Eur J Nutr (2012) 51:791–799
DOI 10.1007/s00394-011-0257-5

ORIGINAL CONTRIBUTION

Coenzyme Q_{10} supplementation ameliorates inflammatory signaling and oxidative stress associated with strenuous exercise

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Received: 5 July 2011 / Accepted: 30 September 2011 / Published online: 12 October 2011

Abstract
Background Exhausting exercise induces muscle damage associated with high production of free radicals and pro-inflammatory mediators.
Aim The objective of this study was to determine for the first time and simultaneously whether oral coenzyme Q_{10} (CoQ_{10}) supplementation can prevent over-expression of inflammatory mediators and oxidative stress associated with strenuous exercise.
Methods The participants were classified in two groups: CoQ_{10} group (CG) and placebo group (PG). The physical test consisted in a constant run (50 km) that combined several degrees of high effort (mountain run and ultra-endurance), in permanent climbing.
Results Exercise was associated with an increase in TNF-α, IL-6, 8-hydroxy-2′-deoxyguanosine (8-OHdG), and isoprostane levels, revealing the degree of inflammation and oxidative stress induced. Oral supplementation of CoQ_{10} during exercise was efficient reducing oxidative stress (decreased membrane hydroperoxides, 8-OHdG and isoprostanes generation, increased catalase, and total antioxidant status), which would lead to the maintenance of the cell integrity. Data obtained also indicate that CoQ_{10} prevents over-expression of TNF-α after exercise, together with an increase in sTNF-RII that limits the pro-inflammatory actions of TNF. Moreover, CoQ_{10} supplementation reduced creatinine production.
Conclusions CoQ_{10} supplementation before strenuous exercise decreases the oxidative stress and modulates the inflammatory signaling, reducing the subsequent muscle damage.

Keywords High-intensity (strenuous) exercise · Coenzyme Q_{10} · Oxidative damage · Inflammation

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Introduction

The beneficial effects of regular, non-exhaustive physical exercise have been known for a long time. The regular exercise is associated with diverse health benefits such as reduced threat of cardiovascular disease, cancer diabetes, and in general with a lower risk of all-cause of mortality [1]. However, these beneficial effects are lost with strenuous exercise [2]. In recent years, strenuous physical sports such as the ultra-marathon, cross-country running, and iron man triathlon are becoming increasingly popular around the world [3]. This type of exercise causes structural damage to muscle cells indicated by muscle soreness and swelling, prolonged loss of muscle function, and leakage of muscle proteins into circulation, among other effects [4].

The strenuous exercise-related muscle damage has been associated with a high degree of oxidative aggression and an increase in the pro-inflammatory mediators [4, 5]. Therefore, some of this damage may be prevented by optimizing nutrition, particularly by increasing the dietary content of nutritional antioxidants [6].

The increase in the rate of free radical production associated with strenuous exercise can be due to many factors such as an increase in body temperature [7] or increase in whole body oxygen consumption [8], among others. In this sense, increased oxygen consumption in muscle fibers lowers intracellular oxygen tension during exercise [9], which increased reactive oxygen species (ROS) production [10]. In addition, during exercise, many tissues can produce reactive oxygen species such as muscle, heart, lungs, or blood [5, 11]. Mitochondria is often cited as the predominant source of ROS in muscle cells [12], and many authors have reiterated early reports that 2–5% of the total oxygen consumed by mitochondria may undergo one-electron reduction with the generation of superoxide, fact that implies a 50- to 100-fold increase in superoxide generation by skeletal muscle during aerobic contractions [13]. Finally, together to this increase in free radical production associated with strenuous exercise, there is an over-expression of inflammatory cytokines such as IL-1, IL-6, and TNF-α, among others [4, 14].

Given the importance of oxidative stress, inflammation, and muscle damage associated with high-intensity exercise, it would be interesting to assess the effect of oral supplementation with an antioxidant substance capable of diminishing muscle aggression, ROS generation, and inflammatory signaling associated with this performance. Coenzyme Q_{10} (CoQ_{10}), an obligatory component of the mitochondrial electron transport chain is essential for ATP generation, particularly in cells with high metabolic demand such as muscle cells during high-intensity exercise. CoQ_{10} acts as a redox electron carrier in the mitochondria [15]. This mitochondrial component has been used for many years as a dietary supplement intended to promote good health by trapping free radicals and the interest for this molecule comes from the fact of this role as a redox link in the mitochondrial electron transport chain, where also has important antioxidant properties under lipophilic conditions [16]. The data available have provided a direct link between physical performance and blood and muscle tissue CoQ_{10} levels [17]. However, most of these studies are focused mainly in the exercise performance and radical-scavenging activity of CoQ_{10} during low-intensity exercise [17], being scarce the studies about the influence of CoQ_{10} supplementation during the performance of high-intensity strenuous exercise and inflammatory signaling. Therefore, the purpose of the present study was to determine for the first time and simultaneously whether oral CoQ_{10} supplementation may be efficient ameliorating the oxidative stress and pro-inflammatory effects induced by the strenuous exercise.

Materials and methods

Subjects

The subjects who took part in this study were male amateur athletes, were highly trained and performed regular exercise on a daily basis. All of them had performed the high-intensity exercise test associated with running to The Sierra Nevada from the city of Granada (50 km) in the previous 2 years. All participants were classified in two groups: CoQ_{10} group (CG) and placebo (control) group (PG). The age of the runners was similar in both groups (41.25 $\pm$ 2.84 years in the CG and 39.75 $\pm$ 2.92 years in the PG). Each individual in CG received oral administration of 5 capsules of 30 mg of CoQ_{10} (2,3-Dimethoxy-5-Methyl-6-Decaprenyl benzoquinone) in the following manner: 1 capsule 2 days before the test with the dinner, 3 capsules on the previous day (breakfast, lunch, and dinner), 1 capsule the same day of the run, 1 h before beginning the physical test. The subjects that assigned to the PG took the same number of capsules using the same scheme. The content of the placebo capsules consisted on beer yeast, cellulose, acacia, silica stearic acid, magnesium stearate, cellulose gum, and maltodextrin. We decided to use these capsules as placebo because this was a totally innocuous product, with the same excipients and externally had similar characteristics to the assayed product (the CoQ_{10} capsules). The study was approved by the Regional Ethics Committee, University of Granada. Informed consent was obtained from all subjects with written consent to participate in this study. Once finishing the selection process, both groups were defined as follows: experimental group (10 subjects) receiving a supplement of CoQ_{10} (CG) and control group of 10 runners, receiving a placebo (PG).

Intense physical exercise performance program

The test consisted of a combination between mountain run and ultra-endurance. This physical challenge consisted run to the top of "Pico Veleta" (Sierra Nevada), in a constant run that combined several degrees of high effort. It is considered one of the hardest trial worldwide. The total distance of the test was 50 km across the highest road of Europe with initial altitude of 640 m and final altitude (arrival) of 3,393 (increase of 2,800 m). The run was almost on a continuous incline, with small flat areas at km 17 (1,300 m of altitude), 21 (1,450 m of altitude), and 30 (2,100 m of altitude). The run also involved highly

changing climatic conditions with many temperature oscillations making the run highly stressful. The time spent in the physical test was similar in both groups (5.34 ± 0.31 h in the CG and 5.74 ± 0.29 h in the PG).

Blood and urine sampling

Blood samples were collected from the participants via venous catheter into heparinized tubes before and immediately after the physical test. Blood was immediately centrifuged at 1,750*g* for 10 min at 4 °C in a Beckman GS-6R refrigerated centrifuge (Beckman, Fullerton, CA, USA) to separate plasma from red blood cell pellets. Plasma samples were immediately frozen and stored at −80 °C until analysis. Erythrocyte cytosolic and membrane fractions were prepared by differential centrifugation with hypotonic hemolysis and successive differential centrifugations according to the method of Hanahan and Ekholm [18]. The final fractions were aliquoted, snap-frozen in liquid nitrogen, and stored at −80 °C until analysis.

Urine samples were collected from the participants into sterilized tubes before and immediately after the physical test.

Biochemical parameters

Total bilirubin, total cholesterol, triglycerides, and phospholipids concentration were determined using a commercial Kit (Spinreact, Barcelona, España) and following the instructions of the manufacturer. Creatinine was measured in urine using a commercial colorimetric kit (Oxford Biomedical Research, Oxford, England). The plasmatic viscosity was determined in samples of plasma obtained from 1 mL of heparinized blood, by centrifugation (2,500 rpm, 4 °C, 10 min) and using a Haake falling ball type C viscometer (Thermo Electron, Karlsruhe, Germany).

Inflammatory parameters

Tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), soluble receptor II of TNF-α (sTNF-RII), and interleukin 1 antagonist receptor (IL-1ra) plasma levels were determined using Biosource Kits (Biosource Europe, Nivelles, Belgium). The TNF-α, sTNF-RII, IL-6, and IL-1ra were solid-phase enzyme-amplified sensitivity immunoassays (EASIA) performed on microtiter plate. The assays are based on an oligoclonal system in which a blend of monoclonal antibodies (MAbs) directed against distinct epitopes of TNF-α, sTNF-RII, IL-1ra, and IL-6 are used. Standards or samples containing TNF-α, sTNF-RII, IL-6, and IL-1ra react with capture monoclonal antibodies (MAbs 1) coated on the microtiter well. The microtiter plate is then read at an appropriate wavelength (450–490 nm) on a microplate reader (Bio-tek, Vermont, USA).

Oxidative stress parameters

Plasma total antioxidant status (TAS) was analyzed with the use of the TAS Randox® kit (Randox laboratories, Ltd, Crumlin, UK). Results were expressed in mM of Trolox equivalents. The linearity of calibration extends to 2.5 mmol/L of Trolox. The reference range for human blood plasma is given by the manufacturer as 1.30–1.77 mmol/L. Measurements in duplicate were used to calculate intra-assay variability.

The erythrocyte membrane hydroperoxide content was determined using the method of Jiang et al. [19]. This technique is based on the rapid oxidation of Fe^{2+} to Fe^{3+} by hydroperoxides under acidic conditions. We performed two separated determinations, the first one, referred to as baseline or basal levels of hydroperoxides, was made using the erythrocyte membrane with no further inductions and the second determination, referred as induced hydroperoxides, was developed after the induction of the sample with 2.5 mM of AAPH (2,2′-azobis-2-methyl-propanimidamide dihydrochloride, a potent generator of free radicals). This measure allows determination of the maximal level of peroxides that a membrane is able to produce. Both determinations were measured spectrophotometrically at 560 nm (Perkin Elmer UV-VIS Lambda-16, Norwalk, Conneticut, USA).

Isoprostanes in urine were measured using a commercial kit Enzyme Immunoassay for Urinary Isoprostane (Oxford Biomedical Research, Oxford, England). This kit is a competitive enzyme-linked immunoassay (ELISA) for determining levels of 15-F2t-Isoprostane (the best characterized isoprostane) in urine samples. Urine samples are mixed with an enhanced dilution buffer that essentially eliminates interference due to non-specific binding. The 15-F2t-Isoprostane in the samples or standards competes with 15-F2t-Isoprostane conjugated to horseradish peroxidase (HRP) for binding to a polyclonal antibody specific for 15-F2t-Isoprostane coated on the microplate. The HRP activity results in color development when substrate is added, with the intensity of the color proportional to the amount of 15-F2t-Isoprostane-HRP bound, and inversely proportional to the amount of unconjugated 15-F2t-Isoprostane in the samples or standards.

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is excised during the repair of oxidative damage to deoxyguanosine sites in DNA and has been widely used as a biomarker for oxidative damage. 8-OHdG was determined using a commercial kit (Oxford Biomedical Research, Oxford, England). This competitive ELISA kit employs a monoclonal antibody specific for 8-OHdG quantification in tissue,

serum, plasma, or urine samples. Results are read at 450 nm on a microplate reader (Bio-tek, Vermont, USA).

Glutathione peroxidase (GPx) activity was measured by the method of Flohé and Gunzler [20]. That method is based on the instantaneous formation of oxidized glutathione during the reaction catalyzed by glutathione peroxidase. That oxidized glutathione is continually reduced by an excess of glutathione reductase and NADPH present in the cuvette. The subsequent oxidation of NADPH to $NADP^+$ was monitored spectrophotometrically (Thermo Spectronic, Rochester, USA) at 340 nm. During the reaction, cumene hydroperoxide was used as substrate.

Catalase (CAT) activity was determined following the method described by Aebi [21], monitoring at 240 nm spectrophotometrically (Thermo Spectronic, Rochester, USA) the H_2O_2 decomposition, as a consequence of the catalytic activity of CAT. The activity was calculated from the first-order rate constant K (sec^{-1}).

Statistical analysis

Data were expressed as the mean ± standard error of the mean of 10 athletes per group. A two-tailed Student's t test was used to determine significant differences. Unpaired Student's t test was used to determine differences between the groups (CG vs. PG), and a paired Student's t test was performed to determine differences due to the physical exercise (before vs. after the physical test). A level of $p < 0.05$ was considered to indicate statistical significance. SPSS version 18.0, 2010 (SPSS Inc., Chicago, IL, USA) software has been used for data treatment and statistical analysis.

Results

Diverse modifications in the plasmatic biomarkers of the sportsmen are produced during exercise, some of which are showed in Table 1. Intense physical exercise resulted in an increase in net protein catabolism and an increase in creatinine excretion in the PG after the physical test ($p < 0.001$) (Table 1); however, the urinary levels of creatinine were lower ($p < 0.05$ before and $p < 0.001$ after the physical test) in the CoQ_{10}-treated group. Other interesting result was that although there was an increase in urinary creatinine in the CG, it was lower than in PG (38.77 ± 10.20 vs. 88.23 ± 11.21, $p < 0.05$). We also observed a decrease in the bilirubin concentrations in the CG after the run ($p < 0.001$) with lower values compared to PG group.

With respect to plasmatic lipids studied, there were no statistical significant difference between groups for cholesterol and phospholipids. In both groups, triglycerides show a decrease associated with exercise ($p < 0.01$ in the PG and $p < 0.05$ in the CG); however, the concentration was higher in CG group after the run.

CAT (Fig. 1a) showed a decreased on its activity associated with strenuous exercise in the PG (0.465 ± 0.021 vs. 0.437 ± 0.016, $p < 0.05$) and increased its activity in the CG compared with the PG ($p < 0.05$ before and $p < 0.01$, after the physical performance). In addition, we found a significant increase in the TAS (Fig. 1b) in both CG compared with the PG ($p < 0.001$ before and after the run). Finally, in our study, we did not found significant difference in GPx (Fig. 1c).

As an indicator of the degree of oxidative stress in the erythrocyte membrane, we have utilized the hydroperoxide content. Basal hydroperoxides (Fig. 2a) increased significantly in both groups during exercise (8.75 ± 0.53 vs. 11.76 ± 0.38, $p < 0.001$ in the PG and 7.14 ± 0.53 vs. 9.60 ± 0.37, $p < 0.01$), although these values were lower in both CG groups compared with the PG ($p < 0.05$ before the physical test and $p < 0.001$ after the exercise). In addition, induced hydroperoxides (Fig. 2b) were lower in both CG groups compared with the PG ($p < 0.001$).

In the present study, after physical intense exercise, the levels of isoprostanes increased significantly in CG and PG ($p < 0.001$ for both groups) (Fig. 3a); however, if we compare the CG with the PG after exercise, there is a clear protective effect of coenzyme Q_{10} (5.83 ± 0.46 vs. 3.88 ± 0.35, $p < 0.001$).

8-OHdG (Fig. 3b) showed a similar pattern as isoprostanes, an increase associated with exercise in both groups ($p < 0.001$), although the increase was much lower in the CG (0.0486 ± 0.0064 in the PG vs. 0.0333 ± 0.0040 in the CG); therefore, CoQ_{10} supplement reduced the degree of oxidative stress damage represented as 8-OHdG after exercise in the CG compared with the PG ($p < 0.001$).

In regard to the inflammatory parameters, we found a significant increase in IL-6 levels cytokine associated with exercise in both groups ($p < 0.001$). CoQ_{10} only decreased significantly the levels of IL-6 compared to CG before the run ($p < 0.001$), but the supplementation had no effect on this cytokine after the exercise. IL-1ra increased significantly in both experimental groups after exercise ($p < 0.001$). Totally, different is the effect of this supplementation on the TNF-α pathway. We found a significant rise in the pro-inflammatory cytokine TNF-α, after competition in both groups ($p < 0.001$) (Table 2); however, CoQ_{10} supplement significantly attenuated this overproduction of TNF-α in the sportsmen after the high-intensity exercise compared with the PG ($p < 0.01$), even before the competition ($p < 0.01$). sTNF-RII increased in CG before and after the physical performance ($p < 0.001$) compared with the PG.

Table 1 Effects of exercise and CoQ_{10} supplementation on biochemical parameters in athletes before and after strenuous exercise

	PG before run	PG after run	CG before run	CG after run
Total bilirubin (μmol/L)	37.01 ± 3.34	39.44 ± 3.45**	36.55 ± 2.87*	30.67 ± 2.95
Total cholesterol (mmol/L)	4.45 ± 0.17	4.61 ± 0.24	4.55 ± 0.24	4.66 ± 0.28
Phospholipids (mmol/L)	2.41 ± 0.08	2.12 ± 0.13	2.45 ± 0.12	2.19 ± 0.07
Triglycerides (mmol/L)	1.71 ± 0.09*	1.31 ± 0.09**	1.91 ± 0.14*	1.75 ± 0.07
Plasma viscosity (mg/mL)	1.20 ± 0.01*	1.08 ± 0.05	1.22 ± 0.03*	1.04 ± 0.04
Urine creatinine (mg/dL)	156.96 ± 29.27*·**	244.18 ± 25.73**	115.58 ± 18.82*	154.35 ± 12.18

Values are means ± S.E.M. Number of experiments = 4 with duplicate estimation. * Mean values differ before and after exercise in the same group, $p < 0.05$; ** Mean values differ between groups either before or after exercise, $p < 0.05$

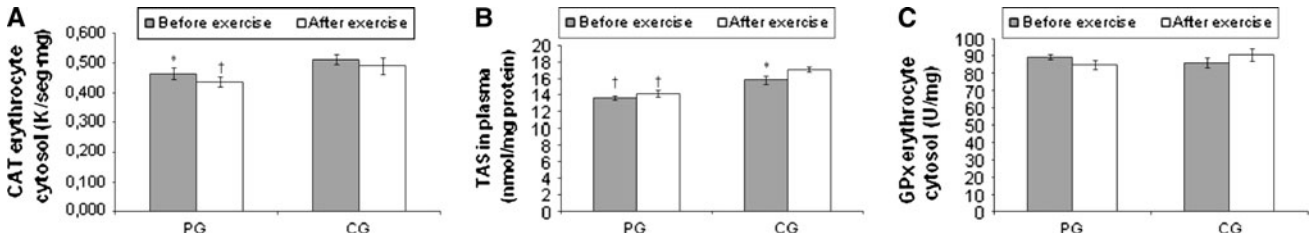

Fig. 1 Effect of exercise and CoQ_{10} supplementation on enzymatic antioxidant status. **a** CAT activity in erythrocyte cytosolic fractions. **b** Total antioxidant status in plasma. **c** GPx activity in erythrocyte cytosolic fractions. Data shown are the mean from 10 subjects and *error bars* show the SEM. Number of experiments = 4 with duplicate estimation (*Mean values differ before and after exercise in the same group, $p < 0.05$; †Mean values differ between groups either before or after exercise, $p < 0.05$)

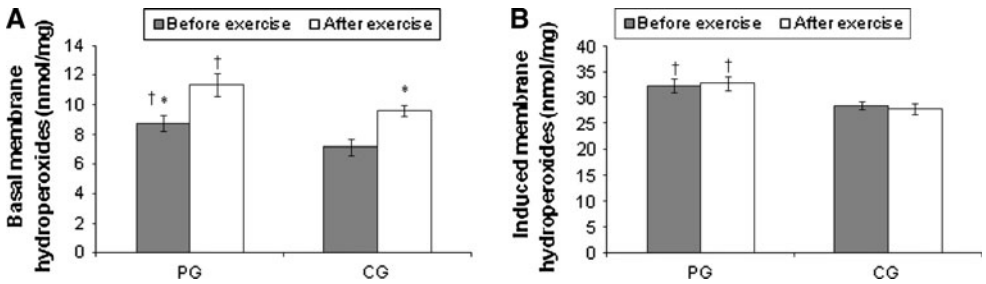

Fig. 2 Effect of exercise and CoQ_{10} supplementation on erythrocyte membrane hydroperoxides production. **a** Basal hydroperoxides. **b** Induced membrane hydroperoxides. Data shown are the mean from 10 subjects and *error bars* show the SEM. Number of experiments = 4 with duplicate estimation (*Mean values differ before and after exercise in the same group, $p < 0.05$; †Mean values differ between groups either before or after exercise, $p < 0.05$)

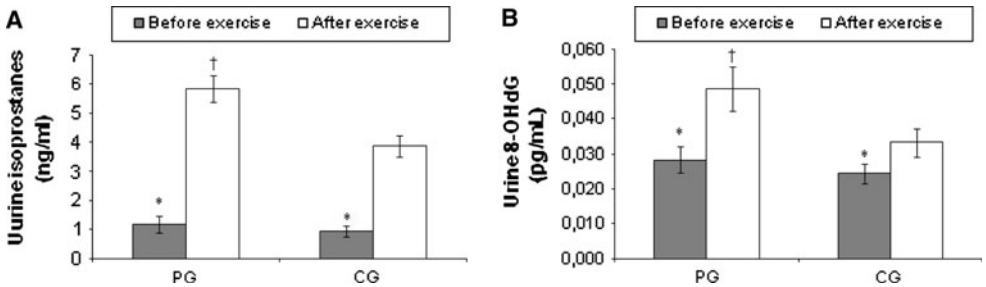

Fig. 3 Effect of exercise and CoQ_{10} supplementation on urine isoprostanes and 8-OHdG concentration. **a** Urine isoprostanes concentration. **b** Urine 8-OHdG concentration. Data shown are the mean from 10 subjects and *error bars* show the SEM. Number of experiments = 4 with duplicate (*Mean values differ before and after exercise in the same group, $p < 0.05$; †Mean values differ between groups either before or after exercise, $p < 0.05$)

Table 2 Effects of exercise and CoQ_{10} supplementation on the inflammatory parameters in athletes before and after strenuous exercise

	PG before run	PG after run	CG before run	CG after run
IL-6 (pg/mL)	5.11 ± 0.73*·**	39.64 ± 5.99	3.47 ± 0.80*	40.86 ± 5.64
TNF-α (pg/mL)	21.93 ± 1.70*·**	28.16 ± 1.76**	16.98 ± 1.36*	21.86 ± 1.70
IL-1ra (pg/mL)	37.99 ± 4.23*	364.73 ± 47.27	33.75 ± 4.57*	318.34 ± 47.50
sTNF-RII (ng/mL)	3.65 ± 0.38**	4.15 ± 0.21**	5.31 ± 0.53	5.07 ± 0.38

Values are means ± S.E.M. Number of experiments = 4 with duplicate estimation. * Mean values differ before and after exercise in the same group, $p < 0.05$; ** Mean values differ between groups either before or after exercise, $p < 0.05$

Discussion

Numerous health benefits including a lower risk of all-cause mortality, reduced threat of cardiovascular disease, cancer, and diabetes are associated with regular exercise [1]. Nevertheless, strenuous exercise especially in amateur athletes promotes oxidative stress together with an inflammatory process, which is the reason for the muscular aggression observed in this type of high-intensity exercise [4, 5]. The administration of substances able to reduce these components will decrease the muscular damage and therefore they will be beneficial for these sportsmen, being the CoQ_{10} a perfect candidate, due to its well known antioxidant and anti-inflammatory effects [15, 16].

Intense physical exercise resulted in an increase in net protein catabolism and an increase in creatinine excretion [22]. In the CoQ_{10}-treated group, creatinine levels were lower, fact that is in agreement with the results reported by Fouad et al. [23].

Plasmatic cholesterol and phospholipids did not show significant differences; however, in both groups, triglycerides show a decrease associated with exercise, being the concentrations higher in CG group after the run. High levels of triglycerides during exercise improve skeletal muscle activity and exercise capacity [24], which could indicate potential ergogenic effect of CoQ_{10}.

With respect to the behavior of antioxidant system, we have observed that CAT, the hydrogen peroxide removing enzyme, increased its activity in the CG compared with the PG, indicating enhanced antioxidant capacity induced by the CoQ_{10} supplement. Increased CAT activity has been associated with greater resistance to oxidative damage [25]. Also, we have found a similar increase in CAT activity after CoQ_{10} supplementation although in rodents and with a different situation of oxidative stress generation [16, 26]. In addition, we found a significant increase in the TAS in both CG compared with the PG. These results in the TAS are due to the supplementation with an antioxidant substance such as CoQ_{10}, which increases its concentration in plasma as it has been shown in other studies [16]. In addition, we have to take into account that CoQ_{10} inhibits the expression of different sources of free radicals [27–29], and therefore, it could increase the antioxidant system in the body because their lower utilization.

In our study, we did not found significant difference in GPx. In other studies, though performed in animal models and under different situations of oxidative aggression, the supplementation with CoQ_{10} shows a clear effect on the activity of the enzyme CAT and almost absence of effect on the activity of the GPx [16, 26].

As an indicator of the degree of oxidative stress in the erythrocyte membrane, we have measured the hydroperoxide content. Basal hydroperoxides were lower in both CG groups compared with the PG. This result is in agreement with those reporting that CoQ_{10} has a protective effect against an excessive reduction in mitochondrial membrane phospholipids during prolonged exercise [30]. This result can be due to two reasons, firstly, the major content of this antioxidant in these membranes as a consequence of the supplementation as it has been observed in other studies [16, 26] and would explain also the lower antioxidant production before the physical test, because this would act as an essential antioxidant and assisting in the regeneration of other antioxidants [31]. On the other hand, there would be a lower free radicals production during the run in the CoQ_{10} supplemented group [27, 28], as it has been previously mentioned. The second measurement performed (Fig. 2b) involves the induction of an oxidative aggression by means of AAPH, which is an azo compound used extensively as a free radical generator, often in the study of lipid peroxidation and the characterization of antioxidants [19]. This measurement shows a clear higher antioxidant defence in the erythrocyte membranes of the CoQ_{10} supplemented group, fact that is of great importance, since it shows that under the same conditions, the erythrocyte membranes of the supplemented group are capable of resisting better an oxidative aggression, and therefore, they would present a major resistance to the hemolysis induced by the free radicals.

Isoprostanes are prostaglandin-like compounds that are produced by free radical-mediated peroxidation of polyunsaturated fatty acids. There is a direct evidence showing that isoprostanes are as an in vivo marker of lipid peroxidation due to the mechanism of their formation (oxidation

of arachidonic acid) [32]. After physical intense exercise, the levels of isoprostanes increased significantly in CG and PG, due to high generation of free radical and ROS during exercise [5, 11]. However, if we compare the CG with the PG after exercise, there is a clear protective effect of coenzyme Q_{10}. This latter result indicates that free radical-induced lipid peroxidation caused by the exercise is diminished, at least in part, by the CoQ_{10} supplement.

8-OHdG is a sensitive indicator of DNA damage as a result of oxidative stress. We observed that CoQ_{10} supplement reduced the degree of oxidative stress damage represented as 8-OHdG after exercise in the CG. Again, our results reveal the high capacity of CoQ_{10} to reduce oxidative stress during strenuous exercise, in this case by protecting the DNA. Bearing in mind that CoQ_{10} is a naturally occurring hydrophobic compound that is not only a critical component of the mitochondrial respiratory chain, but also a powerful antioxidant. CoQ_{10} inhibits the expression of NADPH oxidase [27], which is the one of the main sources of ROS [28], and scavenges lipid peroxidation products during free radical reactions [29]. CoQ_{10} also suppresses excess nitric oxide production and prevents nitrative tissue stress [33], explaining the reduced degree of DNA damage after exercise in the CG.

As it has been mentioned previously, acute exercise increases oxidative stress, especially when the exercise intensity is high, fact that can be correlated with the over-expression of inflammatory cytokines such as IL-1 and IL-6, TNF-α, and C-reactive protein [14]. It has been shown that exogenous administration of CoQ_{10} leads to a remarkable decline in oxidative stress because of its ability to scavenge hydroxyl radicals and pro-inflammatory cytokines [29, 34]. According to this, our results showed a reduction in the oxidative stress parameters and a modulation of inflammatory signaling.

Plasma IL-6 increases in an exponential fashion with exercise and is related to exercise intensity, duration, mass of muscle recruited, and endurance capacity [35]. We found a significant increase in IL-6 levels cytokine associated with exercise in both groups. CoQ_{10} only was able to decrease the levels of IL-6 before the run. This lack of effect on IL-6 levels is in accordance with that found for other authors after supplementation with coenzyme Q_{10} but in different conditions [36, 37]. Other cytokine studied is the IL-1ra, the natural IL-1 antagonist, which has been shown to play a crucial role in the prevention of inflammatory diseases [38]. This cytokine increased significantly in both experimental groups after exercise. According to Lancaster [39], the increase in IL-1ra concentration is similar in magnitude to that of IL-6, results that coincide with the findings of the current study. CoQ_{10} supplementation did not have any effect on this cytokine, which has been observed before although in different conditions [36].

Totally, different is the effect of this supplementation on the TNF-α pathway. We found a significant rise in the pro-inflammatory cytokine TNF-α, after high-intensity exercise in both groups (Table 2), which is in agreement with previous results [4]. However, CoQ_{10} supplement significantly attenuated this overproduction of TNF-α in the sportsmen after the high-intensity exercise compared with the PG, even before the competition. TNF-α seems to have a biphasic effect on muscle: high levels of the cytokine promote muscle catabolism, probably by a NF-kB-mediated effect, whereas low levels of TNF-α, which do not induce NF-kB, stimulate myogenesis [40]. Therefore, TNF-α can be associated with muscle regeneration [41], normally produced by muscle cells, or inhibits myogenesis by activating NF-kB. In addition, this cytokine is known to inhibit contractile function of skeletal muscle, and it may be related to NO production [42] and increased mitochondrial production of ROS that in turn regulate TNF-α/NF-kB signaling. It could be stated that a possible mechanism of protective effect of coenzyme Q_{10} on TNF-α levels can be attributed to its ability to inhibit the activation of nuclear factor-κB signaling pathway that promotes the transcription of NADPH oxidase, TNF-α, and inducible nitric oxide synthase genes [34, 43], although we need to deepen more in these mechanisms.

Other potential mechanism that could be postulated is due to its influence on sTNF-RII. This anti-inflammatory cytokine increased in CG before and after the physical performance compared with the PG. sTNF-RII over-expression limits the detrimental, pro-inflammatory effects of TNF as it has been postulated that, by sequestering TNF, the soluble form of TNF-RII limits TNF availability and binding to TNF-RI, the receptor subtype that mediates the classic pro-inflammatory activities of the cytokine [44]. In support of this mechanism, a recent study has shown that, through sTNF-RII shedding, regulatory T cells inhibit TNF activity both in vitro and in vivo [45]. Also, sTNFR-II signaling exerts neuroprotective and anti-inflammatory functions [46], actives the immunosuppressive IL-10 pathway, and inhibits significantly the effects of several proinflammatory cytokines [47].

In summary, the present study demonstrates a strong correlation between the high-intensity exercise and increased free radical damage as shown by the elevation in hydroperoxides in erythrocyte membrane and isoprostanes and 8-OHdG in urine, and the over-expression in the pro-inflammatory cytokines. In addition, the present findings provide evidence that oral supplementation of CoQ_{10} during high-intensity exercise is efficient reducing the degree of oxidative stress (decrease membrane hydroperoxides, 8-OHdG, and isoprostanes generation with a recovery of antioxidative defense), which would lead to the maintenance of the cell integrity. CoQ_{10} supplementation reduces

creatinine excretion and therefore decrease muscle damage during physical performance. Also, CoQ_{10} administration can modulate the inflammatory signaling associate with exercise by preventing over-expression of TNF-α after the exercise, together with an increase in the sTNF-RII that limits the detrimental, pro-inflammatory actions of TNF. Therefore, the knowledge gained from these findings will provide a foundation for similar CoQ_{10} supplement therapies in athletes performing strenuous exercise in order to reduce the undesirable effects of the evoked oxidative stress and inflammation signaling during high-intensity exercise and reduce the muscle damage induced.

Acknowledgments The authors are grateful to the University of Granada for the personal support of J. Díaz-Castro.

Conflict of interest The authors have declared that no conflict of interest exists.

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