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ORIGINAL ARTICLE

Diphenyl diselenide blunts swimming training on mitochondrial liver redox adaptation mechanisms of aged animals

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Abstract

Background Studies about antioxidant supplementation and exercises combined, especially at hepatic liver tissue, are rare and still controversial. In this study, we aimed to evaluate if the association between a recognized antioxidant compound—Diphenyl Diselenide ([$(PhSe)_2$])—and training can reduce homogenate liver and liver mitochondria oxidative stress in old rats.

Methods Old male Wistar rats were divided into four groups (six animals per group): old-sedentary, old-sedentary [$(PhSe)_2$] supplemented, old-trained, and old-trained [$(PhSe)_2$] supplemented. Trained groups were submitted to swimming training sessions (3% of body weight, 20 min/day during 4 weeks); animals were fed daily with standard feed or standard feed supplemented with 1 ppm of [$(PhSe)_2$] during 4 weeks.

Results Trained and trained + [$(PhSe)_2$] groups decreased reactive oxygen species (ROS) generation, while only the trained group reduces GSSG production and increased GSH/GSSG ratio when compared to trained + [$(PhSe)_2$]. Mitochondrial ROS production was elevated in control sedentary group, but only swimming training prevented its elevation. However, MnSOD activity was found elevated at trained + [$(PhSe)_2$] rats when compared to the trained and [$(PhSe)_2$] supplementation groups. Mitochondrial $\Delta\psi_m$ in trained + [$(PhSe)_2$] was decreased compared to trained group, while ratio (III/IV states) was increased when compared to control sedentary.

Conclusions We conclude that the combination of [$(PhSe)_2$] and swimming training did not manifest synergic effect since it does not prevent the aging-induced hepatic oxidative stress generation, but blunted the induced-exercise adaptations, including at mitochondrial mechanisms.

Keywords Exercise · Supplement · Hepatic damage · Oxidative stress · Aging

Abbreviations

[$(PhSe)_2$]	Diphenyl diselenide
GSH	Reduced glutathione
GSSG	Oxidized glutathione
MnSOD	Manganese superoxide dismutase
$\Delta\psi_m$	Mitochondrial transmembrane electrical potential
H_2DCF-DA	Reduced dichlorofluorescein diacetate
DCF	Oxidized dichlorofluorescein
OPT	O-Phthalaldehyde
KCN	Potassium cyanide

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Introduction

Regular exercise is recognized by health quality improvements, evidenced at all age and gender [1], contributing to the prevention of chronic diseases [2]. Studies indicate that the adaptations associated with exercising can be caused by continuous and moderate reactive oxygen species (ROS)

generation, improving cellular defense systems [3], by raising the expression and activity of antioxidants enzymes [4, 5]. Training also modulates mitochondria bioenergetics' metabolism [6], biogenesis and redox status, leading to a cell increased resistance and better energy synthesis.

Aging process results from continuous bouts of oxygen-free radical attacks at cell components throughout the lifespan [7]. Aging affects liver function through alterations in multiple metabolic pathways. These changes include a reduction in the antioxidant defense [8], decrease of antioxidant protein levels [9], and a chronic inflammatory status, contributing age-related diseases development [10].

Selenium is an essential trace element that is present in several enzymes, such as glutathione peroxidase and thioredoxin reductase [11, 12]; selenium deficiency has been implicated in oxidative stress, inflammatory, and diseases [13, 14]. Most selenium biological functions are attributed to the selenoproteins [15], reducing intracellular ROS content due to its antioxidant property. These proteins can trigger molecular mechanisms related to the anti-inflammatory process [16, 17]. Especially, the diphenyl diselenide [$(PhSe)_2$], is an organoselenium molecule, which is recognized to their low toxicity, high antioxidant, and anti-inflammatory properties [18]. Besides, compound is known for its double face due to a contrasting behavior in biological systems that is dose dependent, whereas low doses have beneficial effects, high doses lead to toxicity. On the other hand, the therapeutic potential of [$(PhSe)_2$] and related compounds seems to be superior to their toxic effects [19, 20]. It has been demonstrated that combined therapy with swimming exercise and diet supplemented with [$(PhSe)_2$] have a favorable effects on levels of pro-inflammatory cytokines [21] and contributed to the hepatic glucose homeostasis in old rats [22].

Over the last years, exercise and dietary supplementation have been longed used as beneficial and not aggressive strategies to prevent aging-related symptoms and disease development. Therefore, this study aims to evaluate if [$(PhSe)_2$] and swimming combined to have greater beneficial against the damages caused by age-related at rat's liver and isolated liver mitochondria than their isolated effects.

Materials and methods supplemented in diet

Animals

Old (27 months y.o.) male Wistar rats were obtained from a local breeding colony and were housed in cages, with free access to food and water. Rats were placed in controlled environment conditions (12:12 h light–dark cycle, with an onset of light phase at 7:00 am, 25 ± 1 °C, 55% relative humidity) with food (Guaiba, RS, Brazil) and water at *libitum*. The experiment was conducted according to the guidelines of the Committee on Care and Use of Experimental Animal Resources 5394050115, the Federal University of Santa Maria, Brazil.

Drugs and $(PhSe)_2$ supplementation in the diet

[$(PhSe)_2$] was prepared according to the method described by Paulmier [23]. The animals were fed daily with a standard feed or standard feed supplemented with 1 ppm of [$(PhSe)_2$] for 4 weeks. The preparation and concentration of supplemented standard chow were based on a previous study [24]. The standard diet was sprinkled with [$(PhSe)_2$] [1 mg of [$(PhSe)_2$]/100 g standard chow] dissolved in ethyl alcohol (1 mg/100 ml) using a spray bottle. The concentration of [$(PhSe)_2$] found in the diet did not cause overt signals of toxicity [25, 26]. The diet was changed daily with 100 g of chows new and the diet was maintained in cages (two animals per cage) for 1 day. The standard and supplemented diets were kept at 60° for 3 h to evaporate ethyl alcohol and then kept at 4° by not more than 1 week.

Experimental procedure and swimming exercise

The animals were divided into five groups (six animals per group): I—old-control (old animals did not perform exercise); II—[$(PhSe)_2$] (old animals supplemented with 1 ppm of [$(PhSe)_2$]; III—trained (old animals performed swimming training); and IV—trained + [$(PhSe)_2$] supplementation (old animals supplemented with 1 ppm of [$(PhSe)_2$] and performed the swimming training) (Fig. 1). Thirty-month-old rats were submitted to the pre-training session 20 min/day for 1 week (III and IV groups). After this adaptation period, the rats performed the swimming training protocol with a workload (3% of body weight) for 20 min/day for 4 weeks [27]. The workload was placed in the back of animal, attached around their chest by an elastic band so that their movements were not restricted. The body weights of animals were measured once a week, for determining the workload. The swimming training occurred at a water temperature of 32 ± 1 °C, the tank used in this study was 80 cm in length, 50 cm in width, 90 cm in-depth and the swimming training was performed in water temperature of 31 ± 1 °C (70 cm depth). Sedentary rats (I and II groups) were placed in the bottom of a separate tank with shallow water (5 cm) at 32 ± 1 °C, without the workload (consists in adapting to water). At the end of the swimming exercise-training period, all animals were towel dried before being returned to their cages.

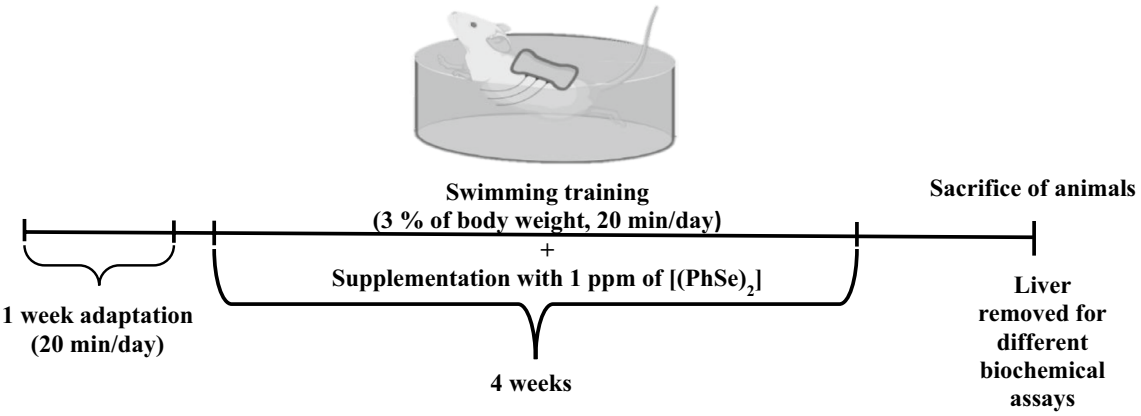

Fig. 1 Experimental design

Liver homogenate preparation

At the end of the protocol, the liver was removed and quickly dissected, placed on ice and immediately homogenized in cold Tris–HCl 10 mM (pH 7.4). Homogenates were centrifuged at 2000×*g* for 10 min to yield the low-speed supernatant fractions that were used for different biochemical assays in all trials. Liver aliquots preparations were frozen (− 80 °C) for later analysis.

Hepatic mitochondria isolation

Rat liver mitochondria were isolated at 4 °C by differential centrifugation [28]. Livers were rapidly removed after sacrificed and immersed in ice-cold 'Ionic Medium' containing 100 mM sucrose, 10 mM EDTA, 46 mM KCl, and 100 mM Tris–HCl (pH 7.4). The tissue was minced using surgical scissors and then extensively washed, then homogenized in power-driven, tight-fitting Potter–Elvehjem homogenizer with Teflon Pestle. The resulting suspension was centrifuged for 10 min at 2500×*g* in a Hitachi CR 21E centrifuge. After centrifugation, the supernatant was centrifuged for 10 min at 10,000×*g*. The pellet was resuspended in 'Ionic Medium + BSA' containing 100 mM sucrose, 10 mM EDTA, 46 mM KCl, 0.1% bovine serum albumin free fatty acid, 100 mM Tris–HCl (pH 7.4), and centrifuged at 10,000×*g* for 10 min. The supernatant was decanted, and the final pellet was gently washed and resuspended in 'Suspension Medium' containing 230 mM mannitol, 70 mM sucrose, and 20 mM Tris–HCl (pH 7.4) [29]. An aliquot of the resulting mitochondrial suspension was separated and rapidly frozen at − 80 °C for following GSH content and mitochondrial enzyme.

Reactive oxygen species (ROS) production

ROS production in liver homogenates was determined by oxidation of reduced dichlorofluorescein diacetate (H_2DCF-DA) [30]. Briefly, homogenates were added to the standard medium containing Tris–HCl as a buffer (10 mM, pH 7.4) and 2′, 7′—dichlorofluorescein diacetate (Sigma-Aldrich, catalogue 35845) (1 mM) for 60 min in a condition without light. Fluorescence quantification was determined at 488 nm for excitation and 525 nm for emission, with slit widths of 3 nm, in a spectrofluorimeter, using oxidized 2′, 7′—dichlorofluorescein (DCF) (Sigma-Aldrich, catalogue 35848) as a standard. Mitochondrial samples (150 µg of protein/ml) were incubated with buffer, and the respiratory substrates glutamate/malate (5 mM) and succinate (5 mM). The reaction was started using H_2DCF-DA, and the medium was kept at constant stirring during the assay period [31].

Reduced glutathione (GSH) and oxidized glutathione (GSSG)

GSH levels were determined in liver homogenate with fluorescence detection after reaction of the supernatants from deproteinized containing H_3PO_4/$NaH_2PO_4^-$EDTA, with O-Phthalaldehyde (OPT) [32]. In brief, 250 mg of liver were homogenized in 3.75 mL phosphate–EDTA buffer (100 mM NaH_2PO_4, 5 mM EDTA, pH 8.0) plus 1 mL H_3PO_4 (25%), resuspended in 1.5 mL phosphate–EDTA buffer and 500 ml H_3PO_4 (4.5%) were rapidly centrifuged at 13000 rpm (Hitachi,) for 10 min. For GSH determination, the supernatant was incubated in phosphate buffer and in the presence of OPT at room temperature for 15 min. GSSG determination, the supernatant was added with N-Ethylmaleimide incubation at room temperature for 30 min. After this was incubated in sodium hydroxide buffer and in the presence

of OPT at room temperature for 15 min, the fluorescence of both was measured at 420 and 350 nm emission and excitation wavelengths, respectively.

Manganese superoxide dismutase (MnSOD) activity

Enzymatic activity in isolated mitochondria was measured after disruption of mitochondria by freeze-thawing (3×), following centrifugation at 2000×*g* for 1 min at 4 °C, and the mitochondrial supernatant (0.1 mg protein/mL) was add to the reaction medium. Manganese superoxide dismutase (MnSOD) activity was measured as described previously [33]. The isolated mitochondria were assayed after incubation with 1 mM potassium cyanide (KCN). At this concentration, cyanide inhibits the CuZnSOD isoform of the enzyme but does not affect the MnSOD isoform [34].

Mitochondrial transmembrane electrical potential ($\Delta\psi_m$)

Mitochondrial $\Delta\psi_m$ was estimated by fluorescence changes in Safranine-O 10 mM recorded by RF-5301 Shimadzu spectrofluorometer (Kyoto, Japan) operating at excitation and emission wavelengths of 495 and 586 nm, with slit widths of 5 nm [35]. The mitochondria (0.5 mg protein) were added, and 30 s later, mitochondrial respiration was initiated by the addition of succinate and glutamate.

Oxygen consumption of liver mitochondria

Oxygen consumption was monitored polarographically with a Clark-type electrode (Hansatech, UK) at 30 °C. Liver mitochondria were incubated in 1 ml of the respiratory medium consisted of 10 mM Tris–HCl (pH 7.4), 320 mM mannitol, 8 mM K_2HPO_4, and 4 mM $MgCl_2$, 0.08 mM EDTA, 1 mM EGTA, 0.2 mg/ml BSA described previously [36]. Respiration of mitochondria (1 mg of protein/ml) was initiated with substrates at 5 mM malate, 5 mM glutamate, and 5 mM Succinate. To induce the phosphorylating (state III) respiration, 200 nmol ADP was used. Next, the oligomycin (1 µg/ml) was used to induce the non-phosphorylating (state IV) respiration. Respiration rates are given in nmol oxygen/min/mg protein. Respiratory control ratio (RCR) was determined by the State III/State IV ratio.

Protein determination

The protein content was determined as described previously [37] using bovine serum albumin (BSA) as standard.

Statistical analysis

Statistical analysis was performed using GraphPad (version 8.0 for Macintosh OSX, GraphPad Software, San Diego, CA). The Shapiro–Wilk test was used to confirm the normality of quantitative variables. With normal data distribution, a two-way analysis of variance (ANOVA), followed by Tukey's Test for post hoc comparison between old groups (sedentary/trained) × (without/with $(PhSe)_2$). Significance was set at $p < 0.05$, and data were expressed as mean ± standard deviation of the mean (SD).

Results

Effect of association $(PhSe)_2$ and swimming on hepatic oxidative damage in old rats

ROS levels showed a significant [$(PhSe)_2$] × training interaction ($F_{(1, 13)} = 10.71$; $p = 0.0061$). The control sedentary group showed an increase in the ROS levels when compared to control trained ($p < 0.0001$; 95% CI of diff. = 9.164–23.34) and [$(PhSe)_2$] + trained ($p = 0.0022$; 95% CI of diff. = 4.159–18.33). Indeed, control trained demonstrated a reduction in ROS levels when compared to $(PhSe)_2$ sedentary ($p = 0.0028$; 95% CI of diff. = − 17.09 to −3.640) (Fig. 2a).

GSH contents revealed a significant main effect of training ($F_{(1, 11)} = 15.51$; $p = 0.0023$). Training alone or association with [$(PhSe)_2$] decreased the GSH levels in comparison to sedentary group ([$p = 0.0265$; 95% CI of diff. = 1.106–18.66], and [$p = 0.0182$; 95% CI of diff. = 1.761–19.32], respectively) (Fig. 2b). We observed also that GSSG contents (Fig. 2c) presented a significant interaction in [$(PhSe)_2$] × training ($F_{(1, 12)} = 12.13$; $p = 0.0045$). Training without association decrease the GSSG levels in comparison to control sedentary ($p = 0.0104$; 95% CI of diff. = 2.075–15.77), and training associated with [$(PhSe)_2$] increased these levels ($p = 0.0488$; 95% CI of diff. = − 13.73 to − 0.03178) when compared to training.

Furthermore, GSH/GSSG ratio revealed a significant [$(PhSe)_2$] × training interaction ($F_{(1, 11)} = 18.19$; $p = 0.0013$). Training alone increased the ratio when compared to control sedentary ($p = 0.0033$; 95% CI of diff. = − 3.211 to − 0.6903), [$(PhSe)_2$] group ($p = 0.0077$; 95% CI of diff. = 0.4709–2.992) and training in association with [$(PhSe)_2$] ($p = 0.0012$; 95% CI of diff. = 0.9545–3.475) (Fig. 2d).

Effect of association $(PhSe)_2$ and swimming on hepatic mitochondrial oxidative damage and $\Delta\psi_m$ in old rats

Mitochondrial ROS levels revealed a significant [$(PhSe)_2$] × training interaction ($F_{(1, 12)} = 6.173$; $p = 0.0225$).

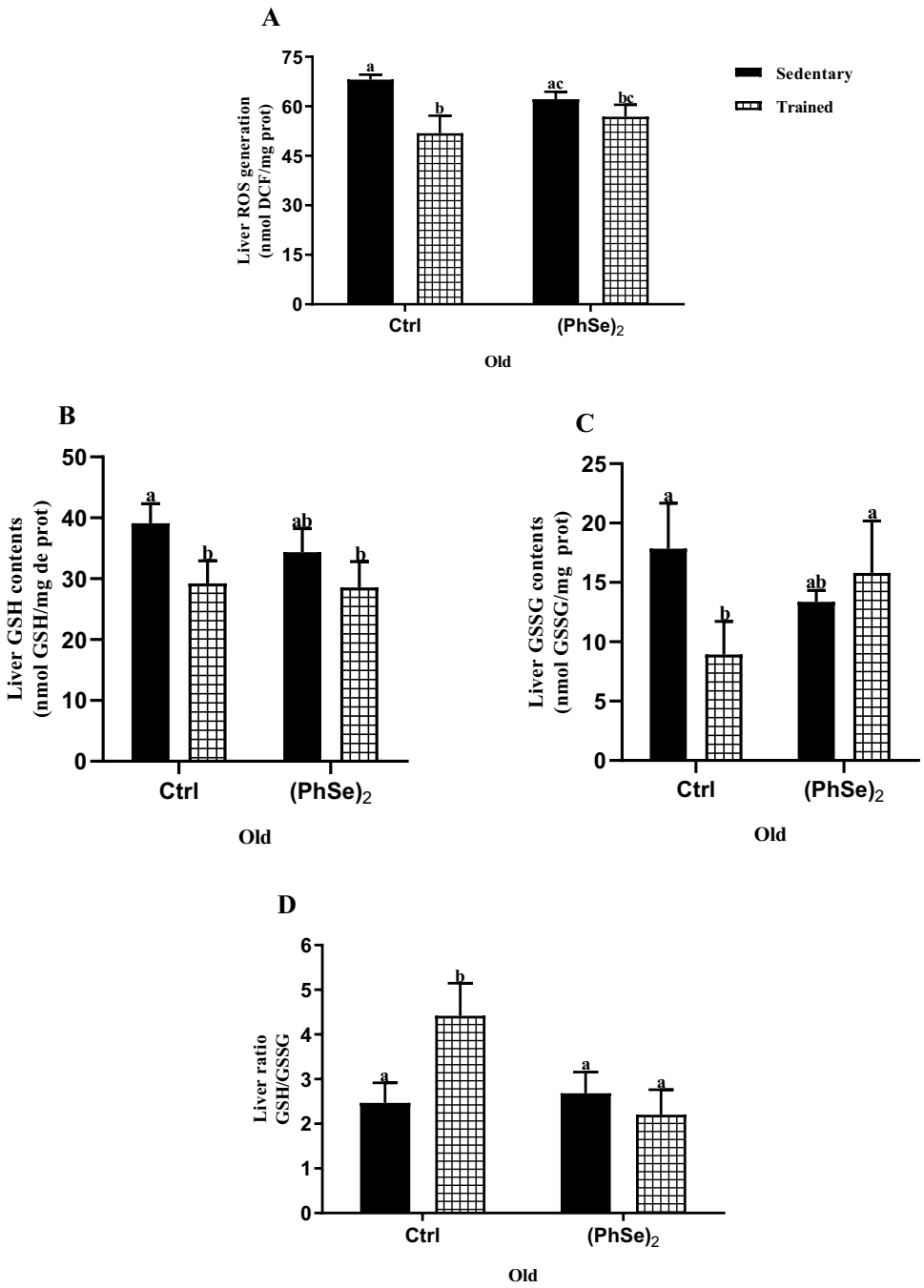

Fig. 2 Effect of association $[(PhSe)_2]$ and swimming training on hepatic oxidative damage markers in old rats. **a** reactive oxygen species (ROS) levels, **b** reduced glutathione (GSH), **c** oxidized glutathione (GSSG), and **d** GSH/GSSG ratio. Different letters indicate significant differences between groups (mean ± SD for $n = 4$–5 in each group, two-way test (ANOVA) followed by Tukey's test, $p < 0.05$)

The analysis demonstrated that sedentary old rats preset higher mitochondrial ROS levels without association ($p = 0.0028$; 95% CI of diff. = 2.185–11.39) or with $[(PhSe)_2]$ association ($p = 0.0013$; 95% CI of diff. = − 12.55 to − 2.894) when compared to the training control group. However, $(PhSe)_2$ association with training was not effective against this increase ($p = 0.0027$; 95% CI of diff. = − 11.43 to − 2.225) (Fig. 3a).

Nevertheless, mitochondrial MnSOD activity (Fig. 3b) showed a significant $[(PhSe)_2] \times$ training interaction ($F_{(1, 8)} = 18.97$; $p = 0.0024$). Analysis demonstrated that $[(PhSe)_2]$ associated with training was effective promoting increased MnSOD activity when compared to trained group ($p < 0.0045$; 95% CI of diff. = − 119.1 to − 26.37) and $[(PhSe)_2]$ sedentary ($p = 0.0138$; 95% CI of diff. = − 106.3 to − 13.61).

Mitochondrial $\Delta\psi_m$ revealed a significant $[(PhSe)_2] \times$ training interaction ($F_{(1, 12)} = 5.685$; $p = 0.0318$). The swimming training associated with $[(PhSe)_2]$ supplementation decreased $\Delta\psi_m$ when

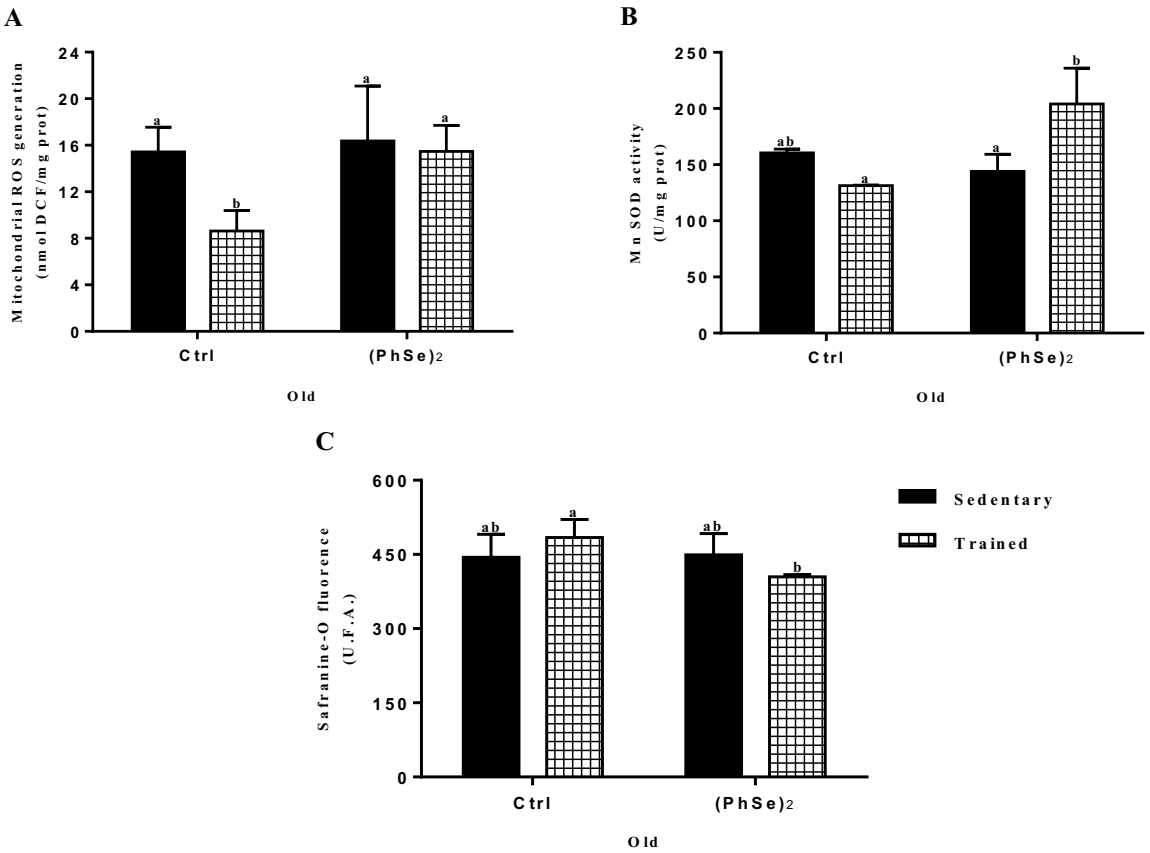

Fig. 3 Effect of association [$(PhSe)_2$] and swimming on hepatic mitochondrial oxidative damage in old rats. **a** Mitochondrial reactive oxygen species (ROS), **b** manganese superoxide dismutase (MnSOD) activity, and **c** mitochondrial $\Delta\psi_m$. Different letters indicate significant differences between groups (mean ± SD for $n = 4$–5 in each group, two-way test (ANOVA) followed by Tukey's test, $p < 0.05$)

compared to control training ($p = 0.0306$; 95% CI of diff. = 6.651–152.2) (Fig. 3c).

$(PhSe)_2$ effects on hepatic mitochondrial respiration rates and phosphorylation efficiency in old rats

Figure 4a shows that the training and treatment with [$(PhSe)_2$] altered the state III of mitochondria respiration in the liver. State III of mitochondria respiration revealed a significant [$(PhSe)_2$] × training interaction ($F_{(1, 12)} = 92.03$; $p < 0.0001$). The training associated with [$(PhSe)_2$] increased the oxygen consumption in the state III of mitochondria when compared to the other groups, control sedentary ($p < 0.0001$; 95% CI of diff. = − 131.0 to − 72.65), control trained ($p < 0.0001$; 95% CI of diff. = − 142.9 to − 84.47), and [$(PhSe)_2$] sedentary ($p < 0.0001$; 95% CI of diff. = − 147.4 to − 91.99.

In addition, state IV showed a main effect of [$(PhSe)_2$] ($F_{(1, 12)} = 5.946$; $p = 0.0253$) and training ($F_{(1,12)} = 8.410$; $p = 0.0095$). Old rats trained associated with [$(PhSe)_2$] showed increased state IV of mitochondria respiration when compared to control sedentary group ($p = 0.0069$; 95% CI of diff. = − 29.94 to − 4.302) (Fig. 4b).

RCR (Fig. 4c) indicated a significant main effect of [$(PhSe)_2$] ($F_{(1, 12)} = 5.852$; $p = 0.0298$) and training ($F_{(1, 12)} = 5.222$; $p = 0.0384$).The supplementation with [$(PhSe)_2$] decreased RCR in the liver of old trained rats when compared to the sedentary group ($p = 0.0229$; 95% CI of diff. = 0.2486–3.691).

Discussion

Here, we appraised the effect of the association between [$(PhSe)_2$] compound and swimming exercise to reduce the oxidative stress in liver homogenate and liver mitochondria from old rats. We used well-known interventions that exert antioxidative properties (i.e. [$(PhSe)_2$] supplementation and swimming exercise), as an associated treatment for better understating the exercise effects integrated with antioxidants and the aging process. It seems that individually, swimming training may have hepatoprotective effects against oxidative damage process (Fig. 2). Regular physical exercise is known

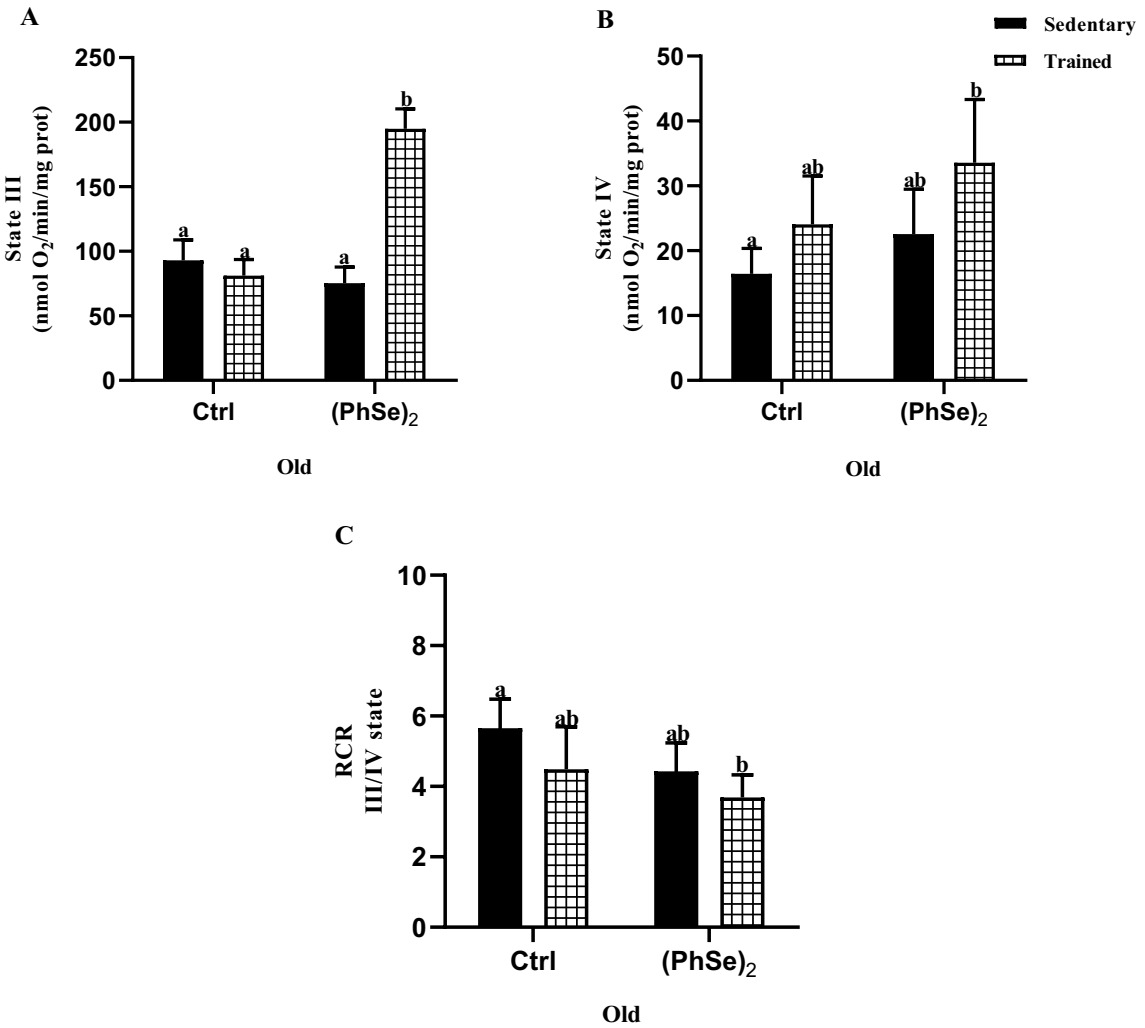

Fig. 4 [$(PhSe)_2$] effects on hepatic mitochondrial respiration rates and phosphorylation efficiency in old trained rats. Mitochondria were incubated in the respiration medium for **a** State III was added ADP (200 nmol) and **b** State IV oligomycin (1 µg/ml). Respiratory control ratio (RCR) (**c**) was determined by State III/State IV ratio. Different letters indicate significant differences between groups (mean ± SD for $n = 4–5$ in each group, two-way test (ANOVA) followed by Tukey's test, $p < 0.05$)

to play an essential role at adaptations mechanisms, reaching cell homeostasis due to the activation of redox-sensitive signaling pathways [38]. However, our results demonstrated that [$(PhSe)_2$] associated seems to blunt this adaptive effects to swimming training.

Briefly, aging is characterized by progressively morphological and biochemical changes, ROS overproduction and accumulation induce an imbalance of the antioxidant defense system, which causes injury in the cellular membrane components interfering on molecular functions [9, 39] and contribute to diseases development [40]. In this sense, strategies to prevent or slow down aging need to be investigated, and this way, it can be applied to improve the health quality of this elderly population.

Previously, [$(PhSe)_2$] was seemed to mimic endogenous antioxidant enzymes, such as glutathione peroxidase (GPx), metabolized by thioredoxin reductase to form a selenol intermediate, performing the same function of the antioxidant seleno-enzymes [12]. Moreover, the decrease in oxidative damage markers has been attributed to exercise-training adaptations in rat liver [41]. In this sense, these antioxidant properties were not replicated here, when combined [$(PhSe)_2$] compound and physical exercise. Despite the association (training + [$(PhSe)_2$]) reduced ROS generation in old rats, the GSSG levels remain higher. On the other hand, in the liver of old trained rats, it was possible to observe a lower ROS generation and an increase at GSH/GSSG ratio, reinforcing the effectiveness of the physical training as an efficient antioxidant strategy. During physical exercise, the liver is a detoxification organ because, when stimulated, induces oxidative stress, and inflammatory responses [42]. Under other conditions, the lack of [$(PhSe)_2$] redox protection may

be related to the fact that our dose remains high to produce a chronic protective effect, and this way, more studies are needed to investigate the threshold dose of [$(PhSe)_2$] with the positive effects expected against age-related damage.

In mitochondria, our results demonstrated that just the old trained rats reduce the ROS levels and, despite no increases in MnSOD activity, it may be linked to antioxidant response modulation against the oxidative alterations caused by physical exercise [41, 43]. Besides, regular exercise down-regulates the rate of mitochondrial ROS generation in the liver [44] and also improves the mitochondrial antioxidant system, resulting in lower oxidative damage markers in several tissues [45]. Besides, old trained rats associated with [$(PhSe)_2$] treatment showed increased activity of MnSOD. However, this does not lead to changes in mitochondrial ROS production, corroborating with studies that have evidenced that previous antioxidant supplementation attenuates the physical training adaptive responses, such as mitochondrial biogenesis and cellular defense mechanisms [44, 46, 47].

To support this, we found that [$(PhSe)_2$] supplementation plus exercise presents a lower liver mitochondrion potential index (Δψm) than those from old trained. In the same way, according to Puntel et al. (2010), the [$(PhSe)_2$] responds negatively to $\Delta\psi_m$ because of a partial depolarization of mitochondria in a concentration-dependent manner [48]. It is known that maintenance of the proton gradient is of vital importance for cell health, bioenergetics, and alterations in mitochondrial functionality compromise the energy balance [49]. Here, our results demonstrated that the old trained plus [$(PhSe)_2$] supplementation rats aggravated the hepatic mitochondrial dysfunction, considering the Δψm decreased, associated to increased mitochondrial ROS generation and MnSOD activity, proving that the combination of antioxidant and exercise training might not be the best strategy. In contrast, our results demonstrated that the training increased Δψm making the mitochondrial membrane more negative and reducing ROS production, thus, once more, the training shows potential benefits to old rats, reducing the production of ROS.

Finally, increases of oxidative stress markers paralleled respiration unbalance, with increases on O_2 consumption (State III) and non-ADP-stimulated respiration (state IV) associated with $\Delta\psi_m$ decrease may be related to mitochondrial dysfunction. We suggest that the exercise plus [$(PhSe)_2$] supplementation may produce a higher O_2 consumption at state III, which is not transmitted to ATP production, and consequently, increase the formation of reactive species (i.e., mitochondrial ROS levels). In this regard, it is known that [$(PhSe)_2$] can inhibit the mitochondrial complex II activity by decreasing the mitochondrial respiration supported by complex I or complex II substrates [50]. Previous studies observed that exposure to acute or chronic toxic doses of [$(PhSe)_2$] can increase selenium deposition in liver [51]. Furthermore, selenium compounds can be toxic to naive animals and cultured cells [52, 53] attributed to the oxidation of thiol groups present in the mitochondrial membrane [54], leading to several alterations in the mitochondrial functions, and this way, interfering on possible mitochondrial adaptation improvements.

Conclusions

In summary, our study is the first to test if [$(PhSe)_2$] supplementation, when associated with swimming training, would interfere in the development of liver oxidative damage during the aging process. Despite [$(PhSe)_2$] and regular physical exercises present individually antioxidant properties, as well as produce tissue adaptations and improve redox mechanisms, in this study, the combination of these two strategies did not manifest the expected benefits. This way, our hypothesis was not confirmed since the association did not present synergic effect, but, surprisingly, blunted the induced-exercise adaptations, including at mitochondrial mechanisms. In future, more researches are needed to investigate the effective dose and possible mechanisms of action, especially at hepatic liver tissue.

Author contributions All authors were involved in the development of this manuscript. As the corresponding author, Rômulo P. Barcelos oversaw the complete manuscript development. The study was designed by José L. Cechella; the training was designed and performed by Marlon R. Leite; data were collected and analyzed by Martin T. B. Leite, Micaela B. Souza, Thayanara C. da Silva and Nelson R. De Carvalho; data interpretation and article preparation were undertaken by Pamela C. Da Rosa, Diane D. Hartmann, and Sílvio T. Stefanello; the study conceived and supervisioned, and review of final version by Félix A. A. Soares, Gustavo O. Puntel. All authors have approved the final version of this manuscript.

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Compliance with ethical standards

Conflicts of interest The author declares that they have no competing interests.

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