indicator of cell size. No statistically significant differences were observed among the groups for soleus muscle area (p= 0.33). Similarly, no differences were found in the extracellular matrix and interstitial space, where collagen fibers, blood vessels, and other connective tissue components are present, and there is no irregularity in the interstitial space. (p= 0.10) (Fig. 2).

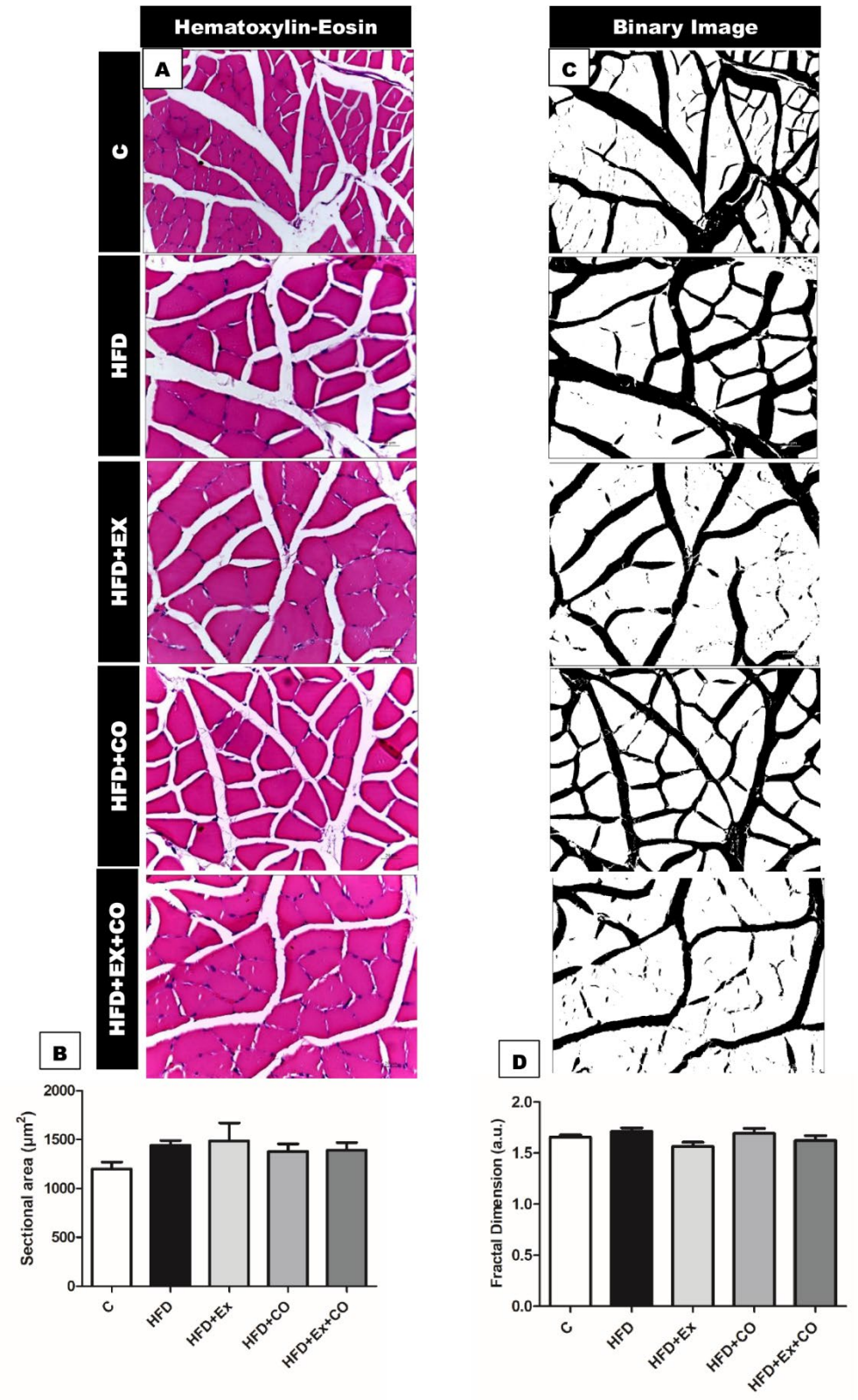

FIGURE 2. Histology analysis of the soleus muscle. Scale bar= 20 µm; Resolution with 40x objective and 400x magnification. A) Soleo stained in haematoxylin–eosin (HE). B) Quantitative analysis of sectional area of Soleus. C) Soleus fractal dimension in HE staining, after binarization. D) Quantitative analysis of fractal dimension. C: control group, HFD: high-

fat diet group, HFD+Ex: high-fat diet and aerobic exercise group, HFD+CO: high-fat diet and chia oil supplementation group, HFD+CO+Ex: high-fat diet, chia oil, and exercise group. The Kruskal-Wallis test was used for mean comparisons.

3.3 | Muscle Collagen

We analyzed collagen deposition in the soleus muscle, and the HFD diet group showed an increased percentage compared to the control group (C: 5.71 ± 1.14% vs. HFD: 9.48 ± 2.29%). However, aerobic exercise and supplemented chia oil was able to reduce collagen levels in the muscle than compared to the HFD group (HFD+Ex: 5.34 ± 0.71%; HFD+CO: 5.69 ± 0.91%) (Fig. 3).

Regarding the fractal dimension, the tissue organization of collagen was not modified by diet and interventions. No differences were observed in the fractal dimension analysis of the Picrosirius-stained slides (p= 0.17). Similarly, no differences were found in type I collagen (p= 0.06) or type III collagen (p= 0.39) among the groups (Fig. 3).

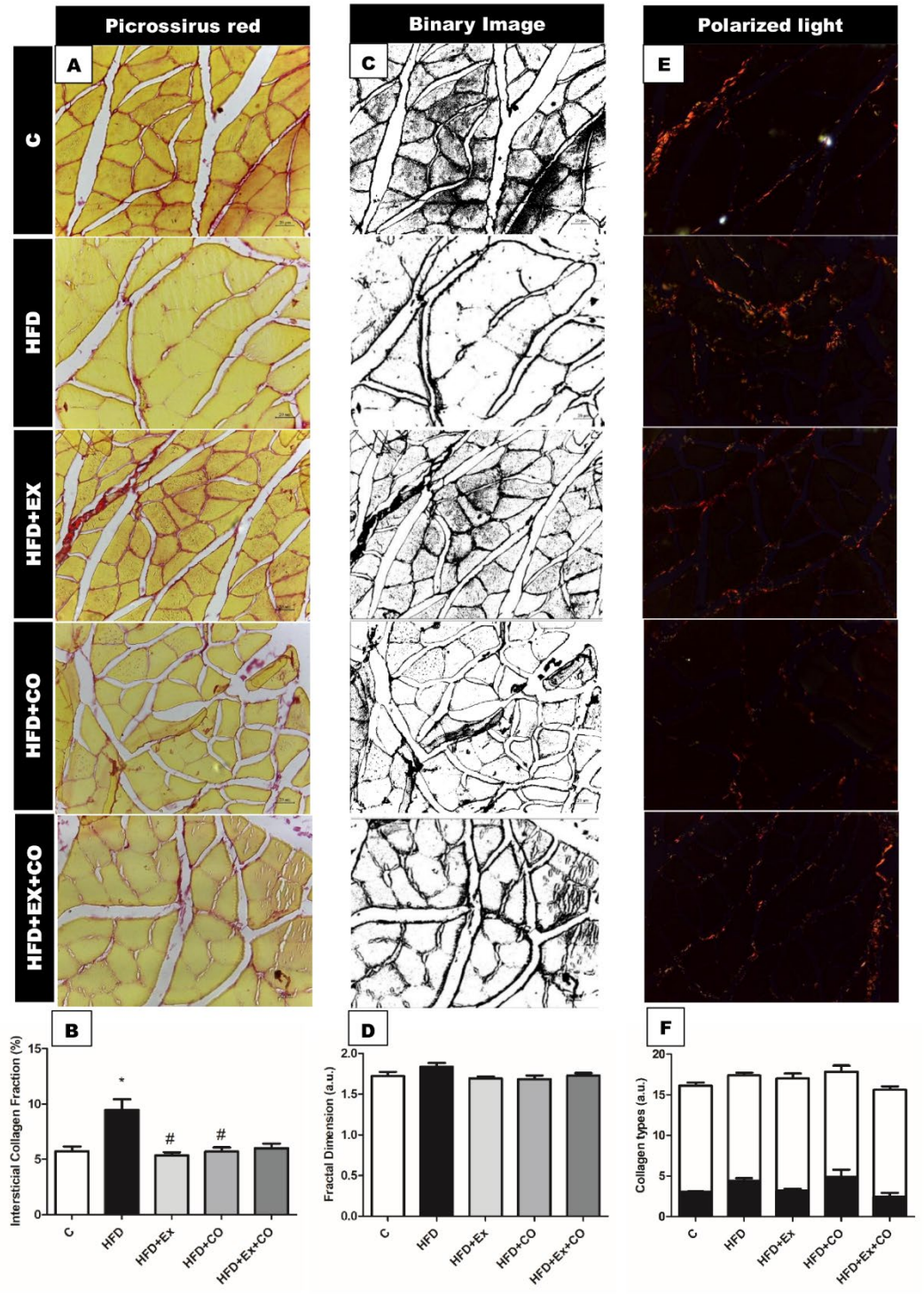

FIGURE 3. Collagen analysis of the soleus muscle. Scale bar= 20 µm; Resolution with 40x objective and 400x magnification. A) Cross-sections of the soleus muscle were stained by the picrosirius red technique (PSR). B) Quantitative analysis of PSR-stained sections. C) Soleus fractal dimension in Picrosirius staining, after binarization. D) Quantitative analysis of fractal dimension. E) Picrosirius red observed under polarized light. The red and green colors are the collagens I and III, respectively. F) Quantitative analysis of collagen types. C: control group, HFD: high-fat diet group, HFD+Ex: high-fat diet and aerobic exercise group, HFD+CO: high-fat diet and chia oil supplementation group, HFD+CO+Ex: high-fat diet, chia oil, and exercise

group. The Kruskal-Wallis test was used for mean comparisons, followed by Dunn's post hoc test. * p< 0.05 vs. C; # p < 0.05 vs. HFD.

3.4 | Arteriolar Analysis

The results of arteriolar analysis are illustrated in Figure 4. No alteration was observed for wall thickness (p=0.28), collagen percentage (p=0.17), and fractal dimension (p=0.53).

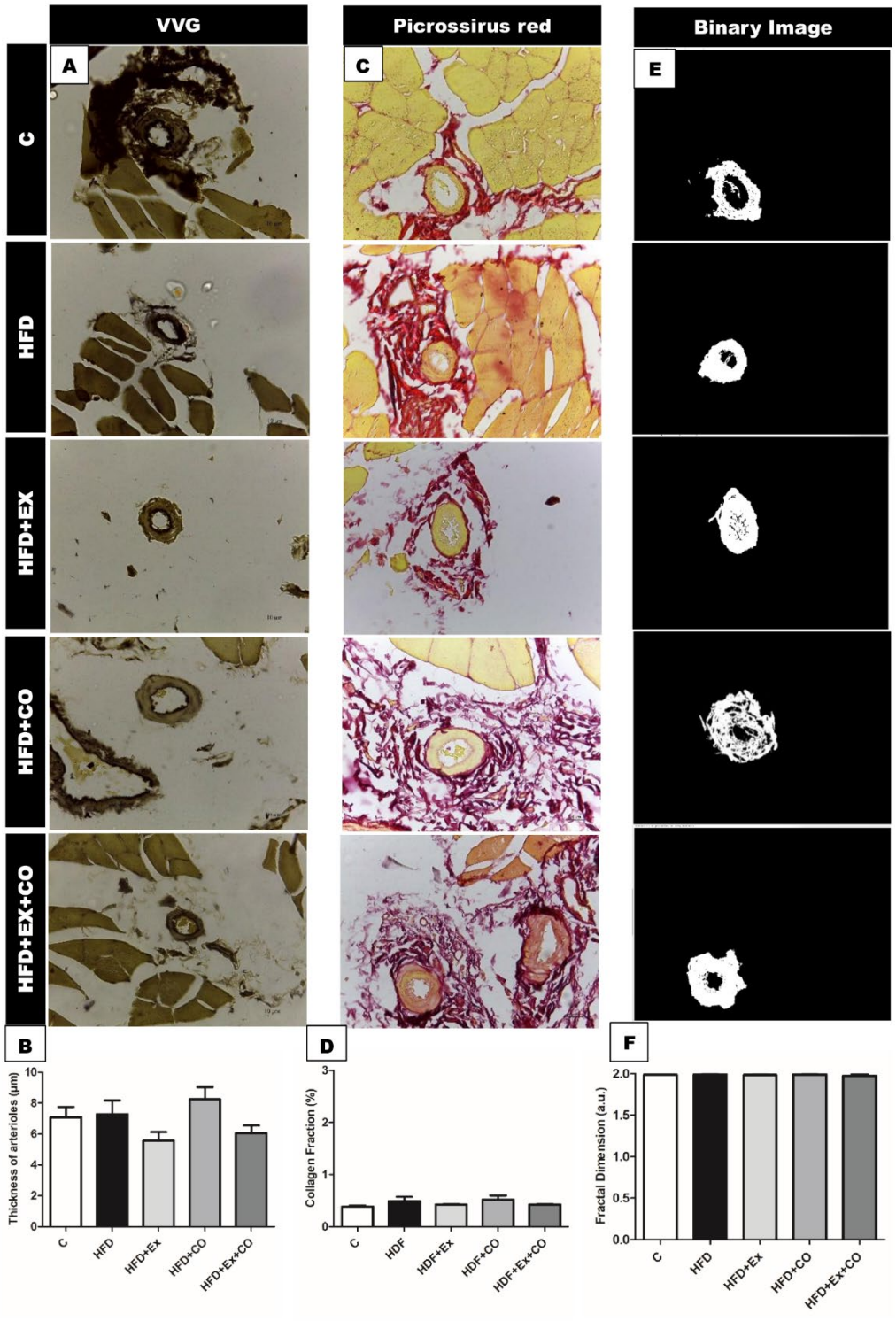

FIGURE 4. Arteriolar analysis of the soleus muscle. Bar= 20 µm; Resolution with 40x objective and 400x magnification. A) Cross-sections of soleus muscle were stained by the Verhoeff Van Gieson (VVG). B) Quantitative analysis of thickness of soleus muscle arterioles C) Cross-sections of the arteriole soleus muscle were stained by the picrosirius red technique. D) Quantitative analysis of arteriole PSR-stained sections. E) Fractal dimension of soleus arterioles in Picrosirius staining, after binarization F) Quantitative analysis of fractal dimension. Control C, HFD high-fat diet, HFD+Ex high-fat diet and aerobic exercise, HFD+CO high-fat

diet and chia oil, HFD+CO+Ex high-fat diet, chia oil and exercise group. The Kruskal-Wallis's test was used to compare means.

3.5 | Oxidative stress markers

The TBARS level was used to evaluate lipid peroxidation, but no difference was observed between the analyzed groups (p=0.27). Another product of oxidative stress is carbonyl and we observed that the HFD diet increased these levels compared to the control group, e exercise alone was not able to reduce these levels (C: 8.21±4.24 nmol/mg vs HFD: 29.62±12.19 nmol/mg; HFD+Ex 27.68±13.55 nmol/mg). Isolated supplementation of chia oil was able to reduce the carbonyl that this diet caused (HFD+CO: 10.08±3.20 nmol/mg). (Fig.5)

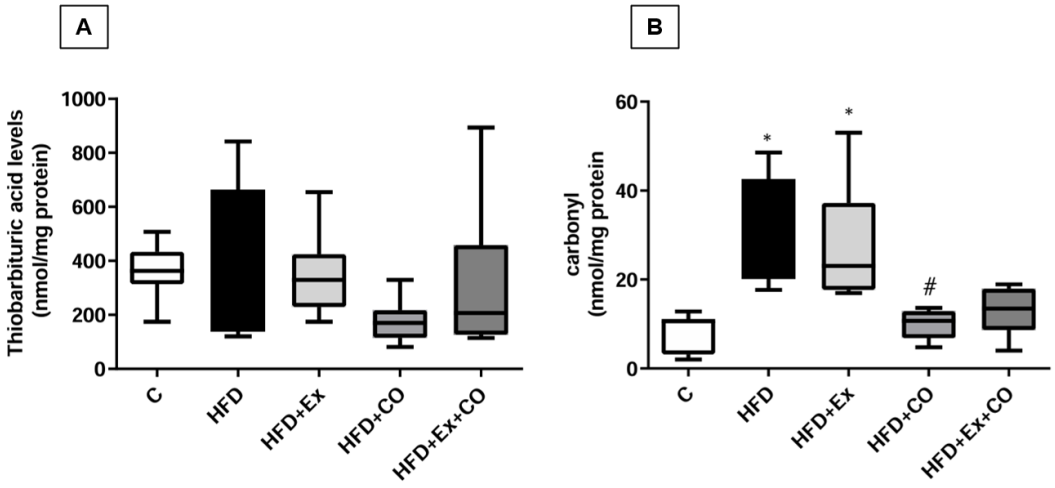

FIGURE 5. Oxidative stress markers in soleus muscle. A) Thiobarbituric acid (TBA) (nmol/mg protein). B) Carbonyl content (nmol/mg protein). C control, HFD high-fat diet, HFD+Ex high-fat diet and aerobic exercise, HFD+CO high-fat diet and chia oil, HFD+CO+Ex high-fat diet, chia oil and exercise group. The Kruskal-Wallis test was used to compare means, with Dunn's post-test. * $p< 0.05$ vs C. # $p< 0.05$ vs HFD

4 | DISCUSSION

The present study induced obesity in rats with 60 days old HFD showing increased weight gain, total fat, relative fat and adipose reserves, increased collagen deposition and carbonyl in skeletal muscle. Aerobic exercise decreased weight gain,

retroperitoneal adipose tissue, collagen, and increased carbonyl in the soleus muscle. Supplementation with chia oil was able to reduce final weight, weight gain, collagen, and carbonyl. The combination of these two interventions for 8 weeks had effects on final weight reduction, weight gain, total fat, epididymis, mesenteric, and retroperitoneal adipose tissue.

The induction of obesity in experimental models by diet usually requires a period of at least 6 to 8 weeks to observe substantial changes in fat stores, and HFD is better able to reproduce the most severe designs of comorbidities up to metabolic syndrome.[45] In one review, 81% reported a significant increase in body weight and 93% in visceral fat in the HFD-fed groups, and this appears to be proportional to the duration of the diet, so the effect of diet on visceral fat becomes more apparent after 10 weeks.[45] The literature recommends the assessment of visceral fat accumulation as a good estimate of obesity in the rat, since body weight does not accurately reflect adiposity in the rat, these parameters are indexes of obesity in these animals.[45,46]

In the present study, 15 weeks of HFD effectively promoted obesity, evidenced by body weight gain, and significantly increased total fat, epididymal adipose tissue, mesenteric adipose tissue, and retroperitoneal adipose tissue. Interventions such as aerobic exercise combined with chia oil supplementation have been shown to reduce these adipose depots and normalize body fat levels, suggesting that these approaches may synergistically enhance the reduction of adipose tissue lipid stores, with a significant decrease in weight gain during long-term HFD consumption.

The results of this research demonstrate that aerobic exercise is effective in reducing retroperitoneal adiposis in obese rats, reinforcing previous findings that suggest a positive interaction between exercise and lipid metabolism regulation. Several experimental designs have explored this relationship. For example, a study by Stanford et al. (2015) used HFD-fed rats for 12 weeks, comparing groups with and without treadmill running intervention, and observed significant reduction in visceral fat in the trained animals. In another approach, 7-week aerobic exercise training reduces adipocyte area, reduces body weight and epididymal fat mass in Wistar rats fed a high-fat diet.[47] Rodrigues et al. (2020) combined different intensities of aerobic exercise and observed that even moderate-intensity workouts were sufficient to attenuate weight gain and the accumulation of subcutaneous adipose tissue. These findings indicate that aerobic exercise acts as a modulator of energy homeostasis, promoting fatty acid

oxidation and inhibiting lipogenesis induced by the high-fat diet. Therefore, different experimental models converge to the understanding that aerobic exercise is an effective strategy in the prevention and control of HFD-induced obesity in animal models.[48]

Although it has been hypothesized that diet-induced obesity would lead to muscle fiber atrophy and decreased muscle transverse sectional areas (TSA), our findings do not indicate significant differences. These results are like those reported by Pincu et al. (2015), who also found no changes in the mean TSA of gastrocnemius-soleus complex fibers after 12 weeks of a diet with 60% fat associated with moderate exercise.[49] Results differ in other studies Sishi et al. reported that 16 weeks of Western diet resulted in reduced muscle fiber TSA in rats[50] On the other hand, Shortreed et al. observed an increase in TSA in soleus muscle after 8 weeks of a 60% fat diet.[51]

In our study, HFD increased the percentage of collagen in soleus muscle, and the groups of isolated aerobic exercise intervention and CO supplementation were able to normalize levels compared to control. Like our results, aerobic exercise, for 12 weeks and HFD, with Sirius Red analysis, a measure of collagen deposition, and associated with mRNA analyses of collagen 1a1 also.[49] The increase in collagen deposition compromises muscle plasticity and increases tissue stiffness, which can reduce the elastic component of force production, resulting in impaired muscle function. [52,53] Evidence shows that the mechanisms underlying collagen regulation by aerobic training are related to the activation of ECM remodeling[54], reduction of inflammatory cytokines[55] and improvement of lipid oxidation by muscles with a higher proportion of type I fibers that use fatty acids as an energy source, thus reducing the lipotoxic effects that contribute to collagen deposition. [56]

Notably, the supplemented group presented collagen levels in soleus muscle similar to the control, suggesting that chia may modulate obesity-induced ECM remodeling. The polyunsaturated fatty acids present in chia oil may act in the regulation of inflammatory cytokines, such as TNF-α, and in the modulation of collagen synthesis and degradation by activating antioxidant and anti-inflammatory metabolic pathways, being a supplementation with benefits in muscle health. [54,55]

In addition, oxidative stress can degrade structural proteins, such as collagen. The reduction in carbonyl levels due to chia oil in rats subjected to a high-fat diet in the

study may also be related to and justify the preservation of collagen levels in skeletal muscles, contributing to the structural and functional maintenance of muscle tissue [28,31]

Our findings indicate that animals submitted to HFD with chia oil supplementation reduced the protein carbonyl content in the muscle. Analysis of protein carbonyl levels is essential for assessing oxidative damage to proteins, which can provide valuable information about the mechanisms of disease progression and aid in the development of antioxidant strategies.[44] Chia has high antioxidant potential and can neutralize reactive oxygen species (ROS), which contribute to the oxidation of proteins and lipids[57] With increased antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), as well as reducing lipid peroxidation in skeletal muscles in diet-induced obesity models.[28] Santos-López et al., demonstrated that supplementation with chia oil decreased the protein carbonyl content, indicating a lower oxidative modification of proteins.[58]

In the present study, we observed that HFD caused an increase in the protein carbonyl content, but not significant in the TBARS content in the soleus muscle. Charradi et al; 2013, found an increase in TBARS and carbonyl content in the plasma of rats that followed a 6-week HFD.[59] The carbonyl content may have different results depending on the tissue analyzed, suggesting an oxidative response and specific compensatory mechanisms.[60] Méndez et al.,2014 Sprague–Dawley fed a high-fat, sucrose (HFHS) diet for 22 weeks found no increase in carbonyl content in skeletal muscle after a high-fat, sucrose diet, only in plasma and liver. [60]

Aerobic exercise for eight weeks had an increase in the carbonyl content compared to control which may indicate an adaptive response of the body, reflecting the ability to cope with the oxidative stress of HFD and exercise. This effect may contribute to the improvement of redox homeostasis and cellular integrity, without associating it with deleterious effects, since it did not cause fibrosis or muscle atrophy. [61,62] In ApoE-KO mice fed HFD for six weeks but not becoming obese, high-intensity interval training and moderate continuous exercise three times a week neutralized the protein carbonyl content in the gastrocnemius muscle.[63] As our model had more repercussions with weight gain, aerobic swimming exercise can cause distinct muscular adaptations in terms of primary function, recruitment pattern, type of contraction, and imposed load.[20,61]

Finally, the lack of significant changes in parameters such as fractal and arteriolar dimensions suggests that the timing and intensity of the interventions may not have been sufficient to impact the vascular structure. Erdei et al. investigated arterioles isolated from the gracilis muscle in male Wistar rats with HFD for 10 weeks and suggest a substantial dysregulation of arteriolar tone by the endothelium mediating nitric oxide (NO) in obesity, which may contribute to disturbed tissue blood flow and the development of increased peripheral resistance.[64] Similarly, Frisbee and Stepp found that in obese diabetic and hypertensive type 2 Zucker rats, NO mediation of arteriolar dilations is impaired due to increased superoxide production.[65] These studies suggested that, in these obese models with complications of type 2 diabetes mellitus, hyperglycemia and hypertension are the main factors responsible for the development of microvascular dysfunction, which was not observed in the present study.

With a low-cholesterol diet for a short period induces vascular dysfunction characterized by reduced relaxation of acetylcholine (Ach), release of NO; endothelium-dependent increase of angiotensin II (Ang II) response [66]; alteration of lipid metabolism and moderate alterations of vascular morphology[67] The inclusion of chia oil in HD partially normalized the response to Ach and the intimate/media relationship, and fully restored NO release.[68] These findings suggest that the increase in ALA levels induced by dietary chia oil may improve vascular function in hypercholesterolemic conditions and thus may serve as a true functional food.[69]

The lack of synergistic effect observed in the combination of chia oil supplementation and aerobic training can be attributed to several biological factors; for example, that the interventions modulate similar metabolic pathways, resulting in a redundancy of effects that prevents additional benefits. Previous studies suggest that chia oil and physical exercise activate AMPK, promote the expression of heat shock proteins (HSPs), and increase endogenous antioxidant capacity, but the combined impact may be limited by the saturation of these metabolic pathways [28,70] As well as methodological factors, such as an experimental protocol longer enough to capture the expected cumulative adaptations from the interventions. Combined interventions may require prolonged periods to elicit measurable synergistic effects, especially in parameters related to muscle and vascular plasticity. [20]

Future studies should consider longer protocols and include more detailed molecular analyses to elucidate the mechanisms involved. These findings contribute to

the advancement of the field by providing a solid scientific basis for the development of integrated, effective and accessible approaches to the management of muscular and metabolic complications associated with obesity.

Under the experimental conditions of this study, we conclude that obesity exerts deleterious effects on skeletal muscle, promoting structural and functional alterations. Our findings demonstrate that chia oil supplementation is effective in reducing protein carbonylation and normalizing muscle collagen deposition. In contrast, aerobic training alone increased protein carbonylation but decreased collagen deposition. The combination of these interventions produced clear synergistic effects, significantly reducing body weight and adipose tissue deposits in the epididymal, mesenteric, and retroperitoneal regions. Therefore, both strategies, whether applied individually or in combination, confer significant benefits in mitigating the muscle alterations induced by obesity.

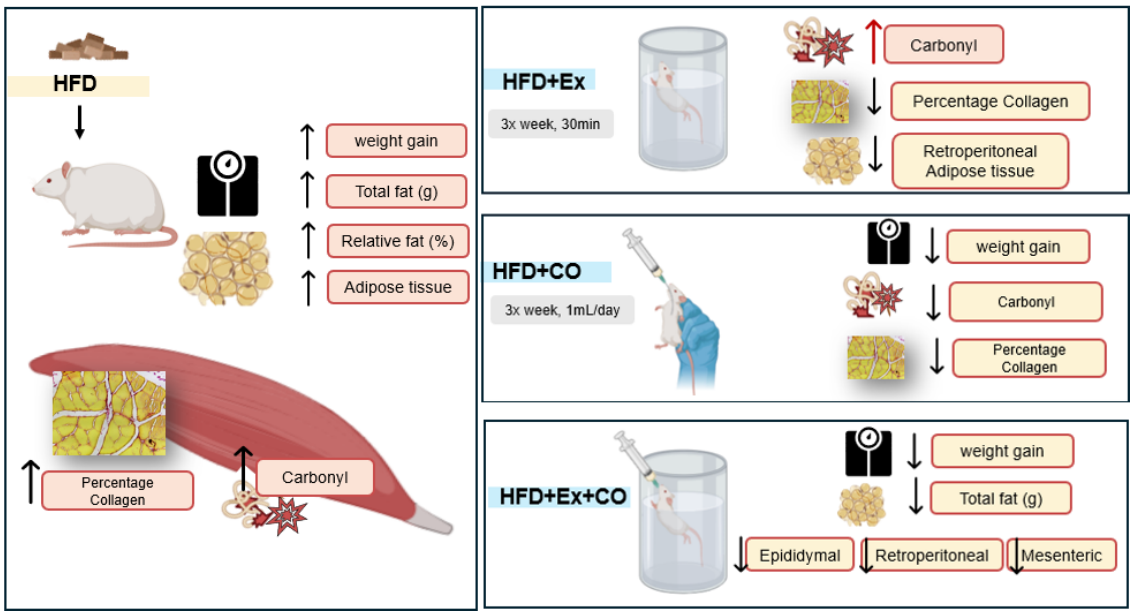

FIGURE 6. Schematic figure summarizing the main findings. C: control group, HFD: high-fat diet group, HFD+Ex: high-fat diet and aerobic exercise group, HFD+CO: high-fat diet and chia oil supplementation group, HFD+CO+Ex: high-fat diet, chia oil, and exercise group.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

N. C. U. Guazi, T. L. S. Pazinato, V. T. Silva, G. R. Teixeira, A. F. Aguiar, S. A. Lanquiste, R. L. Gomes, L. C. M. Vanderlei, made sub stantial contributions to research design; L. E. Engel performed analysis and interpretation of data and drafted the manuscript; I. J. Vechetti, F. L. Pacagnelli revised the manuscript critically for important intellectual content; and all authors approved the final version to be published.

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ANEXO 1 - APROVAÇÃO ÉTICA

UNOESTE - Universidade do Oeste Paulista

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PPG - Programa de Pesquisa de Pós-Graduação
PROBIC - Programa de Bolsas de Iniciação Científica

Parecer Final

Declaramos para os devidos fins que o Projeto de Pesquisa intitulado "**AÇÃO DO ÓLEO DE CHIA (SALVIA HISPANICA L.) E TREINAMENTO AERÓBICO SOBRE A PREVENÇÃO E TERAPÊUTICA DO CATABOLISMO MUSCULAR, DESEQUILÍBRIO REDOX E PROCESSO INFLAMATÓRIO NA OBESIDADE**", cadastrado na Coordenadoria de Pesquisa, Desenvolvimento e Inovação (CPDI) sob o número nº **8570** e tendo como participante(s) **LETICIA ESTEVAM ENGEL (discente), NATALIA CERVANTES UZELOTO GUAZI (discente), MARCELO MOREIRA PIRAJON JUNIOR (discente), TIAGO LYRIA DA SILVA PAZINATO (discente), CAMILA RENATA CORRÊA (participante externo/voluntário), MARINA POLITI OKOSHI (participante externo/voluntário), RAYANA LOCH GOMES (participante externo/voluntário), SABRINA ALVES LENQUISTE (docente), FRANCIS LOPES PACAGNELLI (orientador responsável)**, foi avaliado e **APROVADO** pelo **COMITÊ ASSESSOR DE PESQUISA INSTITUCIONAL (CAPI) e COMISSÃO DE ÉTICA USO DE ANIMAIS (CEUA)** da Universidade do Oeste Paulista - UNOESTE de Presidente Prudente/SP.

Este Projeto de Pesquisa, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro de 2008, do Decreto nº 6.899, de 15 de Julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), tendo sido APROVADO em reunião realizada em 06/03/2024.

MATERIAL ARMAZENADO/DOADO

Protocolo(s)	Data Aprovação	Armazenado (local)	É doação	Detalhes armazenamento
3962	09/06/2017	UNOESTE	SIM	Sala do mestrado Ciência Animal e biofreezer

Presidente Prudente, 9 de Abril de 2024.

Prof. Dr. Jair Rodrigues Garcia Jr.
Docente Responsável pela CPDI

ANEXO 2- NORMAS DA REVISTA INTERNATIONAL JOURNAL OF EXPERIMENTAL PATHOLOGY

Formato do manuscrito

Página inicial:

Uma única 'primeira página' deve conter: (1) o título do manuscrito; (2) um título de curta duração (não excedendo 40 caracteres); (3) o(s) nome(s) do(s) autor(es), incluindo nome próprio e sobrenome; (4) o(s) departamento(s) em que o trabalho foi feito; e (5) o nome, endereço postal completo, número de fax e endereço de e-mail do autor a quem as provas e pedidos de separatas devem ser enviados, com o título 'Correspondência'. O Autor Correspondente deve assumir a responsabilidade de se comunicar com todos os outros autores e obter sua aprovação para que a versão final seja publicada. Durante a submissão on-line, os Autores Correspondentes podem nomear um indivíduo, que pode ou não ser um autor, para 47juda-los na administração do processo de publicação.

O Autor Correspondente deve confirmar que todos os autores listados atendem aos critérios de autoria do ICMJE e que ninguém que se qualifica para autoria foi excluído. Veja a Seção 3.5 Autoria.

Segunda página:

A segunda página deve conter um parágrafo de resumo que deve dar um relato factual do contexto em que o estudo foi realizado, o(s) objetivo(s), métodos e resultados, e uma breve conclusão, em não mais de 250 palavras. Para fins de revisão e indexação, até seis 'palavras-chave' relacionadas aos assuntos discutidos no artigo devem ser identificadas e incluídas no rodapé do resumo.

Artigos de pesquisa originais

Os relatórios do trabalho original geralmente devem ser organizados na ordem convencional de introdução, métodos, resultados, discussão, agradecimentos e referências, com títulos adequados para cada parte. Outras subdivisões, com títulos apropriadamente menos significativos, podem ser usadas. Os resultados podem ser apresentados no texto, em tabelas e figuras, mas o texto deve, em geral, comentar em vez de repetir informações em tabelas.

Cartas ao Editor

Pode ser publicada correspondência relacionada a artigos publicados recentemente na Revista. O Editor reserva-se o direito de solicitar uma resposta dos autores originais para publicação paralela. As cartas devem ser o mais curtas possível (mas não mais do que 1000 palavras de texto, duas figuras ou tabelas ou uma de cada, e até 10 referências). A correspondência para a Revista é aceita no entendimento de que o autor contribuinte autoriza o editor a publicar a carta como parte da Revista ou separadamente dela, no exercício de quaisquer direitos subsidiários relacionados à Revista e seu conteúdo.

Tabelas e Figuras

Cada legenda de tabela/figura deve ser apresentada junto com sua tabela/figura. As figuras/tabelas devem ser inseridas no texto nas posições apropriadas ou agrupadas no final.

Para envio online, as ilustrações devem ser incorporadas ao documento do Word ou carregadas como arquivos separados. A qualidade deve ser suficiente para visualizar a impressão na tela e na área de trabalho.

Sempre que possível, forneça arquivos de arte digital de alta qualidade.

Figuras: JPEG, GIF, EPS, PNG Microsoft PowerPoint, Microsoft Excel, Microsoft Word são todos aceitáveis.

Imagens digitais: As versões digitais das figuras devem ser fornecidas no formato TIFF.

Tanto as tabelas quanto as figuras devem ser numeradas consecutivamente com algarismos arábicos. Cada um deve ter uma legenda descritiva separada. As chaves devem ser dadas nas legendas, não na figura em si. Todas as ilustrações, desenhos e fotografias, devem ser de boa qualidade, pois ocorrerá atraso se os revisores precisarem ver versões melhoradas. Como guia, a resolução/especificação ideal da figura para vários tipos de figuras originais, em seu tamanho final, é a seguinte:

Arte de linha e diagramas – Mínimo de 600 dpi
Meio-tom (fotografias em preto e branco e coloridas) – Mínimo de 300 dpi
Linha e tom (arte de linha e meio-tom combinados) – Mínimo de 600 dpi

É melhor usar o software Illustrator ou Photoshop e salvar o material no formato '.eps' ou '.tif'. Se o autor não puder fornecer esses formatos, salve as figuras no maior número possível de formatos de arquivo diferentes. Para obter mais informações sobre formatos de arquivo, consulte as instruções em http://www.blackwellpublishing.com/bauthor/illustration.as p

Ilustrações a cores

O IJEP é publicado apenas online e não há cobrança pela inclusão de figuras coloridas.

Informações de apoio

Os autores podem fornecer texto ou dados adicionais como 'Arquivos de Informações de Apoio, mas o manuscrito principal deve conter informações suficientes para tornar o trabalho inteligível sem esses arquivos. As informações de apoio são uma parte formal do manuscrito publicado e normalmente não devem ser republicadas em outro lugar.

Nossos tipos de arquivo recomendados para informações de suporte são: .doc/ .xls/ .ppt/ .txt/ .jpg/ .jpeg/ .gif/ .tif/ .tiff/ .png/ .bmp/ .eps/ .ps/ .html/ .pdf/ .mov/ .mpg/ .wav/ .mp3/ .wma

Observe que as informações de suporte não são subeditadas ou revisadas, portanto, os autores devem garantir que os arquivos sejam fornecidos prontos para publicação

online. Os tamanhos dos arquivos devem ser os menores possíveis, para que possam ser baixados rapidamente e não devem exceder 50 MB.

Medidas

As medições devem ser expressas em unidades SI. Se as observações iniciais foram registadas noutras unidades, tal deve ser indicado, juntamente com os coeficientes de conversão adequados.

Abreviaturas padrão

Abreviaturas padrão devem ser usadas e devem seguir as estabelecidas em Units, Symbols and Abbreviations (1994) publicado pela Royal Society of Medicine. As abreviações devem ser usadas com moderação e somente se um nome ou expressão longa for repetido com frequência ao longo do manuscrito. As palavras devem aparecer por extenso na primeira aparição no resumo e no texto, seguidas da abreviatura entre parênteses. Os medicamentos devem ser descritos por seus nomes oficiais, mas os nomes comerciais devem ser indicados entre parênteses na primeira vez que o medicamento for citado no texto.

Agradecimentos

Os autores são obrigados a incluir detalhes de aprovação ética, divulgação de conflitos de interesse financeiros e outros e fontes de financiamento.

Referências

Todas as referências devem ser numeradas consecutivamente em ordem de aparição e devem ser o mais completas possível. No texto, as citações devem citar referências em ordem consecutiva usando algarismos arábicos sobrescritos.

2 ARTIGO DE REVISÃO 2

EFFECTS OF HIGH-INTENSITY INTERVAL TRAINING ON OXIDATIVE STRESS BIOMARKERS ON MYOCARDIAL ISCHEMIA-REPERFUSION INJURY: A SYSTEMATIC REVIEW AND META-ANALYSIS BASED ON PRECLINICAL STUDIES

Leticia Estevam Engel[1], Alessandra Straioto Salomão[1], Rafael Floriano Stuani[1], Jakeline Ortega[1], Andreo Fernando Aguiar[2], Milene Vitória Sampaio Sobral[1], Marina Politi Okoshi[3], José Francisco Cursino de Moura[1], Ivan José Vechetti[4], Francis Lopes Pacagnelli.[1*]

[1] Universidade do Oeste Paulista (UNOESTE), Presidente Prudente, SP, 19.067-175, Brazil

[2] Universidade do Norte do Paraná (UNOPAR), Londrina, PR, 86047-500, Brazil

[3] Universidade Estadual Paulista (UNESP), Botucatu, SP, 18618-687, Brazil

[4] University of Nebraska-Lincoln, Department of Nutrition and Health Sciences, Lincoln, NE, 68583, USA

Short title: Review of myocardial infarcted rats exposed to HIIT

*Correspondence to: F.L. Pacagnelli (francispacagnelli@unoeste.br), Tel.: +55 18 3229 3264, Fax: +55 18 3229 2080

SUMMARY

Introduction: Ischemia-reperfusion (IR) injury, a critical complication resulting from transient interruption of blood flow followed by reperfusion, leads to oxidative stress-mediated cellular damage. Evidence suggests that high-intensity interval training (HIIT) may exert cardioprotective effects by influencing antioxidant defense mechanisms, and attenuating oxidative damage associated with IR. The impact of HIIT on oxidative stress biomarkers in this context requires further investigation to clarify its therapeutic potential and further encourage its indication. Aimed to evaluate, through a systematic review with meta-analysis, the effects of high-intensity interval training on biomarkers of cardiac oxidative stress in animal models with myocardial ischemia-reperfusion injury. **Methodology:** A systematic search was conducted in the Embase, Scopus, PubMed, and Web of Science databases until January 2025 to identify studies that compared markers of oxidative stress in rats undergoing HIIT versus sedentary controls. Statistical analyses were performed using the Review Manager program. Differences between the means (SMD) and 95% confidence intervals (95%CI) were calculated. The risk of bias was assessed using the Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES). **Results:** Six studies involving 88 rats were included in this analysis with biomarkers of myocardial oxidative stress in animal models. The joint analysis showed that HIIT promoted a significant increase in superoxide dismutase levels (SMD-1.88; 95%CI- 0.48 to 3.28; p=0.009) and catalase (SMD-3.47; 95%CI- 0.52 to 6.41; p<0.0001). No significant differences were observed in glutathione peroxidase levels (SMD-0.55; 95%CI- -2.54 to 1.44; p=0.59), reduced glutathione (SMD-6.31; 95%CI- -0.27 to 12.90; p=0.06), malondialdehyde (SMD-0.18; 95%CI- -2.98 to 3.33; p=0.91) and myeloperoxidase (SMD-0.48; 95%CI- -1.11 to 0.15; p=0.13). **Conclusion**: We demonstrated that HIIT has the potential to improve parameters related to oxidative stress myocardial. However, studies of better methodological quality are needed to confirm the findings.

Keywords: HIIT. oxidative stress. Ischemia

NEW DISCOVERIES

What is the central question of the study?

The study investigated the mechanism by which High Intensity Interval Training (HIIT), exerts cardioprotection against ischemia-reperfusion (IR) injury. Although it is known that aerobic exercise attenuates ischemia-induced myocardial damage by reducing cell death and preserving cardiac function, the underlying mechanisms and efficacy of HIIT in this context remain partially elucidated.

What is the main finding and its importance?

During ischemia and subsequent reperfusion, there is an increase in the production of reactive oxygen species (ROS). HIIT promoted adaptations that increase antioxidant defense, demonstrated by the increase of SOD and CAT, demonstrating a cardioprotection of HIIT after IR.

1. INTRODUCTION

Cardiovascular diseases (CVD), including ischemic heart disease, represent the leading cause of global mortality. [1] After myocardial infarction (MI), cardiac remodeling occurs, causing diastolic dysfunction, arrhythmias, and cardiomyocyte hypertrophy that contribute to unfavorable outcomes and can progress to cardiovascular death. [2,3] Although blood reperfusion is necessary to restore oxygen flow to ischemic tissues to decrease mortality in the acute phase of MI, it can also cause ischemia-reperfusion (IR) injury. [4,5]

The process of ischemia-reperfusion occurs when an interruption of blood flow prevents oxygen and nutrients from reaching the tissues, leading to a drastic reduction in cellular function and metabolism [6,7]. When blood flow is restored by reperfusion, the oxygen supply returns to the tissues. However, this process also triggers the excessive production of reactive oxygen species (ROS), such as free radicals and peroxides. These excessive highly reactive molecules can cause significant damage to cellular structures, including proteins, lipids, and DNA. The ROS generated by this process is responsible for a process of oxidative stress in heart cells, which consequently damages cell structures, triggers apoptosis, and compromises myocardial contractile function [8,9,10]

Practice of physical exercise is studied as a potential cardioprotective strategy against IR injury. [7] A systematic review conducted by Calvert and Lefer (2013), analyzing various types of exercises, revealed that exercise training reduced infarct size by 34% in rats with ischemia. [11] Recently, Song et al., also reported that exercise can improve myocardial function after IR injury; However, they stressed that more research is needed in combination with specific questions such as exercise mode, intensity and duration.[12] Several studies show that cardiovascular, muscular, and metabolic adaptations are directly related to training intensity. This is a more important factor than the duration of the activity. [13,14]

High-intensity interval training (HIIT) consists of repeated sets of short, intense exercises, performed at 80-100% of maximum heart rate, alternating with periods of active recovery or light exercise.[15] HIIT training during the IR period can have varying effects on oxidative stress. On the one hand, physical exercise can increase the oxygen demand in the tissues, thereby increasing the production of ROS during ischemia. On the other hand, regular exercise can also induce antioxidant adaptations in the body by increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase, and by promoting the expression of antioxidant transcription factors, such as nuclear factor 2 erythroid 2 (Nrf2). [14,15]

The impact of HIIT on oxidative stress during the IR period can vary according to several factors, such as the intensity and duration of the exercise, the antioxidant status of the body, and the ability of the antioxidant system to adapt. However, despite recent scientific efforts, the mechanism underlying its cardioprotective potential remains unclear. Therefore, a comprehensive systematic review is needed to investigate the influence of HIIT on the response to oxidative stress during the IR period. This study aimed to evaluate, through a systematic review with meta-analysis, the effects of high-intensity interval training on biomarkers of cardiac oxidative stress in animal models with myocardial ischemia-reperfusion injury.

2. METHODS

The meta-analysis adhered to the 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses Criteria (PRISMA) [16], and the International Prospective Register of Systematic Reviews (PROSPERO CRD 504555).

2.1 Systematic review

Different online databases Embase, Scopus, Pubmed, Web of Science, (supplementary materials) were searched in January 2025 to identify experimental studies related to cardioprotection and HIIT training protocol during IR injury. It covers three domains: HIIT (as an intervention), cardiac markers (as a result), and animals with IR injury (as a population). The search strategies for all databases were: ("Injuries, Myocardial Reperfusion" OR "Injury, Myocardial Reperfusion" OR "Myocardial

Ischemic Reperfusion Injury" OR "Myocardial Reperfusion Injuries" OR "Reperfusion Injuries, Myocardial" OR "Reperfusion Injury, Myocardial") AND ("Exercise, High-Intensity Intermittent" OR "Exercises, High-Intensity Intermittent" OR "High Intensity Interval Training" OR "High-Intensity Intermittent Exercise" OR "High-Intensity Intermittent Exercises" OR "High-Intensity Interval Trainings" OR "Interval Training, High-Intensity" OR "Interval Trainings, High-Intensity" OR "Sprint Interval Training" OR "Sprint Interval Trainings" OR "Training, High-Intensity Interval" OR "HIIT"

After excluding duplicate references, we examined the titles and abstracts and identified potentially relevant articles that fit the inclusion criteria. The next step was the full-text analysis of the remaining articles. Two different researchers (LEE and ASS) performed each of these steps independently. Whenever these researchers disagreed, a third researcher (FLP) was consulted. As a complementary search, we also selected eligible studies referenced in the articles retrieved from the literature search and studies suggested by experienced researchers. We summarized the study selection steps in a flowchart (Figure 1) following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA). [16]

2.2. Eligibility criteria

Inclusion in this meta-analysis was restricted to studies that met all of the following eligibility criteria: (1) experimental studies; (2) that included infarcted rats; (3) with rats submitted to different high-intensity interval training protocols, regardless of the duration, frequency, or stage of disease progression; (4) that presented any numerical measure of cardiac adaptation behavior; and (5) who had at least one experimental group or condition not submitted to HIIT, without other concomitant interventions. There were no language or publication date restrictions. In the first selection phase (title and abstract), the exclusion criteria were applied in the following order: (1) reviews/theoretical articles, (2) wrong population (healthy animals), (3) non-exercise intervention, and (4) wrong outcome (no myocardial adaptation). In the second selection phase (full texts), the exclusion criteria were applied in the following order: (1) full text not available, (2) no control group or condition, (3) no IR lesions, and (4) no evaluation of markers in cardiac tissue.

Groups of rats that underwent HIIT and were exposed to ischemia-reperfusion (HIIT+IR) were included and compared with control groups with ischemia-reperfusion from sedentary rats (CTRL+IR).

2.3. Outcomes of interest

The main outcomes of interest are: (1) products related to oxidative stress and (2) biomarkers for cardioprotection. The secondary outcomes of interest are: infarct size, cardiac enzymes, and cardiac structural and functional parameters.

2.4 Risk of bias in individual studies

Bias was assessed using a 10-item checklist from the Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES) [17], with minor modifications [18]: A: peer-reviewed publication; B: Temperature control; C: random allocation for treatment or control; D: blinded induction of the model (group randomly after ischemia induction); E: blinded assessment of the outcome; F: use of anesthetic without significant intrinsic cardioprotective activity; G: appropriate animal model (elderly, diabetic or hypertensive); H: sample calculation; I: compliance with animal welfare standards [preoperative anesthesia, postoperative analgesia, nutrition, environment (temperature, humidity, circadian rhythm) and euthanasia]; and J: declaration of potential conflict of interest. Each item received one point. Two researchers independently assessed the quality of the study, and disagreements were well resolved through consultation with the authors of the correspondence. [19,20,21]

2.5 Data extraction

We used WebPlotDigitizer, when the numerical characteristics were not described, to extract numerical data from images of a variety of data, the results were estimated proportionally to the axes presented in the graphs. The data were extracted directly from the full text by two independent reviewers (LEE and ASS), and the values were averaged. The following data were extracted from each article: title, authors, complete reference, sample size, species, lineage and age, number of animals with IR lesions, number of control animals, type and protocol of physical exercise, duration and

intensity, IR induction method and days of procedure; and the result, methods of cardiac adaptations and cardioprotective markers.

Regarding the comparison of oxidative stress markers, the numerical variable used was extracted as mean ± standard deviation (SD). For studies revealing standard error of the mean, the SD was calculated by dividing it by the square root of the sample size.

2.6 Statistical analysis

An initial descriptive analysis of the HIIT protocol – in addition to the original protocol – was performed with all studies included, analyzing the most important result related to the regression of infarct size in each article (in order: evaluation method, cardiac benefits, and cardioprotective markers after physical exercise). Meta-analyses were performed whenever three or more studies evaluated variables with comparable outcomes. Heterogeneity was evaluated by visual inspection of the Forest Plot, considering the Chi-Square Test (with a significance level of $p<0.05$). In addition, we used the I^2 statistic when performing the fixed-effects meta-analysis, and heterogeneity was considered substantial if the I^2 was greater than 50%. Revman 5.4.1 software was used for statistical analysis.

3. Results

3.1. Database search

The search in the databases provided a total of 82 potentially relevant articles. We demonstrate the screening process by the PRISMA flowchart (Figure 1)

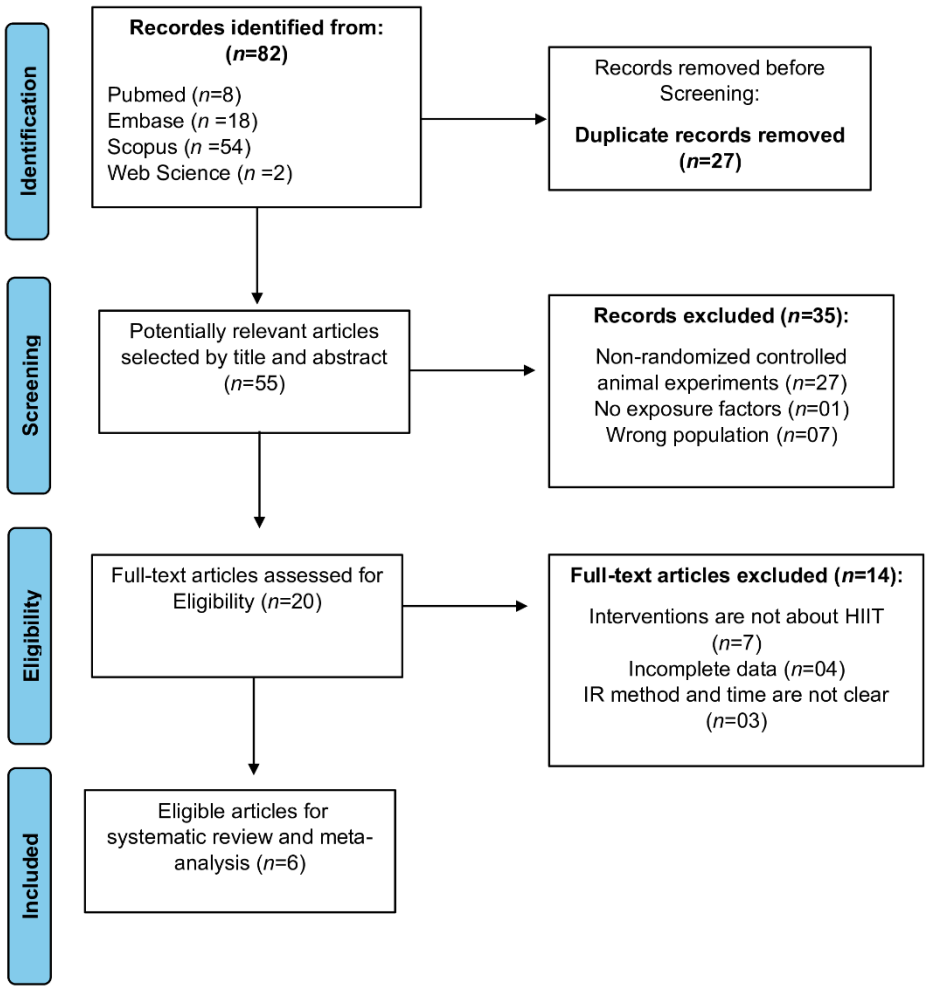


Fig. 1. Flowchart of the literature screening process.

3.2. General characteristics of literature included

In total, 6 articles, involving 88 rats male wistar, were included in this analysis [22-27] Among them, one paper compared old rats with young rats [22]. All studies used the male rat species Wistar. The articles covered two induction methods. Among them, 83.33% (5 studies) used LAD ligation [22-26]. Only 1 article performed the HIIT after the fourth week of surgery IR [24]. The rest of the training articles were before the induction of IR with HIIT on a treadmill for 1 to 8 weeks of training. More specific details about the general features of the articles included are shown in **Table 1.**

Table 1. characteristics of literature included

Autor/ year	sex/ species	Numbe r/ age	Method of myocardial IR	Type exercise and time	HIIT protocol
Ramez et al., 2020	Male Wistar	28/-	LAD Ischemia: 30 min Reperfusion: 24h (24h after the last training session)	Running on treadmill (5 consecutive days)	(1) Warm-up: 50 % VO2max 5 min (2) Main training: (6 ×2 min) at 85 %–90 % VO2max; (5 ×2 min) at 50 %–60 % VO2max. (3) Cool-down: 5 min at 40 %–50 % VO2max
Fallahi et al., 2015	Male Wistar	20/12-16 weeks	LAD Ischemia: 30 min Reperfusion: 90 min (after exercise session	Running on treadmill (8 weeks)	(1) Warm-up: 6 min at 50 %–60 % VO2max. (2) Main training: 7 intervals of 7-min with a slope of 50◦ to 20◦ (4 min with 80%–100% VO2max and 3 min at 50%–60% VO2max); and (3) Cool-down: 5- min at 50%–60% VO2max
Ghardashi et al., 2019	Male Wistar	20/6 weeks	LAD Ischemia: 30 min Reperfusion: 8 weeks (Before exercise)	Running on treadmill (8weeks)	(1) Warm- up: 5 min at 50%–60% VO2max (2) Main training: 30 min: 5 (4 min) of 85 %–90 % VO2max 2 min at 50 %–60 % VO2max. (3) Cool-down: 5 min 50 %–60 % VO2max. 25◦ slope
Fatahi et al., 2022	Male Wistar	20 rats/ young 2-month	LAD Ischemia (30 min/120 min) 3 days after last exercise session	HIIT treadmill running/ 8 weeks (5 days/week, 40 min/day)	(1) Warm- up: 5 min (50% VO2máx) (2) Main training: 10 intervals 2 min (90–95% VO2pico), e 1 min 60–65% do VO2máx (0% slope). (3) Cool-down:5 min (50% VO2máx)
Ranjbar, K. 2022	Male Wistar	20/7-8 weeks	LAD Ischemia (30 min/ 7 days de reperfusion) 3 days before HIIT	HIIT running/ 8 weeks (5 day per week).	(1) Warm- up: 5 min at a speed of 10 m/min (2) Main training: 50% VO2 for 2 min followed by 2 min 90% for 8 sets (3) Cool-down:5 min with 10 m/min.
Rankovic et al.,2021	male Wistar	20/ 11weeks	36h training treatment before in vitro ischemia/reperfus ion injury on the Langendorff apparatus (30min/1h)	HIITr group ran on treadmill 4 weeks, 5 days weekly	Adaptation: 1 week (7 m/min speed for 15 min/day). (1) Warm- up: 5 min, 8 m/min (2) Main training: 2nd week: 5 sprints x 45m/min at 30sec per day; with progression until the 5th week reaching 5 sprints x 55m/min in 90sec; 2min rest between each sprint

3.4.3 HIIT Training Protocols

The duration of the training varies between five consecutive days and eight weeks. **(Table.1)** 66.67% of studies used a period of 8 weeks, also with 5 weekly sessions [22-26]. All of them went through an acclimatization period of three days to a week on the treadmill (speed of 7m-10m/min for 10-15 min/day).

The training protocol was divided with warm-up and cool-down at 5 min., ranging from 50%-60% of VO2peak (**Figure 2**). And the main training was between 5-10 intervals of 30sec. 4 min. (80-100% VO2max) and with active recovery with slower intensity between 1-3min. (50-60% VO2max).

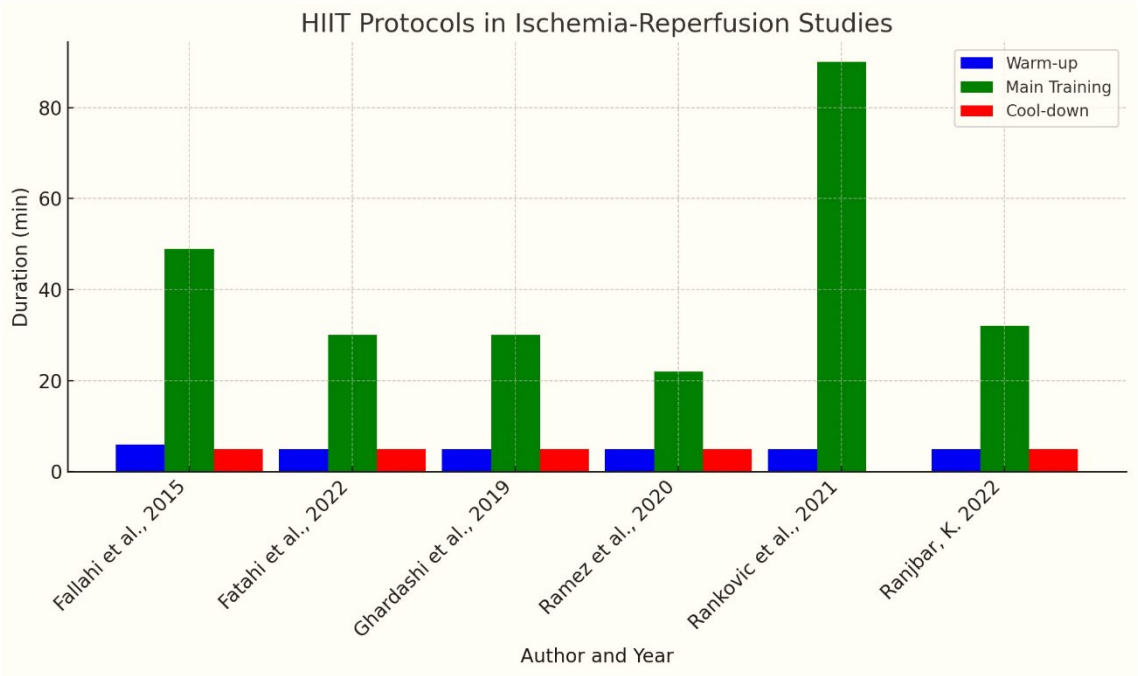


Fig. 2. HIIT training protocols (warm-up, main training, and cool-dow

3.3. Risk of Bias Assessment

To maintain the quality of this systematic review, two researchers independently assessed the included studies, using a collaborative approach to meta-analysis and a review of animal data from the checklist of experimental studies (CAMARADES), which is a 10-item checklist that assesses the risk of bias in pre-clinical animal studies.

The total score 5–8/10. The methodological quality of each study is summarized in Table 2 and risk of bias graph in Fig. 4.

Table 2. The CAMARADES Checklist

		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	Overall
1	Fallahi, 2015	✓	✓	✓	✗	✗	✓	✓	✗	✓	✓	Some concerns
2	Fatahi, 2022	✓	✓	✓	✗	✗	✓	✓	✓	✓	✓	Low risk
3	Ghardashi, 2019	✓	✓	✓	✗	✓	✓	✓	✗	✓	✓	Low risk
4	Ramez, 2020	✓	✓	✓	✗	✗	✓	✓	✗	✓	✓	Some concerns
5	Ranjbar, 2022	✓	✓	✓	✗	✗	✓	✓	✗	✓	✓	Some concerns
6	Rankovic, 2021	✓	✓	✓	✗	✗	✓	✗	✗	✓	✗	High risk

Summary of risk of bias. D1: Journal publication with peer review; D2: Temperature Control Statement; D3: Random Allocation to Groups; D4: Allocation Concealment; D5: Blind evaluation of the result; D6: Use of anesthetic without significant intrinsic cardioprotective activity; D7: Suitable animal model for elderly diabetic hypertensive; D8: Sample size calculation; D9: Compliance with animal welfare regulations; D10: Declaration of potential conflict of interest.

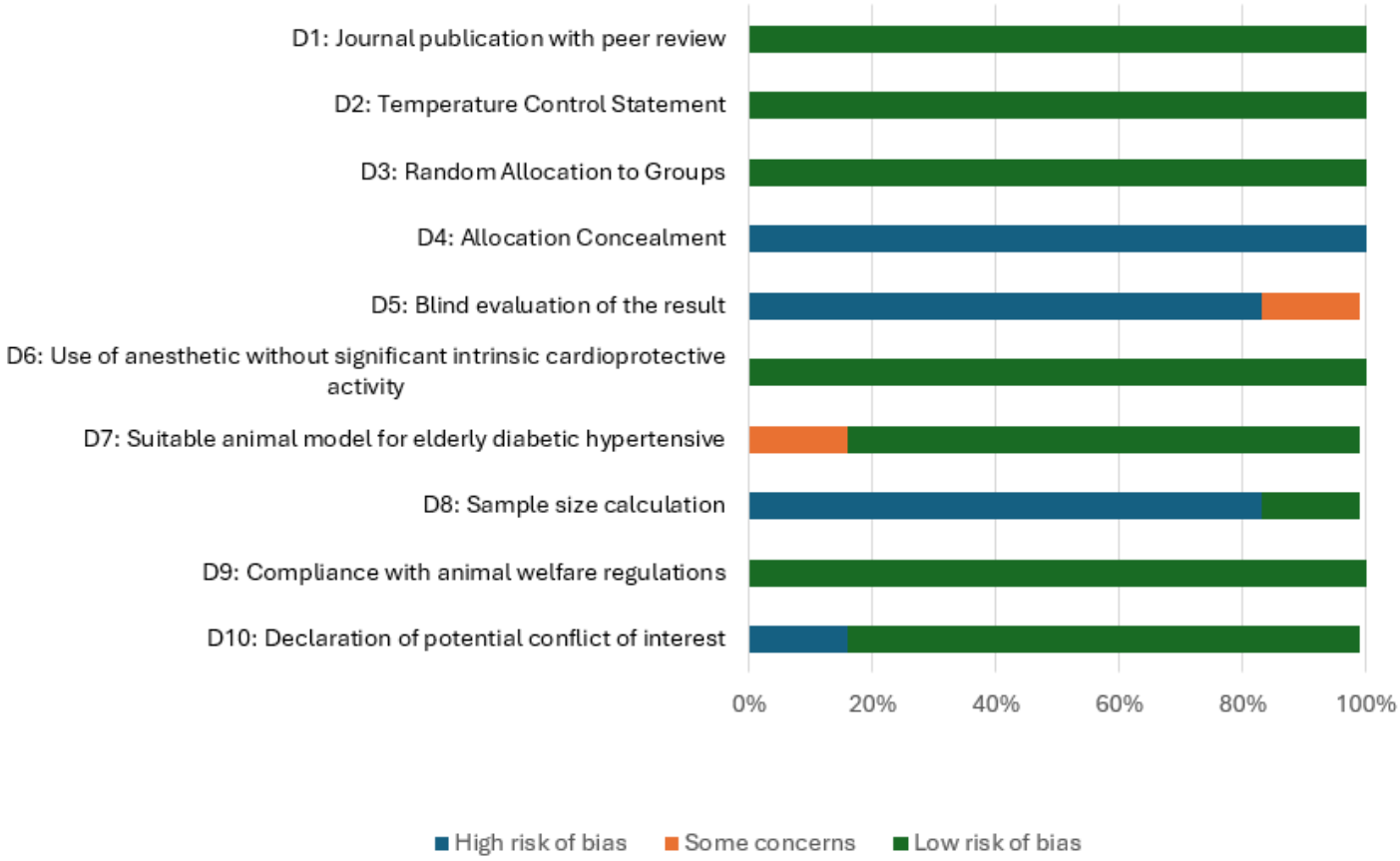


Fig.3 Risk of bias graph

3.4 Outcomes

The authors analyzed the relationship between infarct size, cardiovascular variables such as ejection fraction (EF), cardiac output (CO), and maximal oxygen uptake (VO_2max), as well as antioxidant mechanisms, including levels of enzymes such as SOD, CAT, and GPx. The findings indicate that interventions such as HIIT can modulate these parameters, reducing oxidative stress and promoting cardioprotection. **(Table 3)**

Table 3. Outcomes of literature included

Autor/ year	Infarct size	Cardiovascular Parameters	Mechanism
Ramez et al., 2020	Infarct size ↓ CK-MB, LDH e cTnI:↓	BW: - HW/BW: - LV/BW ratio: - VO2máx: -	1.TAC: ↑ 2.SOD: ↑ 3.CAT: ↑ 4.GPx: ↑ 5. MDA: ↓
Fallahi et al., 2015	Infarct size ↓	Heart mass (mg/g): ↑ Heart mass/body mass: ↑	**Nitric Oxide Metabolites** 1.Nitrite:↑ 2.Nitrate:↑ 3.NOX: ↑
Ghardashi et al., 2019	Infarct size: ↓	Final BW: ↑ HW: ↑ LV /BW ratio: ↑ LV/BW ratio: ↑ EF e FS: ↑ LVIDd e LVIDs:↓	1.GSH: ↑ 2.SOD: ↑ 3.MDA: ↓
Fatahi et al., 2022	Infarct size ↓	HW/BW (Y Ex and O Ex↑) HW (YEx↑) Final body mass (O Ex↓) VO2max, Maximal running speed (Y Ex and O Ex↑) Ventricular ectopy score (Y Ex and O Ex↓)	1.GPX: Y Ex:- 2.GSH: Y Ex:↑ 3.CAT: Y Ex:↑ 4.MDA:Y Ex:- 5.MPO:Y Ex:-
Ranjbar, K. (2022).	Infarct size ↓	SV, CO: ↑ HR: - LVEDd: -LVESd: -	1.GPx – 2.GSH ↑ 3.Cat ↑ 4.MDA ↓ 5. MPO -
Rankovic et al.,2021	-	BW, HW, HW/BW, VE/BW: - BW after: ↓ HR, VPSL: returning to baseline levels. DLVP: ↑ CF: ↑ dp/dt max and dp/dt min: stabilization level the reperfusion period	1. TBARS: - 2. nitrite (NO_2^-) ↑ 3. Super oxide ânion radical (O_2^-) ↑ 4. H_2O_2: -

Body weight (BW), Heart weight (HW), Heart weight/body weight ratio (HW/BW), Ventricular/body weight ratio (VE/BW ratio), Maximal oxygen consumption (VO_2max), Heart mass/body mass ratio (Heart mass/body mass), Left ventricle/body weight ratio (LV/BW ratio), Ejection fraction (EF), Fractional shortening (FS), Left ventricular

internal diameter in diastole (LVIDd), Left ventricular internal diameter in systole (LVIDs), Stroke volume (SV), Cardiac output (CO), Heart rate (HR), Left ventricular end-diastolic diameter (LVEDd), Left ventricular end-systolic diameter (LVESd),Ventricular premature systolic lesions (VPSL), Developed left ventricular pressure (DLVP) ,Coronary flow (CF), Maximum rate of pressure development (dp/dt max), Minimum rate of pressure decline (dp/dt min), Young sedentary (Y Sed); young exercised (Y Ex); old sedentary (O Sed); old exercised (O Ex). lipid peroxidation (TBARS); nitrite (NO^{-}_{2}); Super oxide anion radical (O^{-}_{2}); hydrogen peroxide (H_2O_2). Total antioxidant capacity (TAC), Superoxide dismutase (SOD),Catalase (CAT), Glutathione peroxidase (GPx), Glutathione (GSH), Malondialdehyde (MDA), Myeloperoxidase (MPO).

3.4.1. Myocardial IR lesion infarct size (IS)

As a secondary outcome 5 studies examined the size of myocardial infarction, and 100% studies decrease after HIIT+IR compared in the CTRL+IR [22-26]. The infarct size/risk area (IS/AAR) was measured by evaluating the non-infarcted left ventricular tissue that turned red and the infarcted area that turned white and the ratio between the white portion and the total area of the left ventricle.

Ramez et al., 2020 demonstrated that the HIIT+IR group on after exercise, creatine kinase-MB (CK-MB), cardiac troponin I (cTnI), and lactate dehydrogenase (LDH) decreased. Analysis of plasma levels of CK-MB and LDH compared in sedentary individuals in the CTRL+IR group compared to HIIT+IR confirms the cardioprotective effects of HIIT by reducing the size of the infarction. In addition, it demonstrates effects from one week of HIIT to 8 weeks.

3.4.2. Cardiovascular Parameters

Analysis of final body weight showed a significant increase in the HIIT+IR groups (Y Ex and O Ex), Heart weight, left ventricular weight in relation to body weight (LV/BW) also showed a significant increase. Regarding echocardiographic parameters, ejection fraction (EF) and shortening fraction (FS) showed a significant increase after the HIIT protocol. Concomitantly, the diastolic and systolic diameters of the left ventricle (LVIDd and LVIDs) were reduced, indicating a positive ventricular remodeling and greater efficiency in the ejection of blood volume.

The stabilization of hemodynamic parameters, such as dp/dt max and sd/dt min, during the reperfusion period suggests a protective effect of HIIT against the deleterious changes of RI. In addition, left ventricular systolic pressure (PVSL) and heart rate (HR)

showed a tendency to return to baseline levels, suggesting a better autonomic adaptation to intense physical exertion. Also, the drop in the ventricular ectopy score in the trained groups (Y Ex and O Ex) reinforces the role of HIIT in reducing myocardial electrical instability after RI. Similarly, stroke volume (SV) and cardiac output (CO) were increased, corroborating the efficiency of training in improving global cardiac function.

The results showed a significant increase in VO2max and maximum running speed (Y Ex and O Ex), indicating a substantial improvement in aerobic capacity of the HIIT+IR group compared CTRL+IR. (**Table 2)**

3.4.2. Mechanisms: Oxidative stress

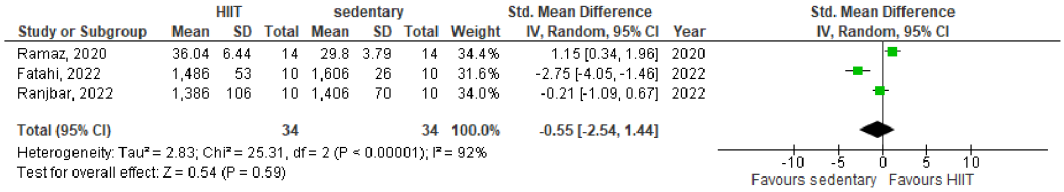
The activity of antioxidant enzymes modified in the HIIT+IR group including SOD, CAT and GSH which were increased, three studies analyzed GPx and only one demonstrated an increase. The level of MDA, a marker of lipid peroxidation and cell damage, was reduced in two of the studies. MPO activity, associated with inflammation and tissue damage, remained unchanged. [22,24,25,27]

The production of nitrite (NO^{2-}) and nitrate (NO_3^-) increased, suggesting greater bioavailability of nitric oxide (NO) and possibly improved vascular function. There was an increase in the production of superoxide (O_2^-) indicating greater mitochondrial activity and response to oxidative stress. [23,26]

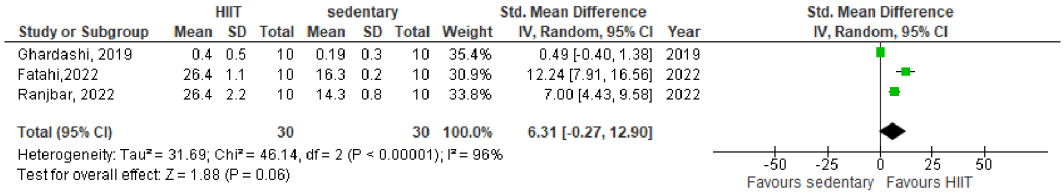
3.4.5. Meta-analysis of oxidative stress

Regarding oxidative stress markers, four studies presented similar parameters. (**Figure 4**). GPX [22,25,27]; GSH [22,24,25], SOD [27,24]; CAT [22,25,27], MDA [22,24,25,27]; MPO [22,25]. HIIT modified SOD levels (SMD = 1.88, 95% CI [0.48 to 3.28], I^2=71% $p < 0.01$; **Figure 4.c**) and CAT (SMD = 3.47, 95% CI [0.52, 6.41] I^2=92%; $p < 0.01$; **Figure 4.d**) compared with a control group, demonstrating cardio protection from exercise after IR. In the other parameters did not differ between the groups

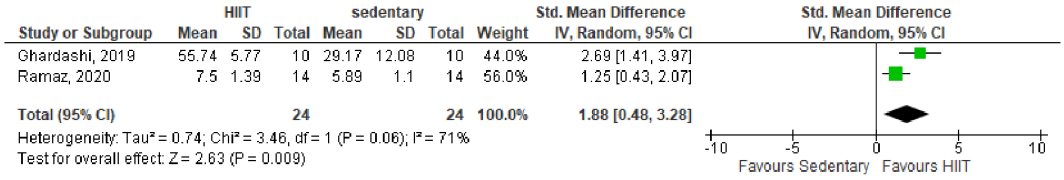
a) GPx



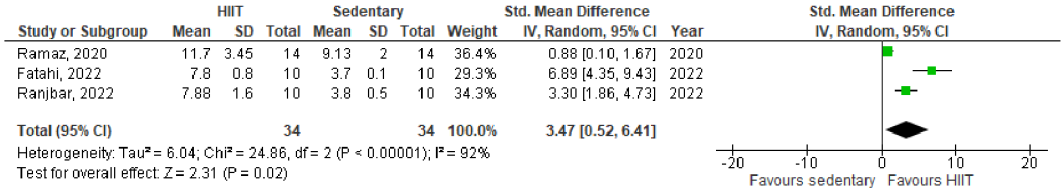
b) GSH



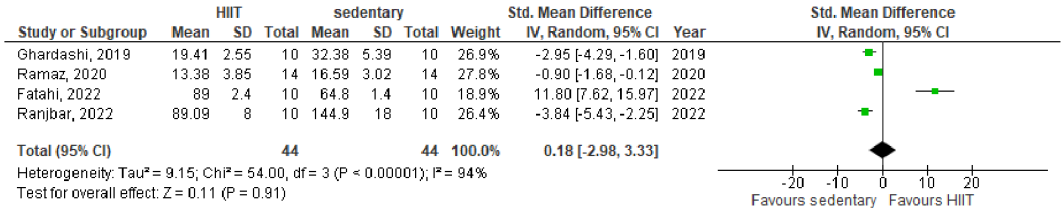
c) SOD



d) CAT



e) MDA



f) MPO

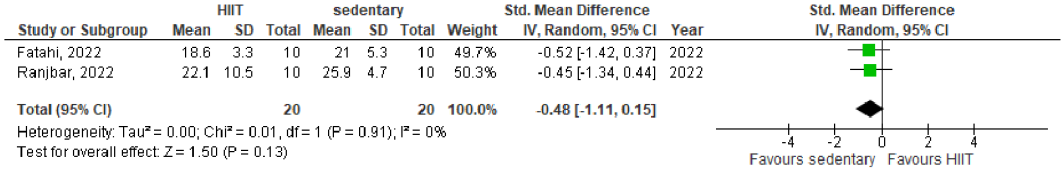


Fig. 4. Forest plot of ischemia-stress oxidative reperfusion in the HIIT group versus control group. a) GPx Glutathione peroxidase, b) GSH Glutathione reduced, c) SOD: Superoxide dismutase d) CAT Catalase, e) MDA Malondialdehyde, f) MPO Myeloperoxidase

4.2 DISCUSSION

This was the first systematic preclinical study to investigate the effects of HIIT on myocardial ischemia-reperfusion injury, including a meta-analysis of oxidative stress. The main contribution of this work is to highlight the potential of HIIT applied before the ischemic event as an effective preventive strategy, which represents a significant advance in the field. Although there are several studies on physical exercise and myocardial ischemia, most focus on conventional exercise protocols, and investigations on the effects of high-intensity training are still scarce. This study, therefore, fills an important gap by highlighting the role of HIIT in cardiovascular protection, opening new possibilities for the preventive application of this type of training in clinical and experimental contexts.

Depending on the length of ischemia, different degrees of heart damage caused by IR can arise. The process of temporary interruption of blood flow to a tissue or organ, followed by its restoration is directly related to the severity of the functional impairment of the myocardium and structural damage. [27,30] The most severe damage from ischemia occurs when the ischemia period exceeds 20 minutes, which can lead to cell death by apoptosis and necrosis [31]. The effects of HIIT in reducing myocardial damage were evidenced by a significant decrease in infarct size, as reported in previous studies (Ramez et al., 2020; Fallahi et al., 2015; Ghardashi et al., 2019; Fatahi et al., 2022; Ranjbar, 2022). In addition, a reduction in the levels of cardiac enzymes, such as CK-MB, LDH and cTnI, was observed, suggesting a lower degree of myocardial cell injury and greater resistance of the heart to ischemic events. These biomarkers are widely used to assess the extent of myocardial damage after an episode of ischemia and reperfusion, and their reduction indicates that HIIT may have a significant protective role. The reduction in troponin I (cTnI), for example, is especially relevant, as it is directly associated with the degree of myocardial necrosis and prognosis after a heart attack.[27]

Structural adaptations are essential to improve cardiorespiratory efficiency and support the increased metabolic demand imposed after HIIT+IR, such as training-induced physiological cardiac remodeling.[24] These findings suggest that HIIT promotes an improvement in the use of oxygen by the tissues, which is essential during

the reperfusion period. [2,22,30]. A systematic review recently revealed that trained rats submitted to ischemia and reperfusion had a smaller infarct area, higher ejection fraction and more pronounced physiological hypertrophy compared to sedentary animals submitted to the same protocol.[32] The relationship between aerobic exercise and the reduction of ischemic damage to the cardiovascular system should be further explored, not only as a therapeutic strategy, but also as a preventive approach.

In our meta-analysis, it was shown that HIIT exerts a significant influence on the reduction of oxidative stress, with a increase in the levels of antioxidant enzymes SOD and CAT. During RI, a significant imbalance occurs in the redox system, leading to excessive production of ROS. HIIT, in turn, imposes additional oxidative stress, further exacerbating ROS production. In response to this acute increase in ROS, the body intensifies the expression and activity of the antioxidant enzymes SOD and CAT, as a defense mechanism to neutralize excess free radicals and minimize cell damage. This phenomenon is consistent with studies that have demonstrated a transient increase in antioxidant enzymes after high-intensity exercise [33] In addition, research on IR injury has observed a similar increase in antioxidant enzyme expression as a response to reperfusion-induced oxidative stress [34] Therefore, the increase in SOD and CAT in rats submitted to IR+HIIT reflects a cellular defense response to acute oxidative stress, seeking to restore the redox balance and protect the myocardium against oxidative damage.

The combination of IR and HIIT in rats may trigger an increase in the antioxidant enzymes SOD and CAT due to the complex interplay between acute oxidative stress and adaptive response. Enhancement of the endogenous antioxidant system, improvement of mitochondrial function, and modulation of the inflammatory response are likely mechanisms that justify these effects. In addition, indicators such as TAC, SOD, CAT, and GPx of the myocardium increased after HIIT, and a lower increase in MDA levels and lipid peroxidation may be associated with a decrease in myocardial necroptosis [22]; and together with the increase in Klotho indicate a defensive role of HIIT against IR injury and attenuation of oxidative stress.[24]

Like our meta-analysis in a review, Veiga et al. (2023) [32] analyzed animal models submitted to IR, comparing several from exercise and sedentary groups. The results indicated an increase in antioxidant capacity due to the upregulation of SOD, CAT and overexpression of HSP70. As well as in the precursor of nitric oxide (NO), in

addition to an increase in the activity of Mn-SOD, which attenuates oxidative modifications in calcium handling proteins and reduces cell death of cardiomyocytes. HIIT even acts as a preconditioning mechanism, protecting the myocardium from immediate damage and regulating pro-inflammatory cytokines, mitochondrial performance, and contractile function.

Exercise intensity plays a key role in modulating antioxidant activity. Song et al. (2023) [21] showed that only high-intensity exercise was able to significantly increase SOD activity after IR injury, while moderate or low-intensity exercise had no significant effects. These results suggest that HIIT may be a promising therapeutic approach to mitigate tissue damage associated with oxidative stress, although factors such as exercise duration and health condition also influence outcomes.

Adaptation to the transient release of ROS during exercise enhances antioxidant capacity. While some studies indicate an increase in the levels of CAT and glutathione peroxidase, others have not detected changes in these indexes. However, there is greater consensus on the stimulation of SOD, particularly its mitochondrial isoform Mn-SOD, in response to HIIT.

During ischemia, the heart intensifies NO biosynthesis as a defensive mechanism. Exercise-induced shear stress stimulates the release of eNOS, a crucial mediator in IR injury cardio protection. Rankovic et al. (2021) [25] observed that four weeks of HIIT resulted in higher levels of NO-2, O-2, and H2O2 in the HIIT+IR group compared to the control group. This phenomenon occurs due to the increased ability of the heart to convert nitrite to NO, contributing to protection against oxidative damage. In the review by Heusch et al.,2020 [35], the authors demonstrated how prolonged exercise acts on nitric oxygen and contractility, bringing benefits to these parameters in the heart after IR.

Despite advances in the understanding of exercise-induced antioxidant mechanisms, there are still disagreements about the specific impact on the activity of myocardial antioxidant agents. Future studies should consider variables such as intensity, duration, and clinical conditions to optimize the cardioprotective potential of HIIT. However, HIIT represents a potent intervention in promoting cardiovascular health, especially in scenarios of high oxidative stress such as IR.

The present research offers significant contributions by systematically compiling and analyzing the effects of HIIT training on oxidative stress biomarkers in animal models submitted to myocardial ischemia-reperfusion injury. The literature indicates that HIIT, compared to sedentary groups or those undergoing other types of training, promotes a consistent improvement in the activity of antioxidant enzymes such as SOD and catalase, in addition to reducing lipid peroxidation, evidenced by lower levels of MDA [36,37]. However, it is important to recognize that differences in the intensity and duration of the HIIT protocols used in the included studies may decisively influence these outcomes. For example, studies with shorter but higher-intensity sessions showed more robust effects on elevating antioxidant capacity [38], while longer protocols with insufficient pauses between sprints resulted in less benefit or even worsening oxidative damage [39]. These findings highlight the importance of standardization and accurate characterization of the HIIT protocol for better interpretation of the results and their subsequent clinical translation. Thus, this meta-analysis not only reinforces the protective potential of HIIT against the deleterious effects of oxidative stress in ischemic events but also highlights the need for greater methodological rigor in preclinical studies.

The rigorous application of the CAMARADES checklist allowed for a critical and transparent analysis of the reliability of the data, highlighting the importance of methodological improvements in future preclinical studies to ensure greater external validity and translational relevance. We can take the results of these studies with caution, but not fully rely on them as conclusive evidence. The presence of selection bias (lack of randomization), measurement bias (no blinding in the evaluation of outcomes), and absence of sample size calculation in most studies compromises internal validity, i.e., the assurance that the results reflect the effects of the intervention studied, and no other external factors. The two studies with low risk of bias (Fatahi, 2022; Ghardashi, 2019) have higher reliability, but still represent a limited fraction of the total sample. The other studies, especially the ones with a high risk of bias (Rankovic, 2021), should be interpreted with greater caution. Therefore, the findings may suggest promising trends, but the methodological basis is insufficient to ensure robust scientific confidence. Ideally, future studies should apply more rigorous designs to strengthen the reliability of the evidence.

4.3. Limitations

Few articles had parameters of molecular analysis and oxidative stress to perform the meta-analysis, taking a small number of samples for comparison. The difference between the methods of articles to induce IR may also be a limitation to compare the results. In addition, animal experiments differ the process of cardiovascular disease, ischemic heart disease in humans is a complex disease, which is usually caused by a combination of several vascular risk factors and comorbidities (e.g. diabetes, heart failure, hypertension, altered coronary circulation, and hyperlipidemia) (Borges and Da, 2017). Therefore, with the differences between animal and human models, more stringent measures need to be adopted in the future, in addition to protocols like clinical practice. Because the average quality of the included studies was not high enough, conclusions should be applied with caution.

5. Conclusion

The studies included in this systematic review showed that HIIT plays a significant protective role, contributing to the reduction of infarct size, cardiac enzyme levels, and structural and functional damage to the myocardium. In addition, the meta-analysis demonstrated the positive modulation of the antioxidant enzymes SOD and CAT, indicating that HIIT acts to reduce oxidative stress, which reinforces its potential as a preventive intervention in ischemic events.

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ANEXO 3- NORMAS DA REVISTA INTERNATIONAL JOURNAL OF CARDIOLOGY, HEART & VASCULATURE

ARTIGOS DE REVISÃO

As resenhas devem ter um resumo não estruturado de até 250 palavras. Os autores são incentivados a usar títulos de seção para facilitar a leitura. Eles não têm uma introdução, métodos, resultados ou seções de discussão. Digite espaço duplo. Para obter instruções sobre referências e figuras, consulte a seção sobre manuscritos originais.

Formato de arquivo

Solicitamos que você forneça arquivos de origem editáveis para todo o seu envio (incluindo figuras, tabelas e gráficos de texto). Algumas diretrizes:

- Salve arquivos em um formato editável, usando a extensão .doc/.docx para arquivos do Word e .tex para arquivos LaTeX. Um PDF não é um arquivo de origem aceitável.
- Disponha o texto em um formato de coluna única.
- Remova qualquer texto tachado e sublinhado do seu manuscrito, a menos que tenha significado científico relacionado ao seu artigo.
- Use as funções de verificação ortográfica e gramatical para evitar erros.

Abstrair

Você deve fornecer um resumo conciso e factual que não exceda 250 palavras. O resumo deve indicar brevemente o objetivo de sua pesquisa, principais resultados e principais conclusões. Algumas diretrizes:

- Os resumos devem ser independentes, pois os resumos geralmente são apresentados separadamente do artigo.
- Evite referências. Se algum for essencial incluir, certifique-se de citar o(s) autor(es) e o(s) ano(s).
- Evite abreviações fora do padrão ou incomuns. Se algum for essencial para incluir, certifique-se de que seja definido em seu resumo na primeira menção.

Keywords

Você deve fornecer de 1 a 7 palavras-chave para fins de indexação. As palavras-chave devem ser escritas em inglês. Tente evitar palavras-chave que consistem em várias palavras (usando "e" ou "de").

Recomendamos que você use abreviações apenas em palavras-chave se elas estiverem firmemente estabelecidas no campo.

Destaques

Você é encorajado a fornecer destaques do artigo no envio.

Os destaques são uma pequena coleção de marcadores que devem capturar os novos resultados de sua pesquisa, bem como quaisquer novos métodos usados durante seu estudo. Os destaques ajudarão a aumentar a descoberta do seu artigo por meio dos mecanismos de pesquisa. Algumas diretrizes:

- Envie os destaques como um arquivo editável separado no sistema de envio on-line com a palavra "destaques" incluída no nome do arquivo.
- Os destaques devem consistir em 3 a 5 marcadores, cada um com no máximo 85 caracteres, incluindo espaços.

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Você é encorajado a fornecer um resumo gráfico no momento da submissão.

O resumo gráfico deve resumir o conteúdo do seu artigo de forma concisa e pictórica, projetada para capturar a atenção de um público amplo. Um resumo gráfico ajudará a chamar mais atenção para o seu artigo online e ajudará os leitores a digerir sua pesquisa. Algumas diretrizes:

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- Nossos tipos de arquivo preferidos para resumos gráficos são arquivos TIFF, EPS, PDF ou MS Office.

Recomendamos que você veja exemplos de resumos gráficos e leia sobre os benefícios de incluí-los.

Unidades, códigos de classificação e nomenclatura

Esta revista exige que você use o sistema internacional de unidades (SI), que segue regras e convenções internacionalmente aceitas. Se outras unidades forem mencionadas em seu artigo, você deve fornecer a unidade equivalente em SI.

Tabelas

As tabelas devem ser enviadas como texto editável, não como imagens. Algumas diretrizes:

- Coloque as tabelas ao lado do texto relevante ou em uma página separada no final do artigo.
- Cite todas as tabelas no texto do manuscrito.
- Numere tabelas consecutivamente de acordo com sua aparência no texto.
- Por favor, forneça legendas junto com as tabelas.
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- Evite regras verticais e sombreamento nas células da tabela.

Recomendamos que você use tabelas com moderação, garantindo que todos os dados apresentados nas tabelas não estejam duplicando os resultados descritos em outras partes do artigo.

Figuras, imagens e obras de arte

Figuras, imagens, obras de arte, diagramas e outras mídias gráficas devem ser fornecidas como arquivos separados junto com o manuscrito. Recomendamos que você leia nossas instruções detalhadas de arte e mídia. Alguns trechos:

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- Por favor, forneça legendas para todas as figuras, imagens e obras de arte.
- Os gráficos de texto podem ser incorporados ao texto na posição apropriada. Se você estiver trabalhando com LaTeX, gráficos de texto também podem ser incorporados ao arquivo.