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Short Communication

Antioxidant effect of exercise: Exploring the role of the mitochondrial complex I superassembly

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ABSTRACT

Mitochondrial respiratory complexes become assembled into supercomplexes (SC) under physiological conditions. One of the functional roles of these entities is the limitation of reactive oxygen species (ROS) produced by complex I (CI) of the respiratory chain. We sought to determine whether the systemic antioxidant effect of exercise is mediated by the assembly of mitochondrial CIs into SCs in rats. Male Wistar rats were exercise trained or remained sedentary for ten weeks; then, blood samples were collected, and the gastrocnemius muscle was isolated. The assembly of mitochondrial SCs and the lipid peroxidation of the mitochondrial and plasmatic fractions were assessed. Our results demonstrate that exercise induced the assembly of CI into SCs in the gastrocnemius and induced a systemic decrease in lipid peroxidation. We also found an inverse association between the superassembly of CIs and mitochondrial lipid peroxidation ($p < 0.01$) and protein carbonyls ($p < 0.05$). We conclude that exercise induces the chronic assembly of CIs into SCs, which provide mitochondrial protection against oxidative damage, at least in the studied muscle. Given the relevant role that mitochondria play in health and disease, these findings should help to elucidate the role of exercise as a therapeutic approach for metabolic diseases.

1. Introduction

Mitochondria are critical organelles that are involved in many aspects of cell activity. The best characterized function of mitochondria is ATP production through the oxidative phosphorylation system and the generation of reactive oxygen species (ROS), mainly by complexes I (CI) and III (CIII) [7,12]. Respiratory complexes have received increased attention because they can undergo superassemblies into supercomplexes (SC) [22]. Rather than mere structural entities, these SCs also have functional roles in the respiratory chain [6], such as the prevention of excessive ROS production; this prevention is conducted by CIs when they are assembled into SCs [20].

Exercise is one of the best-known stimuli for mitochondrial function in skeletal muscle [11]. Accordingly, a recent study reported that exercise induces chronic SC assembly, which is related to mitochondrial respiration and whole-body oxygen consumption [10]. However, an association between SC assembly and mitochondrial redox homeostasis induced by exercise has not yet been tested.

Recent findings from our research group demonstrate that lifelong endurance exercise training induces a systemic decrease in lipid peroxidation [2], as does moderate hypoxic training [4]. Frequently, the antioxidant effect of exercise has been explained by an increased content and activity of antioxidant enzymes [3,8]; however, this is not the case under our experimental conditions [2,4]. Because *ex vivo* studies demonstrate that CI is the main source of mitochondrial ROS production under aerobic exercise conditions [9], our hypothesis states that the CI assembly into an SC may have a primary role in the antioxidant effects of endurance exercise.

We aimed to identify SC formation within the skeletal muscle of chronically exercised rats and evaluate the association between the formation of SC and the oxidative status of the muscle.

2. Materials and methods

2.1. Animals

All experiments were performed according to a protocol approved by the Institutional Animal Care and Use Committee of the University of Granada (procedures Granada, Spain. n°: 28/06/2016/116) and in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (CETS # 123), directive 2010/63/EU on the protection of animals used

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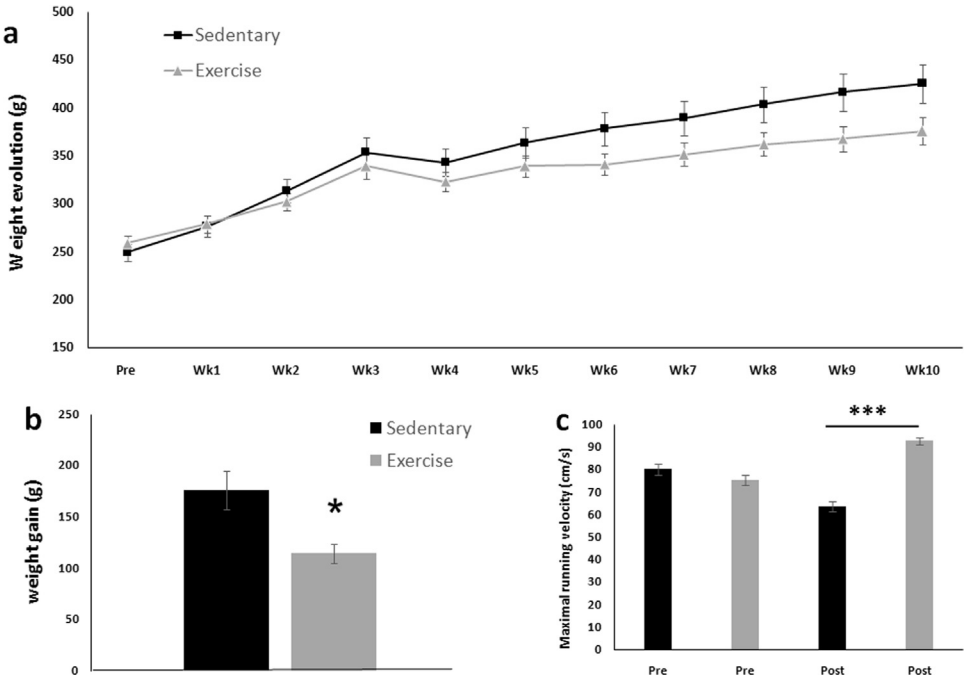

Fig. 1. Weight responses and running performances. a) Weekly evolution of the animals' weights. b) Weight gain was lower in the exercised rats than in the sedentary rats. c) The maximal running velocity was significantly higher in the exercised animals after the 10-week running protocol. $^{*}p < 0.05$ and $^{***}p < 0.001$ *vs.* sedentary rats.

for scientific purposes and the Spanish law (R.D. 53/2013). Male Wistar rats were purchased from Charles River (USA) at six weeks of age. The rats were acclimated to the experimental conditions for two weeks. Next, the rats were randomly allocated into a sedentary (n = 6) or an exercised (n = 6) group for ten weeks, were weighed weekly, and their food and water intakes were also recorded. Seventy-two hours after the last exercise was performed, the rats were fasted overnight, anaesthetised with pentobarbital and euthanized by bleeding.

2.2. Exercise procedures

The animals were acclimated to running on a motorized treadmill (Rat 5-lanes Touchcreen Treadmill for rats, LE8710RTS, PanLab, Spain) for 5 sessions throughout the two weeks of the acclimation period. The exercise programme was divided into two similar 5-wk mesocycles; the rats ran 5 days per week and rested during the weekends (Fig. S1). During the entire protocol, the animals ran at 75% of their maximal velocity as calculated from the maximal velocity test performed at the beginning of each mesocycle. Each mesocycle progressively increased the running volume, the rats began running for 20 min, and this period was increased by 5 min every two days, and when 65 min was achieved, this duration was maintained until the end of the mesocycle. The animals of the sedentary group were daily handled and run for 10 min every two weeks to maintain their acclimation to the treadmill.

The maximal velocity tests were performed before the training period and at week 10 of the training period. These tests started at a velocity of 22 cm/s, and the velocity was increased by 5 cm/s every minute until fatigue was achieved. Fatigue was defined as the point at which the rats remained at the back of the treadmill on an electric shock pad for 5 s.

2.3. Evaluation of supercomplex formation in the skeletal muscle by BNGE

Blue native gel electrophoresis (BNGE) was performed on crude mitochondrial fractions from the gastrocnemius muscles of rats. Mitochondrial isolation was performed as previously described [17]. One aliquot of the crude mitochondrial fraction was used for protein determination. The remaining samples were then centrifuged at 13,000 × g for 3 min at 4 °C. The mitochondrial pellets were suspended in an appropriate volume of medium C (1 M aminocaproic acid, 50 mM Bis-Tris-HCl [pH 7.0]) to create a protein concentration of 10 mg/ml, and the membrane proteins were solubilized with digitonin (4 g/g) and incubated for 10 min in ice. After 30 min of centrifugation at 13,000 × g (4 °C), the supernatants were collected, and 3 µL of 5% Brilliant Blue G dye prepared in 1 M aminocaproic acid was added. Mitochondrial proteins (100 µg) were then loaded and run on a 3–13% gradient native gel as previously described [18]. After electrophoresis, the complexes were electroblotted onto PVDF membranes and sequentially tested with specific antibodies against CI, anti-NDUFA9 (Abcam, ab14713), CIII, anti-ubiquinol-cytochrome c reductase core protein I (Abcam, ab110252) and Vdac1 (Abcam, ab14734).

2.4. Lipid peroxidation in the plasma and mitochondria

The concentration of hydroperoxides (HPx), a specific and direct biomarker of lipid peroxidation, was determined with a Sigma PD1 kit (St Louis, MO, USA). The absorbance changes at 560 nm were monitored by spectrophotometry.

Blood obtained from the bleeding procedure was centrifuged for 10 min at 3000 rpm to isolate the plasma. Then, 40 µL of plasma was used for the quantification of the HPx concentration in plasma. A total of 100 µg of protein from the mitochondrial fraction was used to determine the mitochondrial concentration of HPx.

2.5. Protein carbonyls in the plasma and mitochondria

The formation of protein carbonyl adducts, a marker of oxidative stress [24], was used as an index of oxidative modifications of mitochondrial and plasma proteins. Protein carbonyls were measured in duplicate using an ELISA-based assay according to manufacturer's instructions (OxiSelect Protein Carbonyl ELISA Kit; Cell Biolabs Inc., San Diego, USA).

2.6. Statistics

The statistical analyses were performed using SPSS (version 22 for Windows; IBM Corp., Armonk, NY, USA). The data are presented as the means ± the standard errors of the means (SEM), and $p < 0.05$ was considered significant. Unpaired *t*-tests were used to analyse differences between the exercised and sedentary rats. Pearson correlation analyses