Exercise Physiology

Fish oil supplementation and physical exercise during the development period increase cardiac antioxidant capacity in Wistar rats

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Abstract - Aim: To investigate if treadmill exercise (Ex) associated with fish oil (FO) supplementation during lactation would influence the biochemical profile as well as the oxidative balance in the hearts of male juvenile rats. **Methods:** Fifteen days-old rats were submitted to a daily moderate Ex training (based on their maximal running capacity) and FO supplementation for 4 weeks. Forty-eight hours after the last exercise session, blood fasting glucose and lipid profile were assessed according to the manufacturer's recommendations, while the oxidative status of the hearts was evaluated via colorimetric and absorbance-based assays. **Results:** FO associated with Ex decreased triglycerides (TG-79.27 ± 5.75 to 60.24 ± 6.25 mg/dL) and very-low-density lipoprotein cholesterol levels (VLDL-15.85 ± 1.15 to 12.05 ± 1.25 mg/dL) when compared to sedentary animals. FO, alone, reduced atherogenic index (AI- 1.14 ± 0.03 vs. 1.01 ± 0.04 a.u) while increased high-density lipoprotein cholesterol (HDL-43.90 ± 2.50 vs. 59.43 ± 3.15 mg/dL) of sedentary animals. Additionally, both Ex (67.3 ± 13.5 nmol/mg prot) and FO supplementation (56.6 ± 5.5 nmol/mg prot) decreased the oxidative damage to lipids in non-trained animals (105.8 ± 10.8 nmol/mg prot). The interventions also protected the protein content from oxidative stress (Ex- 5.15 ± 0.46; FO- 4.5 ± 0.5; and vehicle sedentary-7.3 ± 0.6 µmol/mg prot), while increasing the antioxidant defense and oxidative metabolism. **Conclusion:** Our findings suggest that intervention in juvenile rats can improve cardiac metabolism. These are the first findings to show the positive effects of the association between FO and moderate treadmill Ex during the critical period of development. We believe these results can drive early-life origins of heart disease through different avenues and, possibly, assist the development of a heart disease prevention program as well as an adjunctive therapeutic resource.

Keywords: exercise, fish oil, oxidative metabolism, developmental period.

Introduction

Heart diseases are the leading causes of death worldwide, wherein ischemic heart disease and stroke are among the main causes of premature death[1]. Currently, it is well described that the predisposing factors underlying cardiovascular diseases (CVD) in adulthood, nevertheless, may reside in an unappropriated environment still in early life (Developmental Origins of Health and Diseases theory) when external factors can modulate the biochemical milieu, and trigger long-lasting changes seeking for the internal balance[2-4].

In this context, a special class of essential fatty acids (EFAs), called polyunsaturated fatty acids (PUFAs), play an important role in the maintenance of metabolic health, at which point their availability in breast milk is primordial for growth, development, and the establishment of structural elements, especially of the membrane-rich tissues[5,6]. In the heart, it has been proposed that PUFAs may modulate the microenvironment of membrane signaling compounds, wherein their oxygenated products (eicosanoids) have bioregulatory functions in several human life pathways (e.g. prostaglandins, thromboxane, cyclooxygenase, and lipoxygenase)[7,8], as well as in the control of ion channels[9].

Among the different PUFAs classes, the omega-3 fatty acids (n-3 FA) stand out in the CVD protection[10], wherein n-3 FA include: I) alpha-linolenic acid (ALA); II) the eicosapentaenoic acid (EPA); and III) the docosahexaenoic acid (DHA). While ALA is found in vegetable sources such as nuts and seeds, EPA and DHA are predominantly derived from oily fish[11]. In this context, it has been described that FO can protect the heart from both risk factors (e.g. hypertension and atherosclerosis) and events

such as stroke, myocardial infarction, and cardiac arrhythmias[12-15]. Moreover, compelling evidence demonstrates that FO can improve cardiac function by enhancing mitochondria activity and oxidative stress resilience[16-20].

Described as a metabolic condition present in several disorders[21-23], including those from developmental origins[24-27], oxidative stress (OS) is characterized by a chronic imbalance between reactive species production and tissue antioxidant capacity, which leads to oxidative damage to biomolecules (e.g. DNA, proteins, and lipids)[28]. Although the mechanisms involved in the OS-induced cardiac impairments are not precisely known, its relation with cardiovascular disturbances such as ischemic heart disease[29], left ventricular dysfunction[30], cardiac hypertrophy[31], heart failure[32] as well as hypertension[33] is widely accepted.

As a therapeutic strategy, exercise has also been proposed as a useful intervention to maintain health and prevent CVD pathological conditions by decreasing risk factors such as hypertension, obesity, hyperlipidemia, and oxidative stress[34-37]. However, the variety of protocols (i.e. type of exercise, intensity, volume, and periodization) and their different effects throughout the organism still limit the exercise-induced hormesis understanding[38]. Thereby, our laboratory has investigated the benefits of exercise in tissue-dependent oxidative stress[39-42], and recently, we demonstrated that daily moderate physical exercise applied at juvenile ages, can decrease the risk of developing CVD by improving cardiac mitochondrial function and oxidative stress resilience in male rats[43].

Thus, even studies have described the benefits of FO supplementation and Exercise to heart health and the increase in lifespan. To the best of our knowledge, the majority were conducted in adult/aged rats with metabolic impairments. Here, however, we sought a timely intervention scarcely considered (early life), investigating whether a FO supplementation along with a daily moderate exercise would positively influence the cardiac metabolism and lipid profile in healthy juvenile rats.

Methods

Animals and experimental design

This study used Wistar rats obtained from the Nutrition Department at *Universidade Federal de Pernambuco*[44]. Throughout the study, all the experimental procedures were performed to minimize animal suffering and to reduce the number of animals per group. Each experimental phase was performed by the Institutional Ethics Committee guidelines for Animal Research (approval protocol n° 23076.027072/2014-20), complying with the "Principles of Laboratory Animal Care" (National Institutes of Health, Bethesda, USA).

Pregnant rats were reared in a room with a temperature of 23 ± 1 °C and a 12-h light/dark cycle (lights on from 7:00 am to 7:00 pm), with free access to water and food with 23% protein (laboratory chow diet -Presence of Brazil Ltd., São Paulo, Brazil). Twenty-four hours after delivery, the litters were normalized to 8 pups, and, at the 15th post-natal day 4 male pups per litter were randomly assigned into 4 different experimental groups: (1) supplemented with vehicle solution and sedentary (VS, n = 10); (2) supplemented with vehicle solution and exercised (VEx, n = 10); (3) supplemented with fish oil and sedentary (FOS, n = 10); (4) supplemented with fish oil and exercised (FOEx, n = 10).

After weaning, the pups were housed in groups of 3-4 per cage (51 × 35.5 × 18.5 cm) until complete 4 weeks of nutritional and exercise interventions, when they were sacrificed.

Fish oil supplementation

FO supplementation was provided once a day (85 mg/kg) or vehicle solution (V, 1 mL/250 g). The capsules of fish oil (Sundown®) containing PUFAs [docosahexaenoic (DHA; 85 mg/L g) and eicosapentaenoic (EPA; 128 mg/1 g)] were dissolved in Cremophor (Sigma ®) 0.009% and distilled water. The FO group had the PUFAs solution administered via gavage, while the control group received a vehicle solution (same amount of Cremophor and distilled water used to prepare PUFAs solution)[45,46].

Treadmill exercise

Firstly, animals were familiarized with the exercise apparatus for 5 days, then, an incremental running test was performed to provide the maximal running capacity of the animals throughout the training regimen. The physical exercise consisted of a 30 min continuous running on a treadmill (Insight EP-131, 0° inclination), 5 days/week, for 4 weeks, at 50% of the maximal running capacity, which was weekly assessed to readjust the training intensity[43,45]. The animals from sedentary groups were placed on the treadmill for the same period as the exercised animals, but the apparatus remained off.

Blood analysis

Forty-eight hours after the last exercise session, fasted (12-14 h) rats were anesthetized (1 g/kg urethane plus 40 mg/kg chloralose), and blood samples were obtained. The samples from the tail vein were used to measure glucose levels (glucometer: G-Tech Free Sistema NoCode Accumed-Glicomed, Brazil), while the samples obtained by cardiac puncture were placed in 4 mL tubes, incubated for 2 min, and centrifuged at 8,000 RPM for 10 min. Then, supernatants were transferred to 2 mL Eppendorf and stored at −20 °C until the analyses were processed (within 48 h). The serum was used in the total cholesterol (TC),

high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) analyses (Labtest®, Lagoa Santa, MG). The Very low-density lipoprotein (VLDL = triglycerides/5) and atherogenic index were also calculated using the formula [log (triglycerides/HDL-cholesterol)][47,48].

Drugs and reagents

All drugs and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) (Sinc Pernambuco, Brazil).

Heart preparations for biochemical analyses

Once the blood samples were collected, the animals were sacrificed by the collection of the hearts, which were homogenized in 50 mM TRIS buffer, pH 7.4, containing 1 mM EDTA, 1 mM sodium orthovanadate, 200 µg/mL phenylmethanesulfonyl fluoride (PMSF). The homogenate was centrifuged at 4,000 RPM for 10 min at 4 °C, and the supernatant was used in the following biochemical measurements. The supernatant protein concentration was estimated by using bovine serum albumin (BSA) as a standard procedure[26].

Evaluation of lipid oxidation

The lipid oxidation was measured by the thiobarbituric acid (TBA) reactive substances technic, an analysis that evaluates the malondialdehyde content (an ending product from the lipid oxidation)[49]. Briefly, to a 0.3 mg heart homogenate protein, 30% trichloroacetic acid (TCA) and Tris-HCl (3 mM) were added, and centrifuged at 2,500 g for 10 min. Thereafter, the supernatant was transferred to another tube and an equal volume of 0.8% TBA (v/v) was mixed, and boiled for 30 min. After cooling, the pigment was read at 535 nm in a spectrophotometer, and the results were expressed as nmol/mg of protein.

Evaluation of carbonyl content

The protein oxidation was assessed using the procedures highlighted by Reznick and Packer[50]. On the ice, 30% (w/v) TCA was added to 0.3 mg protein homogenate and centrifuged for 14 min at 4,000 RPM. The pellet was suspended in 10 mM 2,4-dinitrophenylhydrazine and immediately incubated in a dark room for 1 h with gentle shakes at each 15 min. Afterward, the final pellet was suspended in 6 M guanidine hydrochloride, incubated for 30 min at 37 °C, and read at 370 nm absorbance. The results were expressed as µmol/mg of protein.

Superoxide dismutase assay

Superoxide dismutase is considered the first line of defense against the reactive species, catalyzing superoxide anion ($^{\bullet}O_{2^-}$) to hydrogen peroxide (H_2O_2)[51]. Herein, the total superoxide dismutase enzyme activity (t-SOD) was performed according to the method developed by Misra and Fridovich[52]. In a sodium carbonate buffer (0.05%, pH 10.2, 0.1 mM EDTA), protein supernatants (0.2 mg/mL heart) were incubated at 37 °C and had the reaction started by 30 mM epinephrine (in 0.05% acetic acid). The epinephrine auto-oxidation inhibition was followed for the 90 s at 480 nm and used to express the enzymatic activity at U/mg protein.

Catalase assay

Once the SOD converts superoxide into hydrogen peroxide, Catalase (CAT) further detoxifies it to water and O_2[51]. Its activity was measured according to Aebi's method[53]. In a milieu compounded by 50 mM-phosphate buffer (pH 7.0), 300 mM H_2O_2, and samples (0.3 mg/mL heart homogenates), the enzyme was determined by measuring the change in absorbance (at 240 nm) per minute over a 4 min period at 20 °C. CAT activity was expressed as U/mg protein.

Glutathione-S-transferases activity

Glutathione-S-Transferase (GST) is a glutathione-dependent enzyme involved in the detoxification of a wide range of toxic agents, including peroxide and alkylating agents present in the tissues[54]. The GST activity was measured by the method described by Habig[55], wherein one unit of enzyme conjugates 10.0 nM of 1-chloro, 2,4-dinitrobenzene with reduced glutathione (GSH). At 30 °C, this reaction was followed at 340 nm during the 90 s, and the results were expressed as U/mg protein.

Glutathione-reduced content

Glutathione reduced is the major intracellular thiol involved in antioxidant reactions. GSH can act both directly, as a reducer agent, and indirectly, as a co-factor in the enzymatic reactions in the glutathione system[56]. By using Hissin's method[57], the GSH content was assessed by incubating 0.300 mg of supernatant protein with a 0.1 M phosphate buffer and 1 mg/mL o-phthaldialdehyde at room temperature for 15 min. Then, the samples had their fluorescence read at 350 nm excitation and 420 nm emission, and the results were compared to a known standard GSH curve.

Total and protein-bound sulfhydryl group content

The sulfhydryl content is inversely correlated to the oxidative damage to proteins and has been widely used as a marker of cellular reducer capacity[58]. This assay was performed in accordance with the previous procedures[59]. Homogenates (0.5 mg/mL) were used to the reduction of 5,5'-dithiobis(2-nitrobenzoic acid) by thiol groups, generating a yellow-stained compound whose absorption is measured spectrophotometrically at 412 nm. Results were calculated as mmol/mg protein.

Oxidative capacity biomarker (citrate synthase activity)

The citrate synthase is the first enzyme in the Krebs cycle, and it has been commonly used as an oxi-

dative metabolic marker in several tissues[60]. It condensates the two-carbon acetate residue from acetyl coenzyme A and a four-carbon molecule (oxaloacetate) to form a six-carbon molecule (citrate). Its enzymatic activity was determined as described previously[26]. Briefly, 0.100 mg of protein was incubated in a reaction mixture containing (in mM) 100 Tris·HCl (pH 8.2), 1 $MgCl_2$, 1 EDTA, 0.2 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB) (ε = 13.6 $\mu mol \cdot mL^{-1} \cdot cm^{-1}$), 3 acetyl-CoA, and 5 oxaloacetate. Citrate synthase activity was measured by assessing the rate of change at 412 nm over 3 min (30-s intervals) at 25 °C.

Statistical analysis

Results are expressed as mean ± S.E.M. Two-way ANOVA test followed by a Tukey test were performed to assess significant differences among the groups. Data were considered statistically significant for $p < 0.05$. All data were plotted and evaluated by GraphPad Prism 6.0 (GraphPad Software Inc., La Jolla, CA, USA).

Results

Fasting glycemia, lipid profile, and atherogenic index

No significant differences were found in the fasting glucose values (VS- 124.38 ± 4.92 mg/dL; VEx- 121.33 ± 4.89 mg/dL; FOS- 114.90 ± 5.35 mg/dL; FOEx- 118.56 ± 6.80 mg/dL), Two-way ANOVA ($F(1,32) = 0.357$, $p = 0.554$). Despite the fasting glucose had not been modulated by either exercise or FO supplementation, the total cholesterol levels ($F(1,23) = 9.81$, $p = 0.005$), triglycerides ($F(1,24) = 10.09$, $p = 0.004$) and very low-density lipid ($F(1,24) = 10.08$, $p = 0.004$) were reduced by their combination (FOEx rats). The comparinsons demonstrated: I) TC (FOS- 109.66 ± 4.77 *vs.* FOEx- 81.72 ± 6.18 mg/dL); II) TG T(Vex- 82.01 ± 4.29 *vs.* FOEx- 60.24 ± 6.25 mg/dL) and III) VLDL (VEx- 16.40 ± 0.86 *vs.* FOEx- 12.05 ± 1.25 mg/dL), as described in Table 1.

Interestingly, HDL and atherogenic index were modulated only by FO supplementation, which increased HDL levels in sedentary animals (VS- 43.90 ± 2.50 *vs.* FOS- 59.43 ± 3.15 mg/dL; $F(1,20) = 5.39$, $p = 0.03$) while reduced atherogenic index regardless the physical status (VS- 1.14 ± 0.03 *vs.* FOS- 1.01 ± 0.04; and VEx- 1.16 ± 0.02 *vs.* FOEx- 1.04 ± 0.05; $F(1,15) = 9.76$, $p = 0.007$) (Table 1).

Oxidative stress biomarkers

The exercise training decreased the MDA (VS- 105.8 ± 10.8 *vs.* VEx- 67.3 ± 13.5 nmol/mg prot; $p < 0.05$) and Carbonyls (VS- 7.3 ± 0.6 *vs.* VEx- 5.2 ± 0.5 µmol/mg prot, $p < 0.05$) levels in the heart of juvenile rats, Figure 1. In addition, FO supplementation also counteracted lipid and protein oxidation, by decreasing both MDA (VS- 105.8 ± 10.8 *vs.* FOS- 56.6 ± 5.5 nmol/mg prot; $p < 0.01$) and carbonyls (VS- 7.3 ± 0.6 *vs.* FOS- 4.5 ± 0.5 µmol/mg prot; $p < 0.001$, Figure 1). Curiously, when the interventions were associated, no incremental protective effects were provided (MDA: FOEx- 63.3 ± 4.6 nmol/mg prot; and Carbonyls: FOEx- 5.4 ± 0.3 µmol/mg prot, Figure 1).

Antioxidant defenses

The antioxidant defense is comprised of the enzymatic and non-enzymatic compounds, which work collaboratively to counteract the reactive species and maintain the oxidative status in equilibrium. Related to the enzymatic defense, FO supplementation increased superoxide dismutase-SOD activity in both sedentary (VS- 5.27 ± 0.28 *vs.* FOS- 6.5 ± 0.2 U/mg prot, $p < 0.05$) and exercised animals (VEx- 5.25 ± 0.33 *vs.* FOEx- 6.7 ± 0.4 U/mg prot, $p < 0.05$), when compared to their respective controls, Figure 2A. Regarding the H_2O_2 detoxification, we observed that exercise upregulates CAT activity in supplemented animals (VEx-1.21 ± 0.04 *vs.* FOEx- 1.51 ± 0.1 U/mg prot, $p < 0.05$) and (FOS- 1.09 ± 0.06 *vs.* FOEx- 1.51 ± 0.1 U/mg prot, $p < 0.01$),

Table 1 - Glycemia, lipid profile, and atherogenic index from animals that were randomly assigned to nine pups on the first day of life, performing the exercised or sedentary experimental groups, respectively. The supplementation with fish oil (FO) or vehicle (V) was carried out from 15 up to 45 days old. Data are presented as mean ± SEM.

Experimental Group (n)	**Blood data**					
	FG (mg/dL)	**TC (mg/dL)**	**TG (mg/dL)**	**VLDL (mg/dL)**	**HDL (mg/dL)**	**AI (arbitrary unit)**
VS (n = 10)	124.38 ± 4.92	97.66 ± 9.66	79.27 ± 5.75	15.85 ± 1.15	43.90 ± 2.50	1.14 ± 0.03
VEx (n = 10)	121.33 ± 4.89	84.69 ± 3.66	82.01 ± 4.29	16.40 ± 0.86	51.14 ± 4.51	1.16 ± 0.02
FOS (n = 10)	114.90 ± 5.35	109.66 ± 4.77	64.97 ± 5.47	12.99 ± 1.09	59.43 ± 3.15[a]	1.01 ± 0.04[a]
FOEx (n = 10)	118.56 ± 6.80	81.72 ± 6.1[c]	60.24 ± 6.25[b]	12.05 ± 1.25[b]	48.85 ± 5.14	1.04 ± 0.05[b]

Legend: FOEx - fish oil and exercised; FOS - fish oil and sedentary; VEx - vehicle and exercised; VS - vehicle and sedentary; FG - fasting glycemia; TC - total cholesterol; TG - triglyceride; VLDL - very-low-density lipid; HDL - high-density lipid and AI - atherogenic index. The letters represent statistical differences: [a]when compared to VS; [b]when compared to VEx; and [c]when compared to FOS.

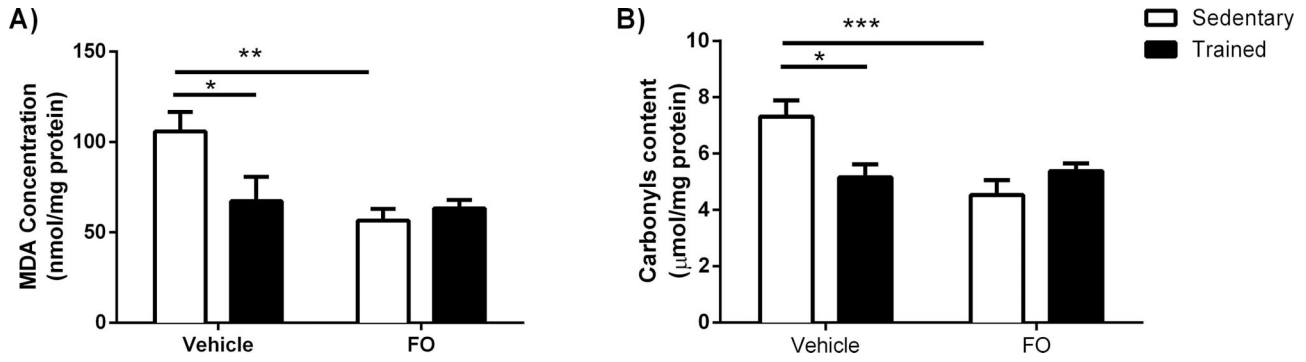

Figure 1 - Oxidative stress biomarkers were evaluated in the hearts of juvenile rats. All data are presented as mean ± SEM. These rats were subdivided into exercised and sedentary groups, and they received one single daily dose of fish oil or vehicle solution (for 4 weeks). A) Lipid peroxidation evaluated by malondialdehyde concentration; B) Protein oxidation evaluated by the concentration of carbonyls. *p < 0.05; **p < 0.01; ***p < 0.001 in a Two-way ANOVA test followed by the Tukey test.

without statistic improvements in glutathione-S-transferase-GST activity, Figure 2B and 2C, respectively.

In the non-enzymatic defense, our results demonstrated that treadmill exercise increased GSH in the juvenile rats (VS- 1.27 ± 0.1 *vs.* VEx- 1.96 ± 0.2 μM/mg prot, p < 0.01, Figure 2D), however, no further improvements were promoted by combining it with FO supplementation. As protein sulfhydryl groups from cysteine residues can be oxidized to form disulfide altering the redox state of proteins, we evaluated the total thiol content[58]. As observed in Figure 2E, the association of Ex and FO supplementation increased sulfhydryl-bound groups, when compared to VEx (VEx- 19.6 ± 1.2 *vs.* FOEx- 30.8 ± 5.4 μM/mg prot, p < 0.05) and FOS (FOS- 17.8 ± 1.2 *vs.* FOEx- 30.8 ± 5.4 μM/mg prot, p < 0.05), indicating their ability to deal with general electrophilic compounds and preserve protein structures.

Oxidative metabolism biomarker

We observed (Figure 3) that both exercise (VS- 0.27 ± 0.02 *vs.* VEx- 0.50 ± 0.05 μM/mg prot, p < 0.05) and FO (VS- 0.27 ± 0.02 *vs* FOS- 0.53 ± 0.07 μM/mg prot, p < 0.05) increased citrate synthase activity around 90%, however, no additional results were verified in animals that trained and received the supplementation.

Discussion

Most studies using FO/PUFAs supplementation address either their protective role in the cardiovascular disturbances already established or their breast milk content impact on health[12-15,61,62]. Here, for the first time, we hypothesized their benefit aspects in healthy young animals, further proposing moderate physical exercise as another tool in the improvement of cardiac metabolism. Thus, we assessed if the combination of exercise and FO supplementation strengthens antioxidant defense and enzymatic activity in the hearts of juvenile rats, wherein the findings indicate that both Ex as FO supplementation improved cardiac oxidative metabolism in juvenile rats.

Nutrition and exercise have been widely used in the improvement of the lipid profile. Here, both interventions modulated positively the parameters evaluated (cholesterol total, TG, VLDL, HDL, and atherogenic index). Interestingly, the lowest atherogenic index and the highest HDL levels were found in the group that received only FO, suggesting that FO supplementation alone can decrease the risk factors for the development of cardiovascular diseases in healthy juvenile rats.

It has been demonstrated that HDL comprises a part of the immune system, playing a role in the antioxidant, anti-inflammatory, and angiogenic activities as well as in endothelial repair[63]. According to the criteria for the diagnosis of metabolic syndrome, high levels of HDL, as well as decreased triglyceridemic levels are both important factors associated with the lowest risk for cardiovascular diseases development[64]. Therefore, our lipid profile data support the idea that FO supplementation can decrease the risk factors for cardiovascular diseases, even in animals without metabolic/cardiac issues.

Furthermore, studies have demonstrated that early physical activity-induced benefits can last for long periods[65, 66]. In accord with Navarro[67], the improvement of oxidative metabolism, by upregulating mitochondrial enzymes, is an important treadmill exercise outcome[67]. Here, we demonstrate that exercise and FO supplementation increase citrate synthase activity in the heart of juvenile rats. Likewise, maternal omega-3 supplementation increases the activity of Krebs cycle enzymes (citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase)[68]. Thus, as the heart energy supply relies mainly on aerobic pathways, interventions that optimize mitochondria-related ATP availability are followed by general improvements in cardiac function.

The effectiveness in the oxidative metabolism allows the heart to use larger amounts of O_2 when required, which is likely followed by an increase in the formation of

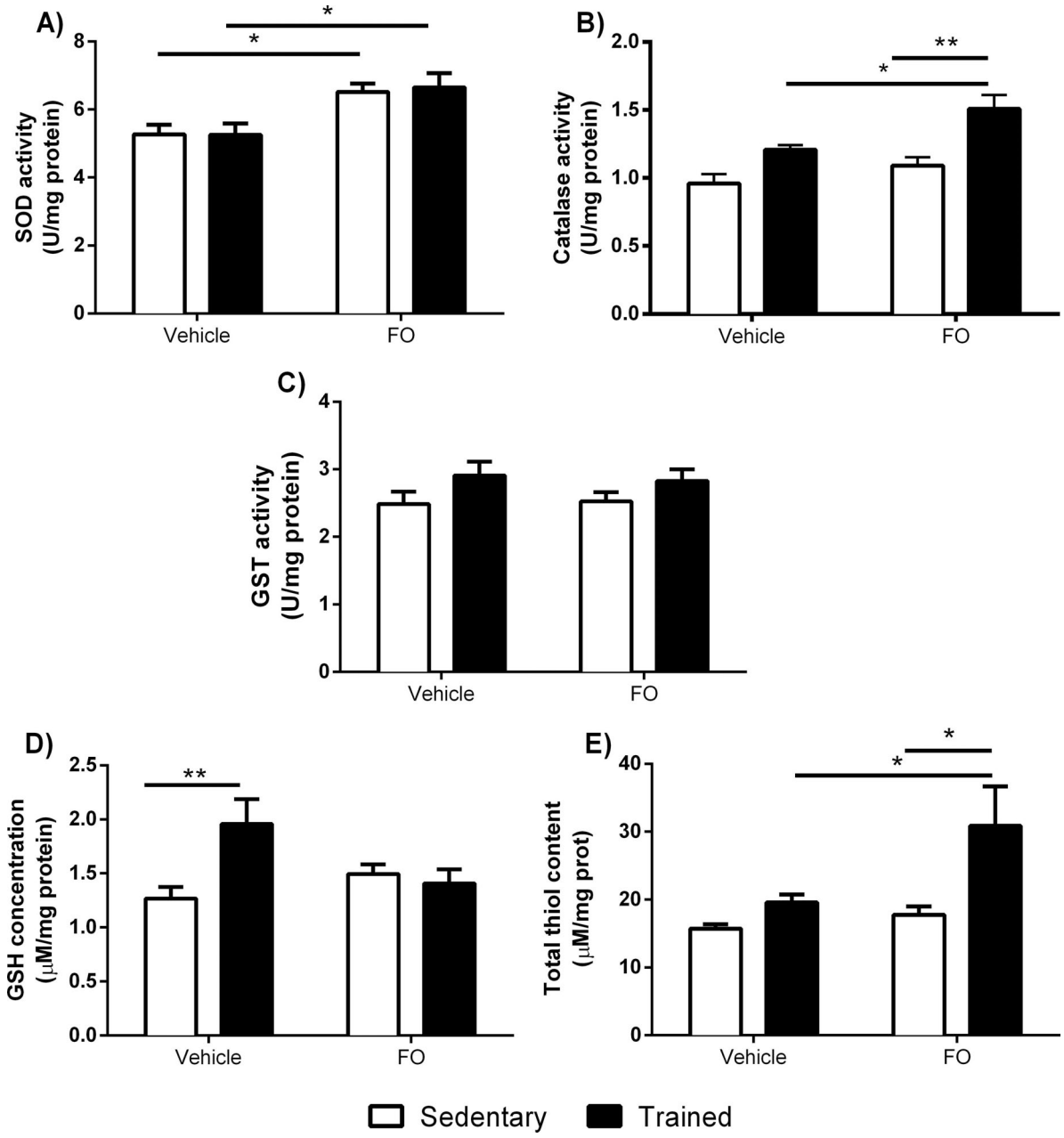

Figure 2 - Evaluation of the enzymatic and non-enzymatic antioxidant defense system in the heart of juvenile rats. All data are presented as mean ± SEM. These rats were previously subdivided into exercised and sedentary groups, and they received one single daily dose of fish oil or vehicle solution (for 4 weeks). A) Superoxide dismutase activity; B) Catalase activity; C) Glutathione-S-transferase activity; D) Reduced Glutathione; E) Thiol levels. *p < 0.05; **p < 0.01 in a Two-way ANOVA test followed by the Tukey test.

reactive oxygen species (~5% of the O_2 consumed by the mitochondrial)[21]. The oxidative burst (compelled production of ROS) had been described in several organic conditions, however, the chronic imbalance between their production and removal is involved in a variety of cardiac impairments[69, 70]. In this context, our data demonstrated that exercise improves oxidative resilience, wherein the cogent decrease in both oxidative damages to lipids and proteins, assures the metabolic advantages of those engaged in regular exercise [37, 71].

Furthermore, it is noteworthy the increase of SOD activity promoted by FO supplementation, which reduces the spontaneous production of peroxynitrite (i.e. superoxide + nitric oxide reaction), increasing the availability of nitric oxide to the cardiac tissue [26, 72, 1]. The increase in the CAT activity only in the FOEx group, however, suggests that the exertional signals triggered by exercise might involve the hydrogen peroxide as a second messenger[74], thus, a higher capacity to deal with this reactive species is required. Interestingly, among the anti-

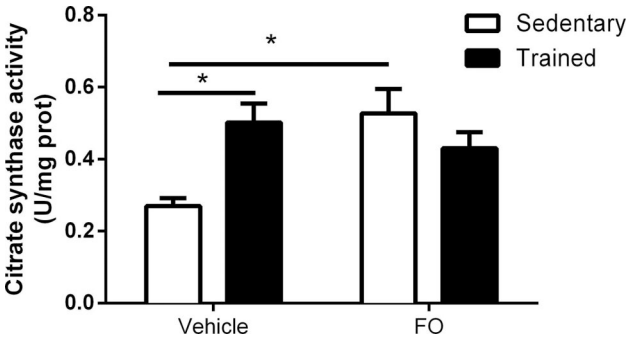

Figure 3 - Oxidative metabolic marker in the heart from juvenile rats. All data are presented as mean ± SEM. These rats were previously subdivided into exercised and sedentary groups, and they received one single daily dose of fish oil or vehicle solution (for 4 weeks). *p < 0.05 in a Two-way ANOVA test followed by the Tukey test.

oxidant compounds evaluated here, only GSH was increased by moderate exercise. As the glutathione pool acts beyond the antioxidant system, we believe that its increase might support the redox balance required in biological processes such as signal transduction and gene expression, especially in animals at this age[75].

Although our FOEx rats showed increased antioxidant and metabolic enzyme activities, our study did not evaluate the physiological impact of this improvement in cardiac variables. As described by Asharf and Roshan[36], it is feasible that the enhancement of the antioxidant capacity would be followed by myocardial resilience to cardiotoxicity. Thus, we believe that FO supplementation along with exercise may decrease the risk of cardiac harm related to stressful events, however, more studies would be necessary to confirm this causal relationship. Taken together, our approach outstands among the FO and Exercise studies by demonstrating that even young healthy animals could have cardiometabolic benefits from FO supplementation, and its combination with moderate physical training increases oxidative stress resilience by enhancing the general antioxidant capacity.

Conclusion

Our data indicate that moderate treadmill Ex and FO supplementation at early ages benefit lipid profile as well as antioxidant and metabolic enzymes even in healthy juvenile rats. Therefore, these pioneer results suggest that an early-life intervention with moderate Ex and n-3 FA supplementation could help to decrease the incidence, and also, the severity of risk factors related to cardiovascular diseases. These beneficial effects seem to be, in part, caused by the reduction of the damage triggered by oxidative stress. However, it would be interesting to include the use of our FO supplementation and treadmill Ex in a controlled protocol of cardiac disease in rats.

Acknowledgments

The authors are thankful to the CNPq and FACEPE that provided financial funds for this work.

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Manuscript received on October 29, 2021
Manuscript accepted on March 18, 2022