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Acute Response of Oxidative Stress and Muscle Damage Biomarkers to a Single Bout of Combined Training (Resistance-Aerobic) with Different Intensities Following Q10 Supplementation

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ABSTRACT

Introduction: Nutritional supplements and controlling exercise intensity are essential to increasing muscle mass. However, the effects of such manipulations on the health of athletes may not be apparent. The present study aimed to compare the effects of combined training (resistance-aerobic) with different intensities and the short-term supplementation of coenzyme Q10 on oxidative stress and muscle damage biomarkers.

Methods: This quasi-experimental study was conducted on 45 male bodybuilders who were divided into three groups of low-, moderate-, and high-intensity combined training (15 per each). Research stages included initial blood sampling, 14 days of Q10 supplementation (400 mg/day), a second blood sampling, a single bout of combined physical activity, and the final blood sampling. In each group, resistance training was initially performed with different intensities (55%, 70%, and 85% 1-RM), followed by aerobic training to consume 300 kilocalories at speeds of 8, 9.6, and 11.2 km/h.

Results: Q10 supplementation had no significant effect on the baseline levels of malondialdehyde (MDA), superoxide dismutase (SOD), lactate dehydrogenase (LDH), and creatine kinase (CK) ($P \geq 0.05$). After performing low-, moderate-, and high-intensity combined activities, a significant increase was observed in the levels of MDA, SOD, LDH, and CK ($P \leq 0.05$).

Conclusion: According to the results, oxidative stress and muscle damage biomarkers increased in response to training intensity, while the increase was not significant at different intensities and fixed times. The performance of the participants may have influenced the obtained results. Given the lower aerobic capacity of male bodybuilders, exercise volume is the primary factor to increase exercise pressure**.** Supplementation may also be effective over long periods.

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Introduction

Today, there is a growing tendency to incorporate physical activity into one's lifestyle to achieve health or championship goals. Meanwhile, the public chooses bodybuilding for several reasons. Achieving well-separated muscles is one of the ultimate goals of bodybuilding. Changing training intensity and volume and combining various training patterns are crucial in this regard. Some optimal approaches are combining training in the form of positive and negative muscles, the combined training of the upper and lower body extremes, and combining resistance and aerobic training to increase the training volume (1). Combined training refers to the combination of several energy-generating pathways and the simultaneous execution of several training types (e.g., resistance and aerobic training) (1). Combined training improves body composition and cardiovascular health factors more effectively compared to aerobic and resistance training alone. Combining aerobic and resistance training may also enhance fitness, body composition, and metabolic health more significantly than each method alone (2). However, the acute response of oxidative stress and muscle damage resulting from such training must be investigated.

The extent of muscle damage and a significant increase in the production of free radicals could

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be determined indirectly by measuring creatine kinase (CK) and lactate dehydrogenase (LDH) (3). Serum CK is a marker of muscle damage, while LDH plays a pivotal role in the metabolism of substances in the body, and fast-twitch fibers catalyze the conversion of pyruvic acid into lactic acid (3). The involvement of these fibers increases the level of lactic acid in the body, and the threshold stage of lactate occurs in the body. Oxidative pressure is induced during the production of free radicals, exceeding the resistance ability of the endogenous antioxidant defense system (3). Oxidative damage yields products such as malondialdehyde (MDA) and superoxide dismutase (SOD) (3, 4).

MDA is an active and highly reactive aldehyde compound produced in the human body through the peroxidation of unsaturated fatty acids, and SOD is the most crucial enzyme that degrades superoxide radicals in the human body (3, 4). During exercise, the body consumes 10-15 times more oxygen, and the oxygen flux in the active muscles increases by up to 100 times (3). Chronic oxidative stress induced by exercise may exceed the protective capacity of the antioxidant defense system. In support of this hypothesis, several studies have shown that aerobic/resistance training significantly increases the production of free radicals (5-7). It has also been reported that after resistance training activities with increased reactive oxygen species and lipid peroxidation markers (including MDA), the imbalance between oxidative pressure and antioxidant defense decreases the total oxidative capacity. After short and intense training, levels of plasma MDA and erythrocytes have also been reported to increase as indicators of lipid peroxidation in red blood cell membranes (8).

In addition to the influential factors in training, using antioxidant supplements reduces the muscle damage and fatigue caused by physical activity. Coenzyme Q10 is a fat-soluble vitamin-like substance and an essential carrier of electrons in the mitochondria (9, 10). Q10 supplementation is an essential electron transporter in the respiratory chain of the inner surface of the mitochondrial membrane for ATP production, as well as an essential antioxidant in the body. This substance may affect maximal oxygen consumption and counteract free radicals, thereby reducing muscle damage (11). Coenzyme Q10 could prevent delayed onset muscle soreness by counteracting free radicals. Therefore, researchers believe that coenzyme Q10 supplementation may also prevent stress or adverse changes in some biochemical parameters due to energy loss during various sports activities. As an antioxidant and anti-fatigue supplement, coenzyme Q10 supplementation is reported to prevent adverse changes in lactate and CK after relatively strenuous activity (12, 13). On the other hand, the findings of Cook et al. (2008) showed that acute coenzyme Q10 supplementation increased coenzyme Q10 concentration without affecting MDA and SOD levels during and after exercise (14).

Objectives

Studies regarding the effects of exercise (especially combined exercise) have proposed contradictory results. Furthermore, exercise programs are generally prescribed so that continuous exercises would be used while performing combined exercises, while also increasing the intensity of exercises and adding more variety. Notably, athletes need to progress quickly and achieve results in today's world, and it is essential to assess the safety of their methods. On the other hand, researchers have mainly investigated the effects of one-step speed training protocols, high resistance, and aerobic intervals on the levels of total oxidative capacity and lipid peroxidation.

Given the importance of maintaining athletes' health and designing training programs to achieve better results, the present study aimed to compare the effects of combined training (resistance-aerobic) with different intensities, along with short-term coenzyme Q10 supplementation, on oxidative stress and muscle damage biomarkers in male bodybuilders.

Materials and Methods

The experimental protocol of the study (based on an MSc thesis) was approved by the Ethics Committee of the Islamic Azad University of Mahallat Branch, Iran (No. 20021404971003). The Ethics Committee initially approved the experimental procedures, and the study protocols were thoroughly explained to all the participants. In addition, a written informed consent form was signed after reading and understanding the details of the experiments. The research was conducted in compliance with the Declaration of Helsinki.

This quasi-experimental study was performed based on predetermined objectives. The research design involved repeated measures ANOVA with three experimental groups, including low-intensity combined training (n=15), moderate-intensity combined training (n=15), and high-intensity combined training (n=15). The sample size was determined based on previous studies. We compared the effects of different intensities of combined training along with short-term coenzyme Q10 supplementation on oxidative stress and muscle damage biomarkers in male bodybuilders. The participants included 45 male bodybuilders aged 19-25 years (Table 1) who volunteered to partake in the study. The independent variables of the research were different intensities of combined training and short-term coenzyme Q10 supplementation, and the dependent variables were oxidative stress biomarkers (MDA and SOD) and muscle damage biomarkers (CK and LDH).

Examinations confirmed the health of all the participants. In addition to signing a written informed consent form, the participants also completed the PAR-Q questionnaires and the daily food registration note. The exclusion criteria of the study were as follows: 1) recent surgery/illness; 2) skeletal and neuromuscular disorders; 3) medical conditions such as cardiovascular diseases, diabetes, hypertension, hepatic diseases, renal disorders, and respiratory disorders; 4) history of food allergies and 5) using tobacco products, alcohol, and medicines, dietary supplements such as antioxidant supplements (e.g., vitamin C and E), and anti-inflammatory drugs (e.g., aspirin subgroups) within three months before the study. None of the participants reported using exogenous anabolic-androgenic steroids, drugs, medications, or dietary supplements with potential effects on redox and inflammatory responses during the study. The subjects were not allowed to participate in the study in case of the contraindications defined and outlined by the American College of Sports Medicine (ACSM) nor if they had ingested any supplements claimed to have ergogenic properties within three months before the study.

Table 1. Mean and standard deviation of the participant's demographic characteristics

Group	Number	Age	Height (cm)	Weight (kg)	BMI (kg/m²)	Vo_2max	Chest press 1-RM
Low intensity	15	22.33±2.3	183.58±4.07	82.01±3.49	24.34±0.26	35.83±2.79	114.75±5.46
Moderate intensity	15	22.58±1.37	183.25±3.04	82.08±3.52	24.44±0.38	36.66±2.14	111.83±10.85
High intensity	15	23.14±1.76	180.89±3.78	81.25±3.54	24.83±0.67	35.75±2.56	115.42±11.27

Based on the records, the health of all the participants was confirmed. The research stages included initial blood sampling, 14 days of Q10 supplementation (400 mg/kg), a second blood sampling, a single bout of combined physical activity (resistance-aerobic), and the final blood sampling. In each group, resistance training was initially implemented with different intensities (55%, 70%, and 85% 1-RM), followed by aerobic training to consume 300 kilocalories at speeds of 8, 9.6, and 11.2 km/h.

A combined training session with different intensities was implemented to compare the intensities of the resistance training session and measure a combined activity of 55% (low), 70% (moderate), and 85% weights (high), as well as the maximum repetition of the resistance training using the following formula:

Amount of weight × number of repetitions × number of sets

The workload of the subjects was the same. Resistance training was performed as a combination of the upper and lower body movements (chest press, leg press, forearm with a barbell, knee bends, lat pulldown, and knee extension) of the upper and lower torso muscles. The number of the training sets of each movement was three repetitions. After each turn in each exercise, the rest time was determined to be 90 seconds to measure the maximum strength in the chest press. A standard formula was used to calculate the 1-RM of the subjects (15).

*1-RM=Lifted weight (kg)/ [1.0278/ (0.0278*re)]*

Aerobic training was implemented based on energy expenditure and consumption of 300 kilocalories for each subject. To compare different intensities of aerobic training in the combined activity, aerobic training was performed on a treadmill (Technogym Run Race HC 1200, Italy) at speeds of 5 mph (8 km/h) for low-intensity training, 6 mph (9.6 km/h) for moderate-intensity training, and 7 mph (11.2 km/h) for high-intensity training. The subjects were trained to 300 kilocalories of energy at the desired speed depending on their body weight.

As such, the training load in the aerobic training was equal for all the subjects.

The Q10 supplement was prepared in the form of two jelly capsules and consumed for 14 days (200 mg each, a total of 400 mg per day; safe dose equivalent to 5 mg/kg/day for a person weighing 80 kg; QSpeed Fast-Melt CoQ10; 200 mg dosage strength, made in Switzerland, manufactured in a cGMP certified facility) (14, 16).

The height and weight of the participants were measured using a Seca device (Seca 714, seca Vogel and Halk Gmbh). One day before the first blood sampling (pretest), the maximum oxygen consumption (VO_{2mx}) of the participants was determined using the Bruce test, and one-repetition maximum (1-RM) was determined based on the chest press. Blood samples were collected in three stages, including the pretest (first stage), 90 minutes after the Q10 supplementation period (second stage), and immediately after combined training (third stage) at the rate of 10 cc of subjects' forearm vein to prepare the serum. All the stages of blood collection from the forearm vein (antibiotic) were performed while the subjects were fasting (8 AM). The blood samples were immediately drained into tubes containing anticoagulants (EDTA). Following that, the samples were centrifuged at 3,000 rpm for five minutes at a temperature of 4°C. The obtained plasma was stored at the temperature of -80°C for subsequent measurements. At the next stage, the samples were preserved at the laboratory temperature for 30 minutes to separate the serum from the plasma and centrifuged (Dlasent, UK model) for 5-10 minutes at 2,000 rpm. A laboratory expert who was unaware of the subjects' condition performed the measurements. The respective kits were used to measure SOD, MDA, CK, and LDH.

MDA was measured using the USA, MI, Cayman Chemical kit. The sensitivity of the method was 0.08 µmol, and the coefficient of variation within the test was 5.9%. The unit of measurement was nanomol per milliliter. SOD was measured using the ELISA method (440 to 460 nm) and the Chemical Cayman kit with a sensitivity of four units per liter and a coefficient of change of 1.8% in liters. CK was measured using the chemical colorimetric method based on the Jaffe reaction with a sensitivity of one unit per liter and a coefficient of change of 1.6% (creatine kinase colorimetric kit, Pars Azmoun Co., Tehran, Iran); the unit of measurement was unit per liter. LDH was also measured using the enzymatic colorimetric method with a sensitivity of five units per liter and a coefficient of change of 2.1% (LDH colorimetric kit, Pars Azmoun Co., Tehran, Iran); the unit of measurement was one unit per liter.

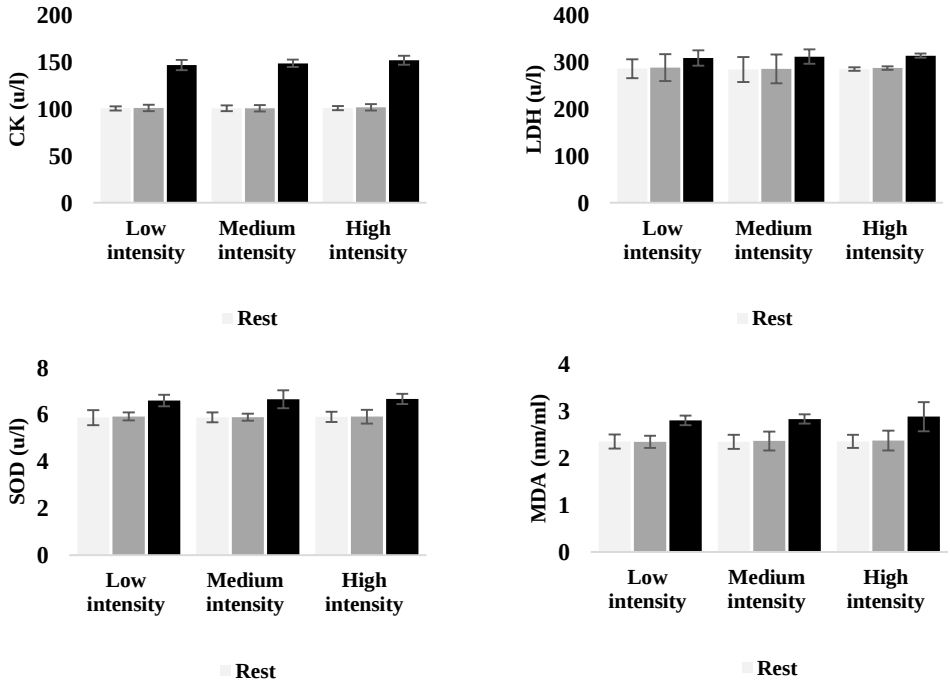

Figure 1. The changes of oxidative stress and muscle damage indices

Data analysis was performed in SPSS version 22 using the Shapiro-Wilk test to assess the normality of the data. Repeated measures analysis of variance (ANOVA) was also applied to evaluate the differences between the study groups. To compare the groups and reduce the effect of the pretest, the covariance option was used.

Results

According to the obtained results, Q10 supplementation had no significant effects on the baseline levels of MDA, SOD, LDH, and CK (P≥0.05). After performing low-, moderate-, and high-intensity combined activities, the levels of MDA, SOD, LDH, and CK increased significantly (F=205.070; P=0001; F=53.158, P=0001; F=6.540, P=0.004; F=50.401, P=0001, respectively). Furthermore, oxidative stress and muscle damage biomarkers increased in response to training intensity, while the increase had no significant difference at different intensities and fixed times. The post-hoc power calculation for each variable was estimated at >0.85 (Figure 1).

Discussion

The present study aimed to compare the intensity of combined training with short-term coenzyme Q10 supplementation in terms of oxidative stress and muscle damage biomarkers in male bodybuilders. The obtained results indicated that Q10 supplementation had no significant effects on the baseline levels of MDA, SOD, LDH, and CK, while after performing low-, moderate-, and high-intensity combined activities, the levels of MDA, SOD, LDH, and CK increased significantly. Oxidative stress and muscle damage biomarkers also increased in response to training intensity, while the increment showed no significant difference at different intensities and fixed times. Differences in research methods play a key role in conflicting and matching results. For instance, various protocols have been implemented in some studies, such as differences in participants' gender, water exercises, or different recovery methods to prevent an increase in oxidative stress indices (7). In the present study, different intensities of combined training were used in young trained men.

A contusion is a reflection of muscle damage in the physiological adaptation of the muscle to strenuous exercise. One of the mechanical causes of muscle contusion is probably damage to the sarcomeres in the muscle structure, which leads to the rupture of the z-plates (17). Current reports indicate that contusions often occur after performing strenuous and unusual activities accompanied by extroverted contractions. Delayed muscle soreness begins 8-12 hours after exercise, peaks within 24-48 hours after exercise, and will eventually resolve within 5-7 days after exercise (17). The etiology of muscle injury and muscle contusion has been extensively discussed in medical and sports communities. This mechanism has also proposed numerous hypotheses, including lactic acid accumulation, muscle spasms, connective tissue damage, and inflammation (17).

Significant oxidative damages initially and mainly occur in the mitochondrial membranes and other tissue membranes. Previous studies suggest that coenzyme Q10 may elicit different antioxidant responses depending on the type, duration, and intensity of exercise at different times after exercise (9, 10, 14). Therefore, it could be inferred that coenzyme Q10 may help athletes by affecting energy storage in the form of ATP, neutralizing free radicals, and reducing the active form of vitamin E by decreasing alpha-tocopherol radicals and increasing strength and endurance (11, 18).

Bagdanis et al. (2013) reported a significant difference in MDA levels in physically healthy men after performing three weeks of resistance training (19). Some studies show that individuals who practice resistance and aerobic training have lower muscle destruction and stress levels compared to non-trained subjects (2). In terms of oxidative stress biomarkers at rest or after the adaptation by exercise training, considered the possibility of improving the antioxidant system in individuals who exercise regularly over a long period, which maintains the redox (reduction-oxidation) system. Long-term anaerobic exercise has also been reported to increase the capacity for anaerobic energy production in the muscle and improve the antioxidant status of the muscle (7).

According to the literature, the concentration of non-enzymatic antioxidants increases with anaerobic exercise. Frequent production of free radicals due to ischemia and blood redistribution on the muscle surface resulting from such exercises seems to enhance the antioxidant

profile (20). In contrast, Gaini et al. (2011) reported that 36 weeks of interval training did not affect MDA levels (21). This lack of change seems to be due to the oxidative defense induced by regular anaerobic interval activity performance. However, studies have shown that intense and irregular physical activity affects the oxidative function of cells and the cell membrane structure by increasing hormones such as catecholamines and macrophage activity, as well as increasing oxidative stress and lipid peroxidation. Reducing local blood flow at the outset of physical activity in organs such as active muscles, kidneys, and liver could also increase lipid peroxidation (20). Regular and continuous exercise probably reduces lipid and protein peroxidation through adaptation and improving the antioxidant defense.

The results of the present study indicated that coenzyme Q10 supplementation did not affect the baseline serum levels of total CK. However, CK levels increased significantly after low-, moderate-, and high-intensity combined activity. This is consistent with the study by Sumida (2007), which indicated a significant increase in the serum levels of CK in non-athlete women after exhausting activity on the treadmill (22). Milias (2005) also investigated the effects of muscle damage caused by extroverted activity on CK levels, reporting that CK levels increased significantly after the activity (23). Our findings are also consistent with the study by Kon et al. (2007 & 2008) in which six weeks of coenzyme Q10 supplementation prevented the elevation of CK levels in male athletes post-exercise (12, 13). In addition, Wang et al. (2004) stated that taking two grams of a Q10 supplement per kilogram of the bodyweight for three months was associated with a significant reduction in CK levels in monkeys (18). Therefore, it could be concluded that the short-term supplementation of Q10 in the present study may not be beneficial.

The possible mechanism of action of coenzyme Q10 is as an antioxidant in reducing CK levels. Q10 also reduces the peroxidation of membrane cells and damages phospholipid membranes by removing free bases and increasing the body's antioxidant capacity. Therefore, it prevents the leakage and penetration of this intracellular enzyme into extracellular fluids (24). Zuliani et al. (2009) conducted research in which coenzyme Q10 supplementation was implemented for four weeks on non-athletes without affecting changes in the serum levels of total CK post-exercise (25). In another study, Kon et al. (2007 & 2008) observed that supplementation with 300 milligrams of coenzyme Q10 per day for four weeks did not increase CK levels (12, 13).

According to the current research, coenzyme Q10 supplementation at baseline did not affect LDH, while the levels of LDH increased significantly after low-, moderate-, and high-intensity combined activity. The study by Deminice et al. (2010 & 2013) consisted of two parts; the findings of the first part regarding intense anaerobic exercise and LDH are in line with the present study. However, the authors also stated that creatine supplementation reduced LDH, which is inconsistent with our findings mainly due to the effect of creatine supplementation (5, 26).

Our findings indicated that short-term coenzyme Q10 supplementation did not affect LDH which is in line with previous studies (4). Meanwhile, this is inconsistent with the findings of Cook et al. (2008) (14). The contradiction may be due to differences in the method of supplementation (type of supplement, purity, amount, and timing of consumption) and the combination of other antioxidant vitamins with coenzyme Q10. In the present study, coenzyme Q10 was administered at the dose of 5 mg/kg/day and alone. Another reason for the contradiction between the results of our study and some other studies may be the duration of the supplementation period (14). It has been reported that the pharmacological properties of coenzyme Q10 (cellular uptake and accumulation) are similar to those of creatine monohydrate is a uniform maximum or a downward trend (in some cases). It has also been observed that it may lead to flattening (uniformity) or even a decrease in muscle concentration during the supplementation period. Although the present study was performed during a 14-day supplementation period, most heterogeneous studies have examined the effect of coenzyme Q10 supplementation in a shorter period.

According to the present study, coenzyme Q10 supplementation had no effect on SOD at baseline, while SOD increased significantly after low-, moderate-, and high-intensity combined activity. In a study on the elderly, Bouzid et al. (2014) reported that during an exhausting activity on an ergometer cycle, SOD and MDA

levels increased significantly, which is consistent with our study (3). Gene Fang Liu et al. (2015) also investigated muscle damage and lipid peroxidation in 36 female weightlifters during one week of strenuous activity, stating that after one week of strenuous activity, MDA, TBARS, and CK levels increased significantly, while SOD levels decreased (27). The results of the mentioned study are not in line with our research, which could be explained by the difference in the gender of the subjects and their experience. In a study of oxidative rats, Hovanloo et al. (2012) reported that after six and nine weeks, no changes were observed in the levels of SOD enzyme in the rats, while continued training for 12 weeks caused a significant decrease in SOD levels (28). This is inconsistent with our research, which could be due to endurance training or research on rats.

As an antioxidant in the body of living organisms, Q10 has not been well elucidated in other studies. In addition, data are scarce regarding the interaction of coenzyme Q10 with other antioxidants during exercise. Kaikkonen et al. (2018) reported that three weeks of Q10 supplementation (90 mg/day) increased coenzyme Q10 concentration and the total antioxidant capacity (29). In the mentioned study, coenzyme Q10 was considered an antioxidant supplement to assess its inhibitory effects on reactive oxygen species and the activity of antioxidant enzymes. The researchers confirmed the antioxidant effects of coenzyme Q10 on inactivating free radicals, while these effects often depend on the amount and duration of the substance, and environmental conditions are also influential.

Conclusion

According to the results, coenzyme Q10 supplementation at baseline did not affect oxidative stress and muscle damage biomarkers. However, muscle injury and oxidative stress rates increased significantly after low-, moderate-, and high-intensity combined training. On the other hand, no significant difference was observed between different intensities of combined training, the level of muscle injury, and oxidative stress biomarkers. Considering the ineffectiveness of short-term coenzyme Q10 supplementation in reducing lipid peroxidation and the cell damage caused by the implemented physical activity, it is recommended that bodybuilders prevent the oxidative stress caused by strenuous exercise and use different antioxidant supplements or long-term supplementation.

Conflicts of Interest

All the authors equally contributed to the reading and approval of the final version submitted. The contents of this manuscript have not been copyrighted or published previously. Currently, the contents of this manuscript are not under consideration for publication elsewhere. The authors declare no conflicts of interest, and there was no funding for this study.

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