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Quercetin supplementation does not enhance cerebellar mitochondrial biogenesis and oxidative status in exercised rats

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ABSTRACT

The present study tested the hypothesis that quercetin may inhibit the mitochondrial and antioxidant adaptations induced by exercise in cerebellar tissue. Thirty-five 6-week-old Wistar rats were randomly allocated into the following groups: quercetin, exercised (Q-Ex; n = 9); quercetin, sedentary (Q-Sed; n = 9); no quercetin, exercised (NQ-Ex; n = 9); and no quercetin, sedentary (NQ-Sed; n = 8). After 6 weeks of quercetin supplementation and/or exercise training, cerebellums were collected. Protein carbonyl content (PCC), sirtuin 1, peroxisome proliferator-activated receptor γ coactivator 1α (*PGC-1α*), messenger RNA levels, citrate synthase (CS), and mitochondrial DNA were measured. When Q-Sed was compared with NQ-Sed, PCC ($P < .005$) showed decreased levels, whereas *PGC-1α*, sirtuin 1 (both, $P < .01$), mitochondrial DNA ($P < .001$), and CS ($P < .01$) increased. However, when Q-Ex was compared with Q-Sed, PCC showed increased levels ($P < .001$), whereas CS decreased ($P < .01$). Furthermore, the NQ-Ex group experienced an increase in *PGC-1α* messenger RNA levels in comparison with NQ-Sed ($P > .01$). This effect, however, did not appear in Q-Ex ($P < .05$). Therefore, we must hypothesize that either the dose (25 mg/kg) or the length of the quercetin supplementation period that was used in the present study (or perhaps both) may impair exercise-induced adaptations in cerebellar tissue.

1. Introduction

Quercetin (3,3,4,5,7-pentahydroxyflavone) is a natural polyphenolic flavonoid that is present in large amounts in onions, garlic, leeks, cabbages, apples, blueberries, tea, and red wine [1]. Despite having quite slow absorption rates [2], quercetin is thought to be significantly accumulated in the lungs, the liver, and the kidneys but can be found in most body tissues [3,4].

Abbreviations: CAT, catalase activity; CS, citrate synthase; mtDNA, mitochondrial DNA; PCC, protein carbonyl content; PGC-1α, peroxisome proliferator–activated receptor γ coactivator 1α; ROS, reactive oxygen species; SIRT1, sirtuin 1; SOD, superoxide dismutase activity; TBARS, thiobarbituric acid reactive substances.

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In the tissues, quercetin exerts some of its biological properties by increasing the transcription of sirtuin 1 (*SIRT1*) and peroxisome proliferator–activated receptor γ coactivator 1α (*PGC-1α*) [5]. Currently, PGC-1α is described in the literature as the master regulator of mitochondrial biogenesis. It coactivates and augments the expression and activity of several transcription factors, which in turn bind the promoters of distinct sets of nuclear-encoded mitochondrial genes [6–8]. In terms of energy/nutrient stress, such as exercise, posttranscriptional activation of PGC-1α is mainly induced by SIRT1, a metabolic sensor that is regulated by NAD+, which in turn induces PGC-1α activation by deacetylation [9–11]. Polyphenolic compounds activate mitochondrial biogenesis by transcriptional regulation of *SIRT1* and *PGC-1α*, which occurs in the brain and in skeletal muscle [5,12,13].

These effects seem to mimic those brought about by exercise in the mitochondrial content of skeletal muscle [8]. However, after a 6-week test of quercetin supplementation on exercised rats, mitochondrial content in skeletal muscle was compromised in response to lower *SIRT1* transcription [14]. Furthermore, the brains of rats that consumed quercetin while exercising showed lower mitochondrial content as a result of the ablation of the *SIRT1–PGC-1α* axis [15]. Furthermore, oxidative damage affected protein structures in the tissues of exercised rats [14,15]. Boots et al [16] described that metabolites (as a result of their antioxidant activity) changed to pro-oxidant agents, which in turn attacked protein structures [16,17]. This paradox might be responsible for cellular and systemic inadaptation to exercise that is observed when quercetin is supplemented during exercise [18].

Exercise improves oxidative status [19] and mitochondrial content [20] in cerebellar tissue. However, quercetin can exert powerful oxidative damage in the cerebellum [21]. Mitochondrial dysfunction and oxidative stress are present in ataxia [22], and the cerebellum plays a primary role in ataxia [23]. It is therefore crucial to determine whether people should be discouraged from consuming isolated quercetin during exercise. Therefore, our research hypothesis is that quercetin may inhibit mitochondrial and antioxidant adaptations caused by exercise in cerebellar tissue. For that purpose, we analyzed mitochondrial content, oxidative stress, and the transcription of SIRT1 and PGC-1α in the cerebellum of rats that were subjected to an exercise regime and concomitantly supplemented with quercetin.

2. Methods and materials

2.1. Animals

This study was performed on male Wistar rats (6 weeks old). The animals were maintained for 8 weeks in individual cages under standard light and temperature conditions. They were allowed ad libitum access to food (Harlan, Indianapolis, IN, USA 2014; maintenance chow) and water. All animals were cared for in accordance with the *Guide for the Care and Use of Experimental Animals*, and all experiments were approved by the committee of ethics of the University of Jaén (Spain).

2.2. Exercise and supplementation

Rats were randomly assigned to quercetin (Q; n = 17) and non-quercetin (NQ; n = 16) supplementation groups. Both groups were further divided into Q-exercised (Q-EX; n = 9), Q-sedentary (Q-Sed; n = 8), NQ-exercised (NQ-EX; n = 8), and NQ-sedentary (NQ-Sed; n = 8). The animals were acclimated to the experimental conditions, as well as to the treadmill, for 2 weeks. Treadmill training took place 5 days a week for 6 weeks (Panlab Treadmills, Hollistion, MA, USA for 5 rats LE 8710R). The rats ran at a constant speed of 44 cm/s and at a 10° angle. The rats ran for 20 minutes on each of the first 2 days and for 25 minutes on the third day. The duration of the training was increased by 5 minutes every 2 days. The animals ran for 80 minutes on the last day of the fifth week and also throughout the last week of training [14]. The rats in the quercetin groups were supplemented via gavage (QU995; Quercegen Pharma, Newton, MA, USA) on alternate days during the experimental period. Quercetin was administered at 8:00 AM in the morning, when the 12-hour dark cycle began and also 2 hours before exercise. A dose of 25 mg/kg of quercetin was diluted in a 1% solution of methylcellulose. This dose has been described in the literature as able to promote mitochondrial biogenesis in the brain [5]. Twice a week, rats were weighed to adjust the quercetin dosage. The no-quercetin groups were also supplemented using the gavage procedure with the vehicle (1% solution of methylcellulose).

2.3. Tissue collection

All rats were anesthetized with pentobarbital and were then bled by cannulation of the aorta 48 hours after the final exercise. We opted for a fresh dissection of the cerebellum. Once collected, the cerebellum was rinsed in saline solution, frozen in liquid nitrogen, and stored at –80°C for later analysis.

2.4. Quantitative real-time polymerase chain reaction

Gene expression of different genes, *PGC-1α* and NAD(+)-dependent histone deacetylases (*SIRT1*), was quantitatively assessed by real-time polymerase chain reaction (PCR) using β-actin as the normalizing gene. Total RNA was isolated from cell extracts using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After treatment with DNase, complementary DNA was synthesized from 1.5 μg of total RNA using reverse transcriptase (SuperscriptTM III RT; Invitrogen) with oligo-(dT) 15 primers (Promega, Fitchburg, WI, USA). Real-time PCR was performed on the Stratagene MxPro 3005P qPCR system using the Brilliant II SyBR Green QPCR Master Mix (Stratagene, La Jolla, CA, USA). The following primer pairs were used: *PGC-1α*, 5′-GCGGACAGAA CTGAGAGACC-3′ and 5′-CGACCTGCGTAAAGTATATCCA-3′; *SIRT1*, 5′-CCTGACTTCAGATCAAGAGATGGTA-3′ and 5′-CTGATTAAAAATA TCTCCACGAACAG-3′; and β-actin, 5′-CTTAGAAGCATTTGCGGTG CCGATG-3′ and 5′-TCATGAAGTGTGACGTTGCATCCGT-3′. Experiments were performed in triplicate, and relative quantities of the target genes corrected with the normalizing gene, β-actin, were calculated using Stratagene MxProTM QPCR Software. Quantification of messenger RNA (mRNA) expression of *PGC-1α* and *SIRT1* was calculated using the ΔΔCT method as previously described [24].

2.5. Mitochondrial DNA quantification

DNA (mitochondrial and nuclear) was extracted from cerebellar samples using a QIAamp DNA minikit (Qiagen, Chatworth, CA, USA), and the concentration of each sample was spectrophotometrically determined at 260 nm. Real-time PCR was performed