Oxidative Medicine and Cellular Longevity

Redox-Mediated Effects of Exercise in Chronic Noncommunicable Diseases

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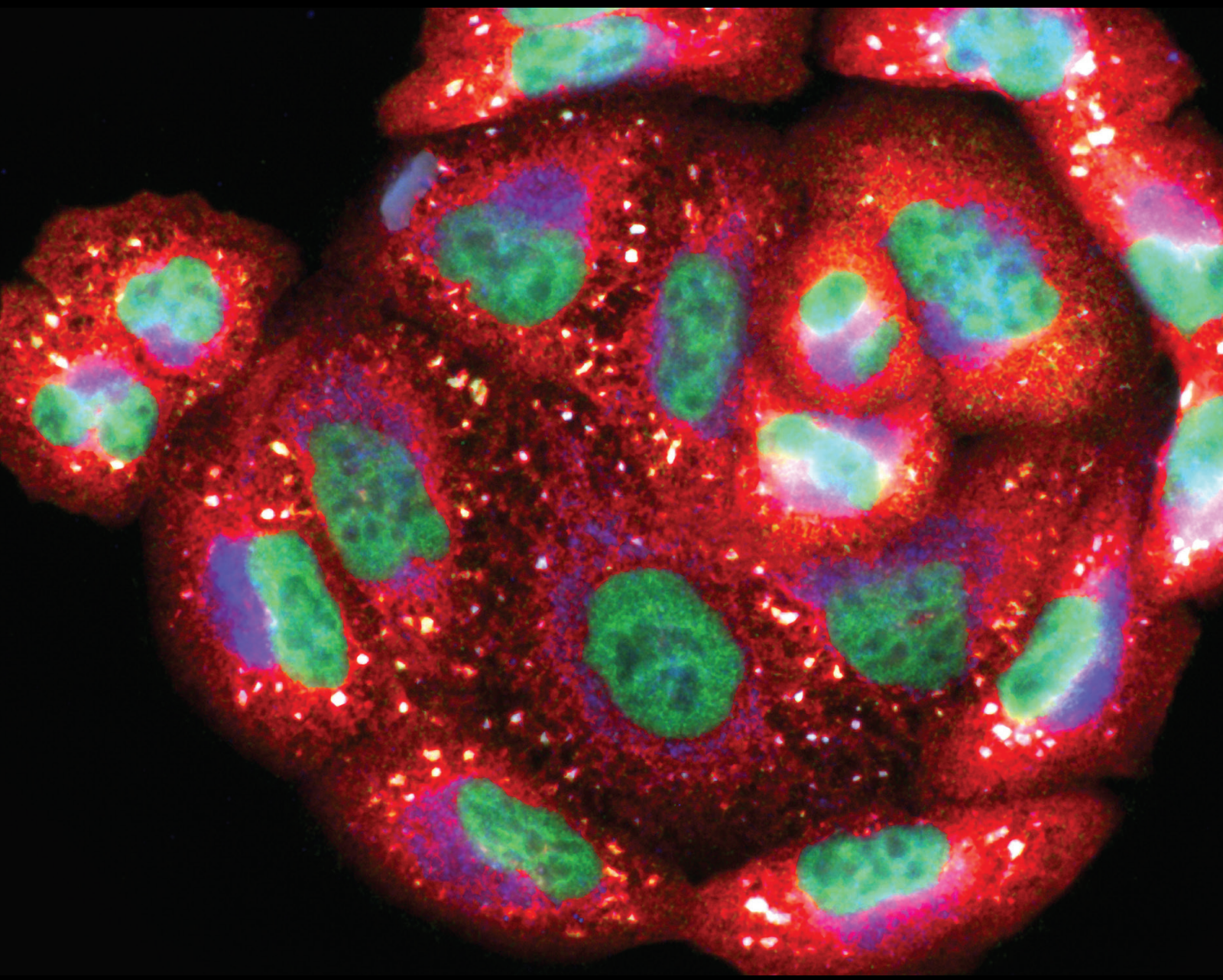

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Review Article

Exercise and Oxidative Stress Biomarkers among Adult with Cancer: A Systematic Review

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Evidence shows that exercise can have a favourable effect in cancer patients. The exercise's clinical benefits are likely to concern multiple interrelated biological pathways, among which oxidative stress plays a key role. Regular training can induce an adaptive response that strengthens the antioxidative status of the body. To formulate public health recommendations regarding the optimal exercise prescription for cancer patients, a detailed understanding is needed regarding the effect of exercise on variables linked to oxidative stress and antioxidant status of patients. The goal of this systematic review, based on PRISMA, was to explore and critically analyse the evidence regarding the efficacy of exercise on oxidative stress biomarkers among people with cancer. Study search was conducted in the following databases: PubMed, Cochrane, CINAHL, Embase, PEDro, and SPORTDiscus. The studies' quality was assessed with the Cochrane risk-of-bias tool and STROBE scale. After identification and screening steps, 10 articles were included. The findings provide an encouraging picture of exercise, including resistance training and aerobic activities, in people with cancer. The exercise improved the indicators of the total antioxidant capacity, increased the antioxidant enzymes' activity, or reduced the biomarkers of oxidative damage in various forms of cancer such as breast, lung, head, and neck. Regarding oxidative DNA damage, the role of exercise intervention has been difficult to assess. The heterogeneity of study design and the plethora of biomarkers measured hampered the comparison of the articles. This limited the possibility of establishing a comprehensive conclusion on the sensitivity of biomarkers to estimate the exercise's benefits. Further high-quality studies are required to provide data regarding oxidative stress biomarkers responding to exercise. This information will be useful to assess the efficacy of exercise in people with cancer and support the appropriate prescription of exercise in anticancer strategy.

1. Introduction

Exercise training is, in general, secure for people that survived after cancer diagnosis, and each of them should remain physically active, as stated in the report of 2018 of the American College of Sport Medicine entitled: "Internationally Multidisciplinary Roundtable on Physical Activity and Cancer Prevention and Control." It should be clarified that physical activity (PA) is defined as any movement of the body generated by skeletal muscles that required energy expenditure. PA can be classified into sports, occupational, conditioning, household, or other activities [1]. Likewise, exercise is a subcategory of PA that is planned and structured, directed also at improving or maintaining cardiofitness status [2].

There is evidence showing that exercise has positive effects in patients diagnosed with cancer [3, 4]. Adapted exercise interventions can diminish the possible resurgence of tumour growth in breast, colon, and prostate cancer [5, 6]. Furthermore, exercise is associated with a better survival and an attenuation of the negative consequences of chemotherapy and radiation [5, 7, 8].

In people with cancer, exercise is linked to positive modifications in cardiorespiratory fitness, physical function, and in anthropometric composition, as well as in patient-reported health benefit in quality of life and manage of fatigue [9–11]. Recent findings indicated that exercise could play a key role in tumour biology. Evidence suggests that exercise could downregulate a group of RAS family oncogenes (RAN, RAB14, and RAB8A) [6–12]. Moreover, the capacity of exercise to enhance the endogenous antioxidant defences has been postulated as a contributing factor to counteract the oxidative stress in various phases of tumourigenesis [6–13]. It is well known that oxidative stress-induced DNA damage can promote the development and progression of cancer. Moreover, cancer cells themselves increase the levels of reactive oxygen species (ROS), inducing cancer progression [14, 15]. In this framework, systematic exercise training can improve physical fitness and capacity of the patients through enhancing the antioxidative status of the body. Although acute and exhaustive training increases in ROS production, the moderate exercise (chronic exercise or aerobic training) induces an organism's response with a decrease in ROS generation and an improvement in antioxidant status [6, 13, 16]. Besides, regular exercise has a hormetic effect given that low levels of oxidative stress deriving from cells as a consequence of exercises may trigger cellular mechanisms which promote the tolerance of acute oxidative stress [6–17]. At a molecular level, exercise training stimulates a transient production of ROS, and it can activate a redox circuit linked [18]. More specifically, ROS arose during exercise and activated the transcription of nuclear factor erythroid 2 (Nrf2). Nrf2 plays a fundamental role in the *trans*-activation of the antioxidant response element (ARE) and in the upregulation of various proteins involved in antioxidant defences [18, 19]; thus, it is pivotal in preserving the balance in the cellular redox process. Most of the Nrf2-dependent target genes encode for enzymes that protect DNA, proteins, and lipids from ioxidative damage. These genes take part in the synthesis of antioxidant enzymes such as catalase (CAT) or superoxide dismutase (SOD), glutathione and other stress response [20–22].

On this basis, physical exercise can be regarded as a regulator of cellular redox homeostasis, which induces an adaptation to overcome the oxidative stress. However, the potential benefits of exercise are influenced by intensity, type, and duration of training [16].

In order to adopt public health recommendations on the optimal exercise prescription for adults with cancer, a more detailed understanding is needed regarding the effect of exercise on variables linked to oxidative stress and antioxidant status. Usually, changes in homeostasis redox are measured by analysing different biomarkers, mainly in the blood or in urine samples collected in test populations. Such biomarkers can detect a specific type of damage on lipids, proteins, and DNA or the concentration of enzymatic and nonenzymatic antioxidants. Thus, different biomarkers can be evaluated for any molecular or cellular damage that can be caused by ROS [23].

In this context, the primary aim of this article is to explore and investigate the evidence regarding the exercise's effect on oxidative stress biomarkers among postdiagnosis cancer patients. Furthermore, a detailed evaluation of previous research is also addressed regarding the association by type, dose, and timing of exercise and cancer location.

2. Methods

2.1. Search Strategy. This systematic review was conducted according to PRISMA [24]. We registered the protocol of the systematic review in the International Prospective Register of Systematic Reviews (ID: CRD42021258326). The primary research objective was addressed, through the development of the PICO question (Patients, Interventions, Comparators, and Outcomes) using the following search terms: (P) people with cancer diagnosis, (I) physical activity exercise intervention, (C) usual treatment and/or no exercise intervention, and (O) the efficacy of exercise on oxidative stress biomarkers.

We conducted a systematic literature search in PubMed, Cochrane Library, CINAHL, Embase, PEDro, and SPORTDiscus up to May 2021 to screen all articles focused on the effect of structured exercises treatment on oxidative stress biomarkers in people with a diagnosis of cancer.

The electronic databases were searched, with a publication date limit of 10 years, because we were focused on recent treatments and approaches. Specific criteria were applied in the search approach: we included randomized controlled trials (RCTs), quasiexperimental study, clinical study, clinical trial, case report, and observational study, with full text available. Search terms was created using the following keywords and Boolean terms: ((Post diagnosis Cancer OR Neoplasia OR Neoplasm OR Tumo* OR Cance* OR Malignan* OR Malignant Neoplas* OR Neoplas*) AND (Exercis* OR Physical Activit* OR Activities Physical OR Activity Physical OR Exercise Physical OR Exercises Physical OR Physical Exercise OR Physical Exercises OR Acute Exercis* OR Exercise Acute OR Exercises Acute OR Exercise Aerobic OR Aerobic Exercis* OR Exercises Aerobic OR Exercise Training OR Exercise Trainings OR Training Exercise OR Trainings Exercise OR Remedial Exercise OR Exercise, Remedial OR Exercises, Remedial OR Remedial Exercises OR Therapy Exercise OR Exercise Therapies OR Therapies, Exercise OR Rehabilitation Exercise OR Exercise, Rehabilitation OR Exercises, Rehabilitation OR Rehabilitation Exercises) AND (Oxidative Stresses biomarkers OR Stress Oxidative markers OR Antioxidative Stress OR Antioxidative Stresses OR Antioxidant enzymes OR Stress, Antioxidative OR Anti-oxidative Stress OR Anti oxidative Stress OR Anti-oxidative Stresses OR Stress, Anti-oxidative OR Antioxidant plasma status OR 8-hydroxy-deoxyguanosine)). When necessary, the search string was adapted to perfectly fit in each database.

A grey literature search was conducted using Medrxiv, and hand searches of key conference proceedings, journals, professional organizations' websites, and guideline clearing houses. Finally, using the snowball technique, we reviewed the primary and most important papers' references in order to find possible more studies.

2.2. Inclusion and Exclusion Criteria. The inclusion criteria were the following: (1) language: articles written in English; (2) study design: randomized controlled trial, quasiexperimental study, clinical trial, clinical study, case report, and observational study with original primary data; (3) population of interest: people with cancer diagnosis; (4) exercise experience: any type of exercise; (5) outcome measurement: oxidative stress biomarkers evaluation, oxidative biomarkers assessed at least once in the paper; additional physical performance measured outcomes, or other indices of physical performance described in each study for example, balance, mobility measured at least once in the study; and (6) comparison: standard treatment and/or no intervention.

The exclusion criteria were as follows: (1) articles not relevant for the research area, (2) people without cancer diagnosis, (3) absence of exercise intervention, and (4) research studies or other papers with no original data. Table 1 summarized the PICOST eligibility criteria.

2.3. Data Extraction and Quality Assessment. The reviewers examined all the papers primarily by reading the titles and abstracts; then, the eligible articles were selected based on our PICOST. All potentially eligible studies were retrieved, after the removal of duplicates, extracted and then reviewed independently by the five researchers (LD, AM, SM, GB, and YL) using a scheduled data extraction format. Disagreements regarding the eligibility of the studies were resolved by discussion among all the authors. The data from the included studies were extracted by the researchers, following the standardized rules for studies collection. The details collected comprised: first's author's name, year of publication, country, study design, study population with ages and number of experimental (EG) and control (CG) groups, type intensity and frequency of the intervention, outcomes, and results. Results were described as mean ± SD where possible. The data extraction was based on the methods provided by the *Cochrane Reviewers' Handbook* [25]. Possible divergences were solved by consensus (LB, FM).

We contacted the study's authors when additional information was necessary [26].

The selected studies were assessed for the risk of bias separately by four researchers (LD, AM, SM, and GB), using the "Cochrane risk-of-bias tool for randomized trials" (ROB) [27] and the "STROBE statement checklists for observational studies" [28]. Any reviewers' disagreement, upon the quality scores, was solved in a schedule meeting manage by a fifth blind reviewer (YL). The risk of bias evaluation was made based on the oxidative stress biomarker outcomes. This methodological approach was endorsed by the PRISMA [24].

The Cochrane risk-of-bias tool for RCTs presents seven categories of bias: (1) how the randomization sequence was generated, (2) the allocation procedures' blindness, (3) selective reporting for reporting bias, (4) blinding of participants and personal, (5) the outcome evaluation procedures' blindness, (6) outcome data partially not reported, and "other bias" category, and (7) evaluated on the possible bias not reported in the previous domains. These categories are translated in a high, low, or unclear (when the authors did not provide enough information about the bias category) value of risk of bias. The STROBE scale is composed by 22 items divided into three different checklists: cohort study, cross-sectional, and case report studies [28]. In line with previous studies [29, 30], we adopted a cut-off for three scores: 0-14 poor quality, 15-25 intermediate qualities, and 26-33 good quality [31].

3. Results

3.1. Study Selection and Characteristics. Overall, 363 articles were detected through the chosen databases and the hand search technique (Figure 1). Studies were published from 2011 to 2021; 87 were duplicated, and 260 studies were excluded in the first step of title and abstract reading. Finally, we considered 16 records as pertinent, 6 of which were subsequently excluded after full-text reading. The principal reasons for exclusion were linked to the nonmatch our review's aim: the effects of exercise interventions on oxidative stress biomarkers in people with cancer diagnosis. The prevalent reason of exclusion was due by the mismatch of the adopted inclusion criteria (people with cancer diagnosis). As shown in Figure 1, only 10 papers were included in the results.

3.2. Risk of Bias Assessment. On the basis of the descriptive analysis, we assessed the risk of bias. Experimental studies were analysed in accordance with the ROB tool for RCTs (Table 2). A RCT [32] showed an overall "good quality," meeting all seven criteria of low risk of bias, the remaining four were evaluated as "poor quality." Regarding items #1 and #2, Katsourakis et al. [33] described in detail how they obtained the random sequence and the allocation process, while this process was unclear for Karimi and Roshan [34] and at high risk of bias for both the studies of Repka and Hayward [35, 36], who adopted a pseudorandomization. All the studies showed a consistency between expected and reported outcomes, resulting in an evaluation of low risk of bias (item #3). Except for Jiang et al. [32], it was unclear the presence of other possible bias (item #4). There was no blinding of participants (item #5), but the researcher judged that the outcome is not likely to be affected by lack of blinding. Considering the blinding of outcome assessment (item #6), Jiang et al. [32] and Karimi and Roshan [34] described and applied techniques and methods that ensured the blindness; this aspect was unclear in both studies of Repka and Hayward [35, 36], while Katsourakis et al. [33] have been judged at high risk of bias. Finally, Jiang et al. [32] and both the studies of Repka and Hayward [35, 36] met the criteria for a low risk of bias in the item #7, while in Katsourakis et al. [33] and Karimi and Roshan [34], it was unclear.

Observational studies were assessed with the STROBE checklist. Quasiexperimental studies were considered comparable to prospective cohort studies. All the five studies showed an intermediate quality (Table 2).

3.3. Data Extraction. Table 3 presents the principal data of the included studies that analysed the effects of exercise on oxidative stress biomarkers, in people with cancer diagnosis. The geographic origin of the articles was as follows: USA

Table 1: PICOST inclusion and exclusion criteria.

Parameter	Inclusion criteria	Exclusion criteria
Population	People with cancer diagnosis	Absence of cancer diagnosis
Intervention	Any type of exercise also combined with pharmacological treatment	Absence of exercise
Comparator	Usual treatment No exercise	
Outcome	Oxidative stress biomarkers levels, physical performance or other indicators of physical fitness	Oxidative stress biomarkers and exercises not assessed
Study design	Experimental or observational study with original primary data	Research studies or other papers with no original data
Timing	English language 10-year publication date limit (May 2011)	Not in English language Published before May 2011

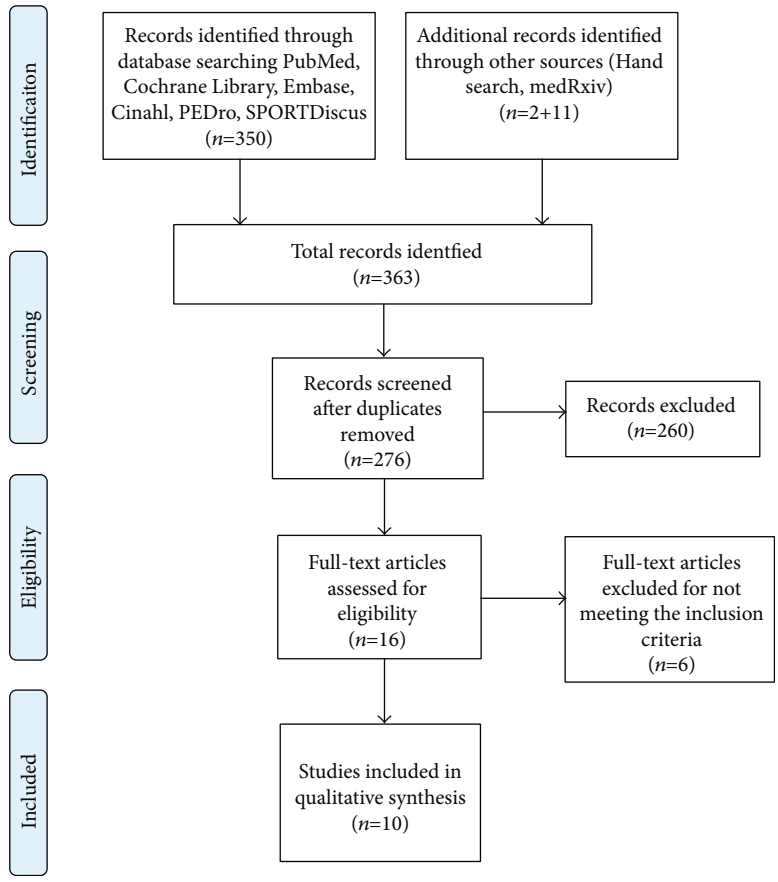

Figure 1: PRISMA flow diagram.

($n = 3$), Iran ($n = 1$), Ireland ($n = 1$), China ($n = 1$), Greece ($n = 1$), Italy ($n = 1$), Poland ($n = 1$), and Taiwan ($n = 1$). Study characteristics were heterogeneous. Within the included studies, five papers presented an observational design [37–41], and five studies were RCT [32–36]. The sample range varied from 12 to 105 people. Ages ranged from 42-54 to 65–72 years. The length of the intervention varied from 6 weeks to 7 months, the frequency from 2 to 7 times a week. The type of exercise was heterogenous: aerobic training [33, 37, 38], combined exercises [35, 36, 40], endurance training [41], water-based exercises [34], dragon boat racing and walking group [39], and Tai-chi practice [37].

Starting from observational studies results, Jones et al. [37] conducted a quasiexperimental, pilot, single arm study. The population was composed of 16 postsurgical non-small-cell lung cancer patients, with the aim to assess the relationship between an aerobic training of moderate intensity on urinary markers of oxidative status in this specific population. Exercise training comprised three aerobic cycle ergometry weekly sessions for 14 weeks. The intensity increased every week starting from 60% of peak workload

Table 2: Quality assessment of RCTs and observational studies.

Authors (year)	Study design	Tool for assessment	Quality
Karimi and Roshan [34] (2012)	RCT	Cochrane ROB tool	Poor
Repka and Hayward [35] (2016)	RCT	Cochrane ROB tool	Poor
Repka and Hayward [36] (2018)	RCT	Cochrane ROB tool	Poor
Katsourakis et al. [33] (2019)	RCT	Cochrane ROB tool	Poor
Jiang et al. [32] (2020)	RCT	Cochrane ROB tool	Good
Jones et al. [37] (2011)	Observational	STROBE	(21/33) intermediate
Guinan et al. [38] (2017)	Observational	STROBE	(18.5/33) intermediate
Tomasello et al. [39] (2015)	Observational	STROBE	(15.5/33) intermediate
Yen et al. [40] (2020)	Observational	STROBE	(18/33) intermediate
Domaszewska et al. [41] (2021)	Observational	STROBE	(16/33) intermediate

in week 1 to 70% in week 14. Interval workouts consisted of 30 s at peak workload followed by 60 s of active recovery for 10–15 intervals. As biomarkers, the investigators assessed F2–isoprostanes, iPF (2 alpha)-III, iPF (2 alpha)-VI, 8,12-iso-iPF (2 alpha)-VI, prostaglandin, 2,3-dinor-iPF (2 alpha)-III, and ametabolite of iPF(2 alpha)-III. An index composed of all the considered F2-isoprostanes isomers increased after the intervention, compared to baseline. Concerning individual isomers, iPF (2-alpha)-III, iPF (2-alpha)-VI, and 8,12-iso-iPF (2 alpha)-VI increased from baseline to postintervention. No change was detected in 2,3-dinoriPF (2 alpha)-III levels.

Tomasello et al. [39] realized a quasiexperimental study, investigating the link between physical exercise on the systemic oxidative status (SOS) in 75 women with breast cancer. The participants were assigned to one of these groups: the control group (resting), dragon boat racing group, and walking group. The walking group consisted in 3-4 hours per week of walking outdoor; the dragon boat racing group attended their session twice a week, for 7 months. All participants followed a supervised fruit/vegetable-rich diet. The investigators assessed oxidant and antioxidant biomarkers, as derivatives of reactive oxygen metabolites (dROMs), determination of lipid hydroperoxides (LPO), biological plasmatic antioxidant potential (BAP) test, total plasmatic thiol groups, SOD activity, and plasmatic glutathione peroxidase (GPx). As secondary outcomes, Tomasello et al. [39] evaluated alkaline and neutral comet assay, human umbilical vein endothelial cell (HUVEC) cultures, isolation of lymphocytes, and DNA repair assay. At the baseline, all women showed high levels of oxidative stress. As major results, exercise kept up the oxidative stress condition, but at the same time, had a positive effect on the antioxidant parameters of the SOS, in particular in the participants who have undergone to the dragon boat racing intervention. DNA fragmentation, according to the levels of single- and double-strand breaks, showed values within the normal range in the participants involved in exercise intervention.

Guinan et al. [38] realized a quasiexperimental, pilot, single arm study, including 12 participants with esophageal cancer. The aim was to verify the impact of a multilevel rehabilitation intervention on inflammation and oxidative stress levels. The intervention was an aerobic training for 12 weeks, 5 times per week. The single session was composed by a warm-up phase, a main aerobic activity, and a cool down phase. Each participant received an individualized dietetic counselling. The assessed biomarkers were as follows: lactate, 8-epimer of prostaglandin F2α (8-iso-PGF2α), 4-hydroxynonenal (4-HNE); tumour necrosis factor- (TNF-) α, interleukin- (IL-) 1β, IL-6, IL-8, and 8-hydroxy-deoxyguanosine (8-OHdG). As major findings, IL-8 reduced significantly from baseline to follow-up, and there was a trend towards lower expression patterns of other inflammatory mediators, even if not significant.

Yen et al. [40] aimed to evaluate if exercise could improve physical capacity and reduce oxidative stress, in 30 participants with head and neck cancer treating with chemotherapy. In this noncontrolled study, all participants received a combined exercise intervention for 8 weeks, 3 days per week with 40 to 45 minutes of training time with the following structure: 5 min warm-up, 30 min of aerobic exercise and a 5 min cool down + TheraBand resistance exercise, 10-12 repetitions for set, three sets per training. The intensity was the 60-70% of the maximum heart rate for the aerobic exercise training; between "somewhat heavy" and "heavy" of the Rating Perceived Exertion scale for the resistance exercise. The authors assessed total antioxidant capacity, malondialdehyde (MDA), carbonyl levels, and 8-OHdG levels. As reported, exercise training significantly raised total antioxidant capacity, while plasma concentrations of carbonyl and 8-OHdG diminished after the exercise session. The levels of malondialdehyde did not change.

Finally, Domaszewska et al. [41] realized a pilot study in order to deepen the link between an endurance training intervention and the prooxidative and antioxidant status in 12 women with breast cancer diagnosis, who received a radical mastectomy. The intervention of this single arm study lasted 2 months, 3 times per week, with a 1 hour of training time with a cycle ergometer. Each session was composed by 5 min of warm-up, 30-45 min of the proper part, 5 min of warm-down, and 15 min of stretching and breathing exercises; the adopted intensity was the 50-60% heart rate maximal for the warm-up phase. Exercise loads were individualized based on ergospirometric test. The investigators evaluated the levels of some indicators related to oxidative stress including total phenolics, ferric reducing ability of

Table 3: Studies included in the review.

Author, year, country	Study design	Study population	Intervention	Outcomes redox status biomarkers of oxidative stress	Results
Jones et al. [37] 2011 USA	Quasiexperimental pilot study, single arm	*N*: 16 Aged: 64 ± 10 Cancer: Lung	Type: aerobic exercise cycle ergometer session included a 5 min warm-up and 5 min cool down Frequency: 3x week Intensity: 60 to ≥70% of baseline peak workload Time: 14 weeks	Primary outcomes F2–isoprostanes iPF(2 alpha)-III, iPF(2 alpha)-VI, 8,12-iso-iPF(2 alpha)-VI Prostaglandin 2,3-dinor-iPF (2 alpha)-III, ametabolite of iPF (2 alpha)-III Secondary outcomes VO_2peak Peak workload	iPF(2-alpha)-III Pre: 0.15 ± 0.13 post: 0.24 ± 0.22 Change: +55% $P = 0, .10$ 2,3-dinor-iPF (2 alpha)-III Pre: 3.05 ± 2.67, post: 3.63 ± 4.02 Change: +19% $P = 0.60$ iPF (2-alpha)-VI Pre: 2.85 ± 1.33, post: 3.66 ± 2.12 Change: +29% $P = 0.04$ 8,12-iso-iPF (2alpha)-VI Pre: 2.12 ± 1.25, post: 2.71 ± 1.84 Change: +28% $P = 0.07$ VO_2peak Change: 1.13 ± 0.21$P = 0.14$ Peak workload Change: 10 Watts $P < 0.001$
Karimi and Roshan [34] 2012 Iran	RCT	*N*: 40 Aged: 48 ± 6 Cancer: breast CG: placebo EG1: water-base exercise CG: ginger supplementation EG2: Exercises + Ginger supplementation	Type: water-based exercise, 10′ warm up, 20-60′ water aerobic exercise and 10′ cool down Frequency: 4 times per week Time: 6 weeks Type: supplementation 3 capsules 750 mg of ginger rhizome powder Frequency: 4 times per day with breakfast, lunch, dinner and afternoon. Time: 6 weeks	Primary outcomes GPx MDA NO Secondary outcome Adiponectin	GPx CG placebo Pre: 25.1 ± 5.47, post: 24.3 ± 4.62 EG1 exercise Pre: 25.9 ± 2.51, post: 36.9 ± 2.42$P < 0.05$ CG supplementation Pre: 27.3 ± 2.26, post: 31.8 ± 3.61$P < 0.05$ EG2 exercise+supplementation Pre: 26.3 ± 2, post: 44.4 ± 4.79$P < 0.05$ MDA CG placebo Pre: 25.39 ± 5.12, post: 25.05 ± 3.23 EG exercise Pre: 25.55 ± 4.83, post: 21.62 ± 3.2$P < 0.05$ CG supplementation Pre: 24.17 ± 3.68, post: 24.07 ± 3.5 EG2 exercise+supplementation Pre: 23.97 ± 4.02, post: 19.55 ± 3.76$P < 0.05$ NO CG placebo Pre: 36.23 ± 5.96, post: 34.46 ± 4.34 EG1 exercise Pre: 35.26 ± 5.51, post: 41.33 ± 6.22$P < 0.05$ CG supplementation

Table 3: Continued.

Author, year, country	Study design	Study population	Intervention	Outcomes redox status biomarkers of oxidative stress	Results
					Pre: 35.47 ± 5.55, post: 37.32 ± 4.90 EG2 exercise+supplementation Pre: 36.06 ± 6.27, post: 45.45 ± 7.01$P < 0.05$ Adiponectin CG placebo Pre: 8.43 ± 0.86, post: 7.84 ± 1.03 EG1 exercise Pre: 8.65 + 1, post: 10.45 ± 1.53$P < 0.05$ CG supplementation Pre: 8.03 + 1.0, post: 8.56 ± 1.07 EG2 exercise+supplementation Pre: 8.18 ± 0.74, post: 11.86 ± 0.74
Tomasello et al. [39] 2015 Italy	Quasiexperimental study	N: 105 Aged: 51 ± 12 (EG); 49 ± 12 (CGhealthy) Cancer: breast EG-exercises dragon boat: 25 EG-walking: 25 CG: 25 CGhealthy: 30	Type EG-exercise: dragon-boat Type EG-walking: walking Frequency: 3-4 hours 2 per weeks Time: 7 months All patients followed a controlled fruit/vegetable-rich diet	Primary outcomes dROMs BAP LPO SOD activity GPx Total plasmatic thiol groups Secondary outcomes Alkaline and neutral comet assay (% TDNA) Human umbilical vein endothelial cell (HUVEC) cultures, isolation of lymphocytes and DNA repair assay (NER analysis)	dROMS (CARR U) 459 ± 61 CARR U for EG-exercise dragon-boat versus 502 ± 76 CARR U for group EG walking ($P = 0.332$); the increase was significant with respect to the control BrC group for each of the two activity groups $P = 0.038$ and $P < 0.001$, respectively. BAP 2,275 ± 337 μmol/l EG-exercise dragon-boat versus 2,236 ± 223 μmol/l EG walking P = ns Following physical training, BAP levels were significantly increased compared with preexercise basal levels and control BrC levels LPO 13.2 ± 3.6 nmol/ml EG-exercise dragon-boat versus 15.08 ± 2.7 nmol/ml EG walking $P = 0.224$ versus 9.7 ± 2.5 nmol/ml control BrC $P = 0.007$ and $P < 0.001$, respectively SOD activity 8.4 ± 1.9 U/ml EG-exercise dragon-boat vs. 6.8 ± 2 U/ml EG-exercise dragon-boat $P = 0.044$ vs. 3.90 ± 2.04 control BrC $P < 0.001$ GPX 246 ± 57.7 nmol/min/ml EG-exercise versus dragon boat, 197 ± 53.3 nmol/min/ml EG-walking versus 147.10 ± 37.6 nmol/min/ml control BrC $P = 0.007$ Total plasmatic thiol groups No differences between EG exercise dragon boat.

Table 3: Continued.

Author, year, country	Study design	Study population	Intervention	Outcomes redox status biomarkers of oxidative stress	Results
					The control BrC values were also significantly lower than those of the two physical activity groups (both $P < 0.001$). Alkaline and neutral comet assay (%TDNA) EG walking 17.10% versus 14.05% EG exercise dragon boat versus 19.59% control BrC group P = ns NER analysis (% TDNA) Dragon boat 31.5 ± 7.6 vs. walking 30.3 ± 8.4% $P = 0.80$ versus 24.5 ± 6% control BrC $P = 0.008$ and $P = 0.045$, respectively, with EG
Repka and Hayward [35] 2016 USA	RCT	N: 22 aged: 64.0 ± 10.8 (EX group); 62.4 ± 9 (CG); 55.1 ± 9.7 (CHhealthy) Cancer: different types EG: 8 CG: 7 CGhealthy: 7	Type: combined exercises 5′ warm up 20′ aerobic training 25′ resistance training 10′ flexibility and balance training. Frequency: 3 days per week, 1-hour session. Intensity: 40%-60% of heart rate reserve or a rating of perceived exertion 4 to 5. Time: 10 weeks No diet prescription	Primary outcomes TEAC, Trolox-equivalent antioxidant capacity (mM Trolox) Protein carbonyls ($nmol^{-1}$) 8-OHdG (8-hydroxy-deoxyguanosine (ng ml) Secondary outcomes: composite arm strength (lb) Composite leg strength (lb) Handgrip strength (lb) VO_2peak (ml)	TEAC EG: Pre: 0.28 ± 0.07$P < 0.01$ than NC Post: 0.39 ± 0.05$P < 0.01$ than baseline CG: Pre: 0.26 ± 0.05$P < 0.01$ than NC Post: 0.32 ± 0.08 CGhealthy 0.37 ± 0.07 Protein carbonyls EG: Pre: 1.30 ± 0.44$P < 0.05$ than NC Post: 0.84 ± 0.33$P < 0.05$ with baseline CG: Pre: 1.18 ± 0.42$P < 0.05$ than NC Post: 1.12 ± 0.22$P < 0.05$ than NC CGhealthy: 0.89 ± 0.25 8-OHdG EG: Pre: 1.47 ± 0.33, post: 0.29 ± 0.18 Significant time by group interaction with control group $P < 0.05$. CG: Pre: 0.35 ± 0.14, post: 0.49 ± 0.22 CGhealthy: 0.33 ± 0.18 Arm strength EX: Pre: 230.4 ± 91.4, post: 324.4 ± 125.2 (significant

Table 3: Continued.

Author, year, country	Study design	Study population	Intervention	Outcomes redox status biomarkers of oxidative stress	Results
					time by group interaction with control group $P < 0.05$ $P < 0.01$ than baseline CG: Pre: 273.3 ± 148.1, post: 280.1 ± 165.7 Leg strength EG: Pre: 332.3 ± 130.9, post: 445.5 ± 157.2 (significant time by group interaction with control group $P < 0.05$ $P < 0.01$ than baseline CG: Pre: 348.1 ± 166.3, post: 346.9 ± 164.4 Handgrip EX: Pre: 25.8 ± 4.8, post: 28.6 ± 6.0 $P < 0.05$ than baseline CG: Pre: 25.6 ± 9.7, post: 26.8 ± 10.0 VO_2 EG: Pre: 20.1 ± 9.7, post: 23.4 ± 10.9 $P < 0.05$ than baseline CG: Pre: 18.1 ± 4.4, post: 18.8 ± 6.1
Guinan et al. [38] 2017 Ireland	Quasi-experimental pilot study, single arm	*N*: 12 Aged: 61 ± 7.29 Cancer: oesophageal	Type: aerobic training. Warm up, aerobic exercise and cool down Frequency: 5 times per week Intensity: from 30% to 60% of heart rate reserve Time: 12 weeks Individualised dietetic conselling Group education session	Primary outcomes 4-Hydroxynonenal (4-HNE) 8-Hydroxy-2-deoxyguanosine (8-OHdG) Secondary outcomes Lactate 8-epimer of prostaglandin F2α (8-iso-PGF2α) Tumour necrosis factor- (TNF-) α Interleukin- (IL-) 1β IL-6 IL-8	4-HNE Pre: 1.32 ± 3.96, post: 3.08 ± 5.22$P < 0.29$ 8-OHdG Almost all 8-OHdg data was below the assay detection, and therefore, no analysis was completed on this outcome Lactate Pre: 153.61 ± 52.45, post: 182.42 ± 64.99 $P < 0.23$ 8-iso-PGF2α Pre: 292.95 ± 134.66, post: 259.45 ± 111.19 $P < 0.20$ TNF-α Pre: 41.73 ± 309.57, post: 35.92 ± 292.12

Table 3: Continued.

Author, year, country	Study design	Study population	Intervention	Outcomes redox status biomarkers of oxidative stress	Results
				CPET 6MWT MVPA Body weight %BF FM SMM FFM	$P < 0.19$ IL-1β Pre: 3.8 ± 13.93, post: 3.05 ± 12.68$P < 0.13$ IL-6 Pre: 8.69 ± 111.45, post: 6.98 ± 109.93$P < 0.52$ IL-8 Pre: 13.16 ± 88.71, post: 10.14 ± 81.25$P < 0.03$ CPET Pre: 20.08 ± 5.2, post: 24.08 ± 4.99$P < 0.004$ 6MWT Pre: 532.17 ± 78.25, post: 588.5 ± 73.14$P < 0.003$ MVPA Pre: 292.91 ± 192.44, post: 317.88 ± 187.05 $P < 0.363$ Body weight Pre: 70.93 ± 19.95, post: 70.28 ± 19.48 $P < 0.28$ %BF Pre: 27.11 ± 5.86, post: 28.22 ± 5.38 $P < 0.09$ FM Pre: 19.48 ± 8.40, post: 19.74 ± 8.04 $P < 0.57$ SMM Pre: 25.44 ± 8.72, post: 24.85 ± 8.97$P < 0.12$ FFM Pre: 50.67 ± 14.99, post: 49.94 ± 15.02$P < 0.16$
Repka and Hayward [36] 2018 USA	RCT	N: 22 Aged: 64.0 ± 10.8 (EG); 62.4 ± 9.7 (CG); 55.1 ± 9.7 (CGhealthy) Cancer: different types EG: 8 CG: 7 Healthy HCG: 7	Type: combined exercise, 5′ warm up, 20′ aerobic exercise, 25′ resistance training, and 10′ flexibility and balance training. Frequency: 1 hour session, 3 days per week Intensity: from 40% to 60% of heart rate reserve or a rating of perceived exertion 4 to 5. Time:10 weeks No diet prescription	Primary outcomes TEAC, Trolox-equivalent antioxidant Protein carbonyls 8-OHdG (8-hydroxy-deoxyguanosine) Secondary outcome Piper Fatigue Inventory	TEAC EG: Pre: 0.75 ± 0.19, post: 1.06 ± 0.13 $P < 0.05$ with baseline CG: Pre: 0.69 ± 0.16, post: 0.85 ± 0.22 CGhealthy 1.0 ± 0.20$P < 0.5$ with cancer Protein carbonyls: EG: Pre: 1.46 ± 0.49, post: 0.94 ± 0.37 $P < 0.05$ with baseline CG: Pre: 1.32 ± 0.48, post: 1.26 ± 0.25 CGhealthy: 1.0 ± 0.28$P < 0.5$ with cancer 8-OHdG EG:

Table 3: Continued.

Author, year, country	Study design	Study population	Intervention	Outcomes redox status biomarkers of oxidative stress	Results
					Pre: 1.45 ± 0.96, post: 0.88 ± 0.55 significant time by group interaction with control group $P < .05$. CG: Pre: 1.08 ± 0.44, post: 1.48 ± 0.66 CGhealthy: 1.0 ± 0.57 CRF: EG: Pre: 5.0 ± 2.2, post: 2.6 ± 1.9$P < 0.05$ with baseline CG: Pre: 4.7 ± 2.5, post: 3.2 ± 2.4 CGhealthy 1.0 ± 1.0$P < 0.5$ with cancer
Katsourakis et al. [33] 2019 Greece	RCT	N: 54 Aged 59.90 EG (range 54.67-65.14) 69.14 CG (range: 65.73–72.55) Cancer: pancreas EG: 28 CG: 26	Type: aerobic exercise Intensity: 60% of maximum heart rate) Frequency: 30 min, 3 per week Time: 12 weeks.	Primary outcomes Uric acid levels Secondary outcomes Haemoglobulin Abumin Blood glucose Glycosylated	Uric acid EG vs. CG $P = 0.069 > 0.05$ The statistics illustrated that exercise can have a positive influence on glycaemic control, but no influence was observed on the levels of uric acid, which represents oxidative stress. Glucose EG vs. CG $P < 0.05$
Jiang et al. [32] 2020 China	RCT	N: 100 Aged: 59.30 ± 7.40 (EG); 57.56 ± 11.23 (CG) EG: 50 CG: 50 Cancer: lung	Type: Tai-Chi 10-min warm-up 40-min practice 10 min cool down Frequency: 60 min, 92 lessons Time: 3 months	Primary outcomes TOS TAS OSI MDA, SOD, CAT, GPx TNF-α, IL-1, and IL-6 Secondary outcome VAS PHS	Pre: no statistical differences for TOS TAS and OSI between the two groups $P > 0.05$. Post: the levels for TOS and OSI in the EG group were lower than those in the CG while TAS level in the EG was higher than in the CG $P < 0.05$. EG vs. CG pre: no statistical differences For SOD, CAT, GSPx and MDA $P > 0.05$. EG vs. CG post SOD, CAT, and GSPx increased while the serum level of MDA was reduced in the EG vs. CG $P < 0.05$ EG vs. CG pre: no statistical differences for TNF-α, IL-1, IL-6 and IL-10 $P > 0.05$. EG vs. CG post TNF-α, IL-1, and IL-6 were reduced while the serum level of IL-10 was increased in the EG vs. CG $P < 0.05$. VAS EG: Pre: 75.86 ± 7.36, post: 50.96 ± 8.23 CG:

Table 3: Continued.

Author, year, country	Study design	Study population	Intervention	Outcomes redox status biomarkers of oxidative stress	Results
					Pre: 82.46 ± 6.52, post: 78.01 ± 7.9$P < 0.001$ PHS EG: Pre: 3.15 ± 0.62, post: 2.03 ± 0.77 CG: Pre: 2.93 ± 0.54, post: 2.68 ± 0.69$P < 0.003$
Yen et al. [40] 2020 Taiwan	Quasiexperimental study, single arm	*N*: 42 Aged: 56.0 ± 12.3 Cancer: head/neck	Type: 5 min warm up, 30 min of aerobic exercise training and a 5 min cool down + TheraBand resistance exercise, 10 to 12 repetitions for one set, three sets per training, both upper and lower extremities. Frequency: 3 days per week. 40 to 45 minutes of training time. Time: 8 weeks Intensity: 60-70% of the maximum heart rate	Primary outcomes Total antioxidant capacity Malondialdehyde Carbonyl levels 8-Hydroxy-20-deoxyguanosine (8-OHdG)	Total antioxidant capacity Pre: 221.7 ± 62.2, post: 443.7 ± 72.1 Malondialdehyde Pre: 4.7 ± 0.8, post: 3.8 ± 1.3 Carbonyl levels Pre: 10.1 ± 2.6, post: 5.5 ± 1.8 8-Hydroxy-20-deoxyguanosine (8-OHdG) Pre: 1031.3 ± 43.8, post: 761.3 ± 66.3
Domaszewska et al. [41] 2021 Poland	Quasiexperimental pilot study, single arm	N: 12, Aged: 50.6 ± 2.9 Cancer: breast	Type: Endurance training 5 min of warm up, 30-45 min of the proper part, 5 min of warm-down, 15 min of stretching and breathing exercises Frequency: 3 times per week. 1 hour of training time with a cycle ergometer. Time: 8 weeks Intensity: 50-60% HRmax for the warm-up phase. Exercise loads were determined individually on the basis of ergospirometric exercise test	Primary outcomes Total phenolics FRAP TBARS Urea Secondary outcomes VT heart rate VT load Peak HR Peak VO_2 Peak load	Total phenolics Pre: 2.44 ± 0.09, post: 2.43 ± 0.28 FRAP Pre: 857.25 ± 147.17, post: 859.67 ± 148.65 TBARS Pre: 5.09 ± 2.09, post: 5.02 ± 1.81 Urea Pre: 3.32 ± 2.09, post: 3.71 ± 1.99 VT heart rate Pre: 127.75 ± 13.07, post: 142.25 ± 13.06 VT load Pre: 76.67 ± 13.37, post: 94.17 ± 14.29 Peak HR Pre: 158.92 ± 15.37, post: 166.50 ± 13.56 Peak VO_2 Pre: 25.74 ± 4.04, post: 27.00 ± 3.68 Peak load Pre: 112.50 ± 23.01, post: 123.33 ± 22.09

Hydroperoxides; SOD: superoxide dismutase; TEAC: Trolox-equivalent antioxidant capacity; 8-OHdG: 8-hydroxy-deoxyguanosine; 8-iso-PGF2α: 8-epimer of prostaglandin F2α; 4-HNE: 4-hydroxynonenal; TNF-α: tumour necrosis factor-α; IL-1β: interleukin-1β; IL-6: interleukin-6; IL-8: interleukin-8; CPET: cardiopulmonary exercise testing; 6MWT: six-minute walk test; MVPA: moderate to vigorous physical activity; %BF: % body fat; SMM: skeletal muscle mass; FFM: fat-free mass; TOS: total oxidant status; TAS: total antioxidant status; OSI: oxidative stress index; VAS: visual analogic scale; PHS: Prince-Henry score method; FRAP: ferric reducing ability of plasma; TBARS: thiobarbituric acid reactive substances; VT: ventilatory threshold; HR: heart rate.

plasma (FRAP), thiobarbituric acid reactive substances (TBARS), and urea, alongside hematological parameters (erythrocytes, hematocrit, hemoglobin, leukocytes, neutrophils, lymphocytes, monocytes, total proteins, and albumins). This type of intervention did not cause a worsening of oxidative stress in women treated for breast cancer. Analysing included RCT studies, in Karimi and Roshan [34], forty women with breast cancer were assigned into four different groups: the placebo, water-based exercise, ginger supplement, and water-based exercise+ginger supplement groups. The water-based exercise consisted in 10 min warm-up, 20-60 min of water aerobic exercise, and finished with 10 min cool down. This exercise program was scheduled with 4 sessions per week for 6 weeks in total. The evaluated biomarkers were adiponectin, GPx, nitric oxide (NO), and MDA. At the end of the 6 weeks, people who has undergone the water-based exercise showed an improvement of adiponectin, NO and GPx and a reduction in MDA levels.

In Repka and Hayward [35], the investigators aimed to assess the effect of an exercise on muscular strength, cardiorespiratory fitness, and oxidative stress biomarkers in 8 cancer survivors compared with a group of 7 nonexercising cancer patients and a group of 8 age-matched individuals without cancer history. The exercise cancer group attended a 10-week combined exercise program, one hour, 3 days per week. Each session was composed of 5 min of warm-up, 20 min of aerobic exercise, 25 min of resistance training, and 10 min of flexibility and balance training. The intensity was established from 40% to 60% of heart rate reserve and a rating of perceived exertion 4 to 5. No specific diet was prescribed. Trolox-equivalent antioxidant capacity (TEAC), protein carbonyls, and 8-OHdG were assessed. The exercise cancer group showed a significant improvement in antioxidant capacity and a decrease in protein carbonyls at the end of the intervention whereas the nonexercise cancer group did not. No significant within-group changes in 8-OHdG occurred. In 2018, in further investigations within the same study, Repka and Hayward [36] evaluated the effect of their intervention on cancer-related fatigue and the possible relationship with the oxidative stress biomarkers, finding similar results in the same parameters.

Katsourakis et al. [33] focused on evaluating if exercise has any benefit on oxidative stress and glucose levels in 54 patients who undergone a radical pancreatic tumour resection. The intervention group started the training 4 weeks after surgery; this involved 30 min on a bicycle (60% of maximum heart rate) 3 times per week for 12 weeks. The control group did not exercise. The authors assessed uric acid levels, glycosylated haemoglobulin, albumin, and blood glucose pre and post the intervention. The results showed the positive effects of aerobic exercise on glycaemic control, while no change was observed on uric acid, an oxidative stress parameter.

Finally, Jiang et al. [32] evaluated the possible effects of a Tai-Chi intervention on blood oxygen level, and the antioxidant and anti-inflammatory activities, in 100 patients with lung cancer. A simplified 24-posture Yang Tai-Chi was taught by specialized instructors in hospital, i.e., specialized nurses. Tai-Chi was conducted in class, early in the morning, for 60 min divided in 10 min warm-up, 40 min practice, and 10 min cool down) for three months. Jiang et al. assessed serum oxidative parameters, such as total oxidant status (TOS), total antioxidant status (TAS), and the oxidative stress index (OSI); the authors also evaluated some biochemical indexes in serum, such as MDA, SOD, cCAT, and GPx. The results suggest that Tai-Chi exercise improves antioxidant properties in lung cancer patients. After three months, OSI and TOS levels were lower if compared to the control group, while TAS showed higher levels. Tai-Chi also increased the levels of antioxidant markers SOD, CAT, and GPx and reduced the levels of MDA.

4. Discussion

Our review systematically analysed ten articles investigating the exercise's effect on oxidative stress biomarkers in adult patients with cancer. In agreement with the evidence outlined in the introduction, three studies [32, 34, 39] have shown the positive effect of exercise on antioxidant enzymes. Karimi and Roshan [34] demonstrated that water-based exercise increased GPx activities in breast cancer patients. Tomasello et al. [39] have also shown that dragon boat racing's exercise significantly raised the levels of GPx and SOD in breast cancer. Jiang et al. [32] observed that Tai-Chi enhanced the blood levels of SOD, CAT, and GPx in lung cancer patients.

It is well known that a rapid and high level of ROS enhances the oxidative damage on DNA that can promote the initiation and progression of cancer [12, 15]. Interestingly, there is evidence that DNA damage promptly arises in white blood cells after an acute endurance exercise training and the DNA damage persist for up 24 h. However, after some postexercise days, the exercise-induced DNA damage is no longer measurable [42]. This biological effect can be due to the effect of exercise in inducing upregulation of DNA repair mechanisms, and it corroborates the concept that exercise causes an adaptive response [6, 42]. 8-OHdG is one of most frequent oxidative DNA lesions that can be observed in various types of cancer [15]. Among the articles meeting the inclusion criteria, only three [36, 38, 40] evaluated the 8-OHdG as biomarker of oxidative stress. Interestingly, Yen et al. [40] found that combined exercise intervention for 8 weeks significantly decreased the 8-OHdG level in people affected by head and neck cancer. In Repka and Hayward's [36] study, a reduction of 8-OHdG level was observed in breast cancer patients after a combined exercise intervention, yet the comparison with the other two study samples is not clear and the results do not allow an exhaustive conclusion. Unfortunately, in the study by Guinan et al. [38], some technical detection problems precluded the fulfilment of the 8-OHdG analysis after exercise. Based on these findings, it is difficult to assess the ability of the exercise intervention to counteract oxidative DNA damage in cancer patients.

To date, different categories of oxidative stress biomarkers are available for assessing the biological effects of exercise in humans [43]. The direct measurement of ROS in the tissue or body fluid is difficult to perform because they are generally too reactive and have a half-life too short [44].

Therefore, to assess the oxidative stress, it is more suitable to investigate the oxidation target products, such as nucleic acid, protein, and lipid or to evaluate the levels of endogenous antioxidants [43]. The most common antioxidant biomarkers include enzymatic antioxidants (CAT, SOD, and GPx), nonenzymatic antioxidants (e.g., GSH and uric acid), and total antioxidant capacity (e.g., TAC) [43]. In this contest, the set of results evaluated in this review gives an encouraging picture of the exercises' benefit in people with cancer. In most studies, the exercise administered to cancer patients improved the indicators of the total antioxidant capacity, enhanced the activity of antioxidant enzymes, or reduced the levels of biomarkers linked to oxidative damage such as MDA and 8-OHdG. Moreover, in our review, the effect of exercise on oxidative stress biomarkers was observed in different types of cancer (lung, breast, head/neck, pancreatic, and oesophageal cancer), and among these, breast and lung cancer are worldwide the most diagnosed [45]. However, the variety of biomarkers found to measure oxidative stress levels (see Table 3) calls for thinking. The heterogeneity of study design hampers the comparison among the articles analysed in this review and limits the ability to draw an exhaustive conclusion on the efficacy of exercise in cancer treatment strategies. This is also in agreement with a previous study indicating that measured oxidative status is variable and sensitive to significant experimental approach differences between research groups [13]. Further research to assess which oxidative stress biomarkers can allow the most valid and reliable measure of change linked to exercise in cancer patients is necessary to broaden outcome integration and to expand the knowledge on this topic of great interest to health care.

Currently, the treatment of cancer is multimodal and includes also PA which is considered a low-cost, safe, and effective strategy [3]. The frequency, intensity, time, and type (FITT) combined to produce total dosage of exercise prescription over a defined period (e.g., weeks or months) and the variation of dosage in the follow-up or within treatment cycles may stimulate greater physiological adaptation also reducing the risk of harm associated to over-training [3]. In the last decades, the scientific community deeply debated the issue if exercise-induced ROS production is a benefit or a disadvantage to health [6]. In connection, a recent study showed that practicing an exercise training regularly does not induce chronic oxidative stress in the involved muscles [23]. Accordingly, different types of exercise in terms of FITT can influence the levels of oxidative stress biomarkers. In the ten studies systematically evaluated, a miscellaneous of exercise interventions were applied in study population affect by different types of cancer. We found differences regarding the FITT of the exercise administered to patients. Combined exercise intervention, including resistance training and aerobic activities, improved the antioxidant status and the profile of the oxidative stress biomarker investigated. The positive influence on oxidative stress level was observed for various types of cancer including breast [34–36, 39, 41], lung [32], pancreatic [33], and head and neck [40]. These findings are promising and support the concept that a combined exercise program, including aerobic and resistance training, should be enclosed in a cancer patient's exercise prescription. Moreover, a combined exercise intervention can well contribute to counter the oxidative damage that arises in the cancer process.

During our investigation about how specific exercise programs can influence the antioxidant status of cancer patients, some issues were identified. One of the limitations of this study is the small number of articles meeting the inclusion criteria and the reduced sample range of the patients enrolled. Additionally, the different study designs (RTC and quasiexperimental study single arm) have restricted the comparison among the selected articles. Finally, the measurement of different biomarkers to evaluate the type of oxidative stress damage or antioxidant capacity of cancer patients has limited the investigators' ability to fully address the study aim.

Despite the limitations observed in the available literature, to our knowledge, this systematic review is the first that have investigated the effects of exercise on oxidative stress biomarkers in various types of cancer. Our findings provide a critical overview of the existing scientific evidence on this topic and point out the need for future studies on this issue.

5. Conclusion and Perspectives

Novel approaches need to be evaluated to enhance the prognosis and the quality of life in people with cancer. Our findings indicate that, in the included studies, a miscellaneous of exercise interventions were investigated in terms of FITT of the exercise administered to patients. In this review, we showed that combined exercise intervention with aerobic activities and resistance training can induce positive effects on some oxidative stress biomarkers and enhance the antioxidant status of patients with different oncological diseases such as breast, lung, head and neck, or pancreatic cancer. However, further well-designed high-quality clinical studies focused on different types of cancer are needed to identify a set of suitable biomarkers able to check the physiological impact of the exercise on the antioxidant defences of oncological patients. In connection, the availability of well-established oxidative stress biomarkers is crucial to analyse the efficacy and safety of FITT exercise in thr cancer control approach. Despite our systematic literature search, the findings on the ability of the exercise to counter oxidative DNA damage in cancer patients are limited and preclude a full conclusion. Since the DNA damage has a pivotal role in cancer development and progression, future studies are strongly recommended to evaluate the decrease of genetic damage induced by exercise in cancer patients.

Overall, oxidative stress biomarkers allow a detailed understanding inside the mechanistic effects of exercise benefit in cancer patients. This promising approach can expand the knowledge on the molecular effects on cancer outcomes that different frequencies, intensities, and types of exercise can induce. Admittedly, this information is important to prescribe an appropriate exercise intervention in anticancer strategy. Besides, the identification of tailored and effective exercises based on the diagnosis and prognosis of individual

patients will offer new perspective for integrated therapy in oncology.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

FM, YL, and LD conceived the study. YL and AM designed methodology and coordinated the activity planning. YL, AM, SM, GB, and LD independently reviewed papers and disagreements were resolved by consensus with CF, LB, and FM. AM, SM, GB, and LD perform the qualitative assessment and disagreements were resolved by YL. YL, AM, SM, GB, and LD acquired, analysed, and interpreted the data. FM checked data extractions. YL, AM, and FM drafted the manuscript. SM, GB, CF, LB, and LD revised the manuscript and contributed with intellectual ideas. FM, LD, CF, and LB supervised the study. All authors have read and approved the final manuscript, including figures and tables.

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Review Article

Multiple Applications of Different Exercise Modalities with Rodents

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A large proportion of chronic diseases can be derived from a sedentary lifestyle. Raising physical activity awareness is indispensable, as lack of exercise is the fourth most common cause of death worldwide. Animal models in different research fields serve as important tools in the study of acute or chronic noncommunicable disorders. With the help of animal-based exercise research, exercise-mediated complex antioxidant and inflammatory pathways can be explored, which knowledge can be transferred to human studies. Whereas sustained physical activity has an enormous number of beneficial effects on many organ systems, these animal models are easily applicable in several research areas. This review is aimed at providing an overall picture of scientific research studies using animal models with a focus on different training modalities. Without wishing to be exhaustive, the most commonly used forms of exercise are presented.

1. Introduction

Sport can be thought of as a therapeutic tool or a prevention strategy for different disorders. It is beginning to be learned that physical exercise exerts its effects via extensive molecular pathways by which it maintains and improves the quality of life. It is well known that regularly maintained training has several beneficial effects on overall health, from cells to whole organ systems [1]. Nonetheless, physical inactivity entails numerous health issues from systemic inflammation to hormonal dysfunctions which raises the risk of wide-ranging noncommunicable diseases, such as type II diabetes, metabolic syndrome, cardiovascular and neurodegenerative disorders, and even cancer [2–5]. Over and above, sedentary lifestyle-related redox disturbance further aggravates preexisting pathological processes [6]. The main purpose of training-related research studies has been targeted on the health benefits of exercise to be able to prevent and treat these conditions. Comprehending the underpinning systemic changes provoked by exercise helps us to develop more efficient treatment methods and prevention strategies against widespread diseases. With the help of animal models, it is possible to study the most complex effects of exercise at all levels of organization. The appropriate animal species and the duration, frequency, and intensity of the training should be chosen according to the purpose of the study

[7]. The most common types of exercise used in animal experiments are voluntary wheel running, forced wheel running, treadmill running, swimming, and resistance training [8, 9]. Aerobic exercise modalities are suitable for almost every noncommunicable disorder-related research area, while anaerobic training modalities are applicable in a much narrower field of research, including muscle formation studies [8–11]. In this review, we discuss the far-reaching benefits of physical exercise and its interpretation in different animal models. Our aim is to provide a comprehensive picture of the different exercise modalities used with rodents and their far-reaching effects on organ systems affecting the most researched noncommunicable diseases.

2. Beneficial Effects of Exercise on Different Organ Systems

2.1. Bones and Skeletal Muscle. Exercise has major effects on body composition. It is well established that physical activity improves bone properties such as bone quality or density. Consequently, it lowers the risk of osteoporosis. Osteoporosis is a condition characterized by low bone mass and bone fragility and mainly occurs among elderly people and postmenopausal women as a result of hormonal changes [12]. Exercise is considered to be the best nonpharmacologic approach in preventing osteoporosis; recent studies discussed that long-term exercise is able to increase bone strength and formation; therefore, it is effective in improving bone quality [13, 14].

Along with bones, exercise is able to increase muscle strength and improve balance and coordination. The most noticeable effect of long-term exercise, especially resistance training, is the increase of muscle mass. This process affects the basal metabolic rate and body composition in a favorable way. Besides, physical activity is proved to be promising in the regeneration and rejuvenation of muscle stem cells [15]. Exercise is also effective in age-related muscle atrophy, called sarcopenia. A recent work of White et al. supported the fact that long-term voluntary exercise can prevent sarcopenia in the hindlimb muscles in female and male rats as well [16]. Taken together, these results indicate that regular exercise has many beneficial effects on skeletal muscle function, regeneration, and bone quality at any age.

2.2. Metabolic Health. In the absence of exercise as a result of chronic positive energy balance, weight gain occurs. In this pathological condition, an increase in the number and size of adipocytes is observed, which leads to the disruption of leptin signaling and eventually to chronic inflammation [17]. On the contrary, exercise promotes metabolic health by decreasing body weight along with the amount of circulating lipids and the concentration of leptin and positively affects glucose tolerance and insulin sensitivity [18, 19]. Studies have shown that regular exercise significantly improved glucose homeostasis in diabetic and prediabetic status [20]. Moreover, regular exercise is efficient enough to reduce plasma leptin and insulin levels in hormone deficiency as well; thus, it plays an important part in the improvement of pathophysiological changes in connection with metabolic syndrome [21, 22]. Furthermore, exercise is able to increase the expression of glucose transporter 4 and contributed to balanced glucose homeostasis and insulin sensitivity in rats [23]. Hence, regular exercise is able to reduce the risk for metabolic disorders and the resulting cardiovascular complications.

2.3. Cardiovascular System. Physical exercise has far-reaching cardiovascular effects as well. Studies have shown that sustained physical activity lowers the individual's resting heart rate and blood pressure while it increases physiological cardiac hypertrophy [24, 25]. Exercise affects the cardiovascular system in different ways; it modulates numerous signaling pathways and improves oxygen delivery throughout the body via angiogenesis and vasodilation [26]. Nitrogen monoxide (NO) production in the endothelium rises significantly as a result of training and causes a well-known vasodilating phenomenon [27]. By enhancing nitrogen monoxide synthase activity, exercise has an undeniable role in the maintenance of normal blood pressure and in the treatment of hypertension [28]. Besides vasodilatation, NO has anti-inflammatory and platelet inhibitory effects as well, thereby contributing to the mitigation of atherosclerotic risk [29]. Additionally, exercise influences blood vessel morphology by extending the capillary network in the cardiovascular system. In a recent study of ours, we proved that a 12-week-long voluntary exercise was an effective therapeutic tool to improve cardiac function in aged rats; we clarified the exercise-moderated genetic modifications that contributed to the functional improvement of the heart [30]. Exercise can serve as a therapeutic tool after myocardial infarction (MI) as well; recent studies supported that after a postmyocardial injury, recreational exercise was able to improve cardiac health and antioxidant status [31, 32].

2.4. Nervous System. Numerous studies demonstrated the effects of physical activity on mental health, cognitive processes, and brain activity [33–35]. It is clarified that exercise affects several complex mechanisms including cerebral perfusion, neurogenesis, and synaptic plasticity [36, 37]. The findings of Kleemeyer et al. discussed that 6 months of exercise is associated with a hippocampal neuron density increase [38], while the work of Ruscheweyh et al. discovered a significant augmentation of the gray and white matter as a result of aerobic training [39]. Interestingly, encouraging results were obtained not only in young rats but also in older animals (19-month-old rodents). Scientists found that 1.5 months of voluntary exercise elevated gliogenesis by 20% and reverted age-related decline in neurogenesis by 50% [40]. Trophic factors such as the brain-derived trophic factor (BDNF) and vascular endothelial growth factor (VEGF) greatly support the cognition in young and older individuals as well. Several studies verified that running exercise at any age increased the expression of BDNF and VEGF in the hippocampus, which the phenomenon was correlated with the improvement of spatial learning and memory [41, 42]. In addition, exercise has been recommended as one of the best lifestyle interventions against neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease)

[43]. It is well established that exercise has a crucial role in the protection of neurons, but recent results have also suggested that it is promising in the prevention of amyloid-β and tau protein plaque formation [44, 45]. According to recent studies, swimming, voluntary wheel running, and treadmill exercise have also proven to treat neuropathic pain in a mouse model [46, 47]. In addition, regular exercise is well known to trigger the release of serotonin and dopamine, which neurotransmitters help to overcome the symptoms of depression and anxiety [48].

2.5. Antioxidant and Anti-Inflammatory Effects of Exercise. A number of studies have shown that besides many favorable effects on different organ system functions (e.g., cardiovascular or metabolic), physical exercise is able to decrease proinflammatory markers and improve antioxidant status systemically [49–51]. Regular exercise mitigates reactive oxygen species-mediated cell damage by boosting antioxidant functions and reducing C-reactive protein, interleukin-6, and tumor necrosis factor-alpha (TNF-α) levels [52, 53]. Furthermore, it is proved to reduce reactive oxygen species (ROS) production; therefore, it plays a key role in the maintenance of redox balance [54]. Regular training leads to the adaptation of the antioxidant capacity and protects the cells against adverse oxidative processes [55]. Sustained exercise has been demonstrated to be essential not only in the elimination of oxidative stress but also in the prevention of the abovementioned complex disorders, such as type II diabetes, metabolic syndrome, and cardiovascular and even neurodegenerative diseases [56]. Numerous animal studies also demonstrated the antioxidant effects of physical exercise by the enhancement of several enzymatic pathways including glutathione (GSH) and the heme oxygenase (HO) system [57, 58]. Szabo et al. supported that 12 weeks of sustained training is an efficient method to enhance the antioxidant GSH and nitrotyrosine-3 levels as well [59]. Moreover, in a hormone-depleted rat model, 6 weeks of physical exercise was proved to be a key process in the amelioration of antioxidant status by enhancing the HO enzyme system [31]. Besides GSH and HO, superoxide dismutase (SOD) is also considered to be a first-line defense participant against oxidative stress. According to animal studies, a significant elevation can be observed in the production of SOD as a result of recreational training [60, 61]. Proinflammatory markers (e.g., myeloperoxidase (MPO), TNF-α, and IL-6) are the main contributors of ROS generation and consequently oxidative stress. Exercise, however, has also been shown to be effective in the reduction of these well-known inflammatory factors. Several studies showed that increased physical activity resulted in diminishing inflammatory processes [49, 62, 63]. It is clarified that 6-week-long voluntary exercise is effective enough to reduce MPO activity and the level of TNF-α in hormone-depleted rats [64]. It can therefore be concluded that exercise represents a potent anti-inflammatory and antioxidant strategy in healthy individuals and under pathological conditions as well. It participates in reducing the risk of morbidity and mortality through its direct and positive impacts on human health.

2.6. Cancer Prevention. Studies provide numerous evidence that physical activity reduces the risk of at least a dozen cancer types, including breast, colon, prostate, or lung cancer [65]. In different chemical-induced or genetic tumor models, all of the training modalities mentioned in this review have been shown to be effective in reducing tumor growth or metastasis [66, 67]. However, the underlying mechanisms of this wide-ranging protection are not yet totally clarified, but the possible mediators are inflammation-, antioxidant-, and immune cell-related [68]. During physical activity, a significant increase in muscle-derived myokines and intensified mobilization of immune cells can be observed in the plasma. While myokines have antiproliferative effects, immune cells can be the most powerful components in the fight against cancer [69, 70]. Pedersen et al. found a marked decrease in tumor incidence and growth as a result of voluntary wheel running in 5 different tumor types. They clarified that natural killer cells have a predominant role in this type of control of tumor growth; with the induction of stimulatory cytokines, enhancement of NK cell-related activated receptors, and their intensified mobilization, they have a major role in the training-related control of tumor growth [71]. Moreira et al. revealed that even a short-term voluntary exercise decreased tumor growth and metastatic processes in a tumor-bearing rat model [72]. A promising observation was made by Hagar et al. as well that 8 weeks of training enhanced antitumor immune processes, thus suppressing tumor growth in mice [73]. Furthermore, aerobic exercise resulted in enhanced tumor cell apoptosis, decreased tumor weight, and diminished cell proliferation in a tumor-bearing rat model compared to sedentary animals [74]. Physical activity has an unquestionable role in the primary prevention of cancerous processes; however, it is also extremely important in terms of health promotion after the diagnosis. With the help of exercise, aerobic capacity and muscle strength increase, while disease-free survival may extend [75].

3. Characteristics of Animal Exercise Protocols

Animal models are essential in basic research including every research field; thereby, choosing a well-designed exercise protocol for the appropriate experiments is fundamental. Before initiating any exercise study, the most important step is the proper selection of the animal model, as the objectives of exercise-related research studies may be different. For exercise training, rodents (rats and mice) are the most commonly used animals due to their many advantages. Rodents are the most affordable species for animal studies, thanks to their low breeding cost. They have high fertility and a relatively short gestational period with many offspring. Another advantage of using rodents is that they require comparatively small living space, and the experimental apparatus designed for mice or rats are also easily accessible [76]. Additionally, the capability of choosing genetically modified strains designed for specific diseases has also popularized these species in every research field. Despite the several advantages of rodents in animal research, few limitations are present in their application. In most animal

studies, including exercise research, gender differences may interfere with the results and make it difficult to generalize data for both sexes, and although human and rodent genes are largely similar, the small but more important differences in the details (e.g., receptors) make rodents unsuitable for some research areas. Considering all aspects, after choosing the applicable animal model, we must consider the elementary factors of exercise. Physical activity can be characterized according to its intensity, duration, modality, and frequency [7, 77] (Figure 1).

Within the intensity of the training, we can distinguish between the low- and high-intensity physical activities; in terms of modality, we can differentiate between dynamic training and static training. According to the duration, exercise can be divided into short- or long-term exercise, while the frequency of training can also be further subdivided into several groups according to the goal of the study [8]. Exercise must meet different criteria according to the purpose of the research. As exercise research studies are designed for assessing the impact of physical activity on several organ systems, it is crucial to optimize the exercise protocol according to the goal of the study. Furthermore, for the successful outcome, exercise training must consist of several fundamental phases including regeneration time. At the beginning of the study (Phase I), animals should be familiarized with the applied training in order to prevent any injuries or exercise-induced stress. In this phase, adaptation to the environment as well as acquaintance with new forms of movement (e.g., swimming or running) takes place. Then, the planned training with the appropriate intensity, modality, and duration is performed (Phase II). Last but not least, in the case of a daily exercise period, resting time is also necessary for the animals (Phase III) in order to restore physical energy after training [8]. These previously described factors fundamentally determine the outcome of the experiment; thus, their understanding and accurate application are essential for adaptation to human physiology. In the following, we summarize the most often used aerobic and anaerobic training models with their possible areas of application.

4. Aerobic Exercise Models

The most commonly used aerobic exercise models in different research fields are voluntary wheel running, forced wheel running, swimming, and treadmill running (Table 1). The aim of these studies can be twofold: to determine the role of sport in disease prevention or to allocate its wide-ranging effects on preexisting disorders.

4.1. Wheel Running. Voluntary wheel running is a form of exercise where animals have free access to a metal wheel for the whole training time. The running wheel is usually built into the cages of the animals; therefore, they can use the apparatus according to their needs at a lower intensity, any time of the day for any length of time [76]. Running wheels are suitable for smaller rodents (e.g., mice or rats) and are nowadays often equipped with an activity tracking device, which allows the scientist to track down the running distance of the animals. This recreational training is the most stress-free modality of exercise; thus, it is suitable for aging studies and also in conditions where it is important to avoid strenuous exercise [30]. As for the duration of the experiment, voluntary wheel running is applicable for short- and long-term interventions as well. Due to its voluntary, nonstrenuous nature, it is often used for cardiovascular and metabolic studies, but it is suitable for almost every research area [78–80]. Long-term voluntary wheel running is considered to be protective against cardiac injury [81] and an effective tool to enhance antioxidant mechanisms [82]. According to Cunha et al., 3-week-long wheel running improved overall antioxidant status in mice [83]. Additionally, 12 weeks of wheel running exercise exerted its positive influence on lipid metabolism by resulting in a significant decrease in the level of plasma triglyceride and leptin [21]. Along with metabolic effects, long-term voluntary wheel running favorably affected bone properties as well in young mice [84]. Voluntary wheel running was also a convenient exercise protocol in neurodegenerative disorders, as it effectively mitigated impaired spatial memory and neuropathological changes in aging rats through complex biochemical processes [85]. This type of aerobic exercise is also a popular therapeutic approach to tumor prevention and treatment in cancer research [86].

A very similar form of movement to voluntary wheel running is forced wheel running. Forced wheel running differs from the previously mentioned form of wheel running in that its wheel is centrally motorized. This automatically rotating wheel is connected to a specific software program, which allows the scientist to adjust training intensity throughout the running from low to intermediate levels. This training modality offers better control of exercise parameters compared to voluntary wheel running. Forced wheel running is suitable for short-term and long-term interventions as well, depending on the purpose of the study. Similar to voluntary wheel running, this type of exercise is applicable in many areas of research.

4.2. Treadmill Running. Treadmill running is considered to be a forced training model, usually applied with smaller rodents or dogs. Unlike voluntary wheel running, during this exercise, animals are removed from their cages and forced to run on a treadmill. Scientists can change several parameters according to the goal of the study; it allows them to perform moderate- or high-intensity training by adjusting speed, duration, or inclination [76]. Treadmill running is a widely used exercise modality, especially in cardiovascular or metabolic research studies. It has been proved that high-intensity treadmill training is an efficient method to reduce cardiovascular risk factors. Haram et al. confirmed that it was able to decrease blood pressure and improve endothelial function and different metabolic parameters as well [87]. It was also reported that high-intensity exercise stimulated mitochondrial biogenesis, thereby contributing to cardiac improvements [88]. Furthermore, this kind of exercise is an applicable method to recreate exercise-induced physiological cardiac hypertrophy. Kemi et al. proved that 4 weeks of intensity-controlled treadmill running caused elevated ventricular weights and normalized the structure and

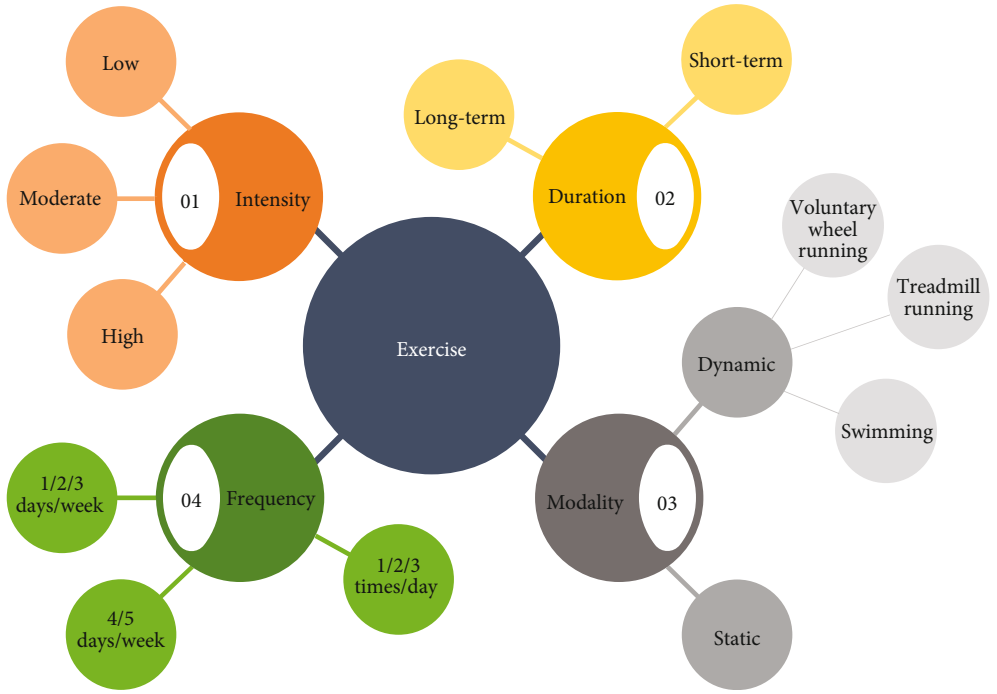

Figure 1: Fundamental elements of the exercise protocol. Intensity, duration, frequency, and modality are the four key components of an exercise protocol. Further variants of these subgroups can be used to refine the form of training.

Table 1: Detailed summary of different exercise modalities (advantages, disadvantages, and areas of application).

Type of exercise	Most common research areas	Advantage	Disadvantage
Aerobic			
Voluntary wheel running	Aging, cardiovascular research, behavioral research, cancer research, metabolic research, stroke, liver and kidney disease, bone and muscle physiology, memory	Nonstressful	Uncontrollable (intensity, duration) Possible paw injuries
Forced wheel running	Aging, cardiovascular research, behavioral research, cancer research, metabolic research, stroke, liver and kidney disease, bone and muscle physiology, memory	Controllable (intensity, duration, frequency)	Stressful Possible paw injuries
Treadmill running	Cardiovascular research, behavioral research, cancer, metabolic research, stroke, liver and kidney disease, bone and muscle physiology, memory	Controllable (intensity, duration, frequency)	Stressful Possible paw injuries Expensive apparatus
Swimming	Aging, cardiovascular research, behavioral research, cancer, metabolic research, stroke, liver and kidney disease, bone and muscle physiology, memory, spinal cord injury	No paw injuries Less expensive apparatus	Stressful
Anaerobic (resistance)			
Ladder climbing	Memory, behavioral research Muscle hypertrophy model	With familiarization, it is less stressful	Long familiarization process
Weight lifting	Muscle hypertrophy model	Similar to human training Quantitative	Stressful to animals Special equipment is needed
Electric stimulation of the muscles	Muscle hypertrophy model Muscle injury	Controlled muscle stimulation Quantitative	Anesthesia Surgery Artificial muscle contraction

function of the heart in female and male mice [89]. The work of Kim and Hwang discussed that a short-term (3 weeks) treadmill training was able to improve oxidative parameters in rats with cardiomyopathy [90], while the work of Cechetti et al. demonstrated that a moderate-intensity treadmill training mitigated oxidative damage in the rat hippocampus, therefore contributing to cognitive improvements [91]. According to the results of Wu et al., 9 weeks of treadmill exercise has beneficial effects against depression-like behavior in rats [92]. In addition, 3 weeks of forced running has also been shown to be beneficial in doxorubicin-induced liver disease through the normalization of oxidative stress markers [93]. Treadmill running is an often-applied exercise modality in cardiovascular therapy-connected research studies; however, conditions occurring during this kind of exercise are generally stressful, which circumstances may interfere with the experimental results, and for that reason, it is not recommended in aging studies. In addition to these areas of use, this type of training at different intensities is able to change the microstructure of the bones, according to Liu et al. [94]. Treadmill running is effective in a hormone-depleted female rat model as well, as its long-term application significantly increased bone mass and strength in young and adult rats [95].

4.3. Swimming. Similar to treadmill running, swimming is also a forced training model. This exercise modality obviously requires a simple swimming apparatus (e.g., a tank), which has to be large enough for the training. It is filled with 30-32°C water, the depth of which must be appropriate to the size of the animal [76]. In order to minimize the water-induced stress response, animals must be familiarized with the environment before the experiment. Unlike in the case of running exercises, here, sedentary control animals should also be placed in shallow water in order to exclude the stressful effects of water [96]. In this type of exercise, both the duration and the frequency can be adjusted according to the purpose of the experiment. Based on these factors, we can distinguish between the moderate- and strenuous-intensity exercises. Moderate training means 1 hour/day, 5 days/week for 8 weeks, while strenuous exercise requires an increasing duration of the sessions, finally reaching a 2.5-hour-long training period/day for also 8 weeks [97]. Furthermore, the swimming procedure can be used as an aerobic exercise with or without an attached weight workload [98]. Swimming with extra weight allows us to study the cardiovascular effects of an exhaustive, strenuous exercise. Olah et al. proved that a 3-hour-long swimming exercise with an extra 5% body weight attached to the animal resulted in elevated plasma troponin T and creatine kinase. Furthermore, they demonstrated that this kind of exhaustive training caused elevated apoptotic signaling and matrix metalloprotease dysregulation in the heart [99].

Moderate-intensity swimming, however, is a suitable exercise protocol to study physiological hypertrophy, similar to treadmill running. The findings of Evangelista et al. demonstrated that 90 min of swimming twice a day, 5 days a week for a 4-week-long period, induced physiological hypertrophy in mice and contributed to normalized heart function [100]. Besides cardiovascular effects, moderate-intensity swimming was proved to be efficient in complex metabolic mechanisms. Moustafa and Arisha clarified the beneficial changes of swimming exercise in terms of metabolic alterations [101]. Short-term swimming could be effective by decreasing blood glucose levels and improving insulin-connected pathways in diabetic rats [102]. Besides metabolic influence, swimming exerts anti-inflammatory effects by reducing proinflammatory cytokines in diabetic rats according to de Lemos et al. [103]. It has also been proven that 8 weeks of swimming training successfully mitigated the oxidative damage of the brain and increased its antioxidant status as well [104]. Similarly, Stone et al. found that moderate-intensity swimming was able to upregulate the expression of GSH, SOD, and catalase enzymes, thus ameliorating the antioxidant properties of the hippocampus [105]. According to the latest findings of Alomari et al., short-term swimming resulted in a significant improvement in short- and long-term memories in rats [106]. In this context, Park et al. proved that swimming ameliorated memory defects and psychological disorders by increasing serotonin expression and neurogenesis [107]. The areas of application of swimming extend to the osteoskeletal system as well, as clear results were obtained by Hart et al., who proved that 12-week-long swimming training was effective in the improvement of bone density, structure, and formation in a hormone-depleted female rat model [108].

5. Resistance (Anaerobic) Training

Resistance training is an exercise modality designed to enhance muscular strength, power, or physical capacity. In this type of training, external assistance (e.g., electric stimuli, surgery, and specific equipment) is essential to provoke the animals to perform the exercise. Resistance training is usually used for studies in connection with cognitive function and muscle hypertrophy or atrophy [11, 109].

5.1. Ladder Climbing. In this type of resistance training, rats are trained to climb a ladder with a load apparatus stabilized to their tail. To perform ladder climbing, no noxious stimuli or motivators are necessarily needed; thus, it can be considered a voluntary exercise. Animals need to be progressively familiarized and trained with climbing the specially designed ladder before the experiment. The intensity of this exercise is defined by how many climbing repetitions are performed during one training phase. It can be applied as a short-term or long-term exercise model as well. Due to the increased muscle workload, in this type of exercise modality, significant muscle hypertrophy is obtained [110]. Jung et al. clarified that 8 weeks of ladder climbing upregulated the muscle hypertrophy-related myokines in young and adult rats [111]. In addition to muscle hypertrophy, ladder models are increasingly used for studies involving the central nervous system. In this context, according to the results of Cassilhas et al., 8 weeks of ladder climbing exercise improved hippocampus-dependent memory tasks in rats [10]. It also increases cell proliferation and the expression of antiapoptotic proteins in the hippocampus [112].

5.2. Weight Lifting. Unlike humans, rodents can perform weight lifting by standing upright and lengthening their hindlimbs. In a specific squat training apparatus, additional weight is added to the animals by using a belt or a shoulder harness. The main disadvantage of this type of resistance training is the use of harmful stimuli in order to motivate the animals to complete the training. This protocol results in nearly 20% hypertrophy of the leg muscles; thus, it is suitable for research on muscle development [113].

5.3. Electric Stimulation of the Muscles. In order to perform this protocol, animals must be anesthetized. This model also requires an implanted electric stimulator placed into the muscle to be examined [114]. Scientists can control the degree of electric stimulation, which can occur bilaterally or unilaterally. This modality of training evokes significant muscle hypertrophy [115]. The advantage of this protocol is that cooperation of the animals is less needed as a result of anesthesia, although this also implies its disadvantage, as anesthetics may influence the physiology of the animal [76].

6. Concluding Remarks

All things considered, the positive effects of physical activity on overall health are unquestionable. Even though animal exercise models have their own limitations, the data obtained through their applications could bring us closer to solving global health issues. As seen, even short-term training can upregulate the antioxidant defense system and induce multifaceted beneficial effects throughout the body. With this nonpharmacological, health-promoting tool, a large percentage of noncommunicable diseases, including metabolic syndrome, CVDs, or even cancer, could be averted. In order to gain a better insight into exercise physiology and its impacts on health status, well-designed animal models are needed.

Conflicts of Interest

The authors declare no conflict of interest.

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Research Article

The Roles of FGF21 and ALCAT1 in Aerobic Exercise-Induced Cardioprotection of Postmyocardial Infarction Mice

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Aerobic exercise mitigates oxidative stress and apoptosis caused by myocardial infarction (MI) even though the precise mechanisms remain completely elusive. In this study, we investigated the potential mechanisms of aerobic exercise in ameliorating the cardiac function of mice with MI. In vivo, MI was induced by left anterior descending (LAD) coronary artery ligation in wild-type mice, *alcat1* knockout, and *fgf21* knockout mice. The mice were exercised under a moderate-intensity protocol for 6 weeks at one week later post-MI. In vitro, H9C2 cells were treated with lentiviral vector carrying *alcat1* gene, recombinant human FGF21 (rhFGF21), PI3K inhibitor, and H_2O_2 to explore the potential mechanisms. Our results showed that aerobic exercise significantly increased the FGF21 expression and decreased the ALCAT1 expression in the hearts of mice with MI. *fgf21* knockout weakened the inhibitory effects of aerobic exercise on oxidative stress, endoplasmic reticulum (ER) stress, and apoptosis in mice with MI. Both/either *alcat1* knockout and/or aerobic exercise improved cardiac function by inhibiting oxidative stress and apoptosis in the MI heart. rhFGF21 inhibited both H_2O_2 and overexpression of ALCAT1-induced oxidative stress and apoptosis by activating the PI3K/AKT pathway in H9C2 cells. In conclusion, our results showed that aerobic exercise alleviated oxidative stress and apoptosis by activating the FGF21/FGFR1/PI3K/AKT pathway or inhibiting the hyperexpression of ALCAT1, which ultimately improved the cardiac function in MI mice.

1. Introduction

Heart failure (HF) is a major public health problem threatening the health and safety of over 23 million people worldwide. Myocardial infarction (MI) is one of the important inducements of HF with a mortality rate close to 5% a year and the incidence rate and mortality rate younger and rising [1]. There is a growing clinical consensus that exercise training after MI can increase the quality of life and prevent future complications and longevity in infarcted patients [2]. Therefore, it is very important to find the effective-targeting molecules of exercise rehabilitation after MI.

Fibroblast growth factor 21 (FGF21), as an endocrine factor, is a unique member of the FGF superfamily and belongs to the subgroup of fibroblast growth factor 19. It is involved in the regulation of metabolic process and has the effects of antioxidative stress and apoptosis [3–6], anti-inflammatory, and promotion of angiogenesis [6, 7]. Oxidative stress and endoplasmic reticulum (ER) stress induce apoptosis as a critical pathophysiological mechanism for cardiomyocyte injury. Many studies have reported that FGF21 significantly inhibited oxidative stress and ER stress-induced apoptosis in damaged myocardium [6, 8, 9]. FGF21 inhibits ER stress-induced cardiomyocyte apoptosis by activating the FGFR1/PI3K/AKT pathway [4]. Additionally, clinical experiments have shown that the content of FGF21 in the blood of healthy subjects increased significantly after exercise [10, 11]. Animal experiments have found that the level of serum FGF21 increased significantly after a single acute exercise in mice [12]. These indicate that FGF21 might play an organ protective role as "exerkines." However, little is known about whether FGF21 mediates

the cardioprotective effects of aerobic exercise on MI and whether the mechanisms are involved in the oxidative stress and ER stress-induced apoptosis.

Cardiolipin (CL) is highly sensitive to oxidative stress injury and oxidized in the early stage of apoptosis [13–15]. Lysocardiolipinacyltransferase-1 (ALCAT1), located in ER, is the key enzyme of CL remodeling. Under pathological conditions, the abnormal increase of ALCAT1 leads to the massive production of ROS, mitochondrial dysfunction, and insulin resistance [16–18], which is the core link of obesity [19], Parkinson's disease [20], diabetes [21], and other chronic diseases and aging process [22]. Furthermore, ALCAT1-targeted inactivation prevents diet-induced obesity and nonalcoholic fatty liver disease (NAFLD) and neurotoxin1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-(MPTP-) induced neurotoxicity, reduces oxidative stress injury, improves mitochondrial function, and mitigates apoptosis [19, 20, 23]. Exercise reduces the expression of ALCAT1 in the skeletal muscle of mice [24]. Aerobic exercise alleviates oxidative stress-induced apoptosis in the kidneys by inhibiting ALCAT1 in MI mice [25]. However, whether ALCAT1 is involved in the effect of cardioprotection of exercise in the post-MI heart has not been elucidated.

In conclusion, we used wild-type (WT) mice, *fgf21* knockout mice, and *alcat1* knockout mice, as well as H_2O_2, lentiviral vector carrying *alcat1* gene, and recombinant human FGF21 (rhFGF21) in H9C2 cells, to explore these problems. This study will provide a theoretical basis and experimental support for the prevention and rehabilitation of ischemic heart disease by aerobic exercise and further elucidate the potential protective mechanism of aerobic exercise on ischemic heart.

2. Materials and Methods

2.1. Animals and Exercise Protocols. 30 wild-type (WT) male C57/B6J mice (20 ± 5 g, 8-weeks old) were purchased from the Laboratory Animal Centre of Xi'an Jiao Tong University (Xi'an, China; animal breeding license number: SCXK (Shaan) 2012-003). *alcat1* heterozygous mice knocked by CRISPR/Cas9 were obtained from Cyagen Biological Co. Ltd (China), propagated to the fourth generation, and homozygous selected for the experiment. Male *alcat1*$^{-/-}$ mice were screened by the mice tail identification method (Fig. S1). The Cre/loxP recombination system was used to conditionally knockout the loxP^{+}/$^{+}$ mice of *fgf21*, (Jackson Laboratory, USA). The genotype of F2 generation after propagation and expansion was identified; then, the tail vein was injected with the adeno-associated CRE virus (rAAV-CMV-EGFP-P2A-CRE-WPRE-bGHpA, AAV2/9, titer: 5.73E+12vg/ml) to the loxP^{+}/$^{+}$ mice of *fgf21* (Fig. S2). All experiment designs and surgical operations were performed in compliance with the guidelines for the use of experimental animals and have been approved by the Ethical Committee of Shaanxi Normal University.

The MI model was established by permanent ligation of the left anterior descending coronary artery (LAD), the ligation sites were located 2 mm below the junction of the left atrial appendage and pulmonary conus. Echocardiography was performed on all mice undergoing MI surgery on the third postoperative day to determine modeling success, and mice with EF values of 40-55% were selected for follow-up experiments. WT mice were randomly divided into three groups ($n = 10$): sham surgery group (S), MI myocardial infarction group (MI), and six-week exercise training with MI group (ME). The grouping of *alcat1*$^{-/-}$ mice was the same as that of WT mice ($n = 10$). *fgf21*$^{-/-}$ mice were divided into the post-MI six-week exercise training group (*fgf21*$^{-/-}$ ME).

Exercise-trained animals were subjected to six weeks of exercise on a motorized rodent treadmill (Model ZH-PT, Anhui Zhenghua Technology Co., China) from the second week after induction of MI. The first week was adaptive training, the mice ran 60 min daily from a speed of 5 m/min to 10 m/min. Formal training was performed later, 10 min with 8 m/min in speed and 55 min with 10 m/min in speed each day, 5 d/week for six-week. The death of two mice was caused by this exercise protocol during the entire study. After the training, the cardiac function of mice was detected by echocardiography and then decapitated. The blood and heart were collected quickly and put into formaldehyde or liquid nitrogen for subsequent experiments.

2.2. Measurement of Echocardiographic and Histological Staining. The cardiac physiological functions were evaluated by the Doppler echocardiography (VINNO 6 VET, VINNO, China). The transthoracic 2D M-mode echocardiographic system was used to obtain M-mode tracings. The left ventricle internal dimension systole (LVIDs), left ventricle internal dimension diastole (LVIDd), ejection fraction (EF), and fractional shortening (FS, FS = (LVIDd − LVIDs)/LVIDd × 100%) were measured in mice anesthetized with 1.5% isoflurane.

To determine the infarct volume, the heart samples were fixed in 4% paraformaldehyde for 48-72 h and embedded in paraffin. 5 μm-thick slices sections were stained with Masson's trichrome. The Image-Pro Plus analysis software was used to analyze the infarct ratio which was expressed as the volume fraction of collagen (CVF% = collagen area/tissue total area × 100%).

2.3. Cell Culture and Treatment. H9C2 cells (Chinese Academy of Sciences Cell Bank, Shanghai, China) were cultured in high glucose DMEM (GIBCO, USA) supplemented with 1% cyan streptomycin double-antibody and 10% (*v*/*v*) FB Sat 37°C under a 5% CO_2 atmosphere. For the present study, H9C2 cells were incubated overnight to reach 70-80% confluence at 37°C before experimentation. H9C2 cells were treated with H_2O_2 at a concentration of 100 μM for 4 hours to construct an apoptosis model [26]. AMPK was activated after exercise [27], so AICAR, an AMPK agonist, was used to simulate the exercise effect of H9C2 cells. Furthermore, H9C2 cells were treated with recombinant human FGF21 (rhFGF21, 75 ng/ml, 15 h; Selleck, USA), FGFR1 inhibitor (PD166866, 100 ng/ml, 15 h, Selleck Chemicals) [8], PI3K inhibitor (LY294002, 10 μM, 1 h, Selleck Chemicals), AICAR (1 mM, 1 h, Selleck Chemicals) [28], and lentiviral vectors carrying *alcat1* gene (ALCAT1 OE, MOI = 1; Brain VTA, China).

2.4. Western Blotting Analysis. Proteins were extracted from myocardial tissues or cultured H9C2 cells using RIPA buffer containing protease inhibitors and phosphatase inhibitor cocktail, and protein concentrations in the lysate were determined using the BCA protein assay kit (Jiancheng Biotech, Nanjing, China), resolved by SDS-PAGE (30-50 μg of protein per sample), and transferred onto polyvinylidene difluoride (PVDF) membranes. The blots were blocked with 5% BSA for 2 h, and the membranes were incubated with primary antibodies diluted in 5% BSA at 4°C overnight. The primary antibodies used in the present study were as follows: FGF21 (1 : 1000, Abcam), FGFR1 (1 : 1000, Abcam), ALCAT1 (1 : 1000, Abcam), p-PI3K(1 : 1000, Cell Signaling), t-PI3K (1 : 1000, Cell Signaling), p-AKT (1 : 1000, Cell Signaling), t-AKT (1 : 1000, Cell Signaling), t-IRE1α (1 : 1000, Cell Signaling), p-IRE1α(1 : 1000, Cell Signaling), t-JNK(1 : 1000, Cell Signaling), p-JNK(1 : 1000, Cell Signaling), CHOP(1 : 1000, Cell Signaling), GRP78 (1 : 1000, Bioworld), c-caspase3 (1 : 1000, Cell Signaling), Bcl-2 (1 : 800, Cell Signaling), Bax (1 : 500, Bioworld), SOD2 (1 : 1000, Cell Signaling), and GAPDH (1 : 5000, Genetex). After the membrane incubation with corresponding HRP-conjugated secondary antibodies (1 : 5000, Jackson), the western blotting bands were performed by image processing and analysis after being visualized with enhanced chemiluminescence reagents under the Gel Imaging System, quantified with the ImageJ software.

2.5. TUNEL Assay. Apoptosis assay was performed using a One-Step TUNEL apoptosis kit (Beyotime, China) according to the manufacturer's instruction. 20 μg/ml of DNase-free protease K was added to each sample of paraffin section of myocardial tissue and incubated in dark for 30 minutes at 37°C temperatures. In the cell experiment, cells were plated on glass coverslips at a density of 1×10^5 cells/well, which were fixed in 4% formaldehyde for 30 min and then permeabilized in 0.3% Triton X-100 for 10 min. Conventional paraffin sections and the cell-loaded sections were labeled with dUTP and TDT enzymes in a humidified box at 37°C for 1 h, and the antifluorescence-quenched sealing agent was used to sealing the film. The red positive particles were observed by a fluorescence microscope.

2.6. Kit Test of Oxidative Stress. We used the assay kits of glutathione peroxidase (GSH-Px), catalase (CAT), total superoxide dismutase (T-SOD), reduced glutathione (GSH), and malondialdehyde (MDA) (Jiancheng Biotech, Nanjing, China) to detect the enzyme activities of GSH-Px, CAT, and T-SOD and the contents of GSH and MDA, which determine the level of oxidative stress in myocardial tissue and cells. The specific experimental method is implemented according to the instructions.

2.7. Statistical Analysis. The percentage of myocardial collagen fibers were analyzed by the Image-Pro Plus 6.0 software (Media Cybernetics, Bethesda, MD, USA), and western blotting results were analyzed and processed by the ImageJ software (Wayne Rasband, National Institutes of Health, USA). All the experimental data were analyzed and processed by the SPSS 21.0 software (IBM Company, USA), and the results were expressed by mean ± SD. Histogram was drawn by GraphPad Prism 8.0 (GraphPad Software, La Jolla, CA, USA). Statistical differences between the groups were evaluated using the one-way analysis of variance (ANOVA) and post hoc least significant difference (LSD) multiple-comparison test. Differences were considered statistically significant at $^{*}p < 0.05$ and $^{**}p < 0.01$.

3. Results

3.1. The Cardioprotective Effect of Exercise in the Heart Was Significantly Reduced in the Post-MI fgf21$^{-/-}$ Mice. In our previous study, we found that the improvement of myocardial function by exercise was related to the expression of FGF21. To further confirm the role of FGF21 on cardioprotection of aerobic exercise in mice with MI, we prepared *fgf21*$^{-/-}$ mice. We quantified the collagen volume of fraction (CVF%) in the heart sections with Masson's trichrome staining. In WT mice with MI, the myocardial tissue was replaced by collagen, CVF% was significantly increased compared with the S group ($p < 0.01$, Figures 1(a) and 1(b)). Aerobic exercise significantly inhibited the further expansion of the fibrotic area of the hearts exposed to MI ($p < 0.01$, Figures 1(a) and 1(b)). However, compared with the ME group in WT mice, the inhibitory effect of exercise on excessive proliferation of myocardial collagen fibers of ME group in the *fgf21*$^{-/-}$ mice was weakened ($p < 0.01$, Figures 1(a) and 1(b)). It indicates that the repression of FGF21 expression inhibited the alleviating effect of partial aerobic exercise on the morphological changes after MI.

Echocardiography was used to detect cardiac function (Figure 1(c)). In WT mice, compared with the S group, we found and analyzed that the LVIDd and LVIDs were significantly increased and EF and FS were significantly reduced in the MI group ($p < 0.01$, Figures 1(c)–1(e)). Compared with the MI group, aerobic exercise significantly enhanced EF and FS and decreased LVIDd and LVIDs ($p < 0.01$, Figures 1(c)–1(e)). However, after inhibiting the expression of FGF21, the changes of aerobic exercise on EF and FS were attenuated ($p < 0.05$, Figure 1(e)). These results showed that aerobic exercise inhibited MI-induced heart injury at least partly via the upregulation of the expression of FGF21.

3.2. Aerobic Exercise Activated the FGF21/FGFR1/PI3K/AKT Pathway and Inhibited Oxidative Stress and ER Stress-Induced Apoptosis in the Heart of MI Mice. The cardiac is not only the target of FGF21 but also can autocrine FGF21 [29]. Previous studies have indicated that FGF21 inhibited oxidative stress and ER stress-induced apoptosis in myocardial ischemia injury [8, 30, 31], which was related to the activation of the PI3K/AKT pathway [4]. Exercise training boosts the level of FGF21 in the heart, liver, and circulation [12, 32, 33]. However, whether and how FGF21 participates in exercise to protect the heart from MI injury remains unclear. Firstly, in WT mice, the protein expression of FGF21, FGFR1, p-PI3K/t-PI3K ratio, and p-AKT/t-AKT ratio were increased in the MI group compared to the S group ($p < 0.05$, $p < 0.01$, Figures 2(a) and 2(b)), which was

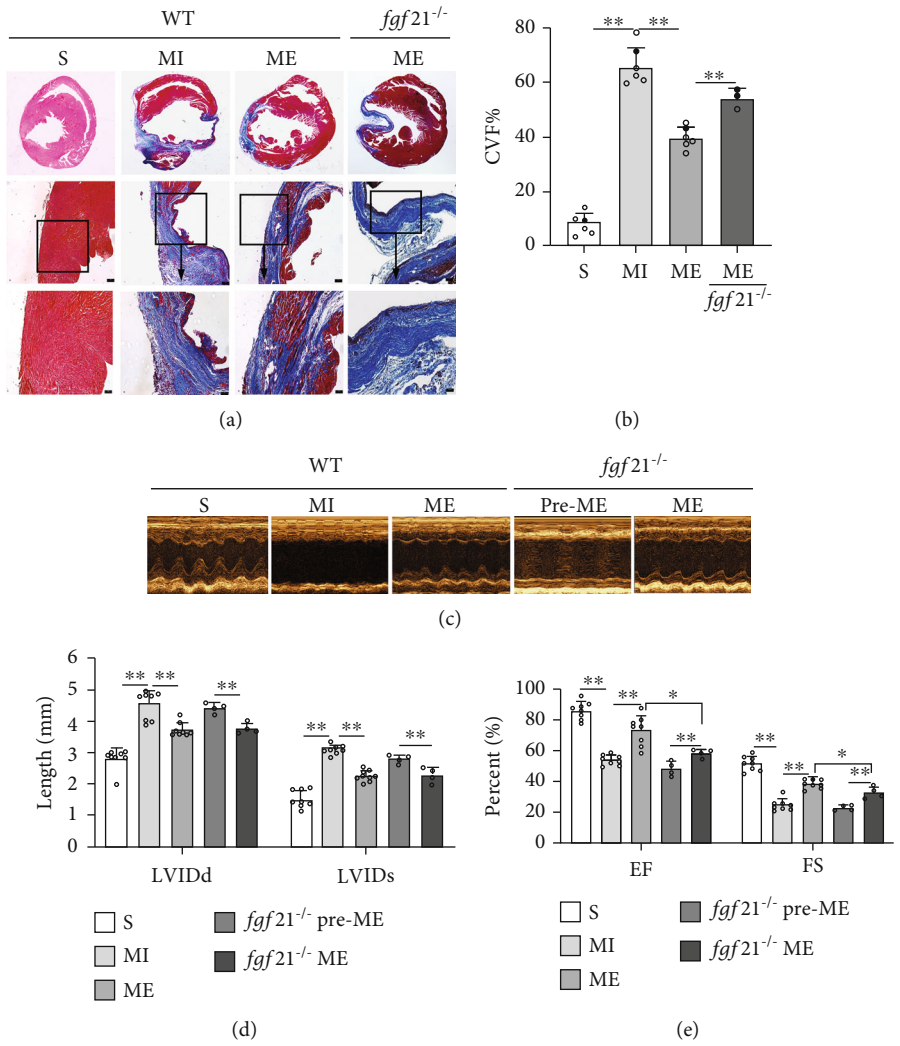


Figure 1: The protective of aerobic exercise on the MI heart in WT and *fgf21*$^{-/-}$ mice. (a) The myocardial infarct size was measured with Masson's trichrome staining. The images show that the fibrosis marker was stained in blue, whereas cardiac muscle fibers in red and nuclei in dark brown. Above: original magnification ×4; middle: original magnification ×10; below: original magnification ×20. Scale bar: 10 μm. (b) Quantification of collagen volume of fraction (CVF%). (c) Echocardiography. (d) Left ventricular end diastolic diameter (LVIDd) and left ventricular systolic diameter systole (LVIDs). (e) Left ventricular ejection fraction (EF) and left ventricular short axis (FS). CVF: collagen volume fraction; EF: ejection fraction%; FS: fractional shortening%; LVIDd: left ventricular internal diameter diastole; LVIDs: left ventricular internal diameter systole. S: sham group; ME: MI + aerobic exercise group. Data presented are means ± SD. One-way ANOVA with post hoc LSD multiple-comparison test. $^{*}p < 0.05$, $^{**}p < 0.01$.

same as that of the previous research [29]. Compared with the MI group, these changed more significantly in the ME group ($p < 0.01$, Figures 2(a) and 2(b)). It was indicated that aerobic exercises upregulated the FGF21 protein expression and activated the FGFR1/PI3K/AKT signaling pathway in the heart of mice with MI.

On the other hand, ALCAT1 protein expression increased significantly, whereas SOD2 decreased significantly in the MI group compared to the S group. The six-week aerobic exercise significantly reversed these changes ($p < 0.01$, Figure 2(b)). Under the conditions of hypoxia, ER stress may lead to cardiomyocytes apoptosis [34]. And the IREα/JNK pathway is a classical ER stress-induced apoptosis [35]. We observed that the p-IRE1α/t-RE1α ratio, p-JNK/t-JNK ratio, GRP78, CHOP, Bax/Bcl-2 ratio, c-caspase3 protein expression, the number of TUNEL-positive particles were significantly increased in the MI group compared to the S group. However, aerobic exercise significantly inhibited these changes ($p < 0.05$, $p < 0.01$, Figures 2(c)–2(e)). Overall, these results showed that aerobic exercise upregulated the expression of FGF21, activated the FGFR1/PI3K/AKT signaling pathway, and inhibited oxidative stress and ER stress-induced apoptosis to protect cardiac structure and function in mice with MI.

3.3. Knockout of fgf21 Reversed the Effect of Aerobic Exercise on the Inhibition of Oxidative Stress, ER Stress, and Apoptosis in the Heart of Mice with MI. To confirm the role of FGF21 in the inhibition of MI injury on the heart by aerobic exercise, we observed the oxidative stress, ER stress, and apoptosis in the hearts of *fgf21*$^{-/-}$ mice. Compared with the ME

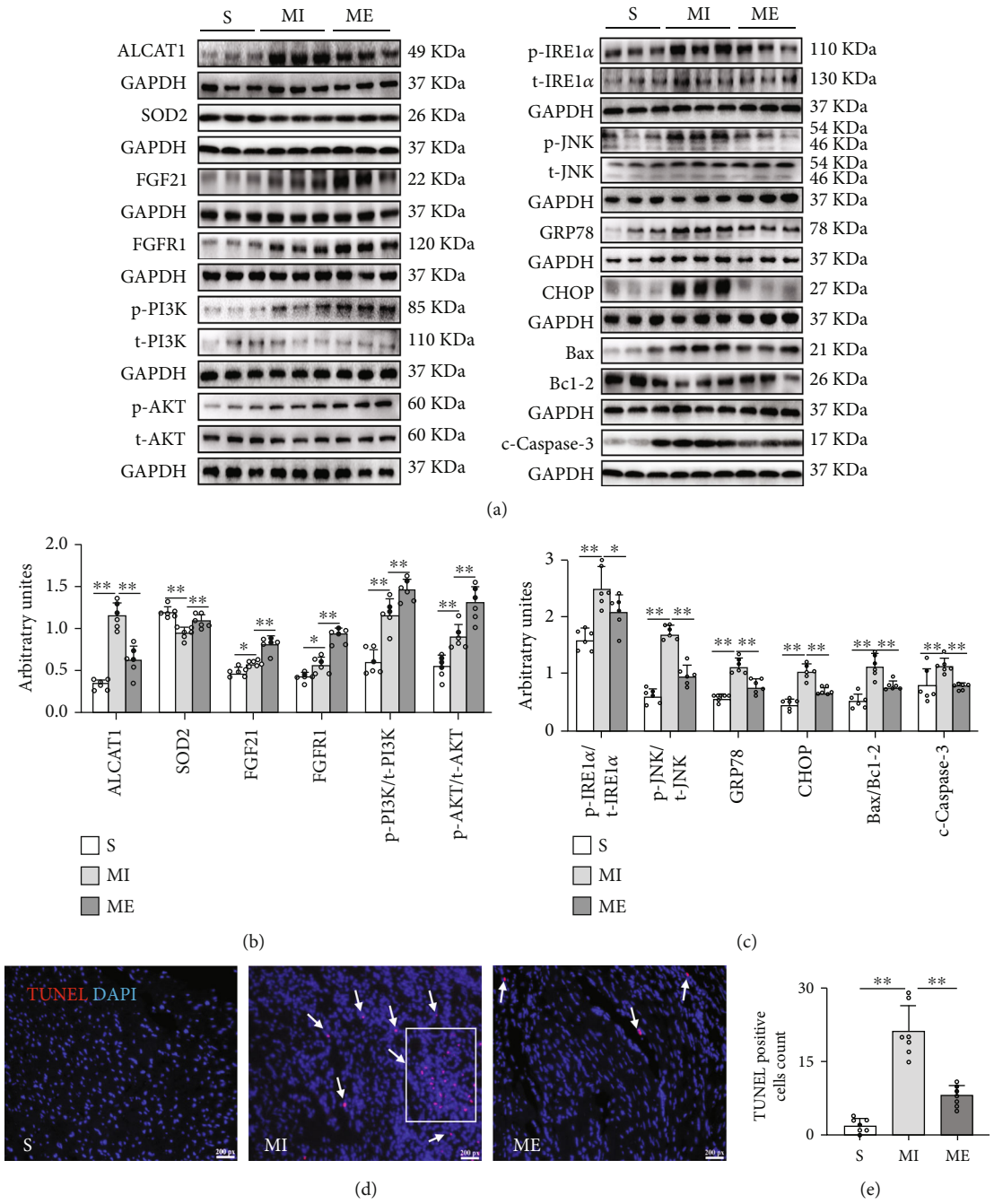


Figure 2: Aerobic exercise significantly inhibited oxidative stress and ER stress-induced apoptosis and activated the FGF21/FGFR1/PI3K/AKT pathway. (a–c) Western blotting images and their densitometric quantitative analysis of ALCAT1, SOD2, p-PI3K/t-PI3K ratio, p-AKT/t-AKT ratio, p-IRE1α/t-IRE1α ratio, p-JNK/t-JNK ratio, GRP78, CHOP, Bax/Bcl-2 ratio, and c-caspase3. (d) TUNEL staining: TUNEL-positive granules in red and DAPI in blue. Original magnification ×10. Scale bar: 200 px. (e) TUNEL-positive cells count. S: sham group; ME: MI + aerobic exercise group. Data presented are means ± SD. One-way ANOVA with post hoc LSD multiple-comparison test. $^{*}p < 0.05$, $^{**}p < 0.01$.

group in WT mice, the higher MDA, p-IREα/IREα, p-JNK/JNK, GRP78, CHOP, Bax/Bcl-2 ratio, c-caspase3, ALCAT1 protein expression and more TUNEL-positive particles with lower enzyme activity of CAT and SOD2 protein expressions after post-MI exercise in *fgf21*$^{-/-}$ mice were observed ($p < 0.05$, $p < 0.01$, Figures 3(a)–3(f)). These results indicated that inhibition of FGF21 expression reduced the inhibition of aerobic exercise on oxidative stress, ER stress, and apoptosis in the MI heart.

3.4. Aerobic Exercise and/or alcat1 Knockout Protected the Damaged Heart in Mice with MI. Aerobic exercise alleviates

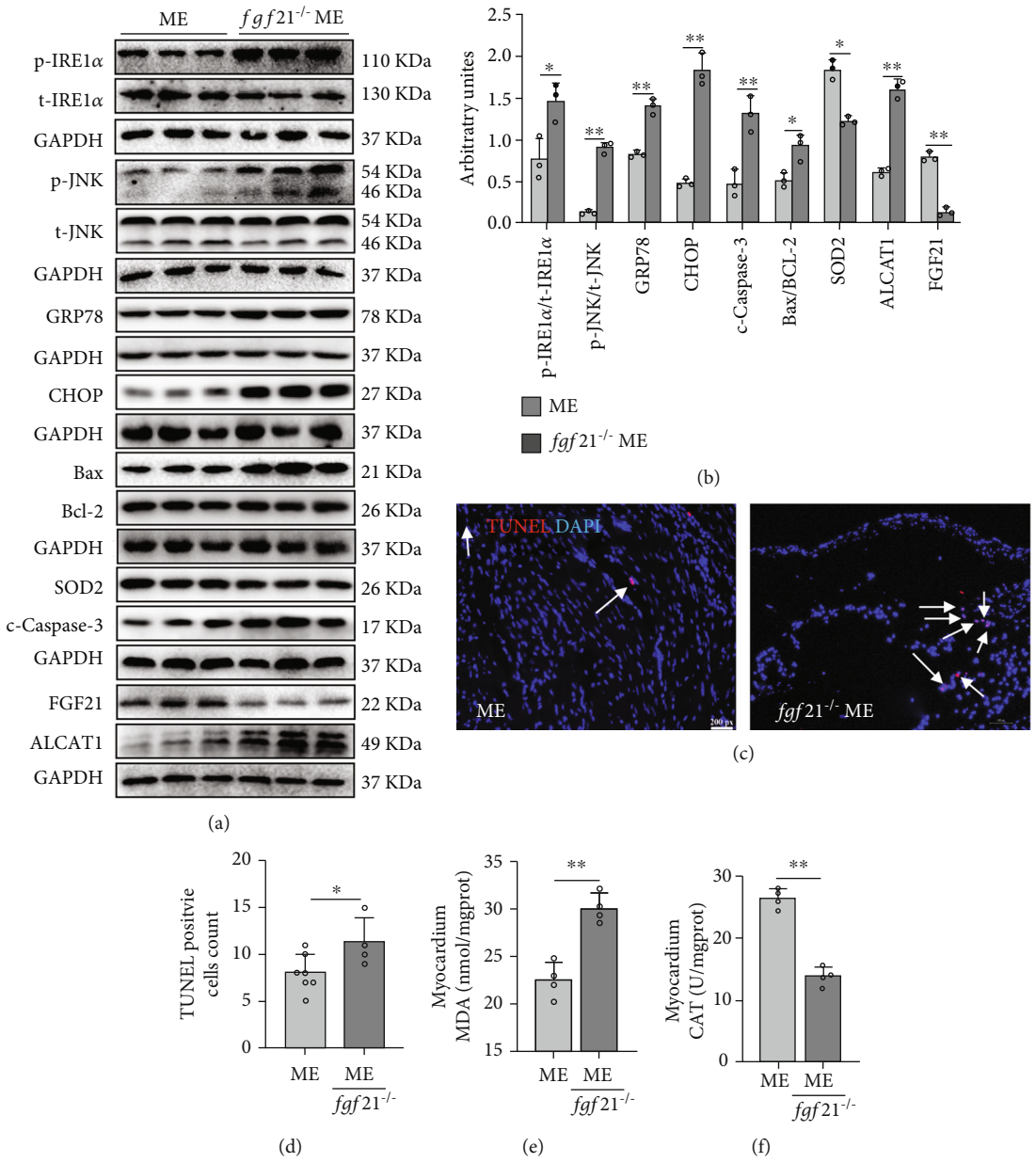
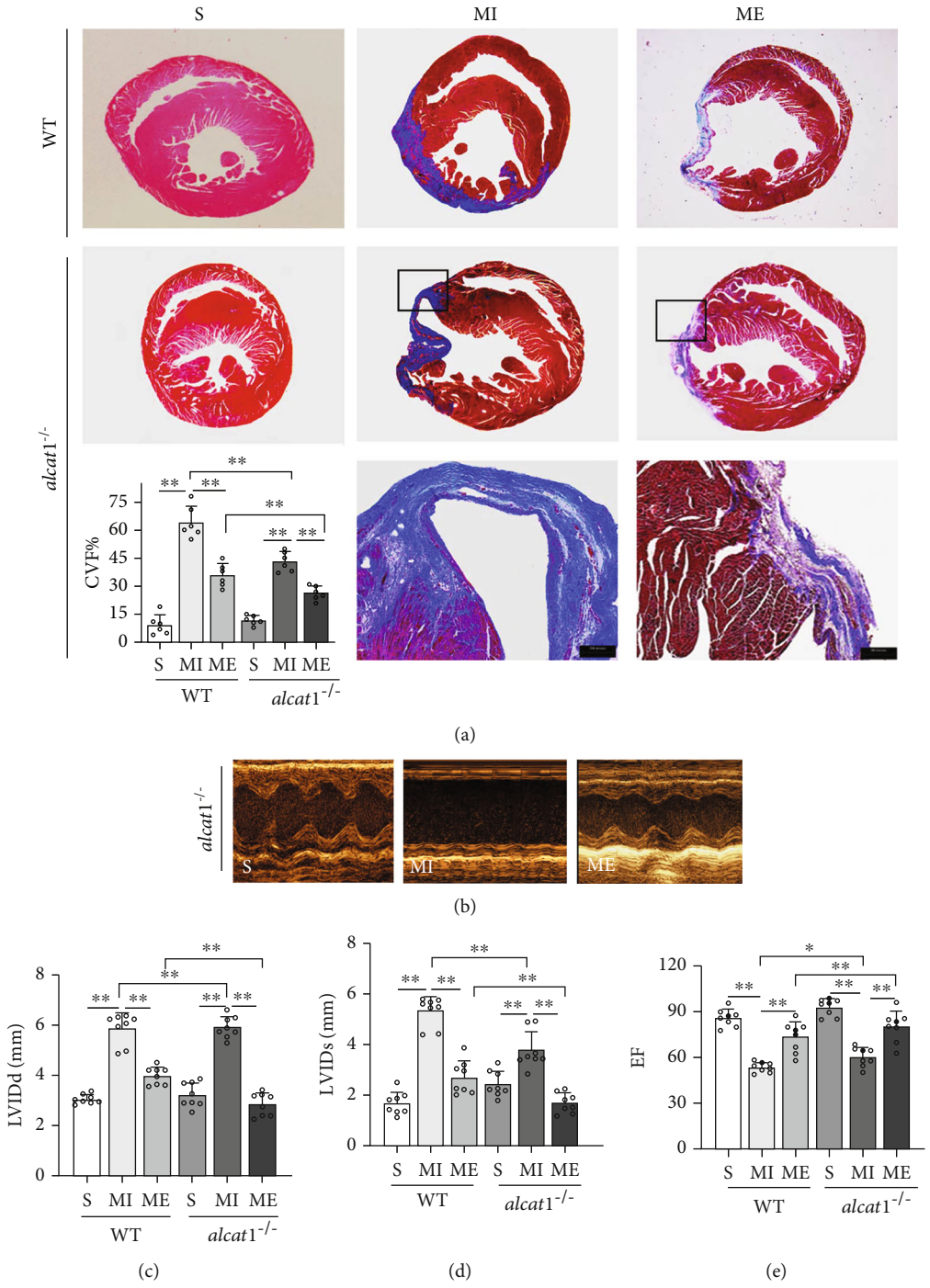


Figure 3: Aerobic exercise training significantly inhibited oxidative stress and ER stress-induced apoptosis partly via FGF21. (a, b) Western blotting images showed the phosphorylation levels of IRE1α and JNK, and the protein levels of IRE1α, JNK, GRP78, CHOP, Bax/Bcl-2 ratio, c-caspase3, SOD2, and ALCAT1. (c, d) TUNEL staining: TUNEL-positive granules in red and DAPI in blue. Original magnification ×10. (e) MDA content in the myocardium. (f) Enzyme activity of CAT in the myocardium. Scale bar: 200 px. ME: MI + aerobic exercise group. Data presented are means ± SD. One-way ANOVA. $^{*}p < 0.05$, $^{**}p < 0.01$.

oxidative stress-induced apoptosis in kidneys of MI mice by inhibiting ALCAT1 expression [25]. However, the role of ALCAT1 in cardiovascular diseases remains unclear. We found that aerobic exercise reduced the increased expression of ALCAT1 in the hearts of mice with MI ($p < 0.01$, Figures 2(a) and 4(g)). To further examine the role of ALCAT1 on the exercise protection of MI, we used *alcat1* knockout (*alcat1*$^{-/-}$) mice. We quantified the collagen volume of fraction (CVF%) in the heart sections with Masson's trichrome staining. In post-MI, the myocardial fibrosis in *alcat1*$^{-/-}$ mice was still increased which was significantly lower than that in the WT mice ($p < 0.01$, Figure 4(a)). Our results showed that six weeks of aerobic exercise training and/or *alcat1* knockout significantly reduced the fibrotic area of the hearts in MI mice and that the combined effect was better than the single effect ($p < 0.01$, Figure 4(a)). Detection of cardiac function by echocardiography (Figures 4(b) and 4(c)). In the *alcat1*$^{-/-}$ mice, LVIDd and LVIDs significantly increased, and EF and FS were significantly decreased in the MI group compared to the S group



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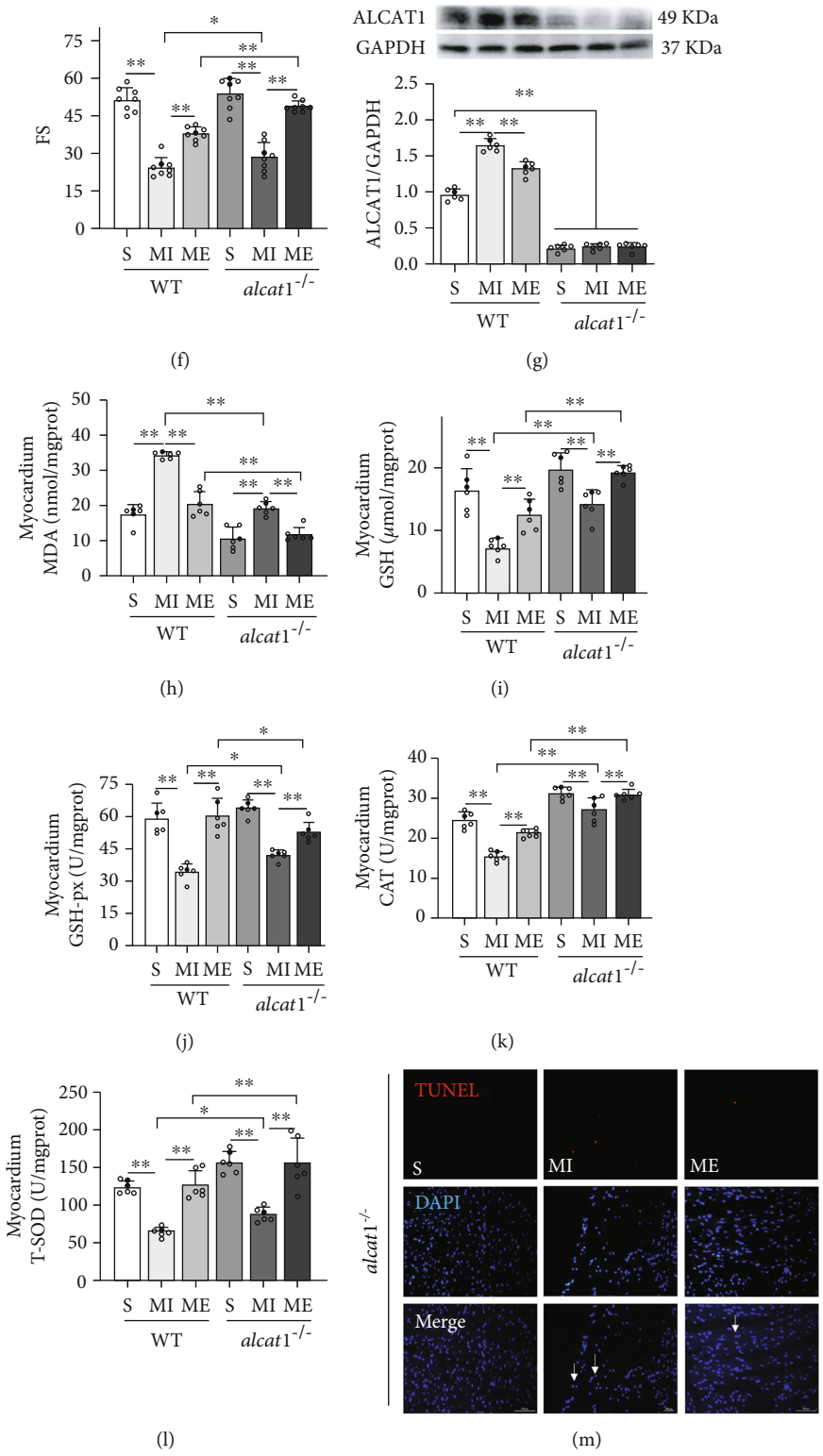

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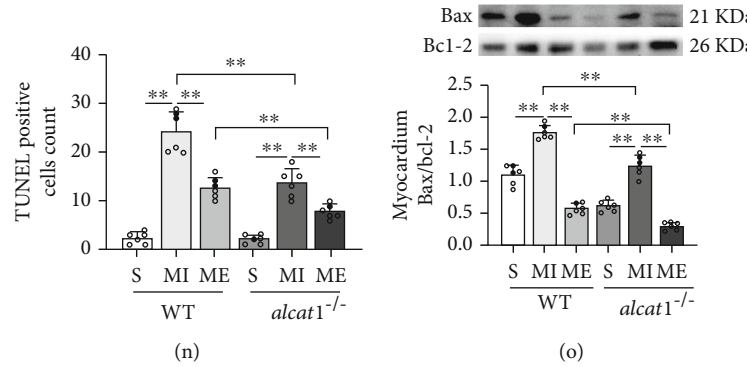


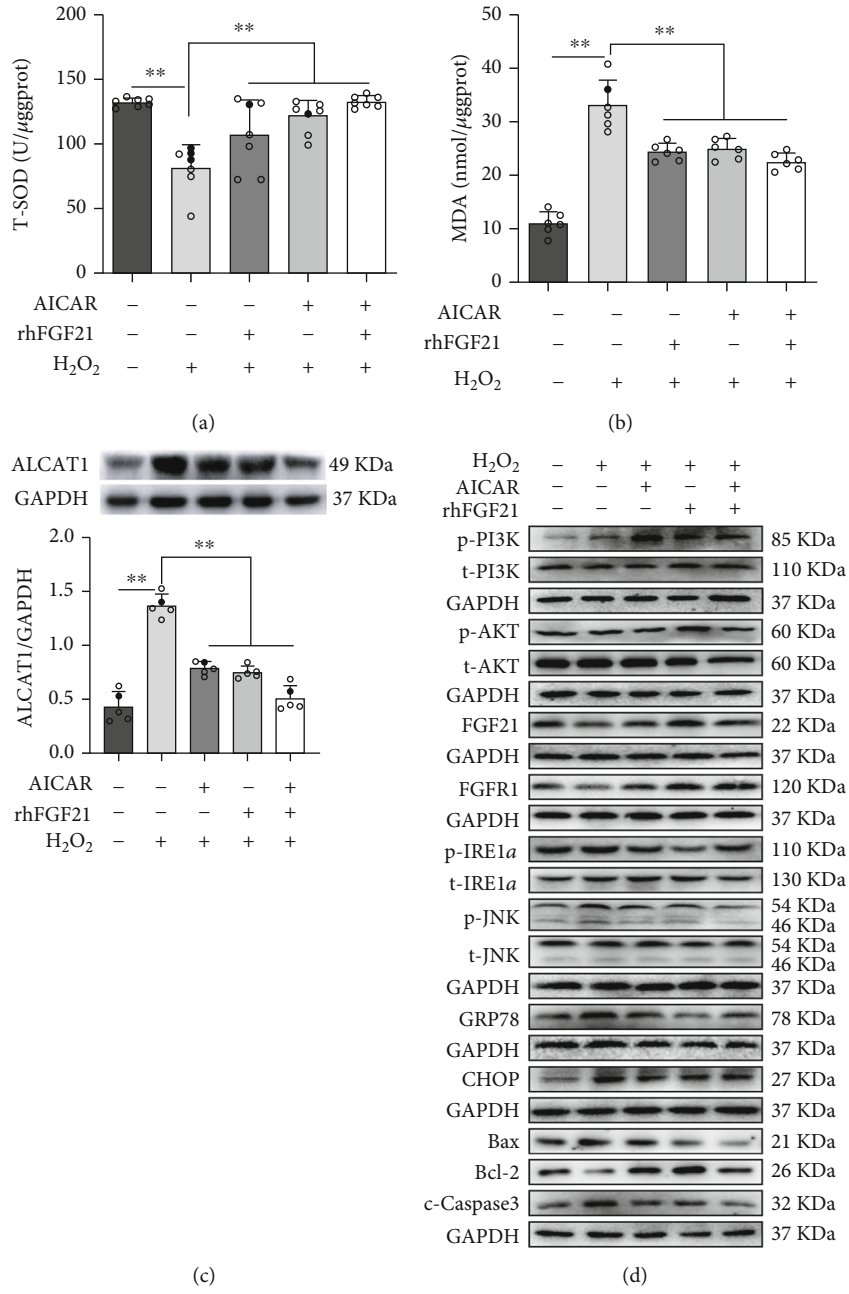
Figure 4: Aerobic exercise and knockout *alcat1* reduced cardiac oxidative stress and apoptosis in MI mice. (a) Myocardial infarct size was measured with Masson's trichrome staining. The images showed that the fibrosis marker was stained in blue, whereas cardiac muscle fibers in red and nuclei in dark brown. Scale bar: 200 μm. Quantification of collagen volume of fraction (CVF%). (b) Echocardiography. (c) Left ventricular end diastolic diameter (LVIDd). (d) Left ventricular systolic diameter systole (LVIDs). (e) Left ventricular ejection fraction (EF). (f) Left ventricular short axis (FS). (g) Protein expression levels of ALCAT1. (h) MDA content in the myocardium. (i) GSH content in the myocardium. (j) GSH-Px activity in the myocardium. (k) CAT activity in the myocardium. (l) T-SOD activity in the myocardium. (m) TUNEL staining: TUNEL-positive granules in red and DAPI in blue. (n) TUNEL-positive cells count. (o) Protein expression levels of the Bax/Bcl-2 ratio. CVF: collagen volume fraction; EF: ejection fraction%; FS: fractional shortening%; LVIDd; left ventricular internal diameter diastole; LVIDs: left ventricular internal diameter systole. S: sham group; ME: MI + aerobic exercise group. Data presented are means ± SD. One-way ANOVA with post hoc LSD multiple-comparison test. $^{*}p < 0.05$, $^{**}p < 0.01$.

($p < 0.01$, Figures 4(c)–4(f)). Furthermore, compared with the MI group in WT mice, the LVIDs was decreased significantly, and EF and FS were significantly increased in the *alcat1*$^{-/-}$ MI group ($p < 0.01$, $p < 0.05$, Figures 4(c)–4(f)). Compared with the WT mice in the ME group, we found that LVIDd and LVIDs significantly decreased, with the increased EF and FS in the *alcat1*$^{-/-}$ ME group. It indicated that both *alcat1* knockout and/or aerobic exercise could improve the cardiac function in mice with MI, and the combined effect of both was better.

Furthermore, the contents of MDA and GSH were significantly reduced, and the enzyme activity of the CAT, GSH-Px, and T-SOD was significantly increased in the myocardium after aerobic exercise under conditions of MI in WT mice ($p < 0.01$, Figures 4(h)–4(l)). The *alcat1*$^{-/-}$ mice obtained similar results ($p < 0.01$, Figures 4(h)–4(l)). Furthermore, the lower MDA and the higher GSH, GSH-Px, CAT, and T-SOD were observed in *alcat1*$^{-/-}$ mice compared to WT mice during MI ($p < 0.05$, $p < 0.01$, Figures 4(h)–4(l)). In exercise after post-MI, MDA was significantly reduced, and GSH, CAT, GSH-Px, and T-SOD were significantly increased in the *alcat1*$^{-/-}$ mice compared to WT mice ($p < 0.05$, $p < 0.01$, Figures 4(h)–4(l)). The TUNEL assay and western blotting analysis of Bax and Bcl-2 protein levels indicated that both *alcat1* knockout and aerobic exercise significantly inhibited apoptosis in the hearts of mice with MI ($p < 0.01$, Figures 4(m)–4(o)). Thus, aerobic exercise reduced oxidative stress and apoptosis on the MI heart in mice by inhibiting ALCAT1 hyperexpression. *alcat1* knockout ameliorated MI-induced oxidative stress and apoptosis and improved the cardiac function in the MI mice. It suggests the potential role of ALCAT1 in cardiovascular disease.

3.5. AICAR or Exogenous rhFGF21 Inhibited H_2O_2-Induced Oxidative Stress and ER Stress-Induced Apoptosis in H9C2 Cells. We have found that aerobic exercise upregulated FGF21 expression, downregulated ALCAT1 expression, and inhibited oxidative stress and apoptosis in the hearts of mice with MI. To explore the possible mechanisms, we treated H9C2 cells with AICAR, rhFGF21, and H_2O_2 for mimicking exercise and hypoxia separately. Compared with the control group, MDA content and ALCAT1 protein expression were increased significantly, and T-SOD activity was significantly decreased in the H_2O_2 group ($p < 0.01$, Figures 5(a)–5(c)). All of these results were reversed by AICAR or exogenous rhFGF21 intervened. Western blotting results exhibited that the p-PI3K/t-PI3K ratio, p-IRE1α/t-IRE1α ratio, p-JNK/t-JNK ratio, GRP78, CHOP, Bax/Bcl-2 ratio, c-caspase3 protein expression, and number of TUNEL-positive particles increased significantly and p-AKT/t-AKT, FGF21, and FGFR1 protein expression decreased significantly in H_2O_2 group compared to the control group ($p < 0.01$, Figures 5(d)–5(h)). The rhFGF21 and/or AICAR significantly reversed these changes, the expression of p-PI3K/t-PI3K protein was further significantly increased. These data showed that exogenous rhFGF21 or AICAR activated PI3K/AKT signaling and inhibited oxidative stress and ER stress-induced apoptosis in H9C2 cells.

3.6. FGF21 Attenuated Oxidative Stress and ER Stress-Induced Apoptosis of H9C2 Cells by Activating the FGFR1/PI3K/AKT Pathway. Our animal experiments have shown that aerobic exercise training activated the FGF21/FGFR1/PI3K/AKT pathway and inhibited oxidative stress and apoptosis on heart in post-MI mice. To further determine the underlying mechanism, we used PD166866 (an inhibitor of FGFR1) and LY294002 (a PI3K inhibitor) to treat with H9C2 cells. Exogenous rhFGF21 protected H9C2 from oxidative stress and ER stress-induced apoptosis under the H_2O_2 condition, but PD166866 inhibited the



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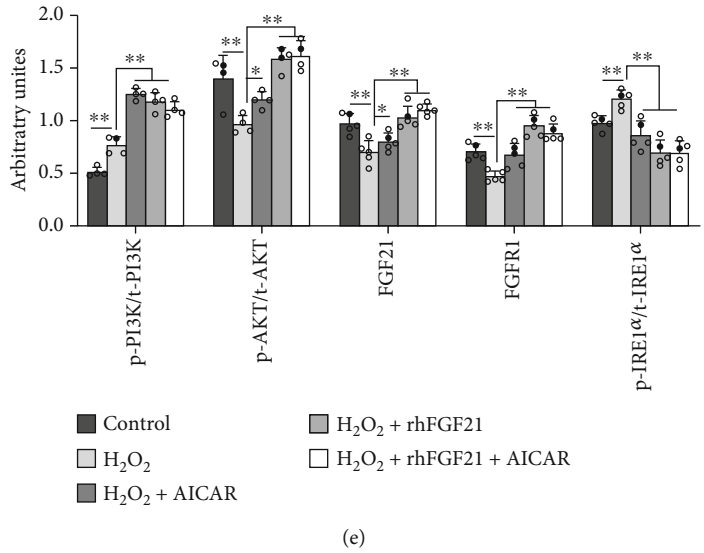
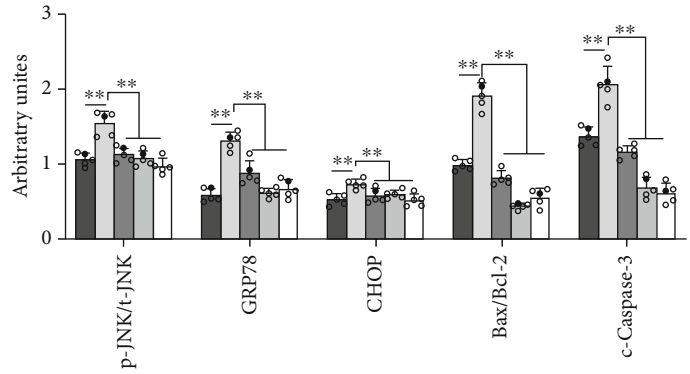
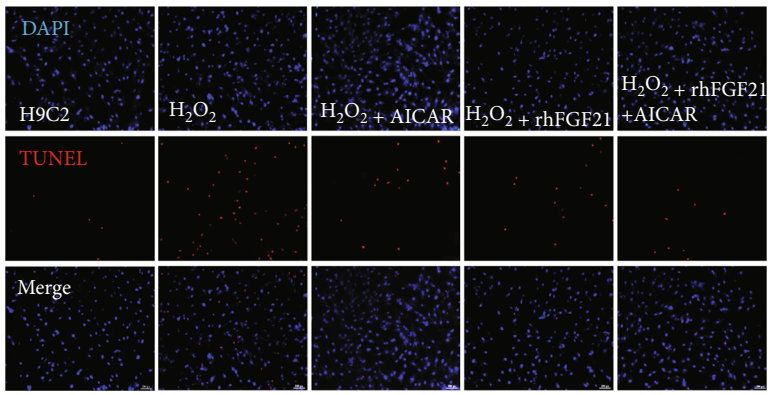


(e)



(f)



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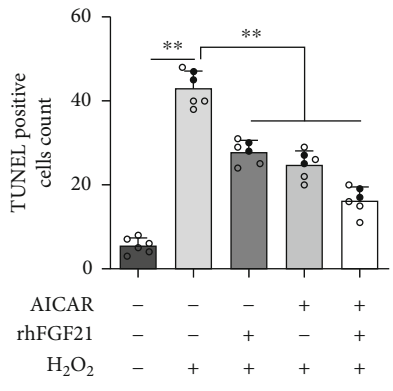

(h)

Figure 5: AICAR or rhFGF21 reduced the oxidative stress and ER stress-induced apoptosis by H_2O_2 in H9C2 cells. (a) T-SOD activity in H9C2 cells. (b) MDA in H9C2 cells. (c–f) Western blotting images and their densitometric quantitative analysis of ALCAT1, p-PI3K/t-PI3K ratio, p-AKT/t-AKT ratio, p-IRE1α/t-IRE1α ratio, p-JNK/t-JNK ratio, GRP78, CHOP, Bax/Bcl-2 ratio, and c-caspase3. (g) TUNEL staining: TUNEL-positive granules in red and DAPI in blue. (h) TUNEL-positive cells count. Data presented are means ± SD. One-way ANOVA with post hoc LSD multiple-comparison test. $^{*}p < 0.05$, $^{**}p < 0.01$.

protective effect of rhFGF21 ($p < 0.05$, $p < 0.01$, Fig. S3). On the other hand, under the H_2O_2 condition, LY294002 intervention attenuated FGF21-mediated downregulation of p-IRE1α/t-RE1α, p-JNK/t-JNK, GRP78, CHOP, Bax/Bcl-2, and c-caspase3 expression, meanwhile inhibiting the upregulation of SOD2 and p-AKT/t-AKT expression ($p < 0.05$, $p < 0.01$, Figures 6(a)–6(c)). All the above data revealed that FGF21 inhibited oxidative stress and ER stress-induced apoptosis by stimulating the FGFR1/PI3K/AKT signaling pathway.

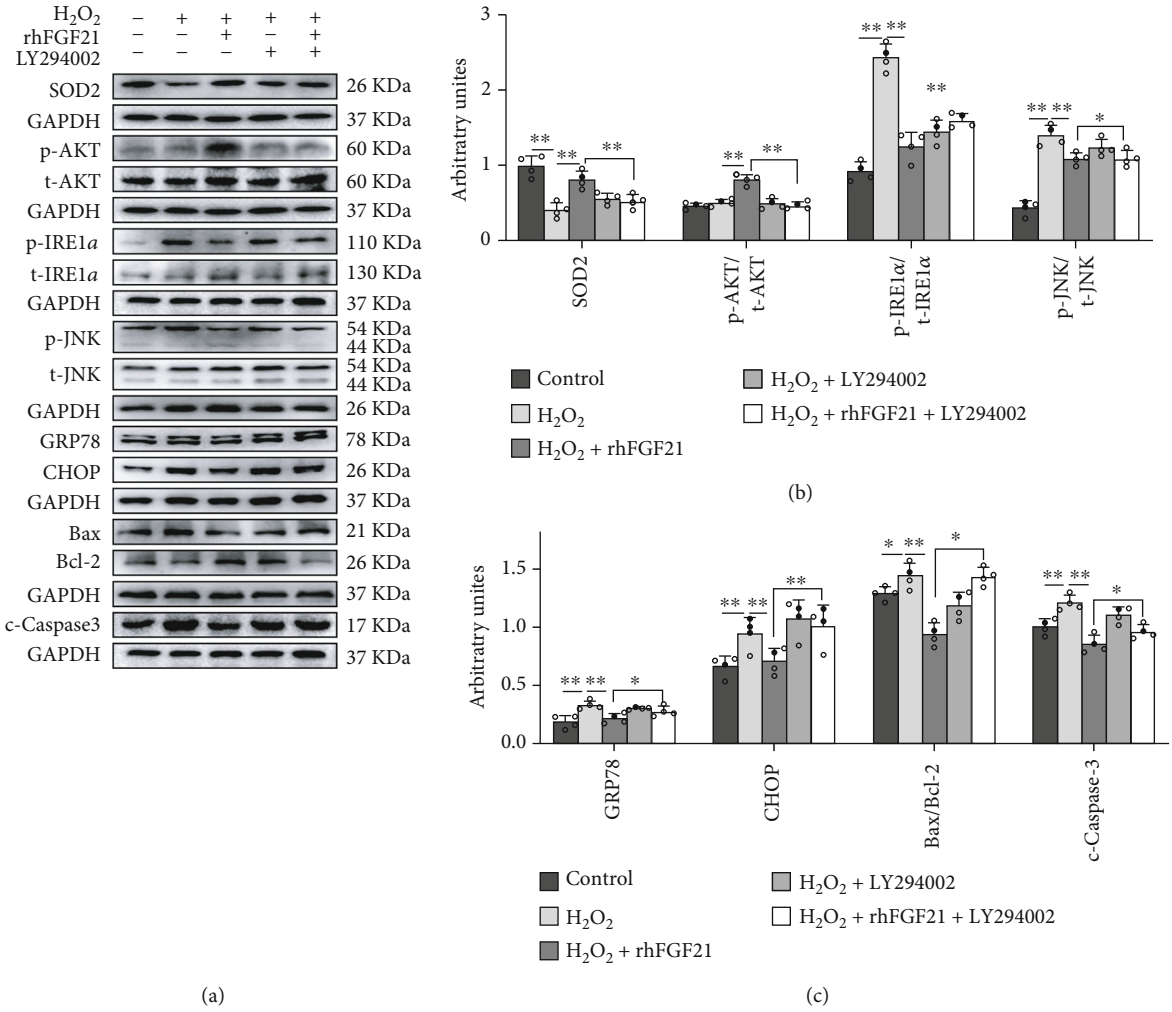

Figure 6: PI3K inhibitor inhibited the FGF21 overexpression-mediated oxidative stress and antiapoptotic effect on ER stress injury. (a–c) Western blotting images and their densitometric quantitative analysis of SOD2, p-AKT/t-AKT ratio, p-IRE1α/t-IRE1α ratio, p-JNK/t-JNK ratio, GRP78, CHOP, Bax/Bcl-2 ratio, and c-caspase3. Data presented are means ± SD. One-way ANOVA with post hoc LSD multiple-comparison test. $^{*}p < 0.05$, $^{**}p < 0.01$.

3.7. FGF21 Activated the PI3K/AKT Pathway to Ameliorate ALCAT1-Induced Oxidative Stress and Apoptosis. ALCAT1 has been proved to be involved in oxidative stress and apoptosis. We speculated FGF21 might inhibit oxidative stress and apoptosis by regulating ALCAT1 expression. H9C2 cells were transfected with lentivirus containing *alcat1* gene for overexpressing ALCAT1 protein ($p < 0.01$, Fig. S4A-B). Compared with the control group, Bax/Bcl-2 ratio and c-caspase3 protein expressions were significantly increased, and the expression of SOD2 protein reduced significantly in the transfected H9C2 cells. All of these results were reversed by rhFGF21 ($p < 0.05$, $p < 0.01$, Figures 7(a) and 7(b)). It was indicated that FGF21 inhibited the hyperexpression of ALCAT1-induced oxidative stress and apoptosis in H9C2 cells.

Then, we examined whether FGF21 inhibited oxidative stress and apoptosis induced by ALCAT1 overexpression through the PI3K/AKT pathway. Compared with the control group, we found p-PI3K and p-AKT/t-AKT ratio protein expression increased significantly after rhFGF21 intervention in H9C2 cells transfected with lentivirus containing *alcat1* gene. The opposite results were observed in LY294002 and rhFGF21 cointervention group in H9C2 cells with lentivirus transfection ($p < 0.01$, Figures 7(a) and 7(b)). Therefore, exogenous rhFGF21 inhibited ALCAT1-induced oxidative stress and apoptosis by activating the PI3K/AKT signaling pathway in H9C2 cells.

4. Discussion

The post-MI heart undergoes extensive oxidative stress, cardiomyocytes loss, accumulation of fibrous tissue, and ultimately ventricular systolic dysfunction. Exercise-based cardiac rehabilitation is an effective treatment in attenuating post-MI apoptosis. Growing clinical consensus believes that exercise training improves myocardial oxygenation and ventricular function in patients with MI and confers sustained improvement in quality of life and reduction in cardiomyocyte apoptosis [2, 36]. In this context, our present study showed that aerobic exercise

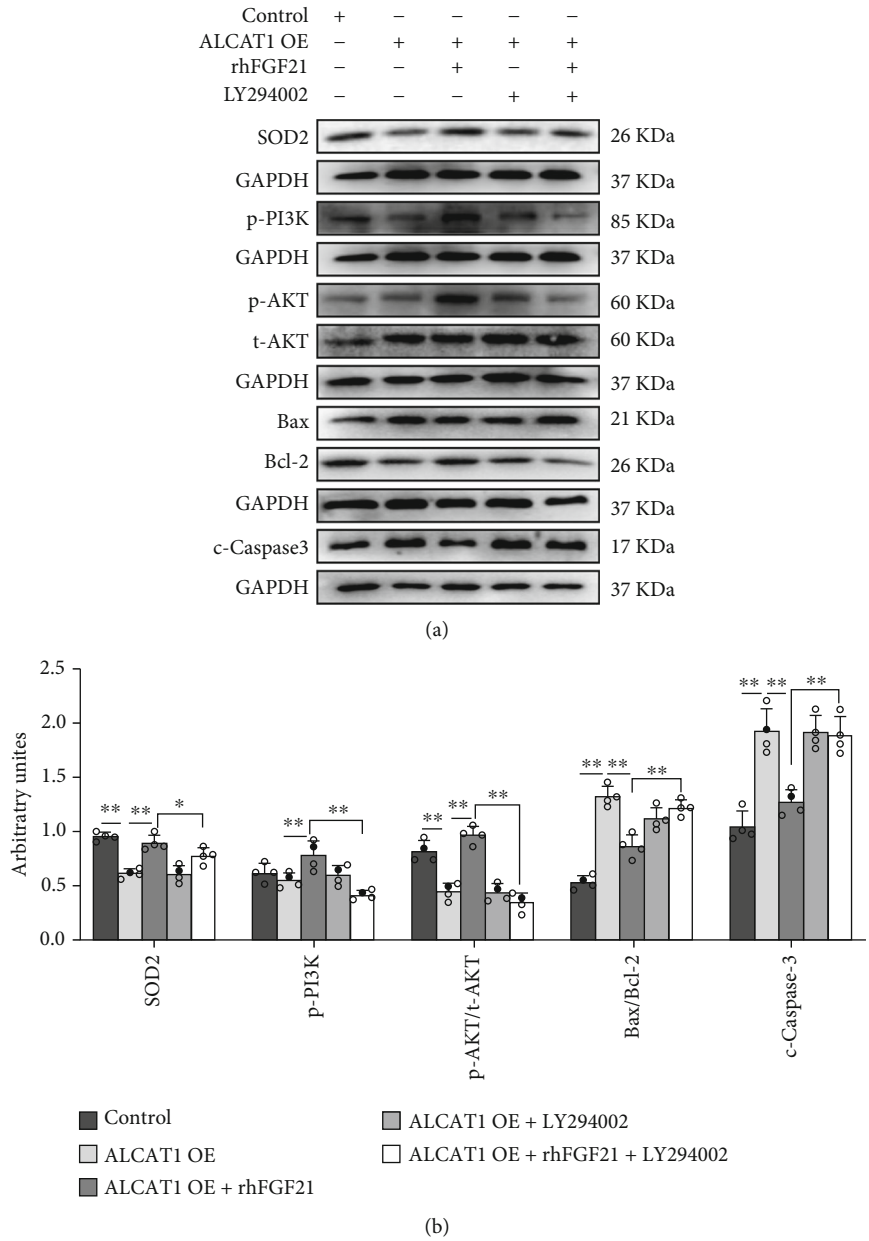

Figure 7: FGF21 inhibited oxidative stress and apoptosis induced by ALCAT1 overexpression through the PI3K-AKT pathway. SOD2, p-PI3K, p-AKT/t-AKT ratio, Bax/Bcl-2 ratio, and c-caspase3 protein levels after rhFGF21 or/and LY294002 intervention in H9C2 cells transfected with lentivirus containing *alcat1* gene. Data presented are means ± SD. One-way ANOVA with post hoc LSD multiple-comparison test. $^{*}p < 0.05$, $^{**}p < 0.01$.

improved the cardiac structure and function through inhibiting oxidative stress and ER stress-induced apoptosis by activating the FGF21/FGFR1/PI3K/AKT pathway in post-MI heart, but *fgf21* knockout partially weakened the cardioprotective effect of aerobic exercise in mice with MI. Additionally, aerobic exercise alleviated oxidative stress and apoptosis and improved the cardiac structure and function by inhibiting the hyperexpression of ALCAT1 in the post-MI mice heart. Knockout *alcat1* alleviated oxidative stress, apoptosis, and cardiac fibrosis and improved left ventricular function in mice heart with MI. Furthermore, exogenous rhFGF21 intervention activated the PI3K/AKT pathway and significantly inhibited H_2O_2 or ALCAT1-induced oxidative stress and apoptosis. These results suggested that aerobic exercise can alleviate oxidative stress and apoptosis by activating the FGF21/FGFR1/PI3K/AKT pathway or inhibiting the hyperexpression of ALCAT1 and ultimately improve the cardiac structure and function of mice with MI.

FGF21 was initially found to play a role in glucose and lipid metabolism and insulin secretion (Nishimura T et al., 2000; Kharitonenkov et al., 2011; Zhang J et al., 2014). A series of subsequent clinical studies have shown that

FGF21 binds to FGFR1 in the form of autocrine or paracrine in the heart [9, 37], which can be used as a predictor of cardiovascular diseases such as acute myocardial infarction and coronary heart disease and play a potential role in prevention and rehabilitation of cardiovascular diseases [38–41]. Deficiency of FGF21 leads to cardiac hypertrophy and adversely affects the ischemic heart. Studies have shown that the secretion of FGF21 in liver tissue increased during myocardial ischemia, and it played a cardioprotective effect through blood circulation [4, 42]. Further studies have found that the intervention of exogenous FGF21 significantly improved the survival of cardiomyocytes under ischemia and decreased the areas of myocardial infarction [29], whereas it inhibited the expression of FGFR1 increased the infarct area of ischemic myocardium [43]. Animal experiments have shown that both one-time acute exercise and exhaustive exercise significantly increased FGF21 expression in serum and skeletal muscle [12, 32, 44]. Clinical studies found that exercise promoted a significant increase in serum FGF21 levels in healthy adults [11, 32]. It suggested that FGF21 may play an organ protection role as an "exerkines." However, it is not clear whether FGF21 is related to the cardioprotective effect of exercise on MI. First of all, in the WT mice, we found that after 6 weeks of aerobic exercise, the expressions of FGF21 and FGFR1 in the heart of the mice with MI were significantly increased, and the cardiac function was significantly improved. Therefore, it was believed that the improvement of cardiac function in MI hearts by exercise was related to FGF21. Then, we performed the same experiment on *fgf21* knockout mice and found that the improvement effect of exercise on the function of hearts in mice with MI was reduced. Considering this, it seems that the improvement of cardiac structure and function in mice with MI by exercise was at least partly mediated by FGF21. But its underlying mechanism remains to be further confirmed.

Oxidative stress and ER stress are highly correlated biological processes, and apoptosis induced by both of them is an important pathophysiological mechanism for cardiomyocyte injury. ER is highly sensitive to intracellular homeostasis and external stimulation. Hypoxia, DNA damage, and calcium depletion induce ER stress and eventually lead to cardiomyocytes apoptosis [36]. UPR is the initial step of ER stress, which helps to maintain ER homeostasis in an early stage. However, when UPR cannot cope with long-term or severe ER stress, the apoptosis pathway mediated by CHOP, caspase-12, and JNK is activated [35, 45]. During ER stress, JNK is phosphorylated by IREα, which activates proteins from the B-cell lymphoma-2 (Bcl-2) family and caspases, which induces cell death [31, 46]. Cardiomyocytes show severe ischemia and hypoxia after myocardial infarction, which results in cellular oxidative stress, ER stress, and apoptosis [47]. Previous studies have shown that FGF21 has inhibited atherosclerosis [48], vascular calcification [49], and cardiomyocyte apoptosis by reducing ER stress [4, 8]. It was reported that overexpression of FGF21 significantly reversed the apoptosis of H9C2 cells induced by TM via inhibiting the IRE1α/JNK pathway [8]. There is a growing consensus that the mechanism of FGF21 in the prevention and treatment of cardiovascular diseases is related to ER stress and oxidative stress. Cardiac tissue antioxidant genes (UCP3 and Sod2) are upregulated in humans with failing hearts which may have a relation that FGF21 mRNA is upregulated [29]. In addition, FGF21 prevents type-2 diabetic lip toxicity-induced cardiomyopathy through activation of the antioxidant pathway effect in the hearts of mice [50]. FGF21 inhibits hypoxia and ER stress-induced apoptosis by activating the PI3K/AKT pathway [29, 51]. Our animal experimental study found that the FGF21/FGFR1/PI3K/AKT pathway was activated, and the oxidative stress and the expression of ER stress-related apoptosis proteins such as IREα, JNK, GRP78, CHOP, c-caspase3, Bax/Bcl-2 were significantly increased in the heart of post-MI mice, whereas the phenomenon was reversed by aerobic exercise. However, the inhibitory effect of exercise on oxidative stress and ER stress-induced apoptosis were weakened after the *fgf21* gene was knocked out. To investigate its mechanisms, H9C2 cells were treated with H_2O_2, AICAR (AMPK agonist), FGFR1 inhibitor (PD166866), PI3K inhibitor (LY294002), and exogenous rhFGF21. We observed that AICAR and rhFGF21 promoted the secretion of FGF21 and FGFR1, activated the PI3K/AKT pathway, and inhibited oxidative stress and ER stress-induced apoptosis in H9C2 cells under the H_2O_2. Furthermore, the rhFGF21 intervention-mediated effect of cardioprotective was reversed by PD166866 and LY294002. Collectively, these results indicated that the protective effect of aerobic exercise on inhibiting oxidative stress and ER stress-induced apoptosis via elevating FGF21 expression in the post-MI heart was mediated, at least in part, by the FGFR1-PI3K/AKT signaling pathways.

Cardiolipin (CL) is the only phospholipid in mitochondria and rich in linoleic acid and is concentrated near the reactive oxygen production site of the mitochondrial inner membrane. CL is highly sensitive to oxidative damage, participates in the regulation of mitochondrial function and oxidative stress, and is oxidized in the early process of apoptosis [13–15]. ALCAT1, as a membrane protein with 414 amino acids, is located in the endoplasmic reticulum and mitochondrial membrane, which is very important in the process of CL remodeling. ALCAT1 catalyzes CL pathological remodeling, which leads to ROS generation, mitochondrial dysfunction, and insulin resistance under the pathological conditions of diabetes, obesity, and cardiomyopathy [16, 18, 19]. ALCAT1 overexpression results in mitochondrial division and fusion disorders, accompanied by mtDNA deletions, while the quality of mitochondria in *alcat1* knockout mice improves significantly, which is not affected by oxidative stress-induced mitochondrial swelling and fragmentation [52]. In addition, ALCAT1-targeted inactivation prevents diet-induced obesity, NAFLD, MPTP-induced neurotoxicity, improves motor deficiency, and inhibits apoptosis [19, 20, 23]. Therefore, ALCAT1 plays an important role in the process of oxidative stress-related diseases, but the role of ALCAT1 in cardiovascular diseases has not been clarified. Exercise inhibits the expression of ALCAT1 in the skeletal muscle of mice [24] and also inhibits the expression of ALCAT1 in the kidney of mice with MI and reduces renal oxidative stress and apoptosis [25]. Aerobic

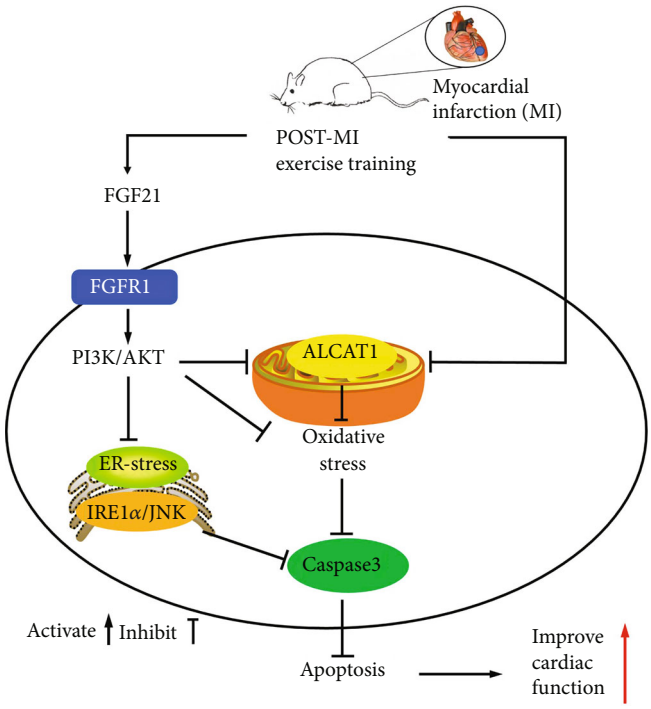

Figure 8: Possible mechanism of aerobic exercise in improving cardiac function in myocardial infarction mice.

exercise significantly inhibits the level of oxidative stress in the ischemic myocardium [53], but ALCAT1 whether involved in its mechanism has not been fully elucidated. Our study showed that in WT mice, the expression of ALCAT1 was significantly increased in the hearts of mice with MI, and aerobic exercise mitigated oxidative stress and apoptosis induced by MI through inhibiting the hyperexpression of ALCAT1. To further determine the role of ALCAT1, we prepared *alcat1* knockout mice and found that *alcat1* knockout significantly improved cardiac structure function by alleviating oxidative stress and apoptosis in mice with MI. In cell experiments, H9C2 cells were transfected with lentivirus containing the *alcat1* gene. Exogenous rhFGF21 intervention inhibited the oxidative stress and apoptosis induced by ALCAT1 overexpression, while PI3K inhibitor (LY294002) attenuated the inhibitory effect of FGF21. These results indicated that aerobic exercise improved cardiac structure and function of mice with MI through inhibiting oxidative stress and apoptosis by suppressing the expression of ALCAT1. Meanwhile, exogenous FGF21 inhibited oxidative stress and apoptosis of H9C2 cells induced by ALCAT1 overexpression by activating the PI3K/AKT pathway.

5. Conclusion

In conclusion, aerobic exercise alleviated oxidative stress and apoptosis by activating the FGF21/FGFR1/PI3K/AKT pathway or inhibiting the hyperexpression of ALCAT1, which improved the cardiac structure and function of the heart in mice with MI (Figure 8). This study further elucidates the protective mechanism of exercise on the ischemic heart and suggests that FGF21 and ALCAT1 may be important molecular targets for clinical treatment or prevention of myocardial infarction.

Data Availability

The [DATA TYPE] data used to support the findings of this study are included within the article.

Additional Points

(1) Aerobic exercise inhibited oxidative stress and ER stress-induced apoptosis of cardiomyocytes by activating the FGF21/FGFR1/PI3K/AKT pathway in post-MI heart, and *fgf21* knockout partially weakened the protective effect of aerobic exercise on the myocardial injury in MI mice. (2) Aerobic exercise alleviated oxidative stress and apoptosis, improved cardiac function by inhibiting the hyperexpression of ALCAT1 in the heart of post-MI mice. Knockout *alcat1* alleviated oxidative stress, apoptosis, and cardiac fibrosis and improved left ventricular function in mice heart with MI. (3) Exogenous rhFGF21 activated the PI3K/AKT pathway and significantly inhibited H_2O_2 or ALCAT1-induced oxidative stress and apoptosis in H9C2 cells.

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions

Z.T. and W.B. contributed the original concepts and designed the experiments; W.B., Y.M., and M.C. conducted the experiments and analyzed the study results; W.B., Y.X., Q.L., and Z.T. drafted the manuscript; Z.T. and W.B. critically revised and finalized the manuscript. All authors reviewed the manuscript and approved its submission.

Acknowledgments

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Supplementary Materials

Supplementary data to this article can be found in Supplemental Files. The supplementary materials include four figures, including the identification results of mouse tail, the protein expression results after cell culture intervention, and the results of alcat1 lentivirus transfection of H9C2 cells. Fig. S1: the electropherogram of *alcat1*$^{-/-}$ mouse tail DNA. Fig. S2: the electropherogram of fgf21 *loxp*$^{+/+}$ mouse tail DNA. Fig. S3: FGFR1 receptor inhibitors inhibited the protective effect of FGF21 on H2O2-induced H9C2 cell injury. Fig. S4: The lentiviral vector containing *alcat1* gene was transfected into H9C2 cells. *(Supplementary Materials)*

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Research Article

Renoprotection Induced by Aerobic Training Is Dependent on Nitric Oxide Bioavailability in Obese Zucker Rats

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Aerobic training (AT) promotes several health benefits that may attenuate the progression of obesity associated diabetes. Since AT is an important nitric oxide (NO^-) inducer mediating kidney-healthy phenotype, the present study is aimed at investigating the effects of AT on metabolic parameters, morphological, redox balance, inflammatory profile, and vasoactive peptides in the kidney of obese-diabetic Zucker rats receiving L-NAME (N(omega)-nitro-L-arginine methyl ester). Forty male Zucker rats (6 wk old) were assigned into four groups ($n = 10$, each): sedentary lean rats (CTL-Lean), sedentary obese rats (CTL-Obese), AT trained obese rats without blocking nitric oxide synthase (NOS) (Obese+AT), and obese-trained with NOS block (Obese +AT+L-NAME). AT groups ran 60 min in the maximal lactate steady state (MLSS), five days/wk/8 wk. Obese+AT rats improved glycemic homeostasis, SBP, aerobic capacity, renal mitochondria integrity, redox balance, inflammatory profile (e.g., TNF-α, CRP, IL-10, IL-4, and IL-17a), and molecules related to renal NO^- metabolism (klotho/FGF23 axis, vasoactive peptides, renal histology, and reduced proteinuria). However, none of these positive outcomes were observed in CTL-Obese and Obese +AT+L-NAME ($p < 0.0001$) groups. Although Obese+AT+L-NAME lowered BP (compared with CTL-Obese; $p < 0.0001$), renal damage was observed after AT intervention. Furthermore, AT training under conditions of low NO^- concentration increased signaling pathways associated with ACE-2/ANG1-7/MASr. We conclude that AT represents an important nonpharmacological intervention to improve kidney function in obese Zucker rats. However, these renal and metabolic benefits promoted by AT are dependent on NO^- bioavailability and its underlying regulatory mechanisms.

1. Introduction

The gaseous signaling molecule nitric oxide ($NO^{\cdot}$) plays a key role in the body, including renal hemodynamics regulation. $NO^{\cdot}$ bioavailability is regulated by an increase of $NO^{\cdot}$ production via NO synthase (NOS) activity, using L-arginine as a substrate, and/or lower $NO^{\cdot}$ degradation [1, 2]. In obesity-related disorders, such as type 2 diabetes (T2DM), the renal $NO^{\cdot}$ bioavailability is impaired, which increases renin secretion, intraglomerular pressure, tubuloglomerular feedback, and renal sodium reabsorption, while renal blood flow perfusion is reduced [3–5]. These effects induce kidney damage leading to the development of diabetic nephropathy, chronic kidney disease (CKD), and cardiovascular disease associated with the pathogenesis of systemic hypertension [2, 6, 7].

Physical activity and/or exercise training is the cornerstone nonpharmacological adjunct for treating and preventing several medical conditions, including obesity-related disorders [8, 9]. It is already established that exercise improves glucose homeostasis by reducing chronic inflammation and oxidative stress [3, 9, 10]. For instance, Ito et al. showed that regular running exercise upregulated NOS expression, while suppressing NADPH oxidase and α-oxoaldehydes in the kidneys, which at least in part improved renal protection in the early progression of diabetic nephropathy in Zucker diabetic fatty rats [11]. Similar results were also observed by other authors, where aerobic exercise during 4 weeks promoted protective effects in a diabetic kidney by reducing renal oxidative stress and inflammation in female Wistar rats [12]. Exercise also decreases mitochondrial oxidative stress by reducing the mitochondrial permeability transition pore (MPTP) [13, 14]. Interestingly, this effect regulates the action of vasoactive peptides, such as angiotensin 1-7, bradykinin, and vasopressin, which are associated with improvements in $NO^{\cdot}$ bioavailability. Restoring $NO^{\cdot}$ bioavailability has been considered a key mechanism to improve endothelial dysfunction in obesity-related disorders [15–19].

In endothelial dysfunction, the elevated vascular oxidative stress decreases NOS expression, especially the endothelial NOS isoform (eNOS), thus reducing $NO^{\cdot}$ bioavailability [20, 21]. Notably, exercise training decreased renal oxidative stress and systemic blood pressure even when chronic inhibition of eNOS was experimentally induced by N(omega)-nitro-L-arginine methyl ester (L-NAME) [22, 23]. L-NAME has been widely applied for several decades in basic and clinical research as an antagonist of NOS [24–29], reducing $NO^{\cdot}$ bioavailability in tissues such as the kidney [30, 31]. However, some studies have shown that while exercise improves systemic blood pressure under reduced eNOS activity, it may increase renal injury in animal models [23, 32]. Thus, exercise has clear health benefits; however, the renal effects of aerobic exercise training (AT) and biological action of $NO^{\cdot}$ in obesity-related conditions, such as T2DM, require further elucidations.

Renoprotection is a complex phenomenon involving the cross-talk between multiple mechanisms and $NO^{\cdot}$ signaling pathways. Functional assessments, morphological, biochemical, and molecular approaches might aid understanding of the complete picture of cellular adaptation in response to AT in a kidney structure. A mechanistic framework of these responses could provide valuable insights for therapeutic approach development and treatment guidance to attenuate kidney diseases and disorders, besides mapping of key biomarkers in physiological responses inherent to obesity conditions. Since exercise is an important NO-inducer mediating kidney function, the present study is aimed at investigating the effects of 8 weeks of AT on metabolic parameters, morphological parameter, redox balance, inflammatory profile, and vasoactive peptides in the kidney of obese-diabetic Zucker rats receiving L-NAME. We hypothesized that Zucker rats exposed to L-NAME display no improvements in glucose homeostasis, blood pressure, kidney structure, redox balance, and inflammatory state.

2. Materials and Methods

2.1. Animals. Forty 6-wk-old male homozygous obese (fa^{-}/fa^{-}) and lean (Fa^{+}/fa^{-}) Zucker rats obtained from the Animal House were used in this research and maintained under a 12 h light/dark cycle (lights on at 07 : 00) at room temperature of $22 \pm 2^{\circ}C$ and relative humidity of $55 \pm 10\%$. After one week of acclimation, the animals were distributed into four groups ($n = 10$, each): sedentary lean rats (CTL-Lean), sedentary obese rats (CTL-Obese), AT-trained obese rats without blocking the NOS (Obese+AT), and AT-trained obese rats with NOS blocked (Obese+AT+L-NAME). During the study, animals were maintained in separate cages (4-3 animals per cage according to their phenotype), fed a standard chow (NUVILAB CR1, Nuvital® Nutrients, Curitiba, Brazil), and received water *ad libitum*. All procedures followed the NIH Guide for the Care and Use of Laboratory Animals (U.S. National Research Council, Washington D.C., USA) [33, 34] and international principles for research involving animals (ARRIVE 2.0) [35]. The present study was approved by the Animal Research Ethics Committee (protocol number: 007/19).

2.2. Determination of the Exercising Lactate. Lactate threshold (LT) was assessed 48 h after the first insulin tolerance test (ITT) [36]. All animals running at 6 m/min speed for 10 min three times per week for two weeks to familiarize the rats with the AT training ergometer (treadmill developed for rats customized model, AVS Projects, Brazil).

The electric shock (5 mV) was conducted according to the NIH Guide for Care and Use of Laboratory Animals [33, 34]. Stimulus was used in familiarization, physical examinations, and exercise training. This familiarization protocol was intended to reduce the animals' stress when performing the required exercise tasks. Intensity started at $6\,m{\cdot}min^{-1}$ and was increased by $2\,m{\cdot}min^{-1}$ for each 3 min stage until exhaustion. In addition to lactate evaluation, the investigators also determined maximum running velocity (Vmax) achieved during the last stage in the graded exercise test. Vmax was obtained in the incremental test, as the velocity of the last complete stage was supported by the animal [37].

Blood (5 μL) obtained from the tail was collected at the beginning of the test (rest) and the end of each complete stage using a portable lactometer (Accutrend® PLUS-Roche, USA) to quantify lactate concentration. After local antisepsis with 70% alcohol, 25 μL of blood was collected from a small incision in the distal tail portion using a calibrated capillary tube. The blood sample was rapidly deposited in Eppendorf® microtubes (0.6 mL), containing 50 μL of 1% sodium fluoride (NaF), and stored at −80°C for further biochemical analysis [38].

To determine LT by the visual inspection method, lactate levels were plotted on individual graphs (lactate versus exercise intensity). The evaluators determined the lactate curve's tipping point as the moment when lactate exponentially increased relative to the exercise intensity. These incremental tests were previously described by Rosa et al. applied in all rats pre- and posttraining [38].

These incremental tests were also used to evaluate the intensity closest to the maximal lactate steady state (MLSS) and reduce the number of sessions required to determine the MLSS. The criterion used to identify MLSS was a blood lactate range of up to 0.5 mmol·min^{-1} during the last 10 min of exercise proposed for rats [38].

2.3. Aerobic Training. The study began with treadmill running familiarization, according to a protocol adapted from Copp et al. [39]. Rats ran at 6 m/min, 10 min per day, 5 days a week, during 2 weeks on a treadmill with individual lanes and electrical stimulation at the rear (customized model, AVS Projects, Brazil). 48 h after the last MLSS assessment, rats in the AT groups started the exercise protocol in the same treadmill of the MLSS: 60 min at the measured 100% MLSS and 0% grade. Training occurred five days per week for eight weeks as described by Rosa et al. [38].

2.4. Inhibition of Nitric Oxide Synthases by L-NAME. Animals assigned to the Obese+AT+L-NAME group were orally supplemented with L-NAME administered in the drinking water at a 5 mg/100 mL concentration for 8 weeks following the protocol published by Bayls et al. [40] L-NAME is a nonselective inhibitor of NOS, but at this concentration, it is a selective inhibitor of eNOS. L-NAME was administered at the beginning of AT and maintained until the end of the protocol.

2.5. Glucose and Insulin Tolerance Tests. Glucose and insulin tolerance tests were conducted in all animals two days after [36] the beginning of the 8 weeks of L-NAME supplementation and exercise. The glucose tolerance test (GTT) was performed using an intraperitoneal glucose solution (2 g/kg body weight) at baseline and at posttraining (48 h after isolated cage time). Glycaemia was determined at 0, 15, 30, 60, and 120 min after the glucose injection. ITT was also performed at baseline and posttraining (48 h after GTT). The ITT required an injection of 0.1 U/kg of recombinant regular insulin Humulin® intraperitoneally. Glycaemia was measured at 0, 5, 10, 15, 20, and 30 min. Glycaemia in both GTT and ITT tests was measured using an AccuCheck Performa Roche®. Fasting plasma insulin (FPI) was measured a rat-sensitive enzyme-linked immunosorbent assay (ELISA) kit (Millipore Corporation, Billerica, MA). Homeostasis model assessment (HOMA) for insulin resistance (HOMA-IR) and HOMA for β-cell function (HOMA-β) were calculated, as previously described by Ito et al. [11]: HOMA-IR : [fasting insulin (ng/mL) × fasting glucose (mg/dL)]/405 ; HOMA-β : [fasting insulin (ng/mL) × 20]/[fasting glucose (mg/dL) – 3.5].

2.6. Blood Pressure Measurement. Systolic blood pressure (SBP) was measured for all animals before and after the AT intervention. Blood pressure measurement was performed according to Neves et al. [41]. Briefly, the SBP was measured using the tail-cuff method with the rats under the conscious condition with the PowerLab system (ADInstruments, Inc., Sydney, Australia). This tail-cuff method is a sensitive and accurate approach for the noninvasive measurement of BP in conscious SHR [41]. SBP was measured once a week at the same time each day (between 6:00 and 8:00 p.m.) to allow the animals to become adapted to the procedure.

2.7. Euthanasia and Tissue Harvest. One at a time, after 48 h of the MLSS, the animals were euthanized as described by Neves et al. [42], and the kidneys were harvested. The right kidney was weighed and separated for histology, and the left kidney was utilized for molecular and biochemical analyses. Samples obtained from the left kidney were also frozen at -80°C and used for mRNA and protein determination. The kidney was weighted and corrected by the tibial length [43].

2.8. Renal Gene Expression of eNOS, iNOS, and Inflammatory Profile. The total RNA was extracted according to the TRIzol method described by Chomczynski and Sacchi [44]. A NanoDrop® spectrophotometer (ND-1000; NanoDrop Technologies Inc., Wilmington, DE, USA) was used to quantify the RNA concentrations and determining the absorbance rate of 260–280 nm. To assess the eNOS and iNOS gene expression in the kidney, a total of 100-350 ng of RNA extracted from each sample was converted to cDNA (final volume 20 μL) using GoTaq® Probe RT-qPCR (Promega-Cat. A6120X) based on the manufacturer's protocol. The standard cycling conditions to perform reverse transcription and amplification of samples were as follows: (a) reverse transcription (1 cycle)—45°C for 15 minutes; (b) reverse transcriptase inactivation and GoTaq® DNA Polymerase activation (1 cycle)—95°C for 2 minutes; (c) denaturation (40 cycles)—95°C for 15 seconds; and (d) annealing and extension (40 cycles)—60°C for 1 minute. The following TaqMan probes were used for the determination of A-actin (Rn00667869m1), eNOS (Rn02132634s1), and iNOS (Rn00561646m1).

Inflammatory profile was measured in kidney samples following homogenization. Total proteins were extracted from tissues using phosphate-buffered saline (PBS 1%, pH 7.4) supplemented with a protease inhibitor cocktail (Roche, Germany). Tumor necrosis factor-alpha (TNF-α), c-reactive protein (CRP), interleukin 10 (IL-10), and interleukin 4 (IL-4) were assessed by commercial ELISA kits

manufactured by R&D Systems (USA). Interleukin 17a (IL-17a) was measured by a rat-sensitive IL-17a ELISA kit manufactured by Invitrogen (Thermo Fischer Scientific, MA, USA). For this kit, sensitivity was established at 1.0 pg/mL, and the reproducibility intra-assay and interassay were 8.5% and 7.6% CVs, respectively. Interleukin 18 (IL-18) was measured using a rat IL-18 ELISA kit manufactured by Abcam (São Paulo, Brazil), the sensitivity was <1 pg/mL, and the reproducibility intra- and interassays were lower than 6.2% and 7.2% CVs. The kidney protein content was analyzed by the Bradford method [45]. Standard curves for each cytokine were generated using serial dilutions of the mediators supplied, with each sample titrated by linear interpolation. All samples were determined in duplicate to guarantee reliability.

2.9. Histological Analyses. The right kidney was weighted and sectioned in the frontal plane (5 μm); then, it was fixed with 10% formaldehyde (10 mM phosphate buffer, pH 7.4) and embedded into paraffin. The blades were stained with hematoxylin-eosin (HE) for the evaluation of structural changes. Sections were visualized at a magnification of 200x (Leica DM1000, Wetzlar, Germany, 20 × objective and 10 × oculars). Approximately 50 glomeruli and renal tubule sections were evaluated. Each photomicrograph was delimited near the outer edges; individual glomeruli were surrounded to determine the glomerular area using AxioVision Rel, 4.8 software (Carl Zeiss, IL, USA). The tubular diameter was measured at the widest cross-sectional point of the captured images using the same software [46].

2.10. Biochemical Analyses. Renal tissue was defrosted, sectioned, and transferred to ice-cold 0.9% NaCl containers and homogenized in 0.1 mol/L Tris-HCl buffer (pH 7.4). The levels of thiobarbituric acid-reactive substances (TBARS), glutathione (GSH), glutathione disulfide (GSSG), superoxide dismutase (SOD), catalase (CAT), NO^-, and trolox equivalent (TE; total antioxidant capacity) were analyzed following the manufacturer's specifications. Protein carbonyls, 3-nitrotyrosine (3-NT), klotho, angiotensin 1 converting enzyme (ACE-1), angiotensin 2 converting enzyme (ACE-2), angiotensin II type-1 receptor (AT_1R), bradykinin (BK), and FGF23 were determined using rat protein carbonyl, rat 3-NT, rat klotho, rat ACE-1, rat ACE-2, rat AT_1R, rat BK, and rat FGF23 ELISA kits, respectively. The overall intra- and interassays CVs for markers were in a range of 2 to 15% (MyBioSource, Inc., San Diego, USA). Asymmetric dimethylarginine (ADMA) and angiotensin II (ANGII) concentrations were determined in duplicate using the rat ADMA (CVs: intra- and interassays were <15%) and rat ANGII (CVs: intra- and interassays were <8% and <10%, respectively) ELISA kits (Cusabio Technology LLC, MD). Angiotensin II type-2 receptor (AT_2R), angiotensin 1-7 [ANG-(1-7)], and vasopressin dosages were performed using the rat AT_2R (CVs: intra- and interassays were <10% and <12%, respectively) ELISA kit (LifeSpan BioSciences, Inc., Seattle, USA), rat ANG-(1-7) ELISA kit, and rat vasopressin ELISA kit; the overall intra- and interassays CVs for ANG-(1-7) and vasopressin were in a range of 5 to 16% (Biocompare, Inc., South San Francisco, USA). Two days after the last AT session, each animal was relocated to an individual cage, food and water were offered *ad libitum*, and 24 h urine samples were collected. Urinary creatinine levels were evaluated in duplicate (CVs: intra- and interassays < than 5%) by the colorimetric method using a kit (LABTEST Diagnostics, São Paulo, Brazil). Urinary 8-isoprostane was analyzed in duplicate by ELISA; the overall intra- and interassays CVs were 11.7 and 16.4, respectively (Cayman Chemical, Ann Arbor, MI). Urinary protein excretion (UPE) was assessed in duplicate by colorimetric assay using a Sensiprot Labtest kit with CVs: intra- and interassays < 10% (Centerlab Ltda, São Paulo, Brazil). Urinary excretion of 8-hydroxydeoxyguanosine (8-OHdG) was measured in duplicate (CVs: intra- and interassays < 12%) using an ELISA kit (Northwest Life Science Specialties, LLC; Vancouver, WA).

2.11. Renal Mitochondrial Swelling and ROS Production. To isolate mitochondria from the renal tissue, we used the differential centrifugation protocol described by Pedersen et al. [47]. Subsequently, internal mitochondrial membrane permeability studies were performed by the mitochondrial osmotic swelling estimated by the decrease of the absorbance at 540 nm with the aid of a Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan), in the presence of $CaCl_2$ at a concentration of 50 μM. These assays were adapted from Kowaltowski et al. [48]. The amount of mitochondrial protein was determined by the Biuret method [49]. Changes of $2'$,$7'$-dichlorodihydrofluorescin diacetate fluorescence were used to assess the production of ROS by mitochondria. $2'$,$7'$-Dichlorofluorescein (DCF) fluorescence was accompanied at 503/529 nm excitation/emission wavelength pair in a Fluorescence Spectrophotometer Hitachi F-2500 (Hitachi, Tokyo, Japan). As previously described by Mello et al., ROS production was represented by relative fluorescence units (RFU) [50]. The experimental design of this study is presented in Figure 1.

2.12. Statistical Analysis. For F tests, the sample power was calculated from an alpha of 5% ($p < 0.05$) and the power of 95% for large effect size. Results of the a priori power analysis ($p < 0.05$ and 95% power) were conducted using GPower® indicating the need of at least 8 rats per group. All data were presented as the mean ± SD. Normality and homoscedasticity were evaluated using the Shapiro-Wilk and Levene tests, respectively. A 2 × 2 (group × time interaction) ANOVA followed by Tukey's Honestly Significant Difference (Tukey HSD) post hoc test was used to identify group × time differences ($p < .05$) for the following variables BW, SBP FBG, FPI, GTT, ITT, HOMA-IR, HOMA-β, MLSS, and Vmax. One-way ANOVA with Tukey HSD post hoc test were used to identify differences ($p < 0.05$) between the groups for mitochondrial swelling and ROS production by mitochondria, renal and urinary parameters, inflammatory and redox profiles, klotho, FGF23, renin-angiotensin system, BK, vasopressin, and renal morphology. Analyses were performed using GraphPad Prism 6.0 software (GraphPad Software, Inc., CA, USA).

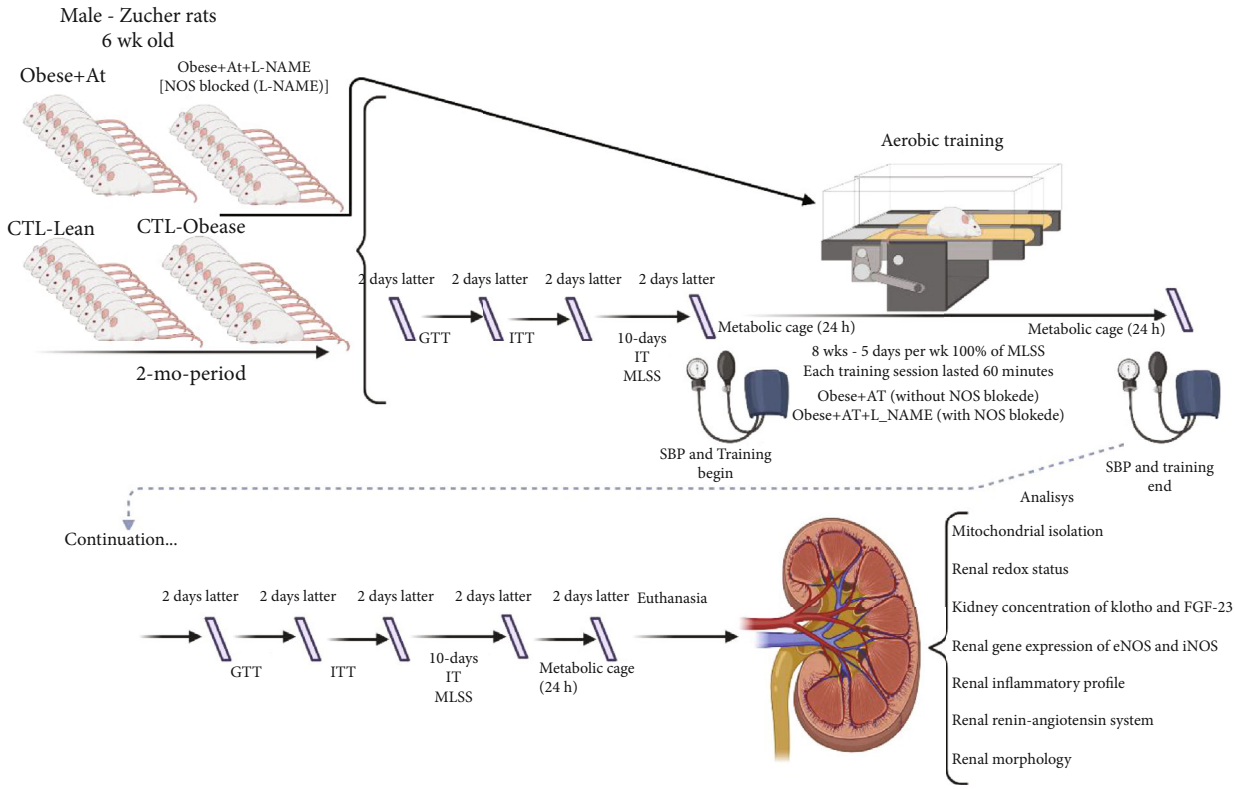

Figure 1: Experimental design. CTL-Lean: sedentary lean rats; CTL-Obese: sedentary obese rats; Obese+AT: obese rats who underwent aerobic training without blocking of nitric oxide synthases; Obese+AT+L-NAME: obese rats who underwent aerobic training with blocking of nitric oxide synthases; L-NAME: N(ω)-nitro-L-arginine methyl ester; GTT: glucose tolerance test; ITT: insulin tolerance test; IT: incremental test; MLSS: maximal lactate steady state; BP: blood pressure; AT: aerobic training; eNOS: endothelial nitric oxide synthases; iNOS: inducible nitric oxide synthases. Male Zucker rats were selected at six weeks old. Rats belonging to the Obese+AT+L-NAME group received L-NAME 8 weeks prior to the beginning of the glycemic homeostasis tests and throughout the experimental design. All rats were analyzed GTT, ITT, IT, MLSS, and BP pre- and posttraining. All these analyses were performed with an interval of two days between them. After two days of the initial physical tests, the animals of trained groups were submitted to AT groups ran for 60 min at an intensity equivalent to 100% of MLSS, with 0% grade, 5 days/wk/8 wk. Afterward, 48 h of the last training session, each rat was isolated in a cage for 24 h urine collection. Euthanasia was performed after two days of the last physical fitness tests. The tibia length was measured (renal weight was corrected for tibial length). The kidney was collected for the biomolecular analyses in the mitochondria, redox and inflammatory profiles in the renal homogenate, and weighed. In addition, evaluations were performed on molecules related to the metabolism of renal nitric oxide, as well as biomarkers of renal health klotho/FGF axis23 and vasoactive peptides.

3. Results

At the baseline, body weight (BW) was higher in the obese groups when compared to the lean group. All groups gained weight from baseline to posttest. As expected, the SBP was higher in the obese groups before and after training than in the CTL-Lean group. Baseline SBP was lower in the CTL-Obese and Obese+AT groups when compared to the Obese+AT+L-NAME group. Post hoc analyses indicated that the Obese+AT and Obese+AT+L-NAME groups had lower SBP than the CTL-Obese group posttraining. The CTL-Obese group was the only group in this study that increased SBP when compared to baseline. Pretraining FBG was higher in the Obese+AT+L-NAME group than in the CTL-Obese and Obese+AT groups, which reinforces the impact of the decrease in the action of NO^- on the increase in blood glucose. Both CTL-Obese and Obese+AT had higher levels of FBG than CTL-Lean. Following the analysis of group versus time interaction, it was observed that AT decreased FBG in obese rats when compared to CTL-Obese. However, the sum of lower levels of NO^- plus AT aggravated the increase in FBG. Furthermore, AT decreased hyperinsulinemia (FPI), glucose intolerance (GTT), and insulin resistance (ITT and HOMA-IR) and improved the pancreatic beta function marker (HOMA-β) compared to CTL-Obese. The reduced levels of NO^- levels promoted by L-NAME are associated with AT appears to increase glucose intolerance (GTT), insulin resistance (ITT and HOMA-IR), and reduction in pancreatic beta function (HOMA-β) caused by obesity and T2DM. AT increases aerobic endurance (MLSS) and maximal running speed (Vmax) in obese Zucker rats. However, the obesity and T2DM observed in Zucker rat cause a decrease in MLSS and Vmax verified in the pretraining period and after the end of the experimental protocol. Data are shown in Table 1.

Kidney mitochondrial swelling at the concentration of $CaCl_2$ 50 μM was higher in the obese groups when compared to the CTL-Lean group. However, the Obese+AT group

Table 1: Responses of body weight, blood pressure, metabolic parameters and physical fitness pre- and posttraining.

Variables		CTL-Lean $N = 10$	CTL-Obese $N = 10$	Obese+AT $N = 10$	Obese+AT+L-NAME $N = 10$	Two-way ANOVA p group	p time	p interaction
BW (g)	Pre	384 ± 12	$534 \pm 9^{*}$	533 ± 11	536 ± 13	<0.0001	<0.0001	0.0005
	Post	$403 \pm 11^{\S}$	$569 \pm 6^{\S,*}$	$571 \pm 11^{\S}$	$564 \pm 10^{\S}$			
SBP (mmHg)	Pre	117 ± 7	$141 \pm 8^{*}$	143 ± 4	$155 \pm 6^{\dagger,\ddagger}$	<0.0001	<0.0001	<0.0001
	Post	121 ± 9	$173 \pm 5^{\S,*}$	$148 \pm 8^{\dagger}$	$152 \pm 7^{\dagger}$			
FBG (mg/dL)	Pre	98 ± 11	$141 \pm 15^{*}$	137 ± 14	$158 \pm 9^{\dagger,\ddagger}$	<0.0001	<0.0001	<0.0001
	Post	105 ± 11	$211 \pm 16^{\S,*}$	$104 \pm 11^{\S,\dagger}$	$285 \pm 45^{\S,\dagger,\ddagger}$			
FPI (ng/mL)	Pre	1.35 ± 0.22	$9.58 \pm 0.30^{*}$	9.49 ± 0.19	$9.81 \pm 0.12^{\dagger,\ddagger}$	<0.0001	<0.0001	0.0002
	Post	1.37 ± 0.12	$9.67 \pm 0.11^{*}$	$9.13 \pm 0.11^{\S,\dagger}$	$9.90 \pm 0.06^{\dagger,\ddagger}$			
GTT (AUC)	Pre	900 ± 95	$1626 \pm 216^{*}$	1618 ± 145	$1798 \pm 67^{\dagger,\ddagger}$	<0.0001	<0.0001	<0.0001
	Post	912 ± 79	$1673 \pm 94^{*}$	$1380 \pm 50^{\S,\dagger}$	$1964 \pm 73^{\S,\dagger,\ddagger}$			
ITT (AUC)	Pre	612 ± 51	$759 \pm 47^{*}$	755 ± 41	$935 \pm 24^{\dagger,\ddagger}$	<0.0001	<0.0001	<0.0001
	Post	641 ± 31	$837 \pm 46^{\S,*}$	$698 \pm 34^{\S,\dagger}$	$995 \pm 40^{\S,\dagger,\ddagger}$			
HOMA-IR	Pre	0.32 ± 0.06	$2.70 \pm 0.86^{*}$	2.98 ± 0.25	$3.82 \pm 0.21^{\dagger,\ddagger}$	<0.0001	<0.0001	<0.0001
	Post	0.35 ± 0.05	$3.02 \pm 0.19^{\S,*}$	$2.40 \pm 0.22^{\S,\dagger}$	$4.26 \pm 0.19^{\S,\dagger,\ddagger}$			
HOMA-β	Pre	0.29 ± 0.06	$1.91 \pm 0.64^{*}$	1.60 ± 0.17	$1.28 \pm 0.07^{\dagger,\ddagger}$	<0.0001	<0.0001	<0.0001
	Post	0.27 ± 0.04	$1.58 \pm 0.10^{\S,*}$	$1.78 \pm 0.16^{\S,\dagger}$	$1.16 \pm 0.04^{\S,\dagger,\ddagger,\S}$			
Parameters related to aerobic fitness (m/min)								
MLSS	Pre	16 ± 2	$12 \pm 4^{*}$	12 ± 3	11 ± 5	<0.0001	0.2491	0.0308
	Post	15 ± 7	$11 \pm 2^{*}$	$15 \pm 4^{\S,\dagger}$	$10 \pm 2^{\ddagger}$			
Vmax	Pre	23 ± 3	$14 \pm 4^{*}$	16 ± 3	$14 \pm 2^{\dagger}$	<0.0001	0.3613	<0.0001
	Post	20 ± 6	$14 \pm 6^{*}$	$26 \pm 5^{\S,\dagger}$	$11 \pm 3^{\ddagger}$			

Data are presented as the mean ± SD. CTL-Lean: sedentary lean rats; CTL-Obese: sedentary obese rats; Obese+AT: obese rats who underwent aerobic training without blocking the nitric oxide synthases; Obese+AT+L-NAME: obese rats who underwent aerobic training with blocking the nitric oxide synthases; BW: body weight; SBP: systolic blood pressure; FBG: fasting blood glucose; FPI: fasting plasma insulin; GTT: glucose tolerance test; ITT: insulin tolerance test; AUC: area under the curve; HOMA-IR: homeostasis model assessment for insulin resistance; HOMA-β: homeostasis model assessment for β-cell function; MLSS: maximal lactate steady state; Vmax: maximum running velocity. Two-way ANOVA, including within and between groups analysis followed by Tukey's post hoc test, was adopted to compare the responses of variables pre- and postexperimental period. § *vs.* Pre; ∗ *vs.* CTL-Lean; † *vs.* CTL-Obese; ‡ *vs.* Obese+AT.

attenuated the mitochondrial swelling when compared to the CTL-Obese and Obese+AT+L-NAME groups (Figures 2(a) and 2(b); $p < 0.0001$). Production of ROS by renal mitochondria (i.e., evaluated by an indicator of oxidative stress DCFH-DA) was lower in the CTL-Lean and Obese+AT groups when compared to the CTL-Obese and Obese+AT+L-NAME groups (Figure 2(c); $p < 0.0001$).

The obesity of the Zucker rat causes oxidative stress in different cellular structures, as observed by the increase in lipid peroxidation markers (TBARS and urinary 8-isoprostane), protein (3-NT) and mitochondrial DNA (urinary 8-OHdG) oxidation, and dysregulation in antioxidant enzymes more present in the cytoplasm (CAT), in the mitochondria (SOD), and in the general antioxidant balance (GSH/GSSG ratio and TE-Trolox equivalent) (CTL-Obese, Obese+AT, and Obese+AT+L-NAME compared to CTL-Lean; $p < 0.0001$). AT reduces these oxidative damages in these different subcellular structures (Obese+AT compared to CTL-Obese and Obese+AT+L-NAME; $p < 0.0001$), but in the absence of $NO^{\cdot}$, AT exacerbates these damages (Obese+AT+L-NAME compared to CTL-Obese; $p < 0.0001$). Values are shown in Figure 3.

AT increases Klotho and decreases FGF23 in Zucker rats (Obese+AT compared to CTL-Obese and Obese+AT+L-NAME; $p < 0.0001$), thus combating the imbalance in the Klotho/FGF23 axis caused by obesity (CTL-Obese, Obese +AT, and Obese+AT+L-NAME compared to CTL-Lean; $p < 0.0001$), but under a low concentration of $NO^{\cdot}$, AT amplifies this lack of control (Obese+AT+L-NAME compared to CTL-Obese; $p < 0.0001$). Data are shown in Figure 4.

In the obese Zucker rat, ACE-1/ANGII/AT_1R pathway hyperactivation and ACE-2/ANG-(1-7)/AT_2R reduction were observed, which are important to induce glomerular and tubulointerstitial lesions (CTL-Obese, Obese+AT, and Obese+AT+L-NAME compared to CTL-Lean; $p < 0.0001$). However, AT decreases ACE-1, AT1R, and vasopressin and increased ACE-2, ANG-(1-7), and AT2R in obese Zucker rats, which culminated in a decrease in kidney damage (Obese+AT compared to CTL-Obese and Obese+AT+L-

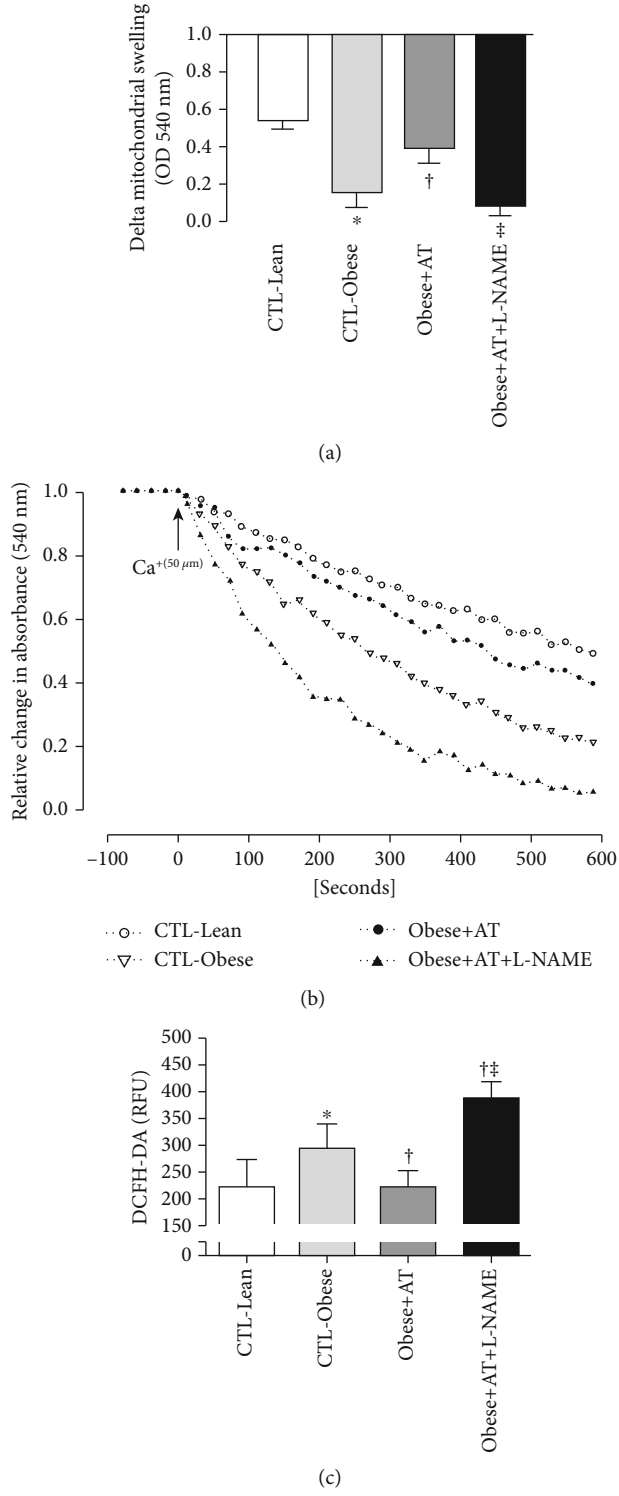

Figure 2: Swelling and production of species reactive to oxygen by the renal mitochondria. Data are presented as the mean ± SD. CTL-Lean: sedentary lean rats; CTL-Obese: sedentary obese rats; Obese+AT: obese rats who underwent aerobic training without blocking of nitric oxide synthases; Obese+AT+L-NAME: obese rats who underwent aerobic training with blocking of nitric oxide synthases. (a) Delta mitochondrial swelling. (b) The figure illustrates the fall in the absorbance of the renal mitochondria (swelling) against the insult with calcium chloride of the groups. (c) Production of species reactive to oxygen by the renal mitochondria. The one-way ANOVA followed by Tukey's post hoc test was adopted. The one-way ANOVA followed by Tukey's post hoc test was adopted. $^{*}p < 0.0001$ *vs.* CTL-Lean; $^{\dagger}p < 0.0001$ *vs.* CTL-Obese; $^{\ddagger}p < 0.0001$ *vs.* AT.

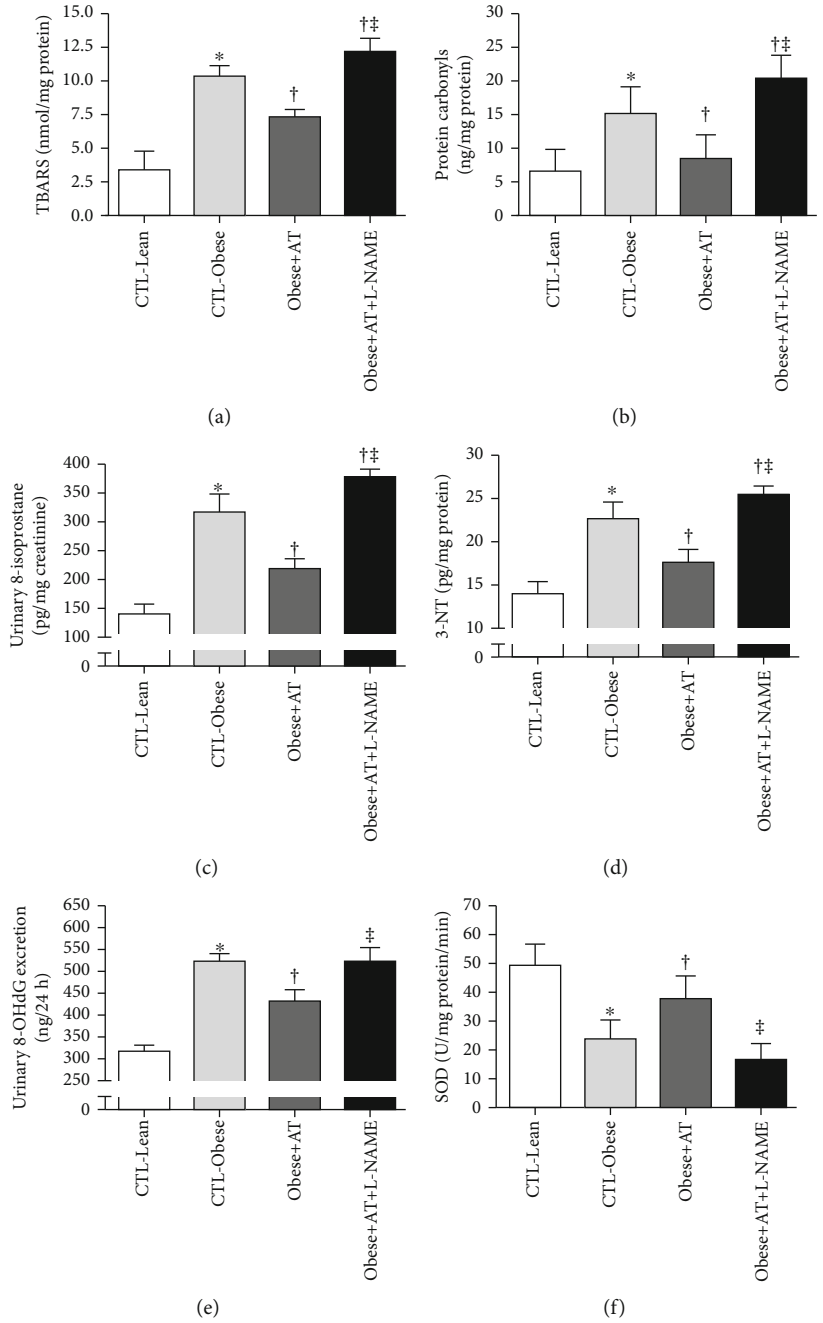

Figure 3: Continued.

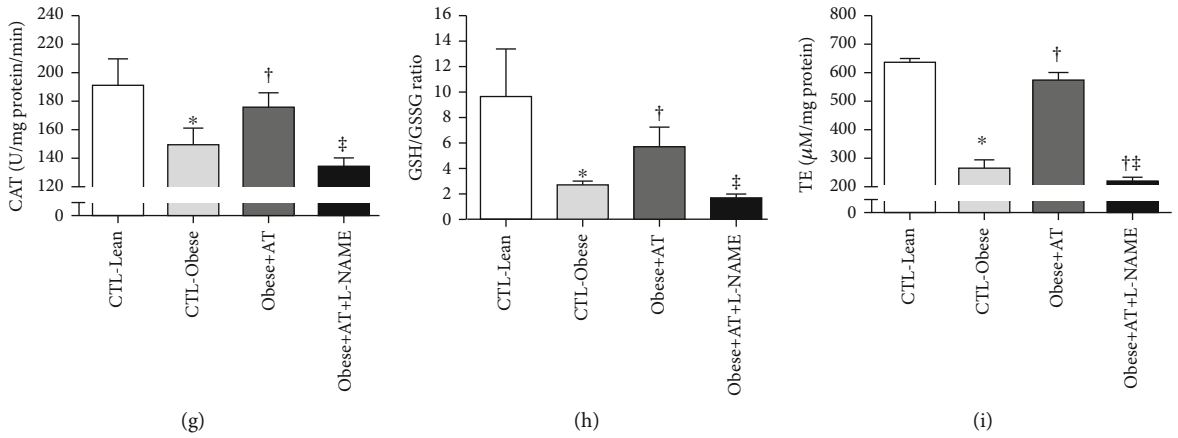

Figure 3: Renal redox balance. Data are presented as the mean ± SD. CTL-Lean: sedentary lean rats; CTL-Obese: sedentary obese rats; Obese+AT: obese rats who underwent aerobic training without blocking of nitric oxide synthases; Obese+AT+L-NAME: obese rats who underwent aerobic training with blocking of nitric oxide synthases; TBARS: thiobarbituric acid-reactive substances; 3-NT: 3-nitrotyrosine; 8-OHdG: 8-hydroxydeoxyguanosine; SOD: superoxide dismutase; CAT: catalase; GSH/GSSG ratio: glutathione/glutathione disulfide ratio; TE: trolox equivalent. The one-way ANOVA followed by Tukey's post hoc test was adopted. $^{*}p < 0.0001$ *vs.* CTL-Lean; $^{\dagger}p < 0.0001$ *vs.* CTL-Obese; $^{\ddagger}p < 0.0001$ *vs.* Obese+AT.

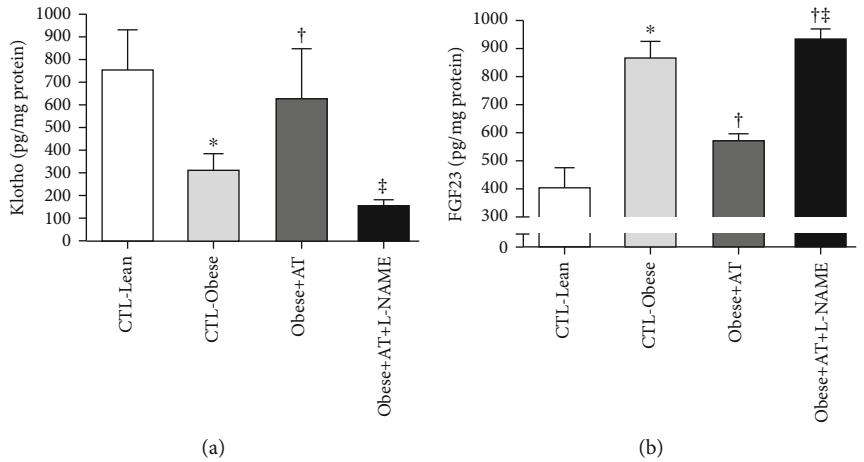

Figure 4: Renal klotho/FGF23 axis. Data are presented as the mean ± SD. CTL-Lean: sedentary lean rats; CTL-Obese: sedentary obese rats; Obese+AT: obese rats who underwent aerobic training without blocking of nitric oxide synthases; Obese+AT+L-NAME: obese rats who underwent aerobic training with blocking of nitric oxide synthases; FGF23: fibroblast growth factor 23. The one-way ANOVA followed by Tukey's post hoc test was adopted. $^{*}p < 0.05$ *vs.* CTL-Lean; $^{\dagger}p < 0.0001$ *vs.* CTL-Obese; $^{\ddagger}p < 0.0001$ *vs.* Obese+AT.

NAME; $p < 0.0001$). Additionally, AT concomitant with a decrease in NO^{-} worsens these pathways of the renin-angiotensin system in the kidney (Obese+AT+L-NAME compared to CTL-Obese; $p < 0.0001$). All these results are presented in Figure 5.

In obese Zucker rats, an increase in proteinuria was observed (CTL-Obese, Obese+AT, and Obese+AT+L-NAME when compared to CTL-Lean), which was reduced by the action of AT (Obese+AT compared to CTL-Obese and Obese+AT+L-NAME). However, the addition of AT with NO^{-} deficiency to proteinuria has been extended (Obese+AT+L-NAME compared to CTL-Obese). Zucker rats with obesity displayed a renal decrease of NO^{-}, gene expression of eNOS, and anti-inflammatory cytokines, such as IL-10 and IL-4. In turn, they presented an increase in the concentration of ADMA, gene expression of iNOS, and proinflammatory cytokines, including TNF-α, CRP, IL-17a, and IL-18 (CTL-Obese, Obese+AT, and Obese+AT+L-NAME compared to CTL-Lean). Nonetheless, AT reverses these molecular adverse scenarios (Obese+AT compared to CTL-Obese and Obese+AT+L-NAME). Again, these mechanistic dysfunctions are aggravated when AT and NO^{-} deficiency are combined (Obese+AT+L-NAME compared to CTL-Obese). Data are shown in Table 2.

Figure 6 shows that obese Zucker rats have an increase in a glomerular area and tubular diameter (CTL-Obese, Obese +AT, and Obese+AT+L-NAME compared to CTL-Lean; $p < 0.0001$). AT minimizes such structural damage to glomeruli and renal tubules (Obese+AT compared to CTL-Obese and Obese+AT+L-NAME). However, given the low concentration of NO^{-}, a glomerular disorganization is observed, with an expressive increase in its area and tubulointerstitial

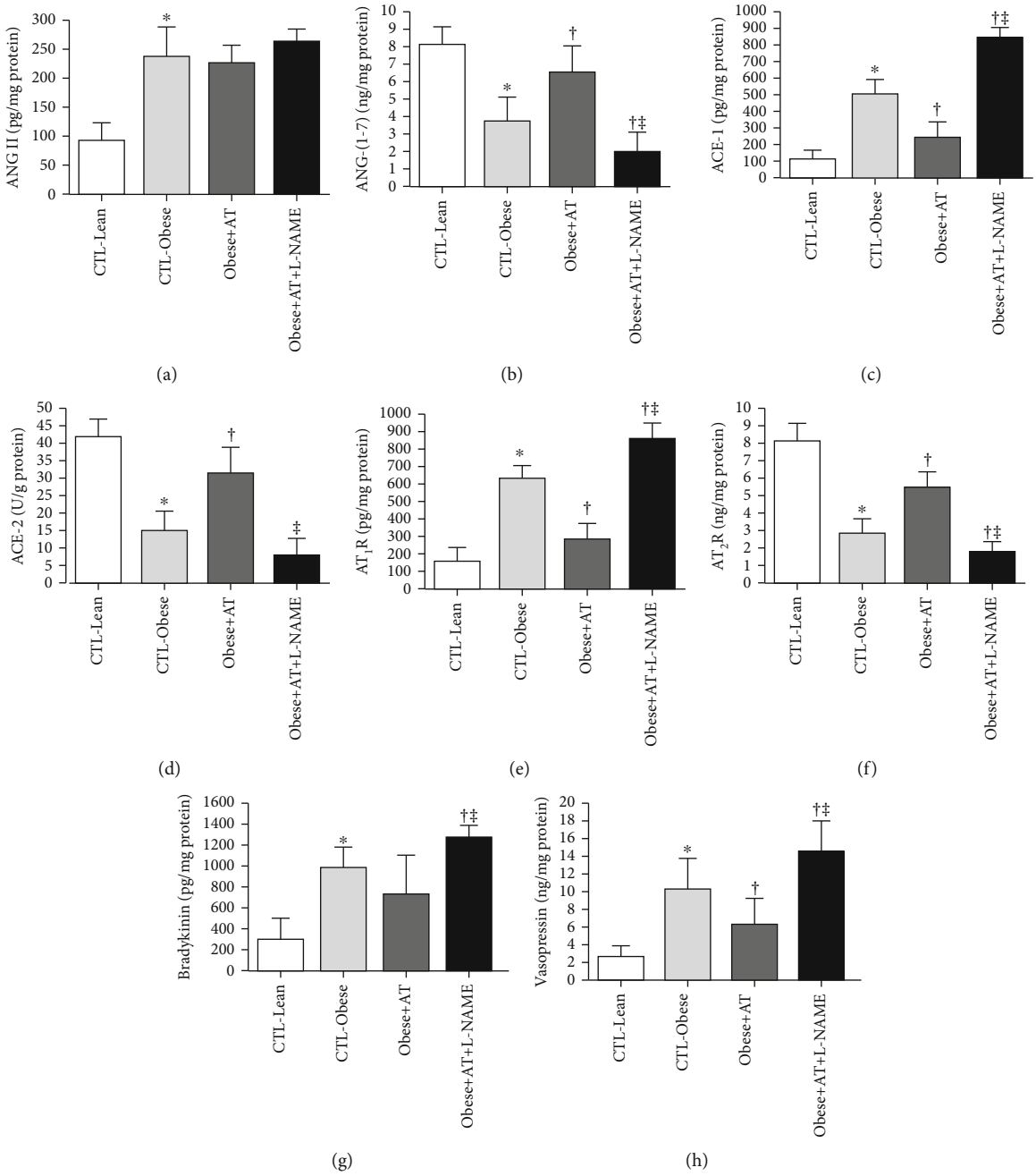

Figure 5: Kidney vasoactive peptides. Data are presented as the mean ± SD. CTL-Lean: sedentary lean rats; CTL-Obese: sedentary obese rats; Obese+AT: obese rats who underwent aerobic training without blocking of nitric oxide synthases; Obese+AT+L-NAME: obese rats who underwent aerobic training with blocking of nitric oxide synthases; ANGII: angiotensin II; ANG-(1-7): angiotensin 1-7; ACE-1: angiotensin-converting enzyme 1; ACE-2: angiotensin-converting enzyme 2; AT_1R: angiotensin II type-1 receptor; AT_2R: angiotensin II type-2 receptor. The one-way ANOVA followed by Tukey's post hoc test was adopted. $^{*}p < 0.0001$ *vs.* CTL-Lean; $^{\dagger}p < 0.0001$ *vs.* CTL-Obese; $^{\ddagger}p < 0.0001$ *vs.* Obese+AT.

disarrangement (Obese+AT+L-NAME compared to CTL-Obese). Representative photomicrograph of renal histology is shown in Figure 6.

4. Discussion

The present study was conducted to investigate AT's effects in obese Zucker rats with low levels of NO^{-} induced by L-NAME. Our results support the initial hypothesis, showing that chronic effects of AT upon the kidney are dependent on renal NO^{-} bioavailability. Although exercise is a well-known NO^{-} inducer associated with several health-related benefits, we have demonstrated that AT increased kidney injury in obese Zucker rats receiving L-NAME. Furthermore, we observed that these animals exhibited no improvements in well-known health parameters associated with

Table 2: Renal morphology, nitric oxide metabolism, and inflammation.

Variables	CTL-Lean $N = 10$	CTL-Obese $N = 10$	Obese+AT $N = 10$	Obese+AT+L-NAME $N = 10$	p value
Renal morphology and protein excretion					
Kidney weight (g)	1.67 ± 0.12	$2.12 \pm 0.18^{*}$	2.14 ± 0.25	2.17 ± 0.24	<0.0001
KW/TL (mg/cm)	307 ± 22	$389 \pm 34^{*}$	392 ± 53	398 ± 50	<0.0001
UPE (mg/24 h)	25 ± 8	$153 \pm 52^{*}$	$72 \pm 31^{\dagger}$	$192 \pm 49^{\ddagger}$	<0.0001
Molecules related to the metabolism of renal nitric oxide					
NO^{-} (μmol/mg protein)	14.6 ± 2.2	$6.8 \pm 1.6^{*}$	$10.8 \pm 1.1^{\dagger}$	$3.7 \pm 1.3^{\dagger,\ddagger}$	<0.0001
ADMA (ng/mg protein)	1.0 ± 0.3	$2.1 \pm 0.3^{*}$	$1.4 \pm 0.1^{\dagger}$	$2.6 \pm 0.2^{\dagger,\ddagger}$	<0.0001
eNOS ($2^{-\Delta\Delta CT}$)	1.12 ± 0.23	$0.40 \pm 0.31^{*}$	$1.35 \pm 0.20^{\dagger}$	$0.13 \pm 0.05^{\dagger,\ddagger}$	<0.0001
iNOS ($2^{-\Delta\Delta CT}$)	1.46 ± 0.19	$2.17 \pm 0.19^{*}$	$1.00 \pm 0.23^{\dagger}$	$2.35 \pm 0.09^{\ddagger}$	<0.0001
Renal inflammatory profile (pg/mg protein)					
TNF-α	217 ± 21	$538 \pm 33^{*}$	$390 \pm 36^{\dagger}$	$611 \pm 47^{\dagger,\ddagger}$	<0.0001
CRP	108 ± 12	$223 \pm 19^{*}$	$164 \pm 24^{\dagger}$	$275 \pm 25^{\dagger,\ddagger}$	<0.0001
IL-17a	18 ± 5	$62 \pm 7^{*}$	$33 \pm 10^{\dagger}$	$81 \pm 130^{\dagger,\ddagger}$	<0.0001
IL-18	180 ± 56	$665 \pm 111^{*}$	$379 \pm 139^{\dagger}$	$862 \pm 89^{\dagger,\ddagger}$	<0.0001
IL-10	29 ± 8	$8 \pm 4^{*}$	$21 \pm 5^{\dagger}$	$3 \pm 2^{\ddagger}$	<0.0001
IL-4	32 ± 8	$15 \pm 6^{*}$	$36 \pm 10^{\dagger}$	$6 \pm 3^{\dagger,\ddagger}$	<0.0001

Data are presented as the mean ± SD. CTL-Lean: sedentary lean rats; CTL-Obese: sedentary obese rats; Obese+AT: obese rats who underwent aerobic training without blocking of nitric oxide synthases; Obese+AT+L-NAME: obese rats who underwent aerobic training with blocking of nitric oxide synthases; KW: kidney weight; KW/TL: kidney weight corrected for the tibial length; UPE: urinary protein excretion; NO^{-}: nitric oxide; ADMA: asymmetric dimethylarginine; eNOS: renal gene expression endothelial nitric oxide synthase; iNOS: renal gene expression inducible nitric oxide synthase; TNF-α: tumor necrosis factor-alpha; CRP: c-reactive protein; IL-17a: interleukin-17a; IL-18: interleukin-18; IL-10: interleukin-10; IL-4: interleukin-4. One-way ANOVA followed by Tukey's post hoc test was adopted to verify the difference between the groups. $*$ *vs.* CTL-Lean; † *vs.* CTL-Obese; ‡ *vs.* Obese+AT.

chronic response to exercise, such as glucose homeostasis, blood pressure decreases, inflammatory, and redox balance control. On the other hand, obese rats not treated with L-NAME have shown a reduction in kidney injury, mitochondrial dysfunction, blood pressure, fasting glucose, and proteinuria, as well as improves oxidative stress, inflammatory profile, Klotho/FGF23 axis, and renin-angiotensin system.

Obesity and associated chronic diseases, such as T2DM, are well-known leading causes of renal injury and CKD. For instance, chronic hyperglycemia is considered a central mechanism in the pathogenesis promoting metabolic changes such as redox imbalance, which increases the intrarenal inflammation [51] and mitochondrial osmotic changes leading to CKD [52, 53]. Moreover, hyperglycemia induce an increase in the flow of electrons in the transport chain, increasing the production of mitochondrial ROS [51]. Thus, elevated ROS production activates cell death downstream pathways, causing kidney damage and inflammation [53]. Dada and Sznajder reported that high levels of proinflammatory cytokines are associated with mitochondrial swelling, which alters permeability transition pore [54]. On the other hand, AT modulates mitochondrial membrane permeability linked to a reduction of oxidative stress and inflammation, hence attenuating the effects of renal damage associated to obesity. Interestingly, Rosa et al. observed an increase in aerobic capacity and maximum speed in obese Zucker rats after eight weeks of AT [38]. In the present study, the lack of NO^{-} bioavailability in the Obese+AT+L-NAME group reduced aerobic capacity improvements. This is possibly due to the fact that NO^{-} plays an important role in controlling several physiological mechanisms that influence the delivery of oxygen to the cells, such as regulating muscle blood flow, mitochondrial biogenesis and respiration, relaxation/contraction cycle, and glucose uptake [55].

Aerobic exercise training has been broadly used as a nonpharmacological antihypertensive therapy decreasing the risk of metabolic and cardiovascular disease [56]. In most research papers, these positive outcomes are associated with improvements in endothelial dysfunction and a decreased eNOS activity [57]. In the present study, trained obese rats not treated with L-NAME have shown less vulnerable nephrons to damage and more capable of coping with oxidative stress due to increased antioxidant enzymes involved in the protection process while reducing prooxidant agents (Figure 3). Several animal and human studies have shown that AT promotes protection via redox balance [11, 17, 19, 58, 59]. Indeed, NO^{-} is an essential contributor to reduce artery stiffness, vascular tone control, and sympathetic activity in response to flow-mediated shear stress [60]. Thus, NO^{-} is a critical determinant of the magnitude of the exercise-induced blood pressure response [19]. Additionally, NO^{-} is also known to have beneficial effects on active myocardial relaxation through diastolic volume maintenance at high heart rates during exercise training [57].

In the present study, AT modulated the Klotho-FGF23 axis, which is considered a key mechanism for renal integrity

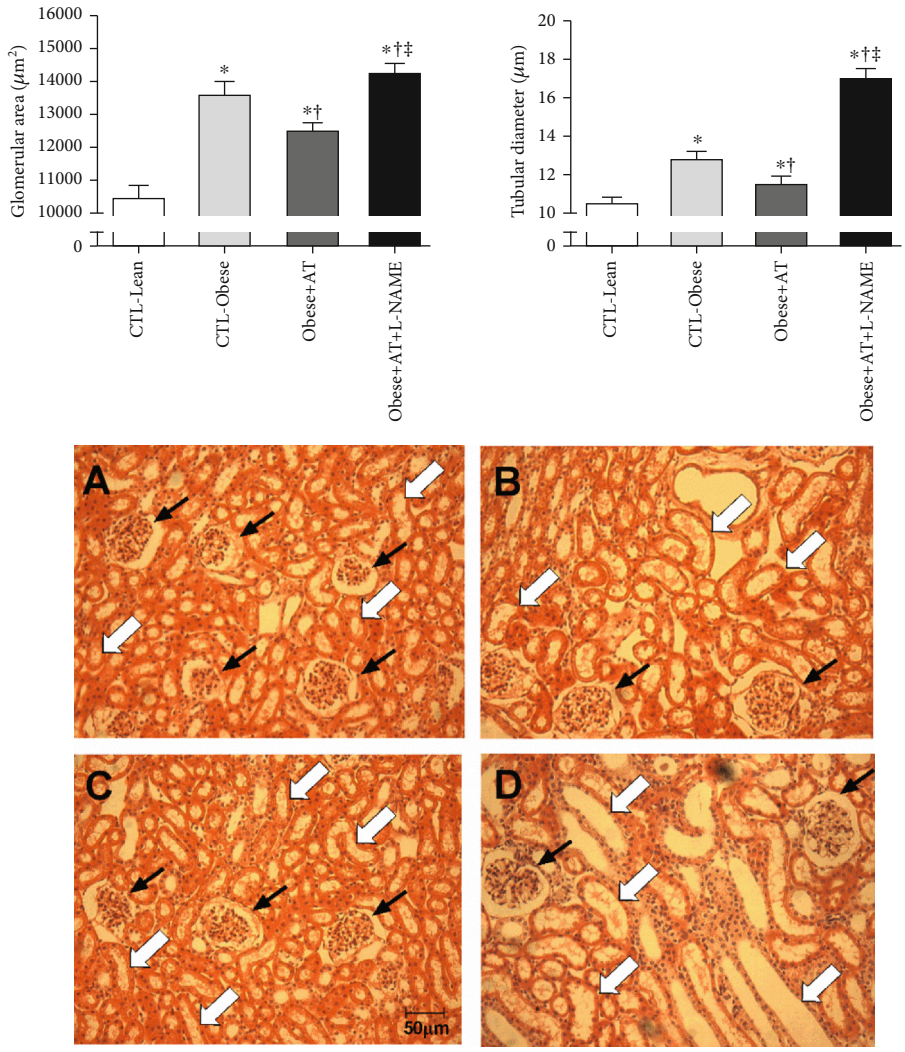

Figure 6: Representative photomicrographs of the kidney. Hematoxylin-eosin staining. (a) CTL-Lean; (b) CTL-Obese; (c) Obese+AT; (d) Obese+AT+L-NAME. Black arrows indicate glomerulus, and white arrows indicate renal tubules. Magnification, 200x. $^{*}p < 0.0001$ *vs.* CTL-Lean; $^{\dagger}p < 0.0001$ *vs.* CTL-Obese; $^{\ddagger}p < 0.0001$ *vs.* Obese+AT.

maintenance [61, 62]. Physiologically, proteins filtered in glomerular capillaries are captured in the proximal tubule and then transferred to the systemic circulation [63]. Disturbances in this process result in glomerular damage, inducing proteinuria and FGF23 upregulation [61]. It has been shown that in patients with T2DM, the use of losartan, a blocker of the binding of angiotensin II (ANGII) to its AT_1R receptor reduced proteinuria and increased Klotho levels, demonstrating the inverse association between these two factors [64]. Additionally, increased Klotho protein expression is beneficial for regenerative tissue response, besides attenuation of proteinuria, secondary hyperparathyroidism, vascular calcification, and ROS damage, improving health span in the kidney disease context. Furthermore, blocking angiotensin-converting enzyme 1 (ACE-1) with ramipril also decreased proteinuria and concomitantly reduced FGF23 levels [62]. The cross-talk between FG23, Kloto, and ACE-2 may explain the AT_1R and AT_2R receptor responses in this study. Therefore, the increase in Klotho levels accompanied by a decrease in FGF23 induced by AT seems to be an important therapeutic target for renal damage attenuation inherent to obesity conditions.

Obese Zucker animals submitted to AT have shown a reduction in asymmetric dimethylarginine (ADMA) and bradykinin (BK) levels, when compared to the CTL-obese group and animals receiving L-NAME (Table 2), which reflect an altered response to blood pressure. Since ADMA is a potent endogenous inhibitor of eNOS and its concentration is inversely correlated with NO^- levels [55], a reduction in ADMA can contribute to increase NO^- levels in AT obese animals. Gamboa et al. showed that ACE-1 inhibitor (ramipril) increased ADMA levels in patients with kidney disease and appears to have been modulated by bradykinin (BK) during ACE-1 inhibition [65]. Here, we have shown that AT decreased BK and ADMA levels in the kidney, and such adjustments were important to increase NO^- and, consequently, renal morphological integrity maintenance. These molecular pathways collectively highlight the importance

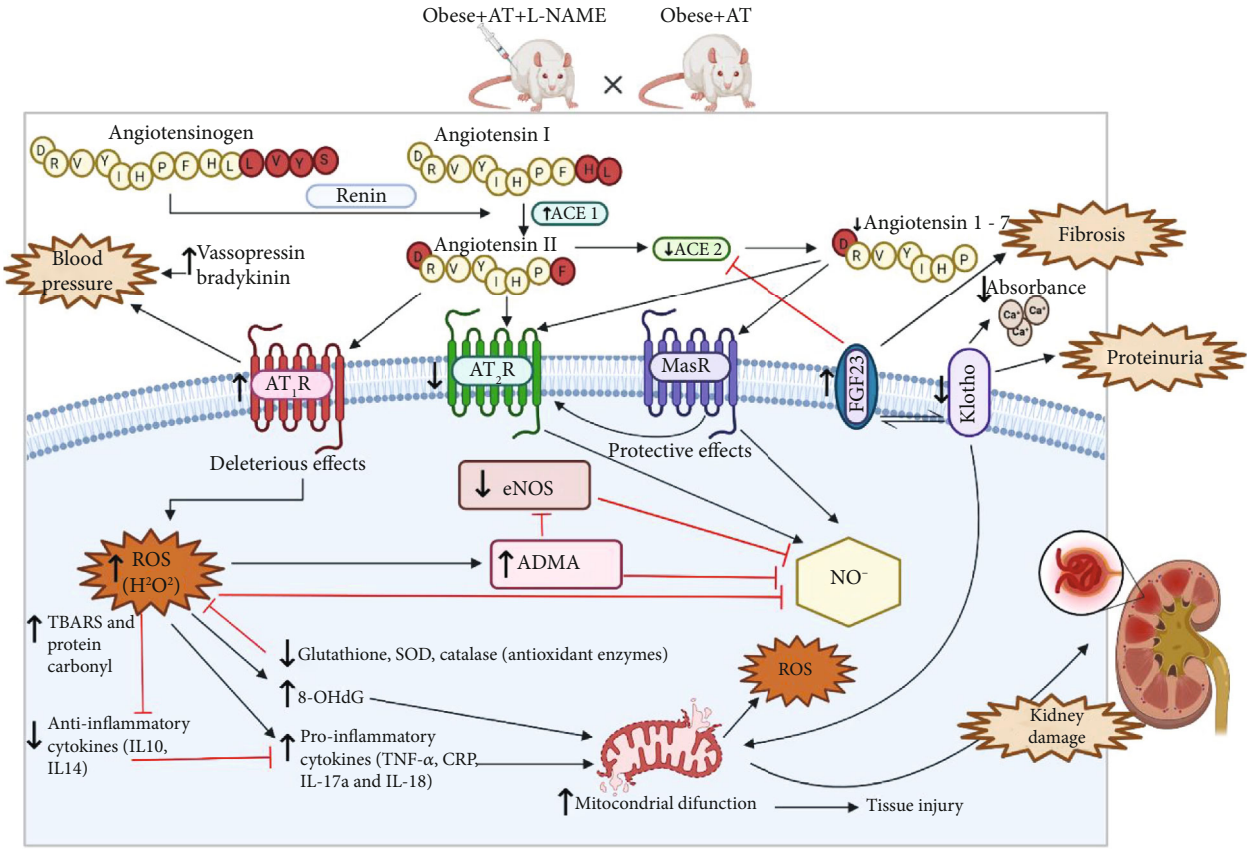

Figure 7: Mechanisms of vasoactive peptide integration. Angiotensinogen is transformed by renin in ANGI, which is cleaved by ACE-1 forming ANGII, interacting on AT_1R increasing intraglomerular pressure, renal fibrosis, mesangial proliferation, proteinuria, vasopressin release, decreased sodium excretion, and activation of the NOX complex, which induces ROS, inflammation, and insulin resistance, which are common complications of cardiometabolic diseases [58, 71, 81]. However, AT decreases ACE-1 and AT_1R in the kidney, which was partly responsible for renoprotection. In addition, AT increased ACE-2, which converts ANGII to ANG-(1-7) that interact with MasR and/or ANGII acting on AT_2R to promote a vasodilatory, antifibrotic, antioxidant, and anti-inflammatory effect, as well as an increase in sodium excretion and maintenance of intrarenal hemodynamics. C-EX: chronic exercise training; ACE-1: angiotensin-converting enzyme 1; ACE-2: angiotensin-converting enzyme 2; AT_1R: angiotensin II type-1 receptor; AT_2R: angiotensin II type-2 receptor; MasR: receptor MasR; ROS: reactive oxygen species; ADMA: asymmetric dimethylarginine; NO: nitric oxide.

of NO^- on kidney cytoprotection and suggest AT as a prerequisite for the protection of cells from oxidative stress and the support of an elongated life span.

The results of the present study agree with a prior investigation that reported that iNOS inhibition reduced proinflammatory markers and prooxidant agents while improving glycemic homeostasis and kidney integrity in a model of obesity-induced kidney disease [66]. Interestingly, we observed an increase in iNOS mRNA levels in the injured kidneys of obese rats, possibly induced by an increase in proinflammatory cytokines (Table 2). We observed that L-NAME administration can induce a downregulation of eNOS mRNA levels and high expression of iNOS mRNA in the kidney. This condition is associated with increased oxidative stress, promoting a vicious cycle and, consequently, exacerbating tissue damage [27–29, 67, 68].

AT reduced the expression of proteins from the renin-angiotensin system (RAS) in the kidney, representing promising candidates to transduce exercise-induced kidney health benefits (Figure 5). The ANGII increases ADMA levels by stimulating AT_1R receptors and increasing oxidative stress, which is an important promoter of ADMA synthesis [69]. Angiotensin I (ANGI) is cleaved by ACE-1 forming ANGII, which interacts with AT_1R receptors and increases of vasodeleterious pathway activity. Additionally, this condition induces ROS formation, inflammation, and insulin resistance [69–71]. On the other hand, glucose metabolism impairments may be restored by exercise via several mechanisms including improvements in insulin secretion and action [72, 73]. These adaptations are essential to maintain enzymatic activity in kidneys, possibly preventing impairments in mitochondrial oxidative capacity.

AT decreased the expression of ACE-1 and AT_1R, while there was an increase in ACE-2. This is particularly important as ACE-2 converts ANGII to ANG-(1-7), which interacts with MasR. ANGII also acts on AT_2R receptors that have a critical vasodilator and antifibrotic, anti-inflammatory, and antioxidant effects [74]. Moreover, it has been established that ACE-2 deficiency in mice induces lower physical fitness, suggesting that this enzyme is crucial to mediate improvements in aerobic capacity [75]. Thus, the lower ANGII content and the higher renal ANG-(1-7)

promoted by AT partly explain renal injury attenuation, blood pressure adjustments, and aerobic performance. Figure 7 demonstrates the possible mechanisms involved in the results of the present study.

Furthermore, the decrease in oxidative stress and mitochondrial structure maintenance induced by AT contributed to eNOS regulation (Figure 2 and Table 2) which is an important factor in preventing the progression of kidney injury [67, 76]. We also observed a downregulation in iNOS mRNA levels after AT. This possibly due to a lower proinflammatory cytokine level, such as TNF-α, C-reactive protein, IL-17a, and IL-18, which are potent stimulators of iNOS activity [77–80]. Furthermore, there was also an increase in key anti-inflammatory cytokine levels, such as IL-10 and IL-4 after AT, which is also essential to reduce kidney damage. Collectively, these results may have important clinical implications for human studies, as AT is key to control the inflammatory response. However, AT effects upon the kidney seems to be, at least in part dependent on NO^- downstream pathways and the mutual crosstalk between oxidative stress and inflammatory responses. More studies, especially in humans, are necessary to improve our understanding of the clinical implications of AT upon the kidney in obesity related disorders, such as T2DM.

5. Conclusion

In obese Zucker rats, the renal and metabolic benefits promoted by AT are dependent on NO^- bioavailability and its underlying regulatory mechanisms, including the structure and production of mitochondrial ROS, redox balance, inflammation, the Klotho/FGF23 axis, ACE-1/ANGII/AT_1R, and ACE-2/ANGII/AT_2R-MasR. Under conditions of low NO^- bioavailability, AT can increase the kidney damage by promoting oxidative stress and inflammation, which contributes to the loss of glucose homeostasis already observed in obesity associated T2DM.

Data Availability

Data are available upon reasonable request to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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