PRÓ-REITORIA DE PESQUISA E PÓS GRADUAÇÃO
MESTRADO EM CIÊNCIA ANIMAL

ISABELLE TIBURCIO PECIN FERREIRA

# INFLUÊNCIA DO TREINAMENTO AERÓBICO E RESISTIDO NA SÍNDROME METABÓLICA, DESEMPENHO FÍSICO, ESTRESSE OXIDATIVO E COMPONENTES DA MATRIZ EXTRACELULAR MUSCULAR: ESTUDO PRÉ CLÍNICO

Presidente Prudente - SP
2025

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Dissertação apresentada à Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciência Animal – Área de concentração: Fisiopatologia Animal.

Orientadora
Prof.ª Dr.ª Francis Lopes Pacagnelli

Presidente Prudente - SP
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**ISABELLE TIBURCIO PECIN FERREIRA**

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Presidente Prudente, 16 de outubro de 2025.

## BANCA EXAMINADORA

Orientador: Prof.ª Dr.ª Francis Lopes Pacagnelli
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Presidente Prudente - SP

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## DEDICATÓRIA

Dedico este trabalho primeiramente a Deus, pela vida, pela força nos momentos de incerteza e pela sabedoria para perseverar diante dos desafios.

Ao meu esposo, pelo amor, paciência e apoio durante toda esta jornada, compreendendo as ausências e celebrando cada conquista comigo.

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*“Suba o primeiro degrau com fé. Não é necessário que você veja toda a escada. Apenas dê o primeiro passo.” (Martin Luther King Jr.)*

## IMPACTO POTENCIAL DESSA PESQUISA

A síndrome metabólica (SM) representa um dos principais desafios de saúde pública do século XXI, caracterizada pela associação entre obesidade, resistência à insulina, hipertensão arterial e dislipidemia. Sua elevada prevalência está diretamente relacionada ao aumento das doenças cardiovasculares e ao impacto socioeconômico decorrente da redução da qualidade de vida e dos custos com saúde.

O exercício físico é reconhecido como uma intervenção não farmacológica de baixo custo e alta eficácia para prevenção e tratamento da SM, mas ainda há lacunas quanto à compreensão de seus efeitos moleculares sobre os mecanismos de estresse oxidativo e remodelação da matriz extracelular muscular. Assim, este estudo contribui para identificar de que forma diferentes modalidades de exercício (aeróbico e resistido) podem modular essas alterações, gerando evidências que subsidiam políticas públicas de promoção da saúde e estratégias preventivas, especialmente em populações de maior risco cardiovascular.

Além do avanço científico, esta pesquisa também possui impacto social e educacional, pois fortalece a formação de recursos humanos no ambiente acadêmico, amplia a disseminação de informações baseadas em evidências e incentiva a adoção de estilos de vida mais saudáveis. Ao utilizar metodologias de análise molecular, o estudo contribui para o fortalecimento da capacidade de inovação científica e tecnológica na área da pesquisa em saúde. Dessa forma, os resultados obtidos podem orientar práticas clínicas, subsidiar políticas públicas e fomentar parcerias institucionais, reforçando o alinhamento da pesquisa com a agenda global de desenvolvimento sustentável, em especial com o objetivo de assegurar saúde e bem-estar para todos.

## RESUMO

### Influência do treinamento aeróbico e resistido na síndrome metabólica, desempenho físico, estresse oxidativo e componentes da matriz extracelular muscular: estudo pré clínico

**Introdução:** A síndrome metabólica (SM) marcada por obesidade, resistência à insulina, hipertensão arterial e dislipidemia, pode ocasionar alterações musculares, estresse oxidativo (EO) e desorganização da matriz extracelular (MEC), com prejuízos funcionais. O exercício físico é indicado como tratamento, entretanto, a modalidade mais eficaz ainda precisa ser definida. **Objetivo:** Investigar o impacto de duas modalidades de exercícios nos componentes da SM e sua influência sobre o músculo esquelético, avaliando os biomarcadores de EO e os componentes da MEC. **Métodos:** Ratos Wistar (n=40) foram distribuídos em dois grupos: dieta controle (C) ou dieta rica em gordura (SM) por 20 semanas. Após caracterização da SM, os animais foram redistribuídos em quatro grupos (n=10): controle (C), SM sedentário (SM), SM+exercício aeróbico (SM+EFA) e SM+exercício resistido (SM+EFR), com 8 semanas de intervenção, 5 dias por semana. EFA: 80%8'+20%2', 30–60 min. EFR: 50/75/90/100%, 4 subidas. Foram avaliados ingestão alimentar, ganho de peso, perfil bioquímico, capacidade funcional, marcadores de EO: carbonilação, malondialdeído (MDA), superóxido dismutase (SOD), catalase (CAT), e expressão gênica dos componentes da MEC (colágenos e metaloproteinases) no músculo sóleo e EDL. A análise estatística utilizou testes paramétricos: t de Student, ANOVA/Tukey, MANOVA/Bonferroni ou não paramétricos: Kruskal-Wallis/Dunn, ($p<0.05$). **Resultados:** SM foi confirmada pois apresentou maior peso, glicemia, pressão arterial e triglicerídeos ($p<0.05$). A SM reduziu a capacidade funcional e as duas modalidades de exercícios melhoraram o desempenho físico entre os momentos avaliados pré e pós-treinamento. Os grupos submetidos aos dois tipos de treino reduziram peso, glicemia e pressão, enquanto somente o aeróbico reduziu % de adiposidade e triglicerídeos. Os protocolos reduziram o MDA e não possuíram alteração na carbonilação para os dois músculos avaliados, aumentaram os níveis de SOD nas duas modalidades de exercício no músculo sóleo, sem alteração para o EDL e ambos os músculos tiveram aumento de CAT somente no exercício resistido. Não houve mudanças na expressão gênica dos músculos. **Conclusão:** Ambas as

modalidades de exercício atenuaram os efeitos da SM nos músculos, o exercício aeróbico promoveu maior benefício na capacidade funcional e o resistido no equilíbrio redox para ambos os músculos.

**Palavras-chave:** Síndrome Metabólica; Músculo Esquelético; Matriz Extracelular; Exercício Aeróbico; Exercício Resistido; Estresse Oxidativo;

## ABSTRACT

### Influence of aerobic and resistance training on metabolic syndrome, physical performance, oxidative stress, and muscle extracellular matrix components: preclinical study

**Introduction:** Metabolic syndrome (MS), characterized by obesity, insulin resistance, hypertension, and dyslipidemia, can cause muscle changes, oxidative stress (OS), and extracellular matrix (ECM) disorganization, with functional impairments. Physical exercise is indicated as a treatment; however, the most effective modality remains to be defined. **Objective:** To investigate the impact of two exercise modalities on the components of MS and their influence on skeletal muscle, evaluating OS biomarkers and ECM components. **Methods:** Wistar rats (n = 40) were distributed into two groups: control diet (C) or high-fat diet (MS) for 20 weeks. After characterization of MS, the animals were redistributed into four groups (n = 10): control (C), sedentary MS (MS), MS + resistance exercise (MS + RE) and MS + aerobic exercise (MS + AE), with an 8-week intervention, 5 days a week. RE: 50/75/90/100%, 4 climbs. AE: 80%8min+20%2min, 30–60 min. Food intake, weight gain, biochemical profile, functional capacity, OS markers: carbonylation, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and gene expression of ECM components (collagens and metalloproteinases) in the soleus and EDL muscles were evaluated. Statistical analysis used parametric tests: Student's t, ANOVA/Tukey, MANOVA/Bonferroni or nonparametric: Kruskal-Wallis/Dunn, ($p<0.05$). **Results:** MS was confirmed because it presented higher weight, blood glucose, blood pressure and triglycerides ($p<0.05$). MS reduced functional capacity and both exercise modalities improved physical performance between the pre- and post-training moments. The groups undergoing both types of training reduced weight, blood glucose, and blood pressure, while only aerobic exercise reduced % adiposity and triglycerides. The protocols reduced MDA and showed no change in carbonylation for both muscles evaluated. SOD levels increased in both exercise modalities in the soleus muscle, with no change in the EDL, and both muscles increased CAT only during resistance exercise. There were no changes in muscle gene expression. **Conclusion:** Both exercise modalities attenuated the effects of MS on muscles;

aerobic exercise promoted greater benefits in functional capacity, and resistance exercise in redox balance for both muscles.

**Keywords:** Metabolic Syndrome; Skeletal Muscle; Extracellular Matrix; Aerobic Exercise; Resistance Exercise; Oxidative Stress.

## LISTA DE SIGLAS

| | |
|---|---|
| **ANOVA** | Analysis of Variance |
| **CAT** | Catalase |
| **cDNA** | Complementary DNA |
| **CEUA** | Ethics Committee on Animal Use |
| **CT** | Cycle Threshold |
| **DNAse** | Deoxyribonuclease |
| **DNPH** | 2,4-Dinitrophenylhydrazine |
| **EDTA** | Ethylenediaminetetraacetic acid |
| **RE** | Resistance Exercise |
| **AE** | Aerobic Exercise |
| **EO** | Oxidative Stress |
| **EROS** | Reactive Oxygen Species |
| **HAS** | Systemic Arterial Hypertension |
| **HSF** | High Sugar-Fat Diet |
| **BMI** | Body Mass Index |
| **MDA** | Malondialdehyde |
| **ECM** | Extracellular Matrix |
| **MMP-2** | Metalloproteinase 2 |
| **WHO** | World Health Organization |
| **SBP** | Systolic Blood Pressure |
| **PBS** | Phosphate-Saline Buffer |
| **PCR** | Polymerase Chain Reaction |
| **qPCR** | Real-Time PCR (quantitative) |
| **RNA** | Ribonucleic Acid |
| **SOD** | Superoxide Dismutase |
| **MS** | Metabolic Syndrome |
| **TCM** | Maximum Load Test |
| **TBARS** | Thiobarbituric Acid Reactive Substances |
| **°C** | Degrees Celsius |
| **cm** | Centimeter |

| | |
|---|---|
| **g** | Gram |
| **g/kg** | Gram per kilogram |
| **g/day** | Gram per day |
| **Kcal** | Kilocalorie |
| **Kcal/day** | Kilocalories per day |
| **kg** | Kilogram |
| **L** | Liter |
| **m** | Meter |
| **m/min** | Meters per minute |
| **mg** | Milligram |
| **mg/dL** | Milligrams per deciliter |
| **mg/kg** | Milligrams per kilogram |
| **mg/mL** | Milligrams per milliliter |
| **mL** | Milliliter |
| **mmHg** | Millimeter of mercury |
| **min** | Minute |
| **ng** | Nanogram |
| **nm** | Nanometer |
| **nmol/mg** | Nanomole per milligram |
| **pmol/min/mg** | Picomole per minute per milligram |
| **rpm** | Rotations per minute |
| **U/mg** | Unit per milligram |
| **µL** | Microliter |

**SUMÁRIO**

## 1 ARTIGO CIENTÍFICO

# Resistance and Aerobic Training Alleviate Oxidative Stress Without Altering Skeletal Muscle Collagen and Metalloproteinase Gene Expression in Rats With Metabolic Syndrome

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## ABSTRACT

*Aim*: This study aimed to investigate the effects of resistance exercise (RE) and aerobic exercise (AE) on different skeletal muscles, with emphasis on oxidative stress biomarkers and the extracellular matrix in rats with metabolic syndrome (MS).
*Methods*: Forty Wistar rats were fed for 20 weeks: control diet (C, n=10) or high-calorie diet (HSF, n=30). After confirmation of MS, they were distributed into three groups (n=10): MS, MS+RE and MS+AE for interventions: eight weeks, 5 days/week. Protocols: RE (50/75/90/100% of maximum load; four climbs/session) and AE (80% 8min + 20% 2min; 30–60 min/session). Food intake, weight, functional capacity, were evaluated. Markers of oxidative stress (protein carbonylation, lipid peroxidation-MDA, superoxide dismutase-SOD and catalase-CAT) and expression of collagens and metalloproteinases were assessed in the soleus and extensor digitorum longus (EDL) muscles.
*Key findings*: The MS was associated to increased weight, blood glucose, blood pressure, and triglycerides. RE and AE improved physical performance and reduced weight, blood glucose and blood pressure; only AE decreased triglycerides and adiposity. A reduction in MDA was observed without changes in carbonylation in both muscles and exercise modalities; RE and AE increased SOD in the soleus, while CAT was elevated in RE in both muscles. Gene expression of collagens and metalloproteinases did not change in both muscles.
*Significance:* Our results indicate that RE and AE are effective non-pharmacologic strategies to improve MS and biomarkers of lipid peroxidation (MDA) in soleus and EDL muscles, without affecting gene expression of collagens and metalloproteinases.


*Keywords:* Metabolic Syndrome; Skeletal Muscle; Extracellular Matrix; Resistance Exercise; Aerobic Exercise; Oxidative Stress;

## INTRODUCTION

Metabolic syndrome (MS) is a serious complex metabolic disorder characterized by obesity, insulin resistance, high blood pressure (HBP), and dyslipidemia [1]. Globally, the average prevalence is 31.8% in the adult population [2], affecting billions of people [3] and potentially leading to diabetes mellitus, certain types of cancer, and cardiovascular diseases [4,5]. Changes in skeletal muscle may occur as a result of MS, which are reflected in atrophy, changes in fiber types, increased oxidative stress, and changes in the extracellular matrix (ECM) [6].

One of the mechanisms associated with muscle dysfunction is oxidative stress (OS) [7]. Under physiological conditions, there is a redox balance in which the production of reactive oxygen species (ROS) is controlled by the action of antioxidant enzymes. However, when ROS production exceeds the capacity of antioxidant defenses, a redox imbalance occurs that is harmful to tissue. Increased ROS production affects cellular function, leading to the oxidation of biomolecules and generating a cycle of tissue damage [8]. One of the targets of ROS is the ECM, which is mainly composed of collagens and metalloproteinases. These components are fundamental in the transmission of force, structural maintenance, and adaptation to the environment [9-12]. Changes in the ECM are accompanied by an increase in collagen and alterations in cell-matrix communication [13]. These changes impair the function and structure of skeletal muscle, cause limitations to exercise, and are associated with increased mortality in people with MS [14-16].

Among the treatments proposed for MS, exercise is widely recommended by the World Health Organization and recent guidelines [17-18] and promotes control of cardiovascular risk factors with weight reduction, improved insulin resistance, reduced blood pressure, and dyslipidemia [19,20]. Exercise can promote adaptations

in skeletal muscle, which include improvement in OS, with reduction of biomarkers and increase in antioxidant enzymes, changes in ECM [21] with an increase in collagen [22], which contributes to functional improvement and prevention of fibrosis. Both aerobic and resistance exercise induce changes in the expression of ECM-related genes [23], with benefits in muscle regeneration and function. Thus, these types of exercise are prescribed for MS, however, the type of exercise with the best impact on these muscle conditions needs to be elucidated.

This approach is essential for directing clinical research and implementing low-cost interventions. Aerobic exercise appears to provide greater benefits in OS related to risk factors such as obesity [14], insulin resistance [23], SAH [24], and dyslipidemia [25]. Therefore, we hypothesize that aerobic exercise would be more beneficial for skeletal muscle in the context of MS. Thus, the objective of this study was to investigate the effects of resistance exercise (RE) and aerobic exercise (AE) on different skeletal muscles, with emphasis on oxidative stress biomarkers and the extracellular matrix in rats with metabolic syndrome (MS).

**METHODS**

Forty adults male Wistar rats (Rattus norvegicus - 254 g) were supplied by the UNESP Central Vivarium and housed at the São Paulo State University (UNESP), Experimental Vivarium. The animals were allocated to individual plastic cages, measuring 30 cm × 16 cm × 19 cm, at a controlled temperature (24 ± 2ºC), relative humidity of 55 ± 5%, with inverted cycles of 12 h of light (light period between 7 p.m. and 7 a.m., dark period between 7 a.m. and 7 p.m.).

The animals were randomly distributed into two groups: the control group (C, n=10), which received control feed + water, and the High Sugar-Fat Diet (HSF) group (n=30), which received feed rich in simple carbohydrates and fat. Drinking water was

supplemented with 25% sucrose (Table 1). The animals were weighed daily and their weight was recorded. After this period, the animals underwent *in vivo* analysis of the components of MS (weight, blood glucose, triglycerides, and systolic blood pressure). After confirmation of MS in the HSF group, the animals were redistributed into three groups: metabolic syndrome (MS n=10), MS+resistance exercise (MS+RE n=10), and MS+aerobic exercise (MS+AE n=10). The rats in the MS+RE group underwent resistance exercise, and the animals in the MS+AE group underwent aerobic exercise, both for eight weeks. At the end of the experiment (28 weeks), the animals were euthanized. Blood and muscle samples were collected and stored in a -80°C freezer. The soleus and extensor digitorum longus (EDL) muscles were analyzed for redox balance and ECM components through gene expression. (Figure 1)

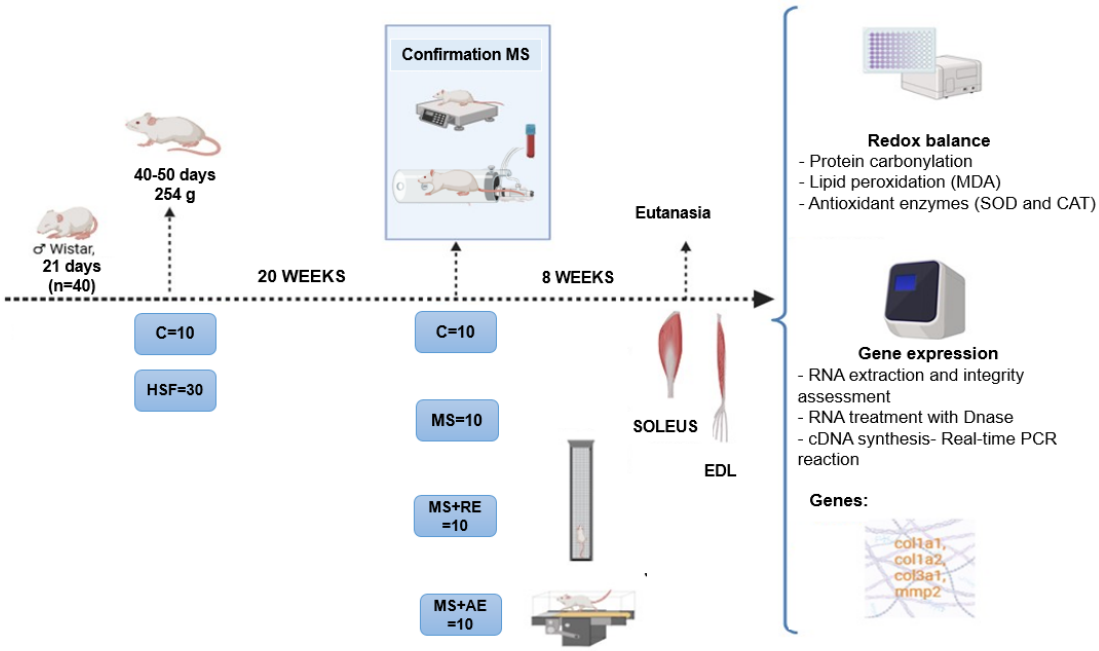


**Figure 1.** Experimental design. C: Control group. HSF: High Sugar-Fat Diet group. MS: Metabolic Syndrome group. RE: Resistance Physical Exercise group. AE: Aerobic Physical Exercise group. Created with BioRender.com.

**Nutritional characterization**

The model chosen for the experiment was a Western diet rich in sugar and fat [24], adapted as shown in Table 1. Feed and water intake was measured daily to obtain the caloric intake (weekly food intake (water and feed) multiplied by the energy value of each diet [g x kcal]) of the animals. The energy calculation included the sucrose intake contained in drinking water (25%) of rats in the MS group. To characterize obesity, final weight and body fat (sum of epididymal, retroperitoneal, and visceral fat deposits obtained at euthanasia) were measured, followed by calculation of the adiposity index (ratio of total body fat/final weight x 100).

**Table 1.** Composition of the HSF diet.

| | **Diets** | |
|---|---|---|
| **Components** | **Control** | **HSF** |
| Soybean meal (g/kg) | 360 | 345 |
| Sorghum (g/kg) | 279 | 75 |
| Soybean hulls (g/kg) | 151 | 113 |
| Dextrin (g/kg) | 157 | 20 |
| Sucrose (g/kg) | - | 80 |
| Fructose (g/kg) | - | 180 |
| Soybean oil (g/kg) | 24 | - |
| Lard (g/kg) | - | 154 |
| Minerals (g/kg) | 25 | 25 |
| Salt (g/kg) | 4 | 8 |
| **Nutritional values** | **Control** | **HSF** |
| Protein (%) | 21.12 | 18.14 |
| Carbohydrate (%) | 60.46 | 53.20 |

| | | |
|---|---|---|
| Fat (%) | 4.20 | 16.49 |
| % Energy from protein | 23.20 | 16.74 |
| % Energy from carbohydrates | 66.42 | 49.02 |
| % Energy from fat | 10.38 | 34.25 |
| Energy (kcal/g) | 3.64 | 4.33 |

HSF: High Sugar-Fat Diet; g: gram; kg: kilogram.

**Characterization of Metabolic Syndrome**

Metabolic syndrome was characterized by weight, blood glucose, systolic blood pressure, and dyslipidemia (measured by triglyceride levels). These data were obtained *in vivo* from the animals. Blood samples for biochemical analysis were collected from the caudal vein from conscious animals after an 8-hour fast.

The collection tubes contained ethylenediaminetetraacetic acid (EDTA) anticoagulant at a ratio of 0.1 mL for every 5 mL of blood. Tubes were centrifuged at 3000 rpm for 10 minutes to obtain plasma and measure glucose and triglyceride concentrations. All these measurements were performed by colorimetric enzymatic method using commercial kits (BioClin®, Belo Horizonte, MG, Brazil) in automated biochemical equipment (BS-200, Mindray, China). Systolic blood pressure (SBP) was measured by the indirect method of tail plethysmography, using an electric sphygmomanometer (NarcoBio-system® model 709-0610; International Biomedical, Inc., USA). After warming the tail in a box heated with lamps, the sensor and cuff were attached around the animal's tail. The cuff was inflated to a pressure of 200 mmHg and then deflated. Arterial pulses were recorded in a computerized data acquisition system, and the results were the average of three recordings.

## Physical training protocol

### Determination of maximum load and acclimatization period

Resistance exercise was performed on a staircase (1 meter high, 0.2 meters wide, 0.5 centimeters between steps, and an 80º incline). To determine the maximum load, the animal was first familiarized with the apparatus, which resulted in three ascents of the staircase. Before the first attempt, the animal was kept in the chamber at the top of the stairs (20x20x20 cm) for 60 seconds to realize that the environment posed no threat. In the first attempt, the animal was placed at the top of the ladder, near the chamber door. In the second attempt, the animal was positioned in the middle of the ladder, and in the third attempt, it was placed at the base of the ladder. Between each attempt, there was a 60-second recovery period with the mouse positioned inside the chamber at the top of the ladder.

The acclimatization period lasted four consecutive days, during which the rats were initially encouraged to climb three steps on the ladder per day without a load, in order to learn the route of the ladder without excessive stress. Subsequently, on the fourth day, the rats climbed four times carrying only the load apparatus, without a load, to acclimate to the sensation of climbing with an object attached to their tails [28]. After the adaptation period, the maximum load test (MLT) was performed in the same week, which required the animals to climb the ladder on the first attempt with a load equivalent to 75% of their weight. Next, the load was increased by 15% of weight until the point which rats were unable to climb the entire ladder. Therefore, the highest load with which the rat was able to climb the entire length of the ladder was considered its maximum capacity. Failure was determined when the rat was unable to progress up the ladder after three successive stimuli on

the tail. MLT was performed before and after the training period for all groups and additionally at weeks 2, 4, 6, and 8 for the RE group to adjust training loads .

**Resistance exercise protocol**

The resistance training protocol used in this study was adapted from Rodrigues et al. [29] and Leite et al. [30] The animals underwent resistance training regimens consisting of four escalating sessions. These sessions involved progressively increasing loads of 50%, 75%, 90%, and 100% of the maximum load capacity, previously established for each rat by MLT, with 1 minute of rest between each climb (Table 2). A one-minute rest period was implemented between each climb, which took place inside the housing chamber located at the top of the staircase. This training protocol was administered over a period of 8 weeks, five days a week—from Monday to Friday.

**Table 2.** Protocol for stair climbing exercise.

| Adaptation Period | | |
|---|---|---|
| Days | Load | Number of Climbs |
| 1º | - | 3 |
| 2º | - | 3 |
| 3º | - | 3 |
| 4º | Loading load equipment | 4 |
| **Resisted Exercise Protocol** | | |
| **Weeks** | **Load (% of maximum load)** | **Number of Climbs** |
| | **Initial maximum load test** | |
| 1ª | 1x 50%, 1x 75%, 1x 90% e 1x 100% | 4 |
| 2ª | 1x 50%, 1x 75%, 1x 90% e 1x 100% | 4 |

| | | |
|---|---|---|
| | **Maximum load test – Intensity adjustment** | |
| 3ª | 1x 50%, 1x 75%, 1x 90% e 1x 100% | 4 |
| 4ª | 1x 50%, 1x 75%, 1x 90% e 1x 100% | 4 |
| | **Maximum load test – Intensity adjustment** | |
| 5ª | 1x 50%, 1x 75%, 1x 90% e 1x 100% | 4 |
| 6ª | 1x 50%, 1x 75%, 1x 90% e 1x 100% | 4 |
| | **Maximum load test – Intensity adjustment** | |
| 7ª | 1x 50%, 1x 75%, 1x 90% e 1x 100% | 4 |
| 8ª | 1x 50%, 1x 75%, 1x 90% e 1x 100% | 4 |
| | **Final test of maximum load carried** | |

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**Aerobic physical capacity and acclimatization period**

Exercise tolerance was assessed by exhaustion speed using a treadmill exercise test (ET) [31]. The ET was conducted on a motorized treadmill for rats (AVS Projects – São Carlos, SP, Brazil) after three days of acclimatization to a low-speed treadmill environment (5 m/min, 5 to 15 min/day). The ET started at 6 m/min and increased by 3 m/min every 3 minutes until exhaustion. Exhaustion was defined as the animal remaining at the rear end of the treadmill for more than 10 seconds. ETs were performed at the beginning and end of the protocol for all groups and additionally during weeks 3 and 5 for the AE group to adjust exercise intensity

**Aerobic exercise protocol**

Aerobic exercise consisted of an interval training protocol, without rest, based on the study by Neves et al. [32] The animals underwent training on a treadmill. An initial adaptation period included a gradual increase in exercise duration, consisting of 30, 40, and 50 minutes during the first, second, and third

weeks, respectively, and 60 minutes from weeks 4 to 8. The running sessions were divided into two intensities: Eight minutes at a speed corresponding to 80% of the exhaustion speed and two minutes at a speed corresponding to 20% of the exhaustion speed. This training regimen was implemented five days a week, Monday through Friday, over eight weeks. (Table 3).

**Table 3.** Protocol for aerobic exercise on a treadmill.

| Acclimatization Period | | |
|---|---|---|
| **Days** | **Speed(m/min)** | **Duration(min)** |
| 1º | 5 | 5 |
| 2º | 5 | 10 |
| 3º | 5 | 15 |
| **Aerobic Exercise Protocol** | | |
| **Weeks** | **% ET + min** | **Duration(min)** |
| | **Initial treadmill test** | |
| 1ª | 80% 8 min e 20% 2 min | 30 |
| 2ª | 80% 8 min e 20% 2 min | 40 |
| 3ª | 80% 8 min e 20% 2 min | 50 |
| | **Treadmill test – Intensity adjustment** | |
| 4ª | 80% 8 min e 20% 2 min | 60 |
| 5ª | 80% 8 min e 20% 2 min | 60 |
| | **Treadmill test – Intensity adjustment** | |
| 6ª | 80% 8 min e 20% 2 min | 60 |
| 7ª | 80% 8 min e 20% 2 min | 60 |
| 8ª | 80% 8 min e 20% 2 min | 60 |
| | **Final treadmill test** | |

ET: treadmill exercise test; min: minutes.

**Preparation of the soleus and EDL muscles**

A 100 mg soleus and EDL muscles samples were weighed and homogenized in 1.0 mL of chilled Phosphate-Buffered Saline (PBS) pH=7.4 using ULTRA-TURRAX® T25 basicIKA® WerkeStaufen/Germany and centrifuged at 800g at 4°C for 10 min. The supernatant was used to perform OE and gene expression analyses.

**Evaluation of OE biomarkers**

**Protein carbonylation**

For carbonylation, 100 µL of tissue supernatant was used for 100 µL of 2.4-dinitrophenylhydrazine (DNPH) (10 mM in 2 M HCl). The samples were incubated for 10 minutes at room temperature, and then 50 µL of sodium hydroxide NaOH (6 M) was added and incubated again for 10 minutes at room temperature. The reading was performed at 450 nanometers (nm) in a Spectra Max 190 microplate reader (Molecular Devices®, Sunnyvale, CA, USA) and the result obtained from the absorbance of the samples and the molar extinction coefficient (22000 $M^{-1}$ $cm^{-1}$). The final result was expressed in nmol/mg of protein [19].

**Lipid Peroxidation (Malondialdehyde - MDA)**

MDA is the result of the degradation of polyunsaturated lipids. Its production is used as a biomarker of lipid peroxidation levels. MDA (250 µL of muscle tissue supernatant) reacts with thiobarbituric acid (TBA), as it is a TBA-reactive substance, in the form of 1:2 MDA-TBA, which is absorbed at 532 nm. Thus, the amount of Thiobarbituric Acid Reactive Substances (TBARS) is proportional to the amount of MDA. The TBARS concentration was calculated using the MDA standard curve and expressed in nmol/mg of protein [19].

**Evaluation of Antioxidant Enzyme Activity (SOD and CAT)**

The activity of antioxidant enzymes was evaluated using superoxide dismutase (SOD) and catalase (CAT) enzymes. SOD activity was measured based on the inhibition of a superoxide radical reaction with pyrogallol, and absorbance values were measured at 420 nm. Values were expressed as units per milligram of protein. CAT activity was evaluated by following the decrease in hydrogen peroxide ($H_2O_2$) levels. Absorbance values were measured at 240 nm. Activity was expressed as pmol of $H_2O_2$ reduced/min/mg of protein [43].

**Evaluation of gene expression in soleus and EDL muscles**

Total RNA was extracted from muscle tissue using TRIzol® (Invitrogen, ThermoFisher Scientific Inc., Carlsbad, California, USA). The concentration of total RNA recovered was measured by spectrophotometry using NanoDrop Lite (Fischer Scientific, ThermoFisher Scientific Inc., Wilmington, Delaware, USA), then treated with Amplification Grade DNAse I (Invitrogen, ThermoFisher Scientific, Foster City, California, USA), following the manufacturer's instructions. The High-Capacity reverse transcription kit (Applied Biosystems, ThermoFisher Scientific, Vilnius, Lithuania) was used for the synthesis of complementary RNA (cDNA) from 1000 nanograms (ng) of total RNA for each sample. Aliquots of cDNA were then subjected to real-time PCR (qPCR) using the following custom assays containing TaqMan probes and primers (Applied Biosystems, Foster City, USA) specific for Rattus norvegicus: type I collagen alpha 1 chain (col1a1, Rn01463848_m1), type I collagen alpha 2 chain (col1a2, Rn06224987_g1), type III collagen alpha 1 chain (col3a1, Rn01437681_m1), and metalloproteinases 2 (mmp2, Rn01538170_m1). Taqman™ Universal Master Mix II (Applied Biosystems) and the QuantStudio system (ThermoScientific) were also used. All samples were analyzed in duplicate. The cycling conditions were 50°C for 2 minutes and 95°C for 10 minutes. This was

followed by 40 cycles of denaturation at 95°C for 15 seconds and final extension at 60°C for 1 minute. Gene expression was quantified relative to the values of group C after normalization by the expression levels of the reference gene cyclophilin74 (Rn00690933_m1) using the 2-ΔΔCt method [33,34].

**Statistical Analysis**

Data were expressed as mean ± standard deviation, median, minimum, and maximum values. Data normality was assessed using the Shapiro-Wilk test. The unpaired t-test was used to compare the development of metabolic syndrome. Performance was assessed using multivariate analysis of variance (MANOVA) for repeated measures, followed by Bonferroni post hoc correction when appropriate.

Group comparisons for clinical and laboratory parameters, oxidative stress, and gene expression were performed using ANOVA followed by Tukey's post hoc test for parametric data, or the Kruskal–Wallis test followed by Dunn's post hoc test for nonparametric data. P <0.05 was considered statistically significant. All analyses were performed using GraphPad Prism®, version 9.0 (GraphPad software, La Jolla, CA, USA).

**RESULTS**

At 20 weeks, nutritional characterization (Table 4) showed that animals in the MS group had lower daily food intake compared with the control group ($p < 0.001$)

Despite lower consumption in grams, caloric intake was significantly higher in the MS group compared to the control ($p<0.001$). Initial weight was similar between groups ($p=0.882$).

**Table 4.** Comparison of food intake, caloric intake, and weight gain between groups over 20 weeks.

| Variables | Groups | | |
|---|---|---|---|
| | **Control** | **MS** | ***p*** |
| **Food Intake (g/day)** | 25.1 ± 2.4 | 11.8 ± 1.7 | <0.001* |
| **Caloric Intake (kcal/day)** | 90.2 ± 8.7 | 110.7 ± 7.7 | <0.001* |
| **Initial Weight (g)** | 254 ± 22 | 253 ± 33 | 0.882 |

Nutritional analysis and weights. g = grams; Kcal = kilocalories; Data are expressed as mean ± standard deviation. Student's t-test for independent samples. [p < 0.05]*

There was an increase in triglycerides, systolic blood pressure, final weight, and blood glucose, characterizing MS at 20 weeks (Figure 2).

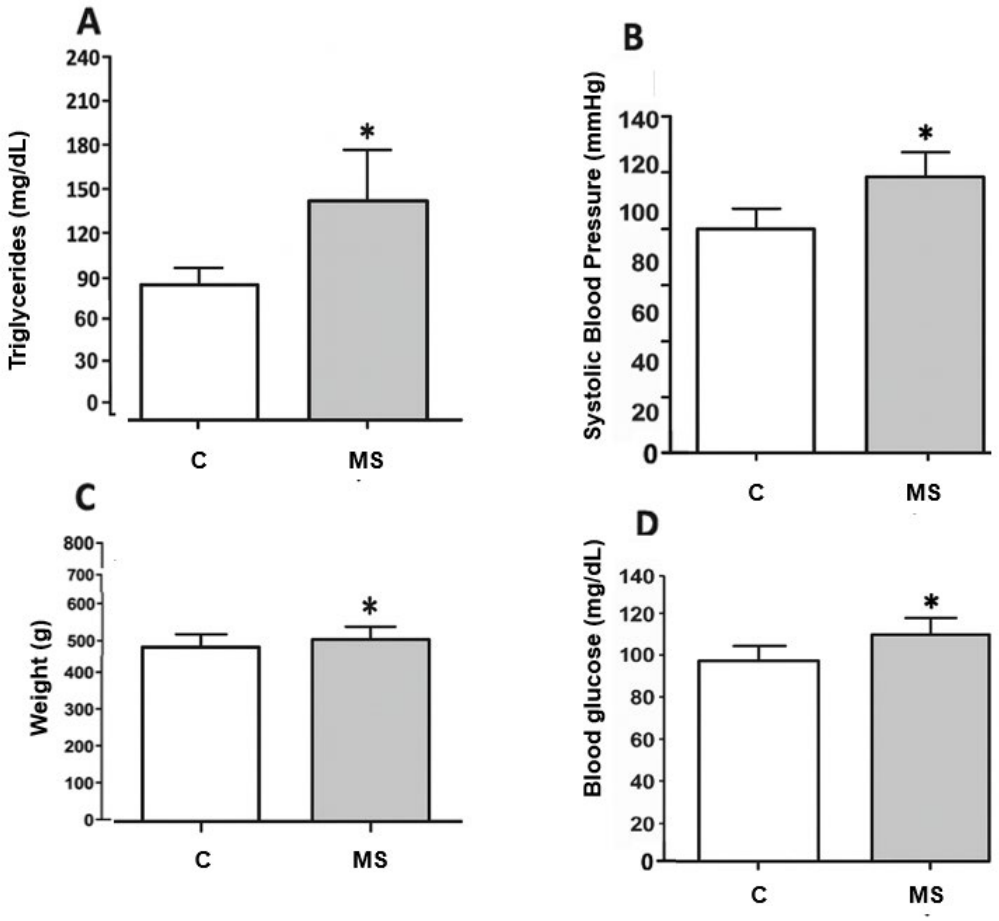


**Figure 2.** Characterization of the experimental MS model at 20 weeks. (A) Triglycerides. (B) Systolic Blood Pressure. (C) Weight. (D) Blood glucose. C=Control,

n=10; MS=Metabolic Syndrome, n=30. Data are expressed as mean ± standard deviation. Student's t-test for independent samples. [p<0.05] *statistically significant difference.

**Physical Performance**

MS reduced functional capacity, as demonstrated by the reduction in exhaustion speed prior to exercise. After eight weeks of aerobic exercise on the treadmill, this functional capacity increased by 110% (Figure 3A). Muscle resistance was not altered by MS, as evidenced at pre-exercise. However, resistance training increased muscle resistance at the end of the protocol by approximately 79% (Figure 3B).

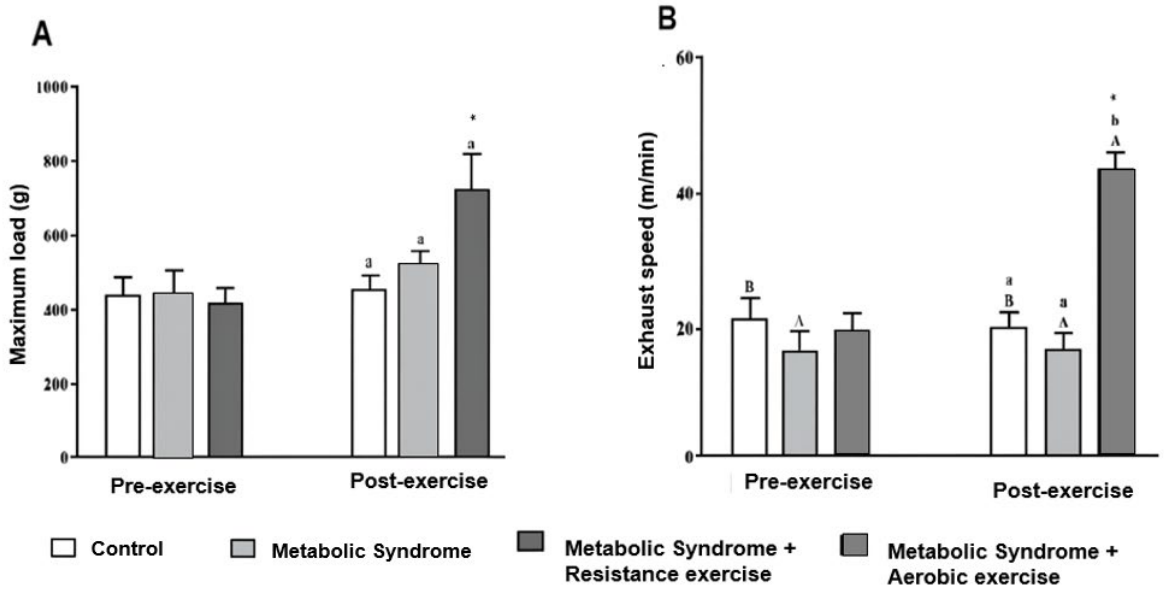


**Figure 3.** Exhaustion speed assessed by treadmill stress test (A) and maximum load test (B) before and after exercise. Two consecutive means with different lowercase letters (a/b) differ in terms of exercise, diet, and time of assessment. Two means followed by a distinct capital letter (A/B) differ in terms of diet, fixed exercise types, and time of assessment. *Indicates statistical difference in terms of time of

assessment, fixed diet, and exercise types. C=Control, n=10; MS=Metabolic Syndrome, n=10; MS+RE=Metabolic Syndrome+Resistance exercise, n=10; MS+AE=Metabolic Syndrome+Aerobic exercise, n=10. Data presented as mean ± standard deviation; multivariate analysis of variance (MANOVA) for repeated measures and Bonferroni. [p<0.05].

**Effects of training on clinical and laboratory parameters**

Aerobic and resistance exercises promoted a reduction in weight compared to the sedentary MS group (p<0.05) (Figure 4A). The adiposity index decreased only with aerobic exercise (p<0.05) (Figure 4B). Both exercise programs resulted in a decrease in blood glucose (p<0.05) (Figure 4C). In addition, there was a decrease in triglycerides only after aerobic training (p<0.05) (Figure 4D). A decrease in systolic blood pressure was also observed after both types of exercise (p<0.05) (Figure 4E).

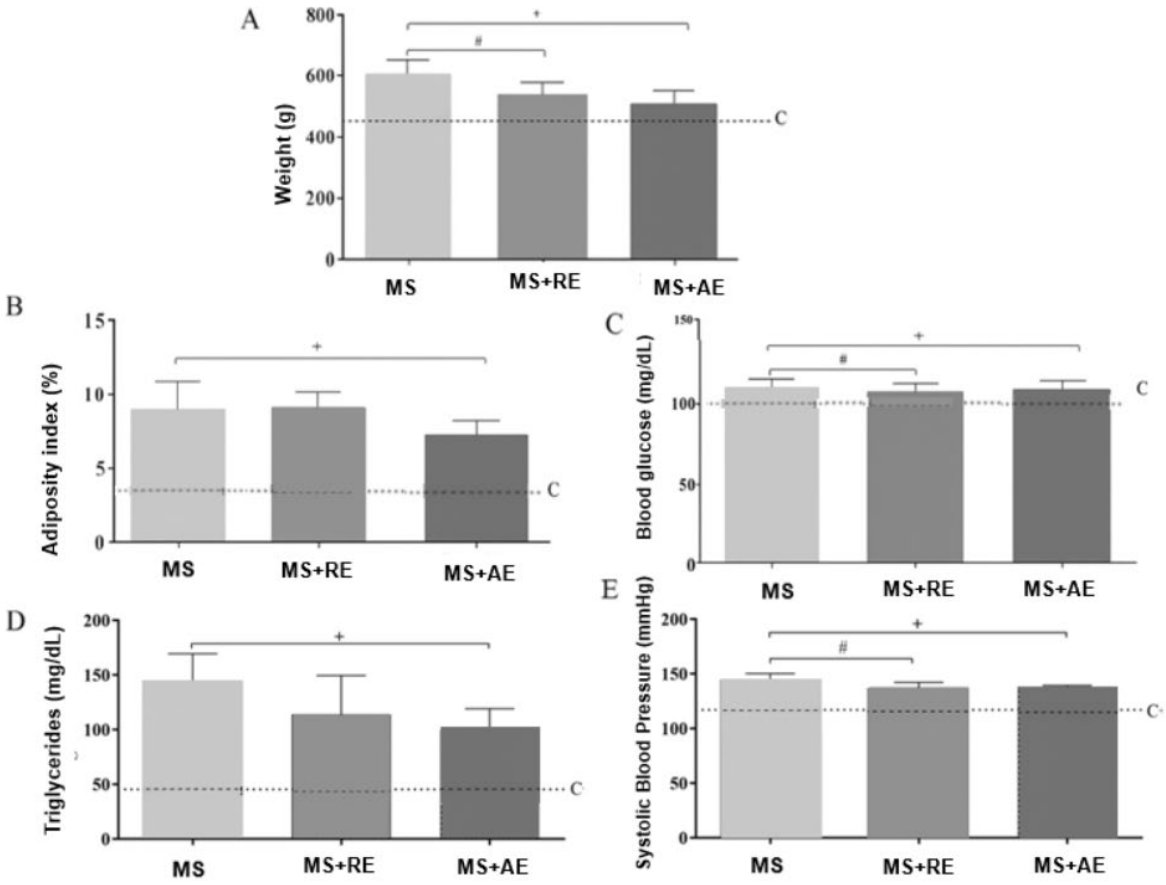

**Figure 4.** Influence of resistance and aerobic training on MS criteria. (A) Weight, (B) Adiposity index, (C) Blood glucose, (D) Triglycerides, and (E) SBP. # MS+RE vs MS, + MS+AE vs MS. C=Control, n=10; MS=Metabolic Syndrome, n=10; MS+RE=Metabolic Syndrome+Resistance exercise, n=10; MS+AE=Metabolic Syndrome+Aerobic exercise, n=10. Data are presented as mean ± standard deviation. Results of one-way ANOVA analysis supplemented by Tukey's post-hoc test at 28 weeks [$p<0.05$].

**Oxidative stress in soleus and EDL muscles**

Protein carbonylation was reduced in the soleus muscle in the MS, RE and AE groups compared to the control group ($p<0.0001$) (Figure 5A). In contrast, increased MDA levels were observed in the MS group compared to the control group and were attenuated by both aerobic and resistance training ($p=0.0002$) (Figure 5B). Regarding SOD, a decrease was observed in the MS group compared to the control, with an increase in both groups subjected to exercise ($p=0.03$) (Figure 5C). CAT activity was reduced in MS compared to the control group and was restored only by resistance exercise ($p=0.002$) (Figure 5D).

Protein carbonylation in EDL decreased in the three MS groups compared to the control group ($p=0.001$) (Figure 5A). MDA did not change in the MS group compared to the control group; however, the MS+RE and MS+AE groups reduced MDA compared to the MS group ($p=0.001$) (Figure 5B). Regarding SOD, MS did not promote changes, nor did the exercise modalities ($p=0.64$) (Figure 5C). CAT decreased in MS compared to the control group and increased only in resistance exercise ($p=0.001$) (Figure 5D).

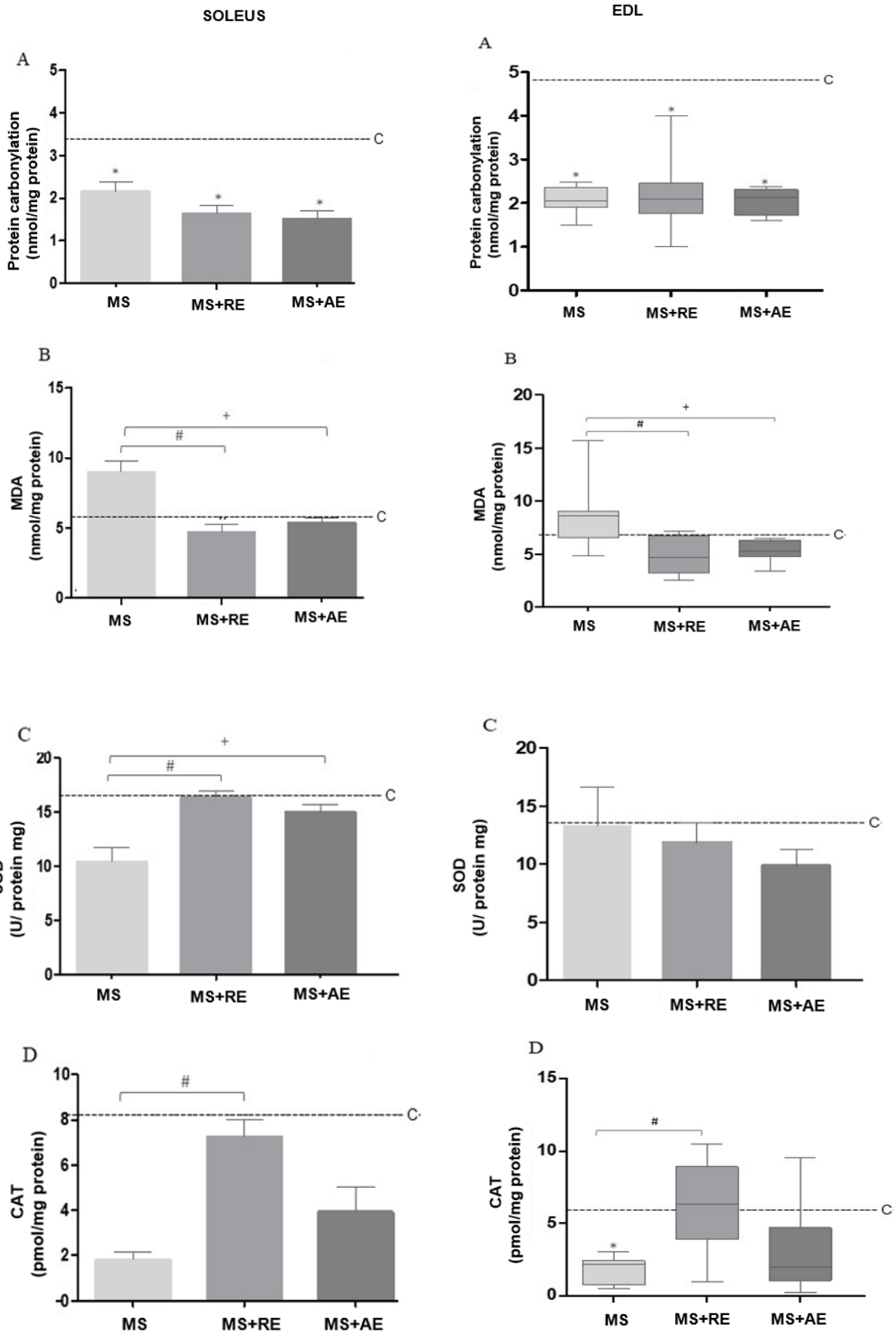
SOLEUS
EDL
A
Protein carbonylation (nmol/mg protein)
C
MS
MS+RE
MS+AE
B
MDA (nmol/mg protein)
#
+
C
D
CAT (pmol/mg protein)
SOD (U/ protein mg)

**Figure 5.** Resistance and aerobic training on redox balance parameters in the soleus and EDL muscles. (A) Protein carbonylation, (B) Malondialdehyde, (C) Superoxide Dismutase, and (D) Catalase. C=Control, n=10; MS=Metabolic Syndrome, n=10; MS+RE=Metabolic Syndrome+Resistance exercise, n=10; MS+AE=Metabolic Syndrome+Aerobic exercise, n=10. # MS+RE vs MS, + MS+RE vs MS, *statistically significant difference compared to the control group. Data are expressed as mean ± standard deviation and median and interquartile range. ANOVA/Tukey and Kruskal-Wallis/Dunn's. [$p<0.05$].

**Gene Expression**

There were no significant changes in the relative quantification of the Col1a1 (Figure 6A), Col1a2 (Figure 6B), Col3a1 (Figure 6C), and Mmp2 (Figure 6D) genes between the groups evaluated in the soleus and ED L muscles ($p>0.05$).

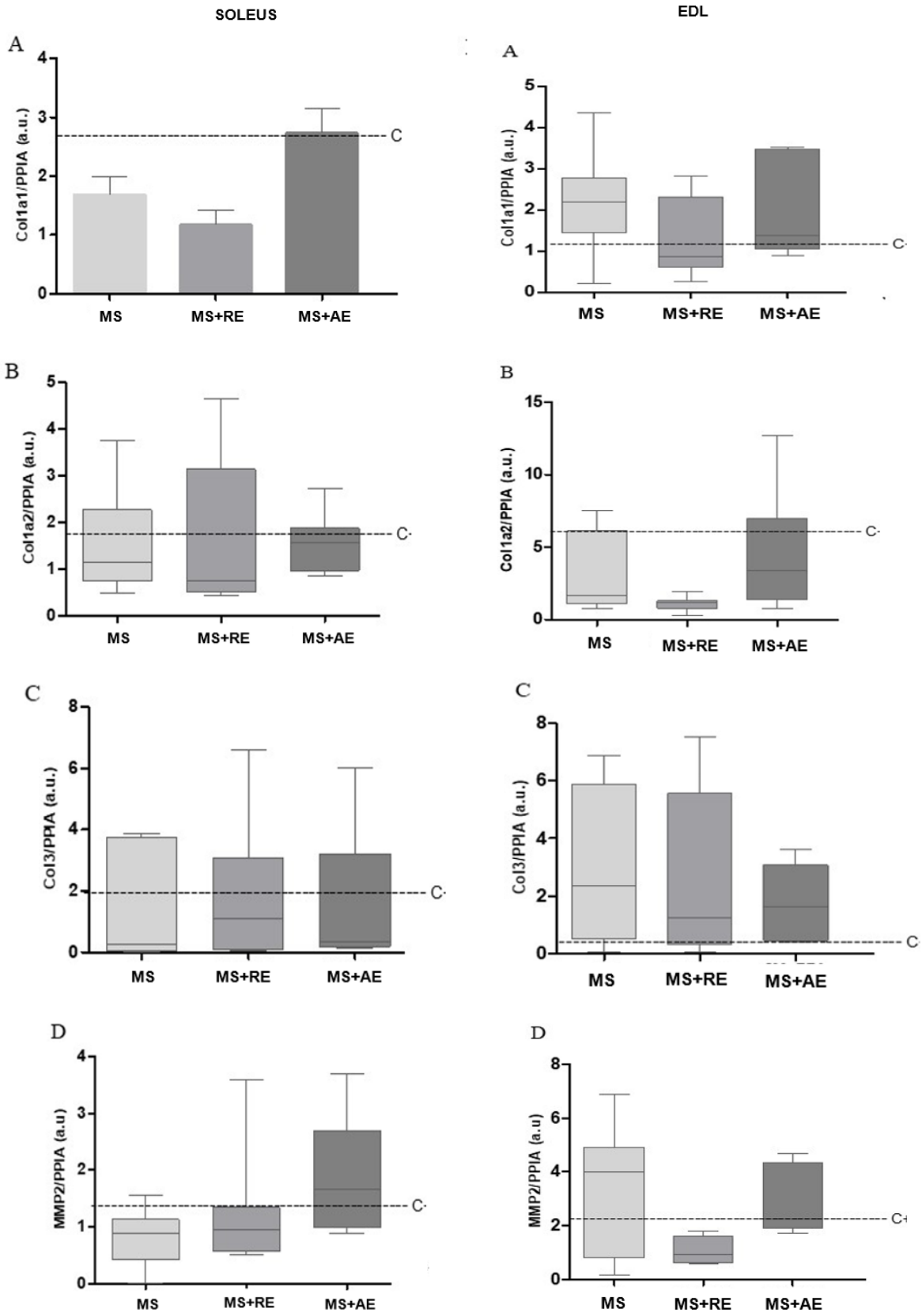
SOLEUS
EDL
A
Col1a1/PPIA (a.u.)
C
MS
MS+RE
MS+AE
B
Col1a2/PPIA (a.u.)
C
MS
MS+RE
MS+AE
C
Col3/PPIA (a.u.)
C
MS
MS+RE
MS+AE
D
MMP2/PPIA (a.u)
C
MS
MS+RE
MS+AE

**Figure 6.** Expression of collagens and metalloproteinases in the soleus and EDL muscles of rats with MS subjected to two types of exercise. (A) Col1a1, (B) Col1a2, (C) Col3a1, (D) Mmp2. C=Control, n=10; MS=Metabolic Syndrome, n=10; MS+RE=Metabolic Syndrome+Resistance exercise, n=10; MS+AE=Metabolic Syndrome+Aerobic exercise, n=10. Data are expressed as mean ± standard deviation and median and interquartile range. ANOVA/Tukey and Kruskal-Wallis/Dunn's. [p<0.05].

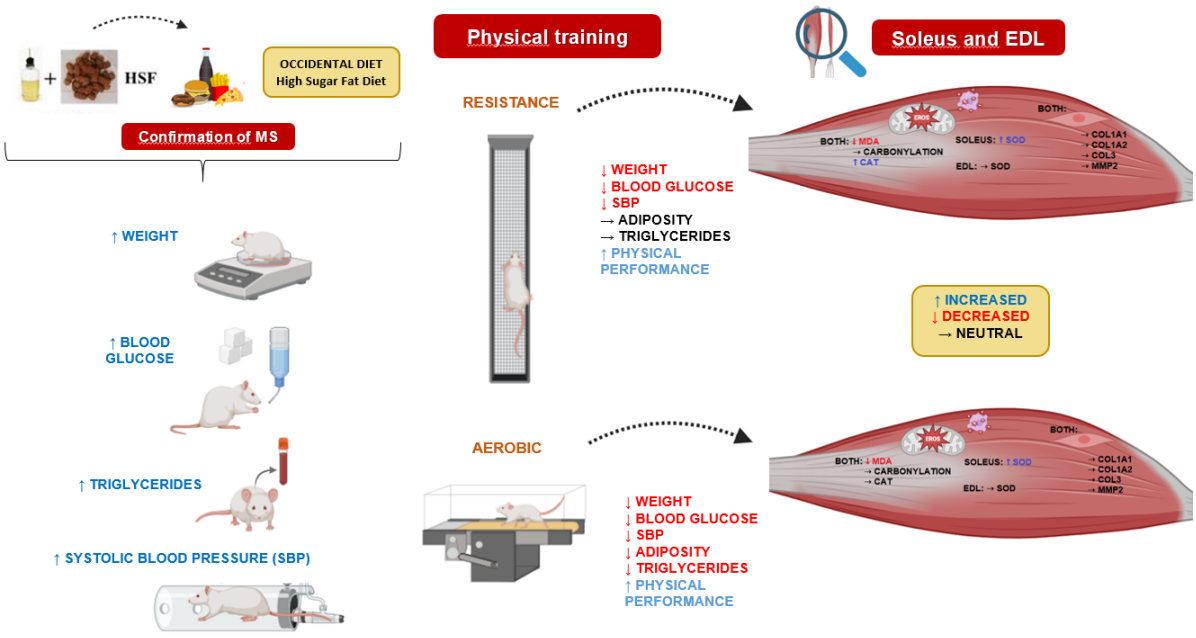


**Figure 7.** Diagram illustrating the main findings. MS findings. Physical performance. Effects on clinical and laboratory parameters. Oxidative stress. Gene expression. MDA=lipid peroxidation. CAT=catalase. SOD=superoxide dismutase. Created with BioRender.com.

## DISCUSSION

This study demonstrated that both resistance and aerobic training promoted metabolic benefits in rats with MS, including weight loss, reduced blood glucose and SBP, as well as improved physical performance and redox balance. Aerobic training

was more effective in reducing adiposity and triglycerides, while resistance training had a greater impact on increasing muscle resistance and restoring CAT activity in all muscles evaluated, highlighting the specific and complementary effects of each modality. MS did not modify gene expression, nor did the different types of exercise influence the muscles evaluated. (Figure 7)

Our model promoted the development of MS, as previously reported by Rodríguez et al.[35], demonstrating that a high-calorie diet is the most suitable for inducing MS in rats, as it simultaneously promotes obesity, insulin resistance, dyslipidemia, and cardiovascular changes. Also, according to the study by Kobi et al.,[36] which compared three models of high-calorie diets—sucrose-rich, fat-rich, and combined—it was concluded that the fat-rich diet induced obesity, glucose intolerance, and hypertension, constituting typical comorbidities of MS.

It was observed that caloric intake was lower in the MS group, a fact that can be attributed to greater satiety induced by the HSF diet, rather than a lipid oxidation process that led the animals to refuse food. It should be noted that the diet was provided under strictly standardized and controlled conditions [24], which ensures that the difference found was related to the effect of the diet composition on the animals' feeding behavior, and not to failures in supply or irregularities in the experimental protocol.

An analysis by Haapala et al. [37] showed that there is a strong inverse association between cardiorespiratory fitness and cardiometabolic risk factors, including those that comprise MS. Individuals with lower cardiorespiratory fitness have an increased risk of developing MS, data that are consistent with the findings of this study, which demonstrated that MS was associated with poorer pre-exercise aerobic functional capacity.

However, the findings of this study indicate that both types of exercise improved physical performance after exercise. According to Sanches et al. [38], an eight-week protocol of moderate aerobic exercise promoted positive metabolic adaptations in rats, increasing exercise capacity and reducing OE and cardiovascular performance. As demonstrated by Antonio et al. [39], also with an eight-week training program, the group subjected to resistance exercise increased their maximum overload capacity, demonstrating robust gains in muscle resistance compared to untrained animals.

According to Liang et al. [40], who analyzed fifteen randomized clinical trials evaluating variables such as glucose, triglycerides, body mass index (BMI), and body fat, it was demonstrated that aerobic, resistance, and combined exercises had a positive impact on reducing body fat. This study confirms that aerobic exercise reduced the adiposity index and triglycerides.

A systematic review of ten studies [41] showed that the combination of aerobic and resistance training produced the greatest positive effects on seven metabolic indicators of MS. However, although our study did not provide a group combining the two modalities, we observed that each modality alone was able to reduce blood glucose levels, weight, and systolic blood pressure.

According to the findings of Er et al. [42], combined interventions involving aerobic exercise five days a week for eight weeks and a high-calorie diet significantly reduced OE, with a decrease in MDA levels and improve metabolic markers in MS conditions. Neves et al. [43], evaluated the effect of dynamic resistance exercise for 12 weeks (5 days per week). Their study found that MDA levels were reduced without a significant reduction in triglyceride levels, which is confirmed in this study. The authors observed a decrease in MDA in the soleus and EDL muscles in both

exercise modalities and no influence of resistance exercise on the reduction of lipid levels. This is possibly because this type of training has a more anabolic and localized effect on the muscle, aimed at increasing resistance and muscle mass, rather than controlling systemic lipid metabolism.

In our study, protein carbonylation was lower in the MS group compared to control group, which may reflect a compensatory antioxidant response. Similar findings were described by Molinar et al. [44], in which there is greater antioxidant capacity, accompanied by reduced protein carbonylation, suggesting that in the early stages of MS, redox defenses may contain tissue oxidative damage.

The review by Xie et al. [45], which included 52 randomized clinical trials, demonstrated that aerobic, resistance, and combined exercise improves muscle antioxidant defenses and reduces signs of ONS. Similar results were found on our study , which demonstrated that both aerobic and resistance exercise were able to increase SOD activity only in the soleus muscle (slow, oxidative fibers), with no changes in the EDL muscle (fast, glycolytic fibers). These observations may be explained by the findings of Pengam et al. [46], who demonstrated that oxidative fiber–dominant muscles respond more robustly to both exercise modalities than glycolytic fiber muscles, which may receive insufficient oxidative stimulation under the applied protocols or exhibit a less sensitive antioxidant response, favoring increased activity of enzymes such as CAT and glutathione peroxidase (GPx).

Resistance training increased CAT in both muscles, restoring redox balance. Mesquita et al. [47], investigated older adults undergoing 6 weeks of resistance training. The authors also observed an increase in CAT in the vastus lateralis muscle. This result suggests that resistance exercise is capable of enhancing enzymatic antioxidant defense, specifically through greater efficiency in the

degradation of hydrogen peroxide, contributing to the reduction of muscle OE. These data reinforce the concept that, even in different experimental models, resistance training promotes consistent antioxidant adaptations, especially regarding CAT activity.

In our eight-week training model, no changes in gene expression were observed in the soleus and EDL muscles. This absence of gene expression changes may be related to the moderate intensity of the aerobic exercise intervention. Jaoude et al. [48], reported that exercise intensity is one of the main factors that can influence the response of ECM metalloproteinases, higher intensities generally lead to increased gene expression.

On the other hand, according to Schweitzer et al. [49], even a single session of resistance exercise is capable of activating collagen remodeling in skeletal muscle. Although our study was not related to the acute effect of exercise, but rather to the chronic effect (24h-48h). Our results suggest that both exercise intensity and the characteristics of the experimental model may influence the adaptive response of ECM.

The selection of muscles in this study was based distinct physiological characteristics. Eduardo et al. [50], demonstrated that the soleus, being a predominantly oxidative muscle, rich in type I fibers, has high mitochondrial density and resistance to fatigue. These features make the soleus more resilient to changes induced by MS. In contrast, the EDL which is primarily composed by type II fibers with glycolytic metabolism, is more susceptible to metabolic dysfunctions and reduced oxidative capacity, but responds to physical training with partial improvement in these parameters. This contrast between muscles highlights the influence of fiber phenotype on adaptations related to oxidative stress and gene

expression. Although our findings showed no difference in gene expression in the soleus and EDL muscles among the groups, this lack of response may be attributable to the exercise intensity employed in the present study.

Onu et al. [51] reinforces the value of exercise as an effective non-pharmacological intervention for the treatment of MS. Our findings further support the potential of exercise to mitigate MS complications and prevent cardiovascular outcomes.

Future investigations should consider higher-intensity training protocols, combine aerobic and resistance exercise, and incorporate histological analyses to confirm morphological changes and collagen deposition in the ECM, as well as to directly associate these structural changes with outcomes such as fibrosis or muscle fiber reorganization

## CONCLUSION

Resistance and aerobic training are effective non-pharmacologic strategies to improve metabolic syndrome and biomarkers of lipid peroxidation in soleus and EDL muscles, without affecting gene expression of collagens and metalloproteinases.

## Authors' Contribution

**Isabelle Tiburcio Pecin Ferreira:** Writing – review & editing, Writing – original draft. **Cristina Schmitt Gregolin:** Writing – review & editing, Writing – original draft. **Bruno Henrique de Paula:** Writing – review & editing. **Felipe Sarzi:** Writing – review & editing. **Camila Renata Correa:** Writing – review & editing. **Natalia Cervantes Uzeloto Guazi:** Writing – review & editing. **Bianca Bento Arruda:** Writing – review & editing. **Lucas Martins Peruque:** Writing – review & editing. **Karen Galvão Valeriano:** Writing – review & editing. **Andreo Fernando Aguiar:** Writing – review & editing. **Artur Junio Togneri Ferron:** Writing – review & editing. **Rafael Floriano Stuani:** Writing – review & editing. **Ana Paula Coelho Figueira Freire:** Writing – review & editing. **Francis Lopes Pacagnelli:** Writing – review & editing, Writing – original draft.

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## Declaration of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# ANEXO A - APROVAÇÃO ÉTICA

UNIVERSIDADE ESTADUAL PAULISTA
CAMPUS DE BOTUCATU
FACULDADE DE MEDICINA

Comissão de Ética no Uso de Animais

Criada através da Portaria DFM nº 611 de 13/12/2012

## CERTIFICADO Nº 1333/2019 – CEUA

Certificamos que o projeto intitulado: **"Análise Proteômica do Miocárdio de ratos com disfunção cardíaca induzida por dieta rica em açúcar e gordura: Papel dos exercícios aeróbico e resistido"**, conduzido pela Pesquisadora: **Cristina Schmitt Gregolin** – Orientadora: Profa. Dra. Camila Renata Correa Camacho, registrada com o **nº 1333/2019**, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica – encontra-se de acordo com os preceitos da Lei n. 11.794, de 08 de outubro de 2008, do Decreto n. 6.899, de 15 de julho de 2009, com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi APROVADA pela Comissão de Ética no Uso de Animais da Faculdade de Medicina de Botucatu, em reunião ordinária de 27 de novembro de 2019.

| | |
|---|---|
| **Finalidade** | ( ) Ensino (**X**) Pesquisa Científica |
| **Vigência da autorização** | 01 de janeiro de 2022 |
| **Espécie/Linhagem/Raça** | Ratos Wistar |
| **Nº de animais** | 60 |
| **Idade/Peso** | 21 dias – 50 gramas |
| **Sexo** | Machos |
| **Origem** | Biotério Central |

Sara Rosa Stanley Sampaio
Secretária da Comissão de Ética no Uso de Animais
Faculdade de Medicina de Botucatu/UNESP

Profa. Associada Bertha Furlan Polegato
Presidente da Comissão de Ética no Uso de Animais
Faculdade de Medicina de Botucatu/UNESP

Distrito Rubião Júnior, s/nº – Botucatu – S.P. CEP: 18.618-970 Fones: (14) 3880.18.80 – E-mail Secretaria: ceua@fmb.unesp.br

# ANEXO B - NORMAS DA REVISTA LIFE SCIENCES

## About the journal: Aims and scope

*Life Sciences* is an international journal publishing articles that emphasize the **molecular**, **cellular**, and **functional basis of therapy**. The journal emphasizes the understanding of mechanism that is relevant to all aspects of human disease and translation to patients. All articles are rigorously reviewed.

The Journal favors publication of full-length papers where modern scientific technologies are used to explain **molecular**, **cellular** and **physiological mechanisms**. Articles that merely report observations are rarely accepted. Recommendations from the Declaration of Helsinki or NIH guidelines for care and use of laboratory animals must be adhered to. Articles should be written at a level accessible to readers who are non-specialists in the topic of the article themselves, but who are interested in the research. The Journal welcomes reviews on topics of wide interest to investigators in the **life sciences**. We particularly encourage submission of brief, focused reviews containing high-quality artwork and require the use of mechanistic summary diagrams.

Manuscripts should present novel preclinical findings addressing questions of **biological significance** to **human disease**. Studies that fail to do so may be rejected without review. Quantitative conclusions must be based on truly quantitative methods. *Life Sciences* does not publish work on the actions of biological extracts of unknown chemical composition. Compounds studied must be of known chemical structure and concentration. The study must be reproducible; materials used must be available to other researchers so they can repeat the experiment. Clinical studies may be considered if they expand understanding of mechanism, but the journal does not encourage clinical trial reports.

Four common reasons for rejection include: out of scope (the manuscript does not conform to the goal of identification of mechanisms related to therapy for human disease); too preliminary (manuscript is based on a limited amount of experimental data diminishing significance); lack of novelty (manuscript is well done but does not address a significant question); unidentified structure (actions of biological extracts of unknown chemical composition).

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Please write your text in good English (American or British usage is accepted, but not a mixture of these). For language assistance, please see Language Services, above. Use decimal points (not decimal commas); use a space for thousands (10 000 and above).

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This journal follows a single anonymized review process. Your submission will initially be assessed by our editors to determine suitability for publication in this journal. If your submission is deemed suitable, it will typically be sent to a minimum of two reviewers for an independent expert assessment of the scientific quality. The decision as to whether your article is accepted or rejected will be taken by our editors.

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This journal will not accept manuscripts that contain data derived from unethically sourced organs or tissue, including from executed prisoners or prisoners of conscience, consistent with recommendations by Global Rights Compliance on Mitigating Human Rights Risks in Transplantation Medicine. For all studies that use human organs or tissues, sufficient evidence must be provided that these were procured in line with WHO Guiding Principles on Human Cell, Tissue and Organ Transplantation. For clinical studies, a statement of informed consent having been obtained from a patient or their nominated representative, paired with ethical approval for the study from a suitable institution, as required by the policies of the journal, may be considered sufficient evidence, but the journal reserves the right to request additional evidence in cases where it feels this is not sufficient. The source of the organs or tissues used in clinical research must be transparent and traceable. If your manuscript describes organ transplantation you must additionally declare within the manuscript that:

- autonomous consent free from coercion was obtained from the donor(s) or their next of kin.
- organs and/or tissues were not sourced from executed prisoners or prisoners of conscience.

**Clinical Trials**

This journal follows the International Committee of Medical Journal Editors (ICMJE) clinical trials registration guidelines.

**Registration**

Clinical trials must be registered in a public trials registry. Purely observational studies, in which the assignment of the medical intervention is not at the discretion of the investigator, do not require registration.

Some important points:

- Trials must be registered at or before the onset of patient enrolment.
- The clinical trial registration number must be included at the end of the article abstract.
- A clinical trial is defined as any research study that prospectively assigns human participants, or groups of humans, to one or more health-related interventions to evaluate the effects of health outcomes.
- Health-related interventions include any intervention used to modify a biomedical or health-related outcome such as drugs, surgical procedures, devices, behavioral treatments, dietary interventions, and process-of-care changes.
- Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events.

**Results**

Authors are required to disclose all posting in registries of results of the same or closely related work. Editors will not consider results to be a prior publication if they have already been posted in the same clinical trials registry in which primary registration resides, as long as the results are presented in the form of a brief structured abstract (fewer than 500 words) or table.

Disclosing results in other circumstances, such as in an investors' meeting, for example, is discouraged and may jeopardize consideration of your manuscript by this journal.

**Reporting**

Authors are encouraged to follow CONSORT guidelines when presenting randomized controlled trials, and provide the CONSORT checklist at manuscript submission, with an accompanying flow diagram illustrating the progress of patients through the trial - including recruitment, enrolment, randomization, withdrawal, completion and a description of the randomization procedure.

- Read the CONSORT guidelines.
- Follow the CONSORT checklist.

**Writing and formatting**

**File format**

We ask you to provide editable source files for your entire submission (including figures, tables and text graphics). Some guidelines:

- Save files in an editable format, using the extension .doc/.docx for Word files and .tex for LaTeX files. A PDF is not an acceptable source file.

- Lay out text in a single-column format.
- Remove any strikethrough and underlined text from your manuscript, unless it has scientific significance related to your article.
- Use spell-check and grammar-check functions to avoid errors.

We advise you to read our Step-by-step guide to publishing with Elsevier.

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You are required to include the following details in the title page information:

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- Author names. Provide the given name(s) and family name(s) of each author. The order of authors should match the order in the submission system. Carefully check that all names are accurately spelled. If needed, you can add your name between parentheses in your own script after the English transliteration.
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- Corresponding author. Clearly indicate who will handle correspondence for your article at all stages of the refereeing and publication process and also post-publication. This responsibility includes answering any future queries about your results, data, methodology and materials. It is important that the email address and contact details of your corresponding author are kept up to date during the submission and publication process.
- Present/permanent address. If an author has moved since the work described in your article was carried out, or the author was visiting during that time, a "present address" (or "permanent address") can be indicated by a footnote to the author's name. The address where the author carried out the work must be retained as their main affiliation address. Use superscript Arabic numerals for such footnotes.

**Abstract**

You are required to provide a concise and factual abstract which does not exceed 250 words. The abstract should briefly state the purpose of your research, principal results and major conclusions. Some guidelines:

- Abstracts must be able to stand alone as abstracts are often presented separately from the article.

- Avoid references. If any are essential to include, ensure that you cite the author(s) and year(s).
- Avoid non-standard or uncommon abbreviations. If any are essential to include, ensure they are defined within your abstract at first mention.

**Keywords**

You are required to provide 1 to 7 keywords for indexing purposes. Keywords should be written in English. Please try to avoid keywords consisting of multiple words (using "and" or "of").

We recommend that you only use abbreviations in keywords if they are firmly established in the field.

**Highlights**

You are required to provide article highlights at submission.

Highlights are a short collection of bullet points that should capture the novel results of your research as well as any new methods used during your study. Highlights will help increase the discoverability of your article via search engines. Some guidelines:

- Submit highlights as a separate editable file in the online submission system with the word "highlights" included in the file name.
- Highlights should consist of 3 to 5 bullet points, each a maximum of 85 characters, including spaces.

We encourage you to view example article highlights and read about the benefits of their inclusion.

**Tables**

Tables must be submitted as editable text, not as images. Some guidelines:

- Place tables next to the relevant text or on a separate page(s) at the end of your article.
- Cite all tables in the manuscript text.
- Number tables consecutively according to their appearance in the text.
- Please provide captions along with the tables.
- Place any table notes below the table body.
- Avoid vertical rules and shading within table cells.

We recommend that you use tables sparingly, ensuring that any data presented in tables is not duplicating results described elsewhere in the article.

**Figures, images and artwork**

Figures, images, artwork, diagrams and other graphical media must be supplied as separate files along with the manuscript. We recommend that you read our detailed artwork and media instructions. Some excerpts:

When submitting artwork:

- Cite all images in the manuscript text.
- Number images according to the sequence they appear within your article.
- Submit each image as a separate file using a logical naming convention for your files (for example, Figure_1, Figure_2 etc).
- Please provide captions for all figures, images, and artwork.
- Text graphics may be embedded in the text at the appropriate position. If you are working with LaTeX, text graphics may also be embedded in the file.

**Artwork formats**

When your artwork is finalized, "save as" or convert your electronic artwork to the formats listed below taking into account the given resolution requirements for line drawings, halftones, and line/halftone combinations:

- Vector drawings: Save as EPS or PDF files embedding the font or saving the text as "graphics."
- Color or grayscale photographs (halftones): Save as TIFF, JPG or PNG files using a minimum of 300 dpi (for single column: min. 1063 pixels, full page width: 2244 pixels).
- Bitmapped line drawings: Save as TIFF, JPG or PNG files using a minimum of 1000 dpi (for single column: min. 3543 pixels, full page width: 7480 pixels).
- Combinations bitmapped line/halftones (color or grayscale): Save as TIFF, JPG or PNG files using a minimum of 500 dpi (for single column: min. 1772 pixels, full page width: 3740 pixels).

Please do not submit:

- files that are too low in resolution (for example, files optimized for screen use such as GIF, BMP, PICT or WPG files).
- disproportionally large images compared to font size, as text may become unreadable.

**Figure captions**

All images must have a caption. A caption should consist of a brief title (not displayed on the figure itself) and a description of the image. We advise you to keep the amount of text in any image to a minimum, though any symbols and abbreviations used should be explained.

Provide captions in a separate file.

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If you submit usable color figures with your accepted article, we will ensure that they appear in color online.

Please ensure that color images are accessible to all, including those with impaired color vision. Learn more about color and web accessibility.

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Please read our policy on the use of generative AI and AI-assisted tools in figures, images and artwork, which can be found in Elsevier's GenAI Policies for Journals. This policy states:

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- The use of generative AI or AI-assisted tools in the production of artwork such as for graphical abstracts is not permitted. The use of generative AI in the production of cover art may in some cases be allowed, if the author obtains prior permission from the journal editor and publisher, can demonstrate that all necessary rights have been cleared for the use of the relevant material, and ensures that there is correct content attribution.

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We encourage the use of supplementary materials such as applications, images and sound clips to enhance research. Some guidelines:

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- Submit supplementary materials at the same time as your article. Be aware that all supplementary materials provided will appear online in the exact same file type as received. These files will not be formatted or typeset by the production team.
- Include a concise, descriptive caption for each supplementary file describing its content.
- Provide updated files if at any stage of the publication process you wish to make changes to submitted supplementary materials.
- Do not make annotations or corrections to a previous version of a supplementary file.
- Switch off the option to track changes in Microsoft Office files. If tracked changes are left on, they will appear in your published version.

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This journal accepts video material and animation sequences to support and enhance your scientific research. We encourage you to include links to video or animation files within articles. Some guidelines:

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- Clearly label files ensuring the given file name is directly related to the file content.
- Provide files in one of our recommended file formats. Files should be within our preferred maximum file size of 150 MB per file, 1 GB in total.
- Provide "stills" for each of your files. These will be used as standard icons to personalize the link to your video data. You can choose any frame from your video or animation or make a separate image.
- Provide text (for both the electronic and the print version) to be placed in the portions of your article that refer to the video content. This is essential text, as video and animation files cannot be embedded in the print version of the journal.

We publish all video and animation files supplied in the electronic version of your article.

For more detailed instructions, we recommend that you read our guidelines on submitting video content to be included in the body of an article.

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We are committed to supporting the storage of, access to and discovery of research data, and our research data policy sets out the principles guiding how we work with the research community to support a more efficient and transparent research process.

Research data refers to the results of observations or experimentation that validate research findings, which may also include software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Please read our guidelines on sharing research data for more information on depositing, sharing and using research data and other relevant research materials.

**Research data deposit, citation and linking**

For this journal **Option B** instructions from our research data guidelines apply. This means you are **encouraged** to:

- Deposit your research data in a relevant data repository.
- Cite and link to this dataset in your article.

- If this is not possible, make a statement explaining why research data cannot be shared.

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To foster transparency, you are encouraged to state the availability of any data at submission.

Ensuring data is available may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you can state the reason why (e.g., your research data includes sensitive or confidential information such as patient data) during the submission process. This statement will appear with your published article on ScienceDirect.

Read more about the importance and benefits of providing a data statement.

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Linking to the data underlying your work increases your exposure and may lead to new collaborations. It also provides readers with a better understanding of the described research.

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- Provide a link to your dataset when prompted during the online submission process.
- For some data repositories, a repository banner will automatically appear next to your published article on ScienceDirect.
- You can also link relevant data or entities within the text of your article through the use of identifiers. Use the following format: Database: 12345 (e.g. TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

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Divide your manuscript into clearly defined sections covering all essential elements using headings.

**Glossary**

Please provide definitions of field-specific terms used in your article, in a separate list.

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Include any individuals who provided you with help during your research, such as help with language, writing or proof reading, in the acknowledgements section. Acknowledgements should be placed in a separate section which appears directly before the reference list. Do not include acknowledgements on your title page, as a

footnote to your title, or anywhere else in your article other than in the separate acknowledgements section.

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Corresponding authors are required to acknowledge co-author contributions using CRediT (Contributor Roles Taxonomy) roles:

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We advise you to read more about CRediT and view an example of a CRediT author statement.

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Authors must disclose any funding sources who provided financial support for the conduct of the research and/or preparation of the article. The role of sponsors, if any, should be declared in relation to the study design, collection, analysis and interpretation of data, writing of the report and decision to submit the article for publication. If funding sources had no such involvement this should be stated in your submission.

List funding sources in this standard way to facilitate compliance to funder's requirements:

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It is not necessary to include detailed descriptions on the program or type of grants, scholarships and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

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*This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.*

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We ask you to use the following format for appendices:

- Identify individual appendices within your article using the format: A, B, etc.
- Give separate numbering to formulae and equations within appendices using formats such as Eq. (A.1), Eq. (A.2), etc. and in subsequent appendices, Eq. (B.1), Eq. (B. 2) etc. In a similar way, give separate numbering to tables and figures using formats such as Table A.1; Fig. A.1, etc.

**Journal specific information**

**Results and Discussion:** The content of the results and discussion should be presented in separate sections within the main text of the manuscript.

**References**

**References within text**

Any references cited within your article should also be present in your reference list and vice versa. Some guidelines:

- References cited in your abstract must be given in full.
- We recommend that you do not include unpublished results and personal communications in your reference list, though you may mention them in the text of your article.
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Linking to cited sources will increase the discoverability of your research.

Before submission, check that all data provided in your reference list are correct, including any references which have been copied. Providing correct reference data allows us to link to abstracting and indexing services such as Scopus, Crossref and

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We encourage the use of Digital Object Identifiers (DOIs) as reference links as they provide a permanent link to the electronic article referenced.

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This journal does not set strict requirements on reference formatting at submission. Some guidelines:

- References can be in any style or format as long as the style is consistent.
- Author names, journal or book titles, chapter or article titles, year of publication, volume numbers, article numbers or pagination must be included, where applicable.
- Use of DOIs is recommended.

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Indicate references by adding a number within square brackets in the text. You can refer to author names within your text, but you must always give the reference number, e.g., "as demonstrated [3,6]. Barnaby and Jones [8] obtained a different result ....".

Number references in the order they appear in your article.

Abbreviate journal names according to the List of Title Word Abbreviations (LTWA).

Examples:

**Reference to a journal publication:**

[1] J. van der Geer, T. Handgraaf, R.A. Lupton, The art of writing a scientific article, J. Sci. Commun. 163 (2020) 51 – 59. https://doi.org/10.1016/j.sc.2020.00372.

**Reference to a journal publication with an article number:**

[2] J. van der Geer, T. Handgraaf, R.A. Lupton, 2022. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2022.e00205.

**Reference to a book:**

[3] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.

**Reference to a chapter in a book:**

[4] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2020, pp. 281 - 304.

**Reference to a website:**

[5] Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/, 2023 (accessed 13 March 2023).

**Reference to a dataset:**

[6] M. Oguro, S. Imahiro, S. Saito, T. Nakashizuka, Mortality data for Japanese oak wilt disease and surrounding forest compositions [dataset], Mendeley Data, v1, 2015. https://doi.org/10.1234/abc12nb39r.1.

**Reference to software:**

[7] E. Coon, M. Berndt, A. Jan, D. Svyatsky, A. Atchley, E. Kikinzon, D. Harp, G. Manzini, E. Shelef, K. Lipnikov, R. Garimella, C. Xu, D. Moulton, S. Karra, S. Painter, E. Jafarov, S. Molins, Advanced Terrestrial Simulator (ATS) v0.88 [software], Zenodo, March 25, 2020. https://doi.org/10.1234/zenodo.3727209.

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When listing web references, as a minimum you should provide the full URL and the date when the reference was last accessed. Additional information (e.g. DOI, author names, dates or reference to a source publication) should also be provided, if known.

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When citing data references, you should include:

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Add [dataset] immediately before your reference. This will help us to properly identify the dataset. The [dataset] identifier will not appear in your published article.

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Where a preprint has subsequently become available as a peer-reviewed publication, use the formal publication as your reference.

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If a template is not available for this journal, follow the format given in examples in the reference style section of this Guide for Authors.

**Journal specific information**

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Life Sciences requires submission of the whole uncropped images of the original western blots in triplicate that contributed to the quantitative analysis, from which figures have been derived. Please submit as Supplementary Figure(s). Please compile all images into the same WORD file. Uploading separate western blots images would delay the editorial speed.

**Please note that this is mandatory when western blots are included in the manuscript**. Please see Example of original western blot for three repeats

**Submitting your manuscript**

**Submission checklist**

Before completing the submission of your manuscript, we advise you to read our submission checklist:

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- All files have been uploaded, including keywords, figure captions and tables (including a title, description and footnotes) included.
- Spelling and grammar checks have been carried out.
- All references in the article text are cited in the reference list and vice versa.
- Permission has been obtained for the use of any copyrighted material from other sources, including the Web.
- For open access articles, all authors understand that they are responsible for payment of the article publishing charge (APC) if the manuscript is accepted. Payment of the APC may be covered by the corresponding author's institution, or the research funder.

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