EXPERIMENTAL STUDY

Effects of CoQ10 supplementation and swimming training on exhaustive exercise-induced oxidative stress in rat heart

Okudan N1, Revan S2, Balci SS3, Belviranli M1, Pepe H2, Gökbel H1

Department of Physiology, Meram Faculty of Medicine, Selçuk University, Meram, Konya, Turkey.
nokudan@selcuk.edu.tr

Abstract: This study examined the combined effects of swimming training and coenzyme Q10 (CoQ10) supplementation on exhaustive exercise-induced oxidative stress in rat heart. The study was carried out with 4-month-old young adult male Wistar rats. Sixty four rats were divided mainly into two groups: trained and control. Each group was further divided into four subgroups: rest, exhausted, rest with CoQ10, exhausted with CoQ10. The training program consisted of swimming one hour each day, five days a week, for six weeks. At the end of sixth week, rats in exhausted exercise group were forced to swim until exhaustion and then they were immediately sacrificed, while rats in rest group were sacrificed at rest. Training alone or in combination with CoQ10 supplementation reduced to increasing MDA levels due to exhaustive exercise in rat heart (p<0.05). The trained-rest with CoQ10 group showed lower 8-OHdG levels than the control-rest with CoQ10 group. Exhaustive exercise effect was significant on SOD activity. Exhaustive exercise increased GSH levels in control groups while decreased GSH levels in training groups (p<0.05). In conclusion, the results suggest that CoQ10 supplementation combined with training may inhibit lipid peroxidation and DNA damage in the heart tissue. Also, it can be said that SOD activity and GSH levels were not influenced by CoQ10 supplementation (Fig. 4, Tab. 1, Ref. 69). Full Text in PDF www.elis.sk.

Key words: CoQ10, swimming training, oxidative stress.

Regular physical activity is associated with enhanced health and reduced risk of all-cause mortality such as cardiovascular disease, hypertension, type 2 diabetes, obesity, osteoporosis, sarcopenia, cognitive disorders, and some forms of cancer (1). However, it is well accepted that acute physical exercise increases oxygen uptake and free-radical production, and consequently could induce oxidative stress and oxidative damage associated with the type, frequency, intensity and duration of exercise (2, 3). Acute strenuous exercise may pose a risk to the heart by generating oxidative stress, while chronic exercise may beneficially influence cardiac antioxidant defenses and support overall cardiac function (4).

Cells continuously produce free radicals and reactive oxygen species (ROS) as part of metabolic processes (5, 6). ROS play a key role in modulating changes in gene expression and cell function, and act through the key signalling and response systems of cells (7). Overproduction of ROS, most frequently either by excessive stimulation of NAD(P)H by cytokines, or by the mitochondrial electron transport chain and xanthine oxidase result in oxidative stress. Oxidative stress is a deleterious process that can be an important mediator of damage to cell structures and consequently various disease states and ageing (8). The harmful effect of oxidative stress is counteracted by the antioxidant action of both antioxidant enzymes and non-enzymatic antioxidants (9). Antioxidant enzymes include superoxide dismutase, glutathione peroxidase and catalase. Non-enzymatic antioxidants include vitamins E and C, β-carotene, glutathione and coenzyme Q10 (ubiquinone, CoQ10) (10).

CoQ10 is a lipid-soluble component of virtually all cell membranes and has multiple metabolic functions (11). The most well-known function of CoQ10 is that it is an essential carrier for the electron transfer in the mitochondrial respiratory chain for ATP generation (12, 13). Another important function of CoQ10 is as a lipophilic antioxidant (14–16). In addition, CoQ10 has an important role in cell signalling and gene expression (17, 18). Supplementation of CoQ10 has also been indicated to increase aerobic or anaerobic capacity (19, 20) and might prevent unfavorable conditions as a result of physical fatigue (21). Besides, it has been used for the treatment of cardiovascular diseases such as congestive heart failure (22) and atherosclerosis (23), cancer (24), diabetes (25), hypertension (26) and neurodegenerative diseases (27, 28).

However, little is known about the effects of CoQ10 and chronic exercise training on acute exhaustive exercise-induced oxidative stress. Thus, we aimed to investigate the effect of regular swimming exercise and CoQ10 supplementation on the levels of MDA, a marker of lipid peroxidation; 8-OHdG levels, a marker of DNA damage; the levels of GSH, an endogenous antioxidant and SOD activity, an antioxidant enzyme after acute exhaustive exercise.

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Materials and methods

Animals

Sixty four young adult male Wistar rats (4 month old) were used in the study and were cared for according to the guiding Principles for the Care and Use of Animals based upon the Helsinki Declaration, 1964. All procedures were approved by the Selcuk University Experimental Animal Medicine Research and Application Center Experimental Animal Ethics Committee. During the study, all rats were housed in polycarbonate cages, four rats per cage, in a temperature-controlled room (23 °C) with a 12:12-h light-dark cycle and were given standard rat chow and water ad libitum.

Exercise procedures

The animals were divided mainly into two groups: trained (T) and control (C). Each group was further divided into four subgroups: trained-rest (TR, n=8), trained-exhausted (TE, n=8), trained-rest with CoQ10 (TR+CoQ10, n=8), trained-exhausted with CoQ10 (TE+CoQ10, n=8), control-rest (CR, n=8), control-exhausted (CE, n=8), control-rest with CoQ10 (CR+CoQ10, n=8) and control-exhausted with CoQ10 (CE+CoQ10, n=8). Swimming protocol was adapted from the protocol developed by Kwon et al. (29). The exercise groups were adapted to the swimming training for five days (20 min/day). After this period of familiarization to exercise, the rats in the trained groups underwent an exercise protocol of five days per week for six weeks, whereas the control groups did not exercise. Swimming was performed in a container (length 100 cm, width 50 cm, depth 50 cm) containing water maintained at 32-34°C and eight rats were allowed to swim together. CoQ10 (Sigma-Aldrich; Cat. No. C-9538, St. Louis, MO, USA) was intraperitoneally applied daily with the ratio of 10 mg/kg/body weight in the CoQ10 treated rats for six weeks. At the end of six weeks training period, the acute bout of exhaustive exercise was performed 48 hours after the last exercise session. In exhaustive swimming groups (TE, TE+CoQ10, CE, CE+CoQ10), the rats were forced to swim until exhaustion and then were sacrificed by heart puncture under ether anesthesia. Exhaustion was determined by the inability of the rat to remain at the surface of water more than 10 seconds (30). The other rest groups were sacrificed 48 hours after the last exercise session and/or last CoQ10 administration.

Tissue preparation and biochemical analysis

Heart tissues were homogenized in 10 volumes of ice-cold Tris-HCl buffer (50 mmol/L, pH 7.4) using a homogenizer (Wise Mix HG-15, Daian Scientific, Seoul, Korea) after cutting the organs into small pieces. Levels of 8-OHdG and GSH and SOD activities were determined in this homogenate. Some of the homogenate was centrifuged and its supernatant was separated. The supernatant solution was extracted with an equal volume of an ethanol/chloroform mixture (5/3, volume per volume [v/v]). After centrifugation at 5000 g for 30 min, the upper layer (the ethanal phase) was used in the protein assays. Lipid peroxidation was estimated using a commercially available kit according to the manufacturer’s recommendations and MDA as a standard (TBARS kit; Cat. #10009055, Cayman Chemical Co., Ann Arbor, MI, USA). Absorbance was measured at 530 nm. GSH was determined by a Cayman’s GSH assay kit (Cat. #703002, Cayman Chemical Co., Ann Arbor, MI, USA) using an enzymatic recycling method. Briefly, GSH reacts with 5,5-dithio-bis-2-nitrobenzoic acid (Ellman’s reagent) using the sulphydryl group and produces a yellow-colored 5-thio-2-nitrobenzoic acid (TNB). The mixed disulfide, GSTNB (between GSH and TNB), which is concomitantly produced, is reduced by glutathione reductase to recycle the GSH and produce more TNB. The rate of TNB production is directly proportional to this recycling reaction, which in turn is directly proportional to the concentration

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Tab. 1. The effects of swimming training, coenzyme Q10 supplementation and acute exhaustive exercise on plasma levels of MDA, 8-OH-dG, GSH and SOD activity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Training</th>
<th>CoQ10 ST</th>
<th>EE</th>
<th>CoQ10*ST</th>
<th>CoQ10*EE</th>
<th>ST*EE</th>
<th>CoQ10<em>ST</em>EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (μM/g tissue)</td>
<td>R</td>
<td>E</td>
<td></td>
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<tr>
<td>R</td>
<td>0.65 ± 0.07</td>
<td>1.05 ± 0.05</td>
<td>0.71</td>
<td>0.25</td>
<td>2.68</td>
<td>0.10</td>
<td>0.39</td>
<td>23.10</td>
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<tr>
<td>R+CoQ10</td>
<td>0.94 ± 0.05</td>
<td>0.95 ± 0.02</td>
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<tr>
<td>E</td>
<td>1.02 ± 0.07</td>
<td>0.61 ± 0.03</td>
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<tr>
<td>E+CoQ10</td>
<td>0.84 ± 0.04</td>
<td>0.89 ± 0.10</td>
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<tr>
<td>8-OH-dG (pg/g tissue)</td>
<td>CR</td>
<td>CE</td>
<td></td>
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<tr>
<td>CR</td>
<td>58.20 ± 4.02</td>
<td>69.80 ± 1.48</td>
<td>2.85</td>
<td>0.00</td>
<td>4.38</td>
<td>4.63</td>
<td>0.05</td>
<td>1.59</td>
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<tr>
<td>CR+CoQ10</td>
<td>62.92 ± 3.25</td>
<td>56.85 ± 2.06</td>
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<tr>
<td>CE+CoQ10</td>
<td>66.35 ± 3.26</td>
<td>63.37 ± 1.02</td>
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<tr>
<td>GSH (μM/g tissue)</td>
<td>CR</td>
<td>CE</td>
<td></td>
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<tr>
<td>CR</td>
<td>1.87 ± 0.17</td>
<td>2.19 ± 0.09</td>
<td>0.01</td>
<td>0.01</td>
<td>0.11</td>
<td>0.08</td>
<td>0.05</td>
<td>6.66</td>
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<tr>
<td>CR+CoQ10</td>
<td>2.08 ± 0.12</td>
<td>2.09 ± 0.08</td>
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<tr>
<td>CE+CoQ10</td>
<td>2.09 ± 0.05</td>
<td>2.02 ± 0.10</td>
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<tr>
<td>SOD (U/mg protein)</td>
<td>CR</td>
<td>CE</td>
<td></td>
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<td></td>
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<tr>
<td>CR</td>
<td>0.26 ± 0.06</td>
<td>0.22 ± 0.08</td>
<td>0.25</td>
<td>0.13</td>
<td>5.71</td>
<td>3.47</td>
<td>1.27</td>
<td>1.99</td>
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<tr>
<td>CR+CoQ10</td>
<td>0.15 ± 0.04</td>
<td>0.36 ± 0.11</td>
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<tr>
<td>CE+CoQ10</td>
<td>0.20 ± 0.07</td>
<td>0.14 ± 0.04</td>
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<tr>
<td>CR+CoQ10</td>
<td>0.43 ± 0.09</td>
<td>0.30 ± 0.05</td>
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CoQ10: main effect of coenzyme Q10; ST: main effect of swimming training; EE: main effect of exhaustive exercise; CoQ10*ST: interaction effect between coenzyme Q10 and swimming training; CoQ10*EE: interaction effect between coenzyme Q10 and exhaustive exercise; ST*EE: interaction effect between swimming training and exhaustive exercise; CoQ10*ST*EE: interaction effect among coenzyme Q10, swimming training and exhaustive exercise. R: rest group; E: exhausted group; R+CoQ10: rest with coenzyme Q10 group; E+CoQ10: exhausted with coenzyme Q10 group. *p<0.05 significant main or interaction effect (three-way ANOVA).
The effects of training, CoQ10 supplementation and acute exhaustive exercise on malondialdehyde (MDA) in the rat heart. The data are presented as means ± SEM. There was a significant interaction effect between training and exhaustive exercise on MDA level (F = 23.10, p < 0.05). A significant interaction effect among the training, CoQ10 supplementation and acute exhaustive exercise was observed on the MDA levels in the rat heart (F = 19.29, p < 0.05). R = Rest group, E = Exhausted group, R+CoQ10 = Rest group with CoQ10 supplement, E+CoQ10 = Exhausted group with CoQ10 supplement. * p < 0.05 significantly different from the control or training rest group, # p < 0.05 significantly different from the control rest group.

Fig. 3. The effects of swimming training, CoQ10 supplementation and acute exhaustive exercise on superoxide dismutase (SOD) in the rat heart. The data are presented as means ± SEM. There was a significant effect of acute exhaustive exercise on the SOD level in heart tissue (F = 5.71, p < 0.05). R = Rest group, E = Exhausted group, R+CoQ10 = Rest group with CoQ10 supplement, E+CoQ10 = Exhausted group with CoQ10 supplement.

Fig. 4. The effects of swimming training, CoQ10 supplementation and acute exhaustive exercise on total glutathione (GSH) in the rat heart. The data are presented as means ± SEM. There was a significant interaction effect of acute exhaustive exercise and training on the GSH level in heart tissue (F = 6.66, p < 0.05). R = Rest group, E = Exhausted group, R+CoQ10 = Rest group with CoQ10 supplement, E+CoQ10 = Exhausted group with CoQ10 supplement.
The swimming time to exhaustion was significantly longer in the trained-exhausted (2.36±0.34 h) and trained-exhausted with CoQ_{10} (2.56±0.16 h) groups than the control-exhausted with CoQ_{10} (1.03±0.01 h) (p<0.05). The time to exhaustion in control-exhausted group (2.11±0.13 h) was not significantly different from other groups.

Training and exhaustive exercise interaction effects were significant on MDA levels (F=23.10, p<0.05). MDA levels after exhaustion were lower compared to the respective rest group only in the training group. However, in the control group, MDA levels were significantly higher (p<0.05). A significant interaction between CoQ_{10} supplementation, training and exhaustive exercise on MDA levels was observed (F=19.29, p<0.05). In the control group, MDA levels were higher in the rest with CoQ_{10} supplement group compared to the rest group (p<0.05). In the training group, MDA levels of rest group and rest with CoQ_{10} supplement group were higher than those of the control rest group (p<0.05) (Fig. 1).

Figure 2 shows that there was a significant main effect of exhaustive exercise on levels of 8-OHdG in rat heart (F=4.38, p<0.05). Exhaustive exercise increased 8-OHdG levels in all groups except training group. There was also a significant interaction effect between CoQ_{10} and training (F=4.63, p<0.05). 8-OHdG levels of trained rest group were higher than those of the control rest and trained-rest with CoQ_{10} supplement groups (p<0.05).

Figure 3 shows that the effect of exhaustive exercise was significant on SOD activity (F=5.71, p<0.05). The exhaustive exercise decreased SOD activity in control-exhausted group, while increased in other exhausted groups. However, post hoc analysis did not detect significant differences between the groups (p>0.05).

Figure 4 shows that swimming training and exhaustive exercise interaction effects were significant on GSH levels (F=6.66, p<0.05). Three-way ANOVA test demonstrated that GSH levels were increased in control-rest group, but decreased in training rest group by exhaustive exercise. However, these increases or decreases were not statistically significant (p>0.05).

**Discussion**

Heart is a unique muscle that works continuously, unlike skeletal muscles. Thus heart may always be exposed to some degree of oxidative stress (32). However, tissues with high oxygen consumption rates, such as liver, heart, and brain, constitutively express higher levels of antioxidant enzymes than those characterized by low oxygen consumption (33). In this study, the effects of training and CoQ_{10} supplementation on the acute exhaustive exercise-induced oxidative stress markers were investigated in rat heart. One of the most important findings of this study was that training alone or in combination with CoQ_{10} supplementation reduced to increasing heart MDA levels due to exhaustive exercise. Besides, a significant interaction effect between CoQ_{10} supplementation and training on 8-OHdG levels was found. GSH levels and SOD activity also showed quite different responses to exhaustive exercise or swimming training in rat heart. However, they were not affected by CoQ_{10} supplementation.

Acute exercise increases MDA levels (34, 35) whereas chronic exercise reduces MDA levels in the heart (36–38). However, some investigators indicate that heart MDA level was not affected by either acute or chronic exercise (39–41). In this study, MDA levels at rest were higher in the training group compared to the control group. It might be suggested that regular physical training may produce oxidative stress, which may lead to generation of free radicals and lipid peroxidation (42). Also, this may be associated with differences in swimming time to exhaustion among the groups. The trained groups had longer time to exhaustion than the untrained groups. Several studies investigated the role of CoQ_{10} supplementation on exercise-induced lipid peroxidation (43, 44).

We found that training reduced MDA levels, also training in combination with CoQ_{10} supplementation prevented to increase MDA levels induced by exhaustive exercise in rat heart. In parallel with these results, Faff and Frankiewicz-Józko (43) found that CoQ_{10} substantially suppressed the exhaustive exercise-induced increase in the heart, liver, and gastrocnemius muscle MDA levels. In addition, Kon et al (44) indicated that CoQ_{10} supplementation reduced exhaustive exercise-induced muscular injury. On the other hand, in studies in humans, Laaksonen et al. (45) reported that neither CoQ_{10} supplementation nor exercise affected serum MDA levels in trained young and older men. In another study, serum MDA levels were also unaffected by treatment of CoQ_{10} in trained cyclists (46). On the contrary, Cooke et al (47) stated that acute supplementation with CoQ_{10} resulted in higher MDA levels during and following exercise. They indicated that acute and chronic supplementation of CoQ_{10} may affect acute and/or chronic responses to various types of exercise. These disparate results may be due to differences in dose and duration of CoQ_{10} treatment and/or the training status of participants. Also, the type of exercise may influence the lipid peroxidation in tissues differently. In this study, swimming was selected because muscle trauma caused by prolonged running, exercise-stimulated electric shock, and plyometric contractions could induce oxidative stress (48). For instance, Nakao et al (32) showed that compared with running, swimming leads to a wide difference of physical responses and mechanical stresses because of effects of water pressure, utilization of different muscles, and reduced effects of gravity. Besides, oxidative stress by acute or chronic exercise elicits different responses depending on the organ tissue type and its endogenous antioxidant levels. The differences among organs may be dependent on several factors, such as oxygen consumption, susceptibility to oxidants and to antioxidant enzyme activation, antioxidant levels, and other repair systems (36).

In addition to lipid peroxidation, ROS are known to cause oxidative modifications of protein and DNA damage (49). Oxidative stress-induced DNA damage and insufficient DNA repair may play an important role in the etiology of cancer, diabetes and arteriosclerosis (50). However, there is one small study regarding the effect of exercise on DNA damage. In this study, exhaustive exercise increased 8-OHdG levels in control groups. Also, we demonstrated that there was a significant interaction between CoQ_{10} supplementation and training on DNA damage showing that CR+CoQ_{10} group had higher 8-OHdG levels than
TR+CoQ10 group. Asami et al (51) found that the 8-OHdG levels in the forced exercise rats were significantly elevated as compared with those in the spontaneously exercised rats in heart and other tissues and they indicate that the intensity of exercise training is an important determinant of oxidative DNA damage. Conversely, other investigators reported that DNA damage in heart tissue was unaffected by either acute or chronic exercise (36, 52). However, Ramirez-Tortosa et al (53) stated that CoQ10 supplementation decreased DNA damage in peripheral blood lymphocytes. These controversial findings may be explained by differences in training intensity, duration and type of training and/or training status (54). Also, the generation of oxidative stress in DNA and lipids is different after exercise training. This is possibly due to different mechanisms or different activities of specific antioxidant and repair systems (48).

SOD is the major defence upon superoxide radicals and is the first defence line against oxidative stress. (49). In the present study, heart SOD activity in the control group (not receiving the training or supplementation) was decreased by exhaustive exercise, but increased in the CoQ10 study, heart SOD activity in the control group (not receiving the training, though the mechanisms remain to be clarified. In addition, whether or not increases in SOD levels actually reduce exercise-induced oxidative stress also remains vague (32).

GSH serves as a sensitive marker of oxidative stress and it plays an important role in maintaining the integrity of the cell system (61). In this study, exhaustive exercise increased GSH levels in only the control group (not receiving the training or supplementation), although it reduced GSH levels in the training group. It is hypothesized that exercise increases the blood flow in the cardiac muscle, which in turn increases the delivery of GSH to this organ. This enhanced intracellular transport of GSH seems to be essential in maintaining the redox state and to cope with the oxidative stress during exercise training. Another possible mechanism for the increase in GSH during exercise training could be hormonal effects. Exercise training triggers a hormonal response that can influence the efflux of GSH from liver to blood (62). Rigorous swim training reduces heart mitochondrial function, making them more susceptible to oxidative stress, and that this damaging effect may be related to a diminished GSH reserve (63), whereas chronic moderate exercise can lead to protective intrinsic adaptations in the myocardium that specifically attenuate myocardial injury and enhance coronary flow when challenged with H2O2 (64). In another study, Liu et al (36) reported that chronic exercise (8-wk treadmill running) and acute exercise (treadmill running to exhaustion) increased GSH in the heart tissue of rats. However, the increment induced by chronic exercise in GSH was not significant. Conversely, Husain and Hazelrigg (37) indicated that training for 8 weeks significantly enhanced cardiac GSH levels in rats. Similarly, Husain and Somani (59) showed that GSH activities in heart tissue were significantly increased by treadmill exercise training for 6.5 weeks. Furthermore, some researchers reported a significant increase in GSH after training exercise (10 weeks) but not after acute exercise (100% VO2max) in rat heart (62). However, Venditti et al (65) found that heart antioxidant capacity remained unchanged after prolonged exercise (210 min), while it underwent a decrease after exhausting swimming. It might be suggested that GSH is actively used in the myocardium during prolonged exercise at moderate intensity and that GSH deficiency is tolerated by the heart, possibly compensated for by an increased GSH uptake from the plasma (66). Another study reported that heart tissue has four times less antioxidant enzyme activity and GSH levels compared to liver and other tissues. Therefore, heart tissue may be more vulnerable to peroxidative damage due to oxidative stress (59).

The levels of GSH in heart were not affected by CoQ10 supplementation in the present study. However, El-Abhar (67) found that GSH levels were restored, while glutathione peroxidase levels were elevated above normal by the administration of CoQ10 in gastric mucosa of rats. Moreover, González et al. (68) indicated that the co-administration of CoQ10 recovered mitochondrial oxidized/reduced GSH ratio, and reduced ROS generation. Another study report showed that supplementation with CoQ10 led to increased erythrocyte GSH concentration in diabetic rats (25). Nevertheless, Nasuti et al (69) stated that supplementation with Vitamin E + CoQ10 could not prevent the reduction in GSH in adolescent rats. This effect could be related to the dual activity of CoQ10 that can act as antioxidant but also as pro-oxidant.

The results of the present study show that CoQ10 supplementation and swimming training have interactive effects on lipid peroxidation and DNA damage in the heart tissue. Also, CoQ10 supplementation alone did not affect antioxidant status.
References


