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PROTECTIVE EFFECT OF COENZYME Q_{10} ON EXERCISE-INDUCED MUSCULAR INJURY

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Summary: The effect of coenzyme Q_{10} administration on exercise-induced muscular injury was examined in rats. Coenzyme Q_{10}-treated and control rats were exercised by 90 min of downhill treadmill running. A part of the animals in both groups were killed immediately after exercise and the others were 40 h postexercise. After the exercise bout, serum creatine kinase and lactate dehydrogenase activities were elevated in the control rats, but not in the coenzyme Q_{10}-treated rats. These enzyme activities in the latter increased to the similar level of the former 40 h postexercise. The muscle coenzyme Q_{10} content increased by the coenzyme Q_{10} treatment. These results suggest that the coenzyme Q_{10} treatment protected skeletal muscles against injury caused during exercise, but not against damage related with inflammatory processes after exercise.

It has been demonstrated that exercise, especially the eccentric muscle contraction, results in injury to skeletal muscle fibers in rats and humans (1-4). This injury is associated with leakage into the bloodstream of several muscle-specific proteins, indicating that exercise causes damage to sarcolemma. CK , LDH, and aspartate aminotransferase are frequently used as indicator for an increased permiability of the muscle membrane (3,4). The detailed mechanism of the muscular injury is not known, but it is suggested that hydrolytic degradation of cell membrane components and production of free radicals are involved in the initial events of exercise-induced muscular injury (4).

CoQ_{10}, a central compound of the mitochondrial respiratory chain, is mainly located in the mitochondrial inner membrane. CoQ_{10} has a hydrophobic

Abbreviations used: CK, creatine kinase; LDH, lactate dehydrogenase; CoQ_{10}, coenzyme Q_{10}; CoQ_9, coenzyme Q_9.

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isoprenoid side chain, thereby having high lipid solubility. It has been reported that CoQ_{10} has a structural stabilizing effect on membrane phospholipids (5-7) and an anti-oxidative effect (8). In the present study, we examined the effect of CoQ_{10} administration on exercise-induced muscular injury in rats. Reported here is the protective effect of CoQ_{10} on the muscular injury.

Materials and Methods

Fifty-seven male Wister rats (10 weeks age), purchased from CLEA Japan, Tokyo, were used in the experiment. The animals weighed 269-304 g. They were housed at 23°C with light from 7:00 a.m. to 7:00 p.m. and with free access to water and food (commercial rat chow). CoQ_{10}, which was suspended at 2.5 mg/ml in an injection solution developed for animal experiments, was supplied by Eisai Co., Ltd., Tokyo.

Rats were divided into a CoQ_{10} group (n=33) and a control group (n=24). CoQ_{10} at 5 mg/kg body weight in the injection solution was injected into the CoQ_{10} group of rats from tail vain. The injection was performed once a day between 6:00 p.m. and 7:00 p.m. for successive 5 days. The control group of rats were treated as the same manner as that for the CoQ_{10} group of rats, except that the injection solution without CoQ_{10} was used. After the treatment, 21 rats randomly selected from the CoQ_{10} group and 16 rats from the control group were exercised for 90 min from 7:00 p.m. according to the method of Armstrong *et al.* (9) using a motor-driven treadmill designed for the mouse and rat (model III, Autome Kogyo, Tokyo); running down a 16° incline at 16 m/min, because the downhill treadmill running was reported as eccentric exercise in rats (9). Three rats each in both groups did not spontaneously run and had injured feet after the exercise bout, therefore these rats were eliminated from each group. After running, 8 rats in the CoQ_{10} group and 5 rats in the control group were immediately anesthetized with ethyl ether (2-3 min), then the abdominal cavity was opened, and a 5- to 10-ml blood sample was obtained from inferior vena cava with syringe. The animals were then killed by exsanguination. Immediately after exsanguination, the triceps brachii muscles were removed, freeze-clamped at liquid nitrogen temperature, and stocked at -80°C until analyses of CoQ_9 and CoQ_{10}. Sedentary rats in each group (the CoQ_{10} group, n=6; and the control group, n=4) were treated immediately after killing the exercised rats by the same procedure described above, and blood and muscle samples were obtained. The rest of exercised and sedentary rats in both of the CoQ_{10} and the control groups received the administration as described above on the next day (22 h after exercise) and were killed 40 h after the exercise bout by the same procedure for rats killed immediately after exercise to obtain blood and muscle samples. A part of blood was heparinized to obtain plasma for determination of glucose (10), and the other part was deproteinized with 0.8 M hydrogen perchloric acid for determination of lactate (11). From the rest of blood, serum was obtained for measurements of CK (12) and LDH (13) activities (at 37°C) and for determination of free fatty acids (14) and β-hydroxybutyrate (15). CoQ_9 and CoQ_{10} in the muscle samples were measured by the method of Hiroshima *et al.* (16).

The statistical significance of the results was analyzed by analysis of variance (ANOVA) followed by Tukey's multiple comparison test, except that the muscle CoQ contents were analyzed by Student's t-test. Differences were considered significant at $p<0.05$.

Results

Serum CK and LDH activities. It has been reported that an eccentric-biased exercise (downhill running) causes elevation of plasma CK and LDH activities with two phases in rats; one of the peaks appears immediately after exercise and another peak 40 h postexercise (9). We used this type of exercise to examine the effects of CoQ_{10} administration on the exercise-induced elevation of the enzyme activities in rat serum. As expected, serum CK activity of the control group was significantly elevated by *ca.* 160% immediately after the exercise bout (Fig. 1). The CK activity of the CoQ_{10}-exercise group immediately after exercise, however, was at the same level as that of the sedentary groups (Fig. 1), indicating that the CoQ_{10} treatment blocked the elevation of the enzyme activity during exercise. The CoQ_{10} administration had no effect on the enzyme activity in the sedentary rats (Fig. 1). The CK activity in the CoQ_{10} group tended to increase 40 h

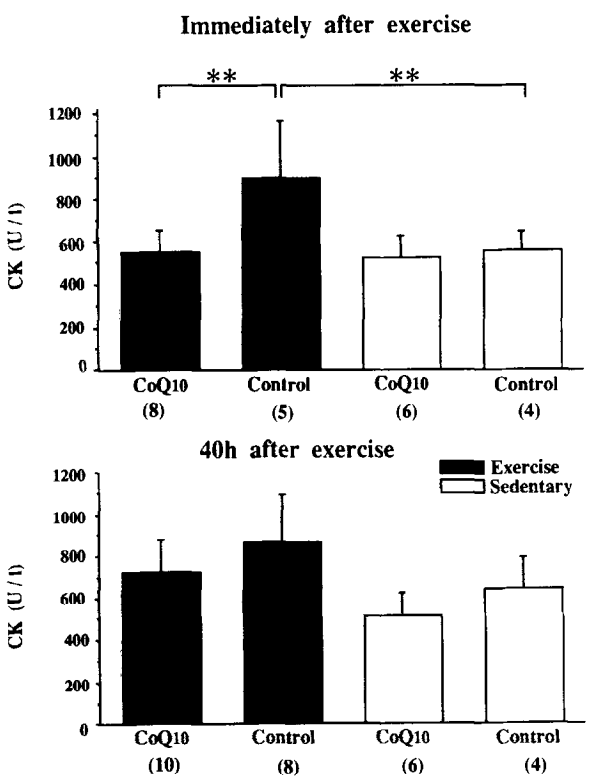

Fig. 1. Effect of CoQ_{10} administration on serum CK activity in exercised and sedentary rats. Values are means ± SD. Rat numbers are given in parentheses. $^{**}p<0.01$.

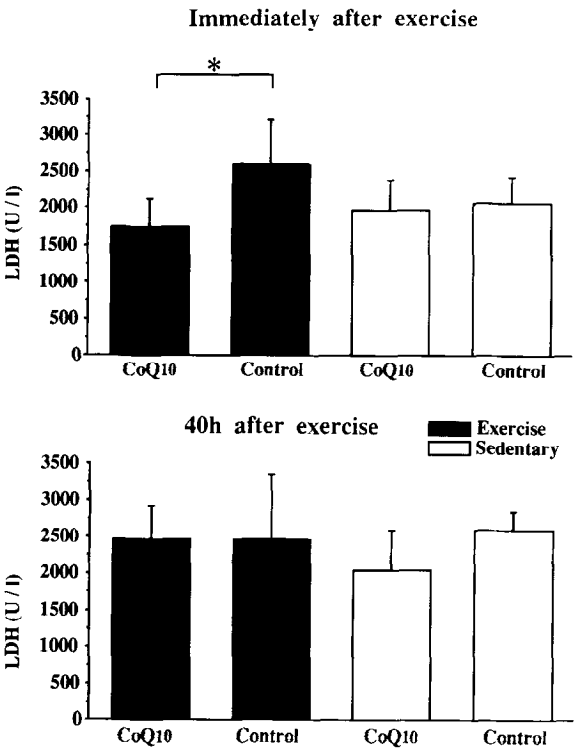

Fig. 2. Effect of CoQ_{10} administration on serum LDH activity in exercised and sedentary rats. Values are means ± SD. Rat numbers are given in Fig. 1. *$p<0.05$.

postexercise, resulting in no significant difference between the CoQ_{10}-exercise and the control-exercise groups 40 h postexercise (Fig. 1). The similar effect of CoQ_{10} was observed on exercise-induced elevation of the serum LDH activity (Fig. 2); the CoQ_{10} treatment blocked elevation of the LDH activity immediately after exercise. These results suggest that skeletal muscles were protected against injury caused during exercise by the CoQ_{10} administration.

Muscle CoQ contents. The CoQ_{10} content in triceps brachii muscles increased by the CoQ_{10} treatment (1.93 ± 0.30 μg/g tissue (mean ± SD, n=30) for the CoQ_{10} group, and 1.71 ± 0.21 (n=21) for the control group, $p<0.01$), whereas the CoQ_9 content in the muscles was not affected by the treatment (25.8 ± 4.1 μg/g tissue for the CoQ_{10} group, and 25.5 ± 3.3 for the control group), indicating that CoQ_{10} administered was, at least in part, incorporated into the muscles.

Table 1. Effect of CoQ_{10} administration on concentrations of plasma glucose and serum free fatty acids, β-hydroxybutyrate and lactate in rats[1]

Group	Glucose (mg/dl)	FFA (mM)	β-HB (μM)	Lactate (mg/dl)
Immediately after exercise				
CoQ_{10}-exercise	132 ± 19	1.04 ± 0.18	994 ± 232**	10.1 ± 1.5
Control-exercise	117 ± 12	1.19 ± 0.22	1216 ± 225**	8.1 ± 1.4
CoQ_{10}-sedentary	144 ± 9	0.88 ± 0.14	307 ± 105	7.2 ± 1.0
Control-sedentary	152 ± 16	0.92 ± 0.17	517 ± 103	8.8 ± 2.3
40 h after exercise				
CoQ_{10}-exercise	150 ± 11	0.42 ± 0.07	109 ± 39	13.2 ± 5.6
Control-exercise	153 ± 9	0.51 ± 0.10	123 ± 10	10.9 ± 2.1
CoQ_{10}-sedentary	153 ± 7	0.54 ± 0.05	115 ± 43	13.4 ± 1.5
Control-sedentary	150 ± 14	0.58 ± 0.10	124 ± 27	12.5 ± 1.7

[1]Values are means ± SD. The rat numbers are given in Fig. 1.
**Significantly different from the sedentary group ($p<0.01$). FFA, free fatty acids; β-HB, β-hydroxybutyrate.

Plasma glucose and serum free fatty acid, β-hydroxybutyrate and lactate concentrations. The concentrations of plasma glucose and serum free fatty acids and lactate were not significantly affected by the exercise bout in either CoQ_{10} or control group, whereas the β-hydroxybutyrate concentration in both of the groups significantly increased immediately after exercise (Table 1). There was no difference of these parameters between the CoQ_{10} and the control groups in either exercised or sedentary rats (Table 1), suggesting that the CoQ_{10} administration had no effect on the energy metabolism in rats.

Discussion

It has been reported that downhill running (eccentric exercise) causes elevations of plasma CK and LDH immediately after exercise (the first peak) and 1.5-2 days postexercise (the secondary peak); the first peak is caused by exercise-induced damage of sarcolemma and the secondary peak is related with inflammatory processes (9). CoQ_{10} administration blocked the appearance of the first peak of enzymes, suggesting protection of muscle cell membranes against exercise-induced injury. Although the detailed mechanisms of

exercise-induced muscular injury is unknown, it is suggested that loss of Ca^{2+} homeostasis in the injured muscle fiber is the central event; a high concentration of intracellular Ca^{2+} induced by the influx from the bloodstream serves to activate a number of proteolytic and lipolytic systems (4). Probably, CoQ_{10} may protect the muscle cell membranes against the proteolytic and lipolytic degradation, because it has been reported that CoQ_{10} has the structural effect that renders membrane components resistant to attack by hydrolytic enzymes (5). This view was supported by the increase in CoQ_{10} content in muscles of rats treated with CoQ_{10}. Furthermore, the anti-oxidative effect of CoQ_{10} might be also involved in the protection of muscle cell membranes, since production of free radicals and lipid peroxidation increase during exercise (17).

It has been reported that muscle fibers about 2 days postexercise, a time when the secondary peak of the plasma enzymes appears, are in the most advanced stages of degeneration associated with accumulations of mononuclear cells (9). The CoQ_{10} administration did not block an appearance of the secondary peak in the present study, suggesting that the CoQ_{10} treatment had little effect on the muscle fiber damage related with such inflammatory processes.

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