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Can fish oil supplementation and physical training improve oxidative metabolism in aged rat hearts?

Anderson Apolonio da Silva Pedroza [a], Andréia Lopes [b], Rosângela F. Mendes da Silva [b], Glauber Ruda Braz [a], Luciana P. Nascimento [a], Diorginis Soares Ferreira [a], Ângela Amâncio dos Santos [c], Manuella Batista-de-Oliveira-Hornsby [b,*,1], Claudia J. Lagranha [a,**,1]

[a] Laboratory of Biochemistry and Exercise Biochemistry, Department of Physical Education and Sports Science, CAV-Federal University of Pernambuco, Brazil
[b] Department of Nutrition, Federal University of Pernambuco, Recife, PE, Brazil
[c] Department of Physiology & Pharmacology, CCB-Federal University of Pernambuco, Recife, PE, Brazil

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ABSTRACT

Aims: It is well known that in the aging process a variety of physiological functions such as cardiac physiology and energy metabolism decline. Imbalance in production and elimination of reactive oxygen species (ROS) may induce oxidative stress. Research shows that oxidative stress is an important factor in the aging process. Studies suggest that −3 polyunsaturated fatty acids (PUFAs) and moderate physical exercise modulate the ROS system. Therefore, the present study aimed to investigate whether −3 present in fish oil supplementation coupled with moderate physical training could improve antioxidant and metabolic enzymes in the hearts of adult and aged rats and, if these effects could be associated to glycemia, plasma lipid profile or murinometric parameters.
Main methods: Adult (weighing 315.1 ± 9.3 g) and aged rats (weighing 444.5 ± 11.8 g) exercised and receive fish oil supplementation for 4 weeks. Then they were used to evaluate murinometric parameters, fasting glucose and lipid profile. After this, their hearts were collected to measure the levels of malondialdehyde (MDA), antioxidant enzyme activity (superoxide dismutase—SOD, catalase—CAT, glutathione peroxidase—GPx) and oxidative metabolism marker (citrate synthase—CS activity).
Key findings: Fish oil supplementation increases HDL concentration and activity of CAT and CS. Moreover, physical training coupled with fish oil supplementation induces additional effects on SOD, GPx and CS activity mainly in aged rats.
Significance: Our data suggest that combined treatment in aged rat hearts improves the antioxidant capacities and metabolic enzyme that can prevent the deleterious effects of aging.

1. Introduction

Aging is a multifactorial process that induces decline in the functions of different tissues [1]. This decline in function may be attributed to many diseases due to alteration in energy metabolism and normal physiology [2,3]. According to biochemical theories of aging, side-reactions of metabolism have been known to result in eroding overall health and well-being of biological systems and their functions [4]. Several theories try to explain the aging process, but the most popular is the free radical theory, the accumulation of oxidative damaged protein, lipids, and nucleic acids provokes cellular damage and death [5]. The heart aging process is directly affected by the accumulation of oxidized molecules and a decrease in antioxidant defense [6]. Antioxidant molecules can be synthesized in vivo or taken from the diet. Decreased levels of antioxidants can result not only in oxidative stress, but also failure in repair or replacement systems, raising levels of oxidative damage biomarkers [7].

Omega-3 supplementation with fish oils results in a significant incorporation of polyunsaturated fatty acids (PUFAs) in the biological membranes, contributing to the maintenance of the structural and functional integrity of cells [8] and lowering the risk of cardiovascular diseases [9,10]. Study from Garrel et al. [11] demonstrates that dietary supplementation with omega-3 enhances the activity of mitochondrial antioxidant enzyme superoxide dismutase (SOD) in the growing tissues of rats born from dams fed a diet with omega-3. The authors suggested that increased SOD activity is a critical determinant in the tolerance to

* Correspondence to: M. Batista-de-Oliveira-Hornsby, Av. Prof. Moraes Rego, 1235, CEP: 50670-901, Departamento de Nutrição, Cidade Universitária, Recife, PE, Brazil.
** Correspondence to: C.J. Lagranha, Rua Alto do Reservatório, s/n, CEP: 55608-680, Núcleo de Educação Física e Ciências do Esporte, Bela Vista, Vitória de Santo Antão, PE, Brazil.
E-mail addresses: batistadeoliveiram@gmail.com (M. Batista-de-Oliveira-Hornsby), lagranha@hotmail.com (C.J. Lagranha).
[1] These authors contributed equally to this work.

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oxidative stress induced by supplemented diet with omega-3 [11]. The omega-3 fatty acid supplement can also decrease the formation of the reactive oxygen species (ROS) and protect the heart [12,13].

Worldwide, cardiovascular disease is the main cause of death [14]. When moderate exercise is practiced regularly, it can decrease the chances of dying from cardiovascular disease by the reduction of risk factors such as hypertension, obesity and hyperlipidemia, and physical activity; it also enhances longevity by mechanisms independent of these risk factors [15,16]. In addition, there are results demonstrating that moderate physical exercise plays a key role on reducing the risk of cardiovascular diseases in elderly subjects [17,18].

It is well known that either fish oil supplementation or moderate physical exercise alone can benefit the quality of life [19–21]. However, the benefits of fish oil supplementation combined with physical exercise, as tested in adult and aged rats, are still not totally clear. Therefore, the present study investigated whether fish oil supplementation combined with moderate physical training could improve antioxidant and metabolic enzymes in hearts of adult and aged rats; with additional improvement in plasma lipid profile and murinometric parameters.

2. Material and methods

2.1. Drugs and reagents

All drugs and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Sovereign (Sao Paulo, Brazil).

2.2. Animals and experimental design

All experiments were carried out with the offspring of an outbred colony strain of Wistar rats obtained from the Nutrition Department of the Federal University of Pernambuco (Brazil). The experimental design was performed in accordance with the guidelines of the Institutional Ethics Committee for Animal Research (approval protocol no. 23 076 016 320/2012-45), complying with the "Principles of Laboratory Animal Care" (National Institutes of Health, Bethesda, USA). All the experimental procedures were careful to minimize animal suffering, and to reduce the number of animals per group.

Rats were reared in a room at 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 — a commercial laboratory chow diet (Purina do Brazil Ltd., Paulinia, São Paulo, Brazil) with 23% protein. After mating and gestation, the pregnant rats delivered 7–12 pups per litter. The pups from 4 to 6 litters were first joined in a big pool. Male pups from this pool were randomly assigned to perform the litters of this study. After weaning, all pups were housed in groups of 3–4 per cage (51 × 35.5 × 18.5 cm), when they were randomly distributed in four experimental groups as described below. The animals were divided into two different age groups: Adult group (from 3 to 4 months old, n = 44) and aged group (16 ± 2 months old, n = 32).

2.3. Fish oil supplementation

The rats received one single dose of fish oil (FO, 85 mg/kg/d and 1 ml/250 g/d) or vehicle solution (V, 1 ml/250 g/d) daily during a period of 4 weeks, from Monday to Friday as previously described by Rachetti et al. [22]. The fish oil capsules (PROEPA — Ache®), containing polyunsaturated fatty acids [docosahexaenoic acid (DHA; 120 mg/1 g) and eicosapentaenoic acid (EPA; 180 mg/1 g)] were dissolved in Cremophor (Sigma®) 0.009%, then in distilled water and administered via gavage. The vehicle solution (V) was offered to the placebo group with the same amount of Cremophor and water.

2.4. Treadmill exercise

All the groups from different ages were subdivided into: V and FO with or without exercise (sedentary—S and exercised—E). The exercised groups were subjected to treadmill running during the same period of supplementation with either fish oil or the vehicle. All rats exercised in a treadmill apparatus (Insight EP-131, 0° inclination) followed the parameters of moderate exercise adapted from the exercise routine previously described by [23]. Periods of treadmill exercise lasted 4 weeks. During the first 3 weeks, the animals were subjected to the treadmill for 30 min/day (from Monday to Friday). The treadmill running velocity was increased from 5 m/min during the first week to 10 m/min during the second week, and increased again to 15 m/min during the third week and fourth week. Rats from the sedentary groups were placed on the treadmill for the same period as the exercised animals, but the treadmill remained off. Table 1 summarizes the group distribution.

2.5. Murimetrical parameters

The heart weight (HW), body weight (BW), body length (BL, muzzle-to-anus length), abdominal circumference (AC, immediately anterior to the forefoot), and thoracic circumference (TC, immediately behind the foreleg) were determined in all rats, as described by Wallen et al. and Novelli et al. [24]. The measurements were made in rats under anesthesia with a mixture of 1 g/kg urethane plus 40 mg/kg chloralose (both from Sigma Co., USA) immediately before blood and tissue collection. The (BW), (BL), (AC) and (TC) were used to determine the following anthropometric index [25]: Body mass index (BMI) = body weight (g) / length^2 (cm^2) and Lee index = cube root of body weight (g) / muzzle-to-anus length (cm) AC/TC ratio.

2.6. Blood analysis

Fasted (12–14 h) rats had blood collected from the tail to measure glucose levels with a glucometer (G-Tech Free Sistema NoCode Accumed-Glicomed, Brazil). Animals were then anesthetized with a mixture of 1 g/kg urethane plus 40 mg/kg chloralose (both from Sigma Co., USA), and blood samples were obtained by cardiac puncture and collected immediately in separate tubes. Approximately 4 ml of blood was placed in a 10 ml tube containing EDTA (ethylenediaminetetraacetic acid) and gently inverted for 30 s to mix. After 20 min, the sample was centrifuged at 8000 rpm for 10 min. The plasma was frozen at −15 °C until assayed for content of lipids. The lipid panel analysis was used as an initial broad medical screening tool for abnormalities in lipids. The analyses of cholesterol (total cholesterol and high-density lipoprotein, HDL) and triglycerides were performed as previously described [26,27]. Very low-density lipoprotein (VLDL = triglycerides / 5) and atherogenic index were also calculated [log (triglycerides / HDL-cholesterol)] [28] [29]. After the blood samples were obtained, the anesthetized animals were then sacrificed and the hearts collected as described previously [30].

Table 1
Summary of the group distribution. Adult and aged rats were divided in sedentary or exercised; and whether they had received supplementation with fish oil or vehicle daily for 4 weeks.

Experimental groups		Data analysis
Exercised (E) ($n = 38$)	Fish oil (FO) ($n = 19$)	Adult rats (3 to 4 months old, n = 11)
		Aged rats (16 ± 2 months old, n = 8)
	Vehicle (V) ($n = 19$)	Adult rats (3 to 4 months old, n = 11)
		Aged rats (16 ± 2 months old, n = 8)
Sedentary (S) ($n = 38$)	Fish oil (FO) ($n = 19$)	The same period as exercised groups
	Vehicle (V) ($n = 19$)	