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Effects of Exercise and Food Consumption on the Plasma Oxidative Stress

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Abstract. [Purpose] The effects of exercise and food consumption on the oxidative stress regulation system were studied using 45 male ICR mice. [Subjects and Methods] The mice were randomly divided into two groups: a control group (CO group) and a group consuming a reduced form of coenzyme Q10 (H_2CoQ10: QH) (QH group). Both groups were made to run on a treadmill for animals (TM) twice and the changes in their running time were measured. For the oxidative stress regulation system, plasma oxidative stresses (d-ROM test) and plasma anti-oxidant potential (BAP test) were measured before and after treadmill running with analytical equipment for reactive oxygen and free radicals, and then the BAP/d-ROM ratio was calculated. [Results] The measurements and calculations showed a significant increase in the running time of the QH group. With respect to the oxidative stress regulation system, no significant change was observed in the BAP/d-ROM ratio. [Conclusion] These results showed that a single consumption of QH produced an effect on exercise, but had no effect on the oxidative stress regulation system.

Key words: Oxidative stress regulation system, Reduced coenzyme Q10 (H_2CoQ10), Exercise

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INTRODUCTION

Several percent of the oxygen taken into the body is derived from the electron transfer system in the mitochondria in cells, and becomes active oxygen (superoxide radical etc., is collectively referred to as oxidant stress), which cannot be reduced to water or hydroxide. As oxygen flux to tissues reaches about 100 times higher than normal at the maximum exercise[1)], the amount of oxidant stress remarkably increases, which causing oxidant stress to increase in the body. In order to protect itself maintain life against such exercise-induced oxidant stresses, the body has various homeostatic mechanisms including the autonomic systems, an endocrine system, immune systems and an oxidative stress regulation system[2)]. Generally, the oxidative stress regulation system is a "balance (latent anti-oxidant potential) between oxidative reaction (oxidative stress) and anti-oxidant reaction (anti-oxidant potential) of the body" and what is important is how the anti-oxidant potential can be increased against oxidative stresses.

As well as exercise, the consumption of foods such as vitamins C and E is known as to produce for anti-aging and stress management effects[3–5)]. Especially, the consumption of a reduced form of coenzyme Q10 (H_2CoQ10, Kaneka Corporation, Japan: QH), which is known to have anti-oxidant effects, has been reported to have such effects as delayed the manifestation of aging[6)], have on impacts on psychological elements[7)], and improve QOL improvement of the elderly[8)] and recovery from fatigue[9)]. However, not many reports have reviewed the effect of QH consumption on exercise and the oxidative stress regulation system. It has been reported that the effect of coenzyme Q10 (CoQ10) consumption on the oxidative stress regulation system results in a difference of anti-oxidative potential, depending on formulation methods of CoQ10[10)], but it was not clearly described whether the CoQ10 used was oxidized CoQ10 or QH.

Generally, oxidized CoQ10 (ubiquinone) has been used for CoQ10. Oxidized CoQ10 is transformed by reduction enzymes into QH (H_2CoQ10) in the body (especially in the blood plasma and the liver) of animals such as humans and rats, which means that QH is a major metabolic form of CoQ10[11)]. As the percentage of QH in the body and the ability to transform oxidized CoQ10 into QH decrease with aging[12)], it has been reported that the action on the body by the consumption of QH has widely different physical effects from that by the consumption of oxidized CoQ10[6)]. Additionally, as the many factors that prevent the accurate evaluation of supplements' effects. It has been pointed out that it is difficult to strictly control the subjects' life-styles, including food consumption, and to determine the appropriate amount of supplement to be consumed due to insufficiency of basic data regarding the validity of dosage[13)]. Therefore, to discuss the effect of QH consumption on the body, it is necessary to strictly regulate

the amounts of QH and food, exercise to be consumed. Extension of forced swimming hours by rats has been reported as an effect of QH consumption on the fatigue[9], but its the effects on the limit of running in gravity or the effect on the oxidative stress regulation system have not been discussed. There is no report, in particular, that has comprehensively discussed the oxidative stress regulation system in the equilibrium state (correction ratio) of oxidative stress and anti-oxidant potential or has examined the effect on the exercise with a regulated exercise load.

In this study, we regulated the amount of QH to be consumed by experimental animals (mice) and examined how QH consumption affected the limit of running (running time on a treadmill) in gravity and the oxidative stress regulation system. Using the method of our preceding study, we made comprehensive evaluations of the oxidative stress regulation system with plasma oxidative stress (reactive oxygen metabolites test: d-ROM test), plasma anti-oxidant potential (biological antioxidant potential: BAP test) and the BAP/d-ROM ratio (BAP test value/d-ROM test value).

SUBJECTS AND METHODS

In the experiment, 45 four-week-old male ICR mice were used. During the one-week habituation period, all of them performed running exercises three times to get acclimated to the treadmill for mice (model TM-R-N1, Osakamicro, Japan**:** TM) (running conditions: 1 speed 20 m/min, tilt 0 degree, time 10 min, 2 speed 10 m/min, tilt 10 degrees, time 30 min, 3 speed 20 m/min, tilt 10 degrees, time 30 min). Running was performed in the order of 1 and 2 and once per day. Then, at the age of five weeks, the mice were randomly divided into two groups, a control group (CO group: n=24) and a QH consumption group (QH group: n=21). All of mice were kept at a room temperature of 20 ± 1°C, and the relative humidity of about 50%, and with a light - dark cycle of 12 hours; and free to take food (CE-2, CLEA Japan, Inc.) and tap water were provided ad libitum.

A treadmill was used for exercise load with its speed set to 25 m/min and its tilt to 20 degrees. This exercise load corresponds to about 80% of the maximum oxygen consumption[14]. The termination criterion for the limit of running decided as the point when the time interval of electro-stimulation delivered to the back of the running surface of the treadmill fell below 5 seconds. The mice in the QH group were made to run on the treadmill to their limit two days before QH consumption (running time 1) and to run on the treadmill to their limit again three hours after QH consumption (running time 2). Then, the change in the length of running time caused by QH consumption was measured (running time 2 – running time 1). The measurement of running time was made twice so that we could consider the effect of the fatigue; the second measurement was made forty-eight hours after the first one to eliminate the effect of the fatigue. The time setting after QH consumption was based on the results of a preceding study in which oxidized CoQ10 (1.5 mg/kg) was orally administered to the rats[15]. The mice in the CO group were also made to run twice on the treadmill to their limit (running time 1 and running time 2) under the same conditions as the QH group and the change in the length of the running time (running time 2 – running time 1) was measured. The amount of QH consumption in the QH group was regulated to 300 mg/kg body weight, which was forcibly administered by mouth (only once), while the CO group took tap water. The amount of QH administered was based on the results of a preceding study[6].

With respect to the oxidative stress regulation system, the d-ROM test value (degree of oxidative stress) and the BAP test value (anti-oxidant potential) were measured by using the analytical equipment for reactive oxygen and free radicals (model FRAS4, H&D, Italy). These values were measured at rest before QH consumption and immediately after the limit of running (immediately after the running time 2), and then the BAP/d-ROM ratio (latent anti-oxidant potential) was calculated. For the d-ROM and BAP values measurement, blood was extracted from the tail vein and, which was centrifuged immediately (for five minutes at a speed of 6,000 rpm) to obtain blood plasma extracted for analysis.

In the d-ROM test, levels of free radicals in the body, especially hydroperoxide concentrations, were measured (unit: U.CARR, 1 U.CARR = 0.08 bmg/dl of hydrogen peroxide) according to the optical measurement method (color reaction) and the measured value indicates the degree of oxidative stress (oxidative reaction). Meanwhile, in the BAP test these levels were measured (unit: μM) by reduction action of anti-oxidant materials in blood plasma and the measured value indicates the degree of anti-oxidant potential (anti-oxidant reaction). That is, the amount of the blood plasma which, when mixed with reagents containing ferric ions, was reduced to ferrous ions was measured at the decoloring level of the color reaction liquid according to the optical measurement method. The content of iron ions to which blood plasma is reduced is the anti-oxidant potential. The BAP/d-ROM ratio, which was calculated on the using of the values obtained in the BAP test and the d-ROM test, indicates the degree of latent anti-oxidant potential. In other words, latent anti-oxidant potential indicates a balance between oxidative stress and anti-oxidant potential. The values of the oxidative stress regulation system on humans (healthy Japanese) have been reported as 284.6 ± 17.5 U.CARR in the d-ROM test, 2137.1 ± 228.3 μM in the BAP test, and 7.541 ± 0.978 in the BAP/d-ROM ratio[16], but there has been no report of those values for ICR mice.

Numerical values are presented in this study as the shown by an average value ± standard deviation. The SPSS (Ver19.0 for win) was used as statistical software and the Mann-Whitney U test was used to tests the significance of differences. This study was conducted with the approval of the Animal Research Committee of the university to which we belong (approval number: 23).

RESULTS

The weights of the mice during running on the treadmill were 29.8 ± 2.8 g in the QH group and 30.8 ± 2.0 g in the

Table 1. Presents Changes in running times

	QH group	CO group
running time (1) (min)	33.96 ± 10.86	34.67 ± 13.29
running time (2) (min)	63.55 ± 21.64***,†††	42.12 ± 16.70
average amount of changes (min)	29.59 ± 16.85***	7.45 ± 10.08
average rates of changes (%)	94.89 ± 65.70***	23.63 ± 32.33

QH group: QH consumption group (reduced form of coenzyme Q10, H_2CoQ10, Kaneka Corporation: QH). CO group: control group. running time (1): running on the treadmill to their limit two days before QH consumption; running time (2): running on the treadmill to their limit again three hours after QH consumption; average amount of changes: running time (2) - running time (1); average rates of changes: average rates of changes (running time (2)- running time (1)). *** $p<0.001$ as compared with CO group. ††† $p<0.001$ as compared with running time (1).

Table 2 Changes in the oxidative stress regulation system

			QH group	CO group
d-ROM test	Rest	(U.CARR)	123.0 ± 18.8*	138.8 ± 22.6
	Post	(U.CARR)	124.8 ± 29.4	123.7 ± 22.2†
	average amount	(U.CARR)	1.8 ± 21.9*	–15.1 ± 23.8
	average rates	(%)	1.4 ± 18.0*	–9.4 ± 17.3
BAP test	Rest	(μM)	2816.0 ± 280.6	2896.3 ± 258.4
	Post	(μM)	3022.7 ± 319.1	3086.8 ± 243.7††
	average amount	(μM)	206.7 ± 225.3	190.5 ± 315.3
	average rates	(%)	7.6 ± 8.3	7.2 ± 11.2
BAP/d-ROM ratio	Rest		23.5 ± 4.8	21.5 ± 4.3
	Post		25.9 ± 7.8	25.8 ± 5.2††
	average amount		2.5 ± 5.5	4.3 ± 5.9
	average rates	(%)	9.8 ± 23.3	23.0 ± 27.8

QH group: QH consumption group (reduced form of coenzyme Q10, H_2CoQ10, Kaneka Corporation: QH); CO group: control group. d-ROM test: Reactive oxygen metabolites test; BAP test: Biological antioxidant potential; BAP/d-ROM ratio: BAP test/d-ROM test. Pre: Before the limit of running; Post: after the limit of running. average amount: average amount of changes (Post-Pre); average rates: average rates of changes(Post-Pre). * $p<0.05$ as compared with CO group.†† $p<0.01$, † $p<0.05$ as compared with Rest.

CO group. These values were not indicate any significantly different between the groups. Table 1 presents the changes in running time. Between the QH group and the CO group there was no significant difference in running time 1, but a very significant increase was shown in running time 2 in the QH group ($p<0.001$). Similarly, there was a very significant increase in the average amount of the changes and the average rate of the changes was shown in the QH group ($p<0.001$). When running time 1 was compared with running time 2 in the QH group, running time 2increased was very significantly ($p<0.001$)longer, but no significant change was observed in the same items in the CO group. These results showed an increase that the running time by QH consumption increased the running time. Table 2 shows the changes in the oxidative stress regulation system. The value of the oxidative stress regulation system of all the mice (when they were at rest before QH consumption) was 131.8 ± 22.4 U.CARR in the d-ROM test, 2792.3 ± 495.5 μM in the BAP test, and 21.620 ± 5.388 in the BAP/d-ROM ratio. This result in the male ICR mice indicates that lower value in the d-ROM test, and higher values in the BAP test and BAP/d-ROM ratio (about 186% of the ratio of humans) in comparison with those of humans. Compared to the QH group, the CO group showed a significantly higher value in the d-ROM test when they were at rest ($p<0.05$). However, no significant difference was observed between these groups immediately after the limit of running. The CO group showed a significant decrease in the average amount of the changes and the average rate of the changes in the d-ROM test. However, there was no significant difference between the two groups with respect to the BAP test value or the BAP/d-ROM ratio. As seen from the result that the values measured at rest in the d-ROM test were higher in the CO group, these results indicate that many of the mice in the CO group had higher oxidative stress levels than those in the QH group. As the standard variation was high values for the average amount of the changes and the average rate of the changes, it was also indicated that there were wide variations of oxidative stress among the individual mice.

In the QH group there was no significant difference between the values measured at rest and right after the limit of running in the d-ROM test, while in the CO group significant differences were observed between these values in the d-ROM test ($p<0.05$~0.01). These results show that in the QH group, there were significant increases were

obtained in the running time, but there was no change in the oxidative stress regulation system evaluated by the d-ROM test values.

DISCUSSION

Generally, CoQ10 is synthesized in the body with mevalonic acids being the starting material, following part of the same route as cholesterol (intrinsic CoQ10, which is derived from biosynthesis by the body itself). And then CoQ10 is supplied to all cells in the body with the CoQ10 which is derived from food and supplements (extrinsic CoQ10) is supplied to all cells in the body and can be found in each organ of the body including the heart[15]. Oxidized CoQ10 has been used as a medical agent for congested heart failure in Japan; and it has been used in Japan for the preservation of health since the classification of pharmaceuticals and non-pharmaceuticals was changed in March 2001. In Europe and the United States oxidized CoQ10 has been widely used as a supplement material [17]. It has been reported that the physiological effects of CoQ10 include the energy through the production of produces ATP with mitochondria activator and the anti-oxidant activity[18,19]. Recently, QH, which is a major CoQ10 in the body, has been put to commercial use and is reported to have such actions as antisenescence activity, which is different from that of oxidized CoQ10[6,11].

This study demonstrated that consumption of QH results in a significant increase in running time. We attribute this increase is attributed to physiological effects, such as the action of producing ATP by the activation of mitochondria and the increase in antisenescence activity, as seen in oxidized CoQ10. In addition, QH has been reported to not only activate the metabolism of the cardiac muscle, but also decrease peripheral vascular resistance and improves cardiopulmonary function and peripheral circulation[20]. Therefore, it is possible that similar effects were induced in this study. Langsjoen et al.[21] reported that among the patients with severe chronic cardiac failure consumption of oxidized CoQ10 had no effects, but consumption of QH was effective. Their report showed that the consumption of QH exhibited the increase of CoQ10 levels in blood plasma in association with the improvements in clinical findings (NYHA class: New York Heart Association) and left ventricular functions (Ejection Fraction). Accordingly, we expected that the consumption of QH would result in an show a higher increase in CoQ10 levels in blood plasma greater than that of oxidized CoQ10, and there was also the possibility that this study made clear the physiologic effects[18-21] shown by the preceding studies would also be apparent.

In general, the production of oxidant stress increases in proportion to oxygen consumption[1]. It has been reported that significant increases in oxidant stress (d-ROM test) and anti-oxidant potential (BAP test) are shown after exhaustive exercises such as cycling races[22]. It has also been reported that in a study using rats, just as in the case in humans, the oxidation product of DNA, 8-OHdG, increased in urine after exercise on a treadmill [23]. With respect to the oxidative stress regulation system, our present showed that the d-ROM test value was lower and the BAP test value and BAP/d-ROM ratio were higher in male ICR mice, than in those of humans. These differences in values occur as mice do not have a genetic mutation in the enzyme located at the end of the biosynthetic route of vitamin C (GLO: gulono -γ-lactone oxidase); and thus, sufficient amounts of vitamin C are synthesized in the mice[24]. We assume this is reason why, it was assumed that the d-ROM test value was lower even though mice have relatively large amounts of oxygen consumption, compared to humans.

In this study the CO group presented higher values when measured at rest in the d-ROM test and showed a decrease in the average amount of the change, compared to the QH group, while no significant change was observed in the running time. These results showed possibilities that the CO group contained some mice whose oxidative stresses were not effectively scavenged[24] by vitamin C while they were at rest and that the exercise with exercise load activated the scavenging activity of vitamin C. However, as wide variations between individuals were seen, it is highly likely that there were individual differences in this scavenging activity. Furthermore, in the QH group the running time increased, but no change was observed in the d-ROM test value. As oxygen is commonly taken into the body for the continuous production of ATP[1], the effect of exercise on oxidant stress was expected to increase as the exercise time was extended. However, no significant differences were observed in the d-ROM test value even though the running time increased, which indicating that QH might have worked protectively against continuously exposure to oxidant stress through promotion of the scavenging activity[24] by vitamin C and the coenzyme Q redox cycle[15,25]. The coenzyme Q redox cycle is a mechanism that maintains QH in the body with reduction enzyme, and it is one of the important protective mechanisms for against exercise-induced oxidant stress which accompanies exercise[15]. It is possible that this protective mechanism prevented breakdown of the oxidative stress regulation system to break down even though the running time increased. On the other hand, it is also possible that the effects of vitamin C and QH consumption were not reflected well in the values of the BAP test, which analyzes the reducing ability of iron ions in blood plasma. It will be necessary to compare oxidant stress markers with a percentage of QH in blood plasma and to study the effects by a long-term consumption of QH to clarify this issue.

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