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Original Article

Long-term Effect of Ubiquinol on Exercise Capacity and the Oxidative Stress Regulation System in SAMP1 Mice

Hiroshi Maruoka, PT, PhD[1)*], Kenji Fujii, PhD[2)], Kazuhisa Inoue, PT, PhD[1)], Satoshi Kido, PT[1)]

[1)] *School of Health and Social Services, Saitama Prefectural University: 820 Sannomiya, Koshigaya City, Saitama 343-8540, Japan*
[2)] *Functional Food Ingredients Group, QOL Division, Kaneka Corporation, Japan*

Abstract. [Purpose] This study examined how exercise capacity and the oxidative stress regulation system are affected by different amounts of dietary Ubiquinol (reduced form of coenzyme Q10, H_2CoQ10: QH) over the long term. [Subjects and Methods] Twenty-three senescence-accelerated mouse P1 (SAMP1) mice were randomly divided into two groups: one consuming a relatively high amount of QH (300 mg/kg; Group A) and the other a relatively low amount (30 mg/kg, Group B). Food and tap water were provided ad libitum. Both groups were made to run on a treadmill until exhaustion, and total running duration was measured. For the oxidative stress regulation system, the d-ROM test value (degree of oxidative stress) and BAP test value (antioxidant potential) were measured in a resting state, and then the BAP/d-ROM ratio (B/R ratio) was calculated. The values of plasma QH and plasma ubiquinone (plasma oxidized form of CoQ10) were also measured, and the reduced ratio was calculated. Measurements were taken 3 times: at the start of the study when the animals were 39 weeks old (baseline), after consumption of QH for 7 months (7 mo), and after consumption of QH for 10 months (10 mo). [Results] The senescence score at 10 mo was significantly lower in Group A. Comparison of the mean percentage change in running time showed a difference of 15.1% between the 2 groups. At 10 mo, the d-ROM test value was significantly increased and the B/R ratio was significantly decreased in Group B. Significant increases in the plasma QH value and reduced ratio were seen in Group A. [Conclusion] Group A showed a greater decrease in the d-ROM test and increase in the reduced ratio than Group B. Thus, a dose-dependent effect of QH consumption was demonstrated.
Key words: Ubiquinol, Exercise capacity, Oxidative stress regulation system, Mice

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INTRODUCTION

Oxygen is essential for maintaining homeostatic function, but small amounts of active oxygen species are constantly produced in all cells with aerobic metabolism. They are normally reduced in an organism's scavenging system, but with breakdown of the scavenging system or accumulation of active oxygen species when the amount of active oxygen produced exceeds the capacity of the scavenging system, highly reactive active oxygen species are produced and oxidative stress occurs[1)]. Oxidative stress is thus a state in which the balance between oxidation reactions (degree of oxidative stress) and anti-oxidation reactions (antioxidant capacity) in a body is disrupted and shifts toward oxidation reactions, which is unfavorable for the body. Generally, oxygen flux to tissues increases during exercise[2)], leading to generation of a marked increase in oxidative stress. Oxidative stress contributes to senescence[3)] and induces lifestyle-related diseases and various other diseases[4)]. Therefore, the level to which defense systems (antioxidant capacity) against oxidative stress can be enhanced through antioxidant enzyme systems and consumption of antioxidant substances becomes important. The effects on oxidative stress and antioxidant capacity in small animals (rats) exposed to exercise have been investigated[5)], but few reports have investigated the relationships of consumption of vitamin C, which is sensitive to oxidative stress, or foods containing ubiquinol (reduced form of coenzyme Q10, H_2CoQ10: QH) radical-scavenging antioxidants[6, 7)] with exercise ability, the oxidative stress defense system, and senescence[8, 9)]. Moreover, there are no reports on the effects of long-term consumption of different foods (antioxidants) on exercise capacity and degree of oxidative stress. Coenzyme Q (CoQ) is synthesized in the same mevalonate pathway as cholesterol and is present in all organs and cells. It has the two following major actions: activation of energy production and antioxidant action[10)]. In humans and other mammals, the main forms of CoQ are plasma ubiquinone (plasma oxidized form of CoQ10) and QH, but nearly all CoQ is present

*Corresponding author. Hiroshi Maruoka (E-mail: maruoka-hiroshi@spu.ac.jp)

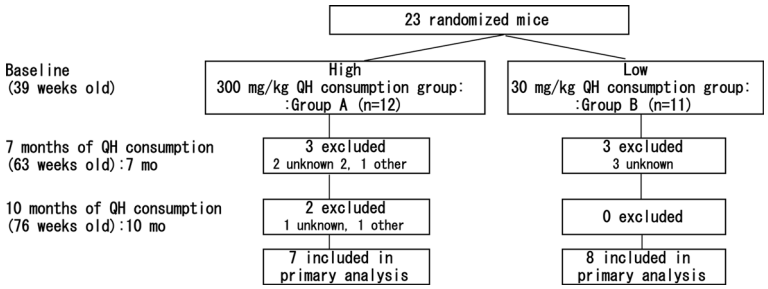

Fig. 1. Flow of individuals with respect to QH consumption in the study. The study was performed on 23 male SAMP1 mice (39 weeks old), which were randomly divided into two groups: one consuming a high amount of QH (300 mg/kg, n=12, Group A) and the other consuming a low amount of QH (30 mg/kg, n=11, Group B). Eight mice were excluded (35%).

in a reduced form Ubiquinol. The oxidized form is converted to the reduced form by NAD(P)H oxidase (oxidation-reduction enzyme) in the body, and the ability to perform this conversion decreases with age[11]. Consumption of CoQ by mice is reported to increase plasma CoQ10 values[12, 13], but there are no reports on the effects of long-term consumption of different foods on the reduced ratio.

In this study, the effects of differences in the amount of QH consumed over a long period on oxidative stress defense systems, including exercise capacity and degree of oxidative stress, and senescence were investigated in an animal experiment.

SUBJECTS AND METHODS

Twenty-three senescence-accelerated mouse P1 (SAMP1/Sku Slc) (39 weeks old) mice were randomly divided into two groups: one consuming a relatively high amount of QH (300 mg/kg, n=12: Group A) and the other a relatively low amount (30 mg/kg, n=11: Group B). At 39 weeks of age, all mice were made to perform running exercise twice to become acclimated to the treadmill (model TM-R-N1, Osaka Microsystems, Ibaraki, Japan) (I, speed 20 m/min, tilt 0 degrees, time 30 min; II, speed 20 m/min, tilt 10 degrees, time 30 min). The first running exercise (I) was done five days before measurement of running time, and the second exercise (II) was performed four days before measurement. All of the SAMP1 mice were kept at room temperature (20±1°C), with a relative humidity of about 50% and a light-dark cycle of 12 hours; food (CE-2, CLEA Japan, Inc., Tokyo, Japan) and tap water were provided ad libitum. The SAMP1 mice used in this study were useful in that senescence was accelerated after the mice grew up, and they were assumed to live for 50% of their survival time (297 days on average)[10].

In this study, running time (exercise capacity), the oxidative stress regulation system, senescence grading scores, and weight were measured at 39 to 76 weeks of age. The number of dropouts by 10 months of QH consumption (age 76 weeks) was eight (35%) overall. The reasons were unknown in six cases and other in two (time of blood sampling or time of senescence level determination) (Fig. 1).

A treadmill with the speed set to 25 m/min and tilt at 20 degrees was used for the exercise load. Both groups were made to run on the treadmill until exhaustion, and the total running duration was measured. The termination criterion for the limit of running was decided as the point at which the time interval of electrostimulation delivered to the back of the running surface of the treadmill fell below 5 seconds[8, 9].

Using analytical equipment for reactive oxygen and free radicals (model FRAS4, H&D, Parma, Italy), the d-ROM test value (reactive oxygen metabolites test: degree of oxidative stress) and BAP test value (biological antioxidant potential) were measured in a resting state, and then the latent antioxidant potential (B/R ratio) was calculated. As an oxidative stress marker, the values of plasma QH and plasma ubiquinone (plasma oxidized form of CoQ10, plasma Q10) were measured using an electrochemical detector (Shiseido Co., Ltd., Tokyo, Japan), and the reduced ratio (plasma QH value / plasma QH value + plasma Q10 value) was calculated. For the d-ROM and BAP measurements, blood was extracted from the tail vein and centrifuged immediately (for five minutes at 6,000 rpm) to obtain blood plasma for analysis.

In the d-ROM test, the levels of free radicals in the body, especially hydrogen peroxide 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 values indicated the degree of oxidative stress (oxidative reaction)[14, 15]. Meanwhile, in the BAP test, the levels were measured (unit: μM) by the reduction action of antioxidant materials in blood plasma, and the measured values indicated the degree of antioxidant potential (antioxidant reaction). That is, the amount of blood plasma reduced to ferrous ions when mixed with reagents containing ferric ions was measured as 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 antioxidant potential[14, 15]. The B/R ratio, which was calculated on the basis of the values obtained in the BAP test and the d-ROM

Table 1. Changes in running time and senescence grading scores

		Baseline (39 weeks old)	7 months of QH consumption (63 weeks old): 7 mo	10 months of QH consumption (76 weeks old): 10 mo	n
Running times (min)	Group A	21.88±6.09	9.59±4.04	7.88±3.98	7
	Group B	19.92±6.81	7.58±2.53	4.28±2.42	8
Senescence grading scores (points)	Group A	2.4±0.4	7.1±0.8	9.1±0.9	7
	Group B	2.5±0.5	8.1±0.5	10.6±1.1*	8
Weight (g)	Group A	36.2±2.0	33.0±3.5	34.6±3.0	7
	Group B	33.7±2.1	31.9±2.4	34.7±2.8	8
d-ROM test (U.CARR)	Group A	113.6±3.8	114.2±20.9	125.8±10.7	7
	Group B	115.1±8.7	118.2±11.6	163.3±42.4*	8
BAP test (μM/l)	Group A	2286.4±84.2	2098.5±546.0	2601.2±280.6	7
	Group B	2189.8±58.8*	2606.7±225.4*	2537.2±154.2	8
BAP/d-ROM (ratio)	Group A	20.2±1.2	18.4±3.0	20.8±2.7	7
	Group B	19.1±1.4	22.3±3.2*	16.5±3.8*	8

Group A, high amount of QH (300 mg/kg); Group B, low amount of QH (30 mg/kg); QH, Ubiquinol (reduced form of coenzyme Q10, H2CoQ10); d-ROM test, reactive oxygen metabolites test; BAP test, biological antioxidant potential; BAP/d-ROM ratio, calculated on the basis of the values obtained in the BAP test and d-ROM test; Values are means±SD.
*$p<0.05$ compared with the value in the intergroup comparison by the Wilcoxon signed-rank test.

test, indicates the degree of latent antioxidant potential. In other words, the latent antioxidant potential indicates the balance between oxidative stress and antioxidant potential. The reduced ratio is affected by decreases (or increases) in the plasma QH value due to oxidative stress and the amount of NAD(P)H oxidase (oxidation-reduction enzyme) needed for re-reduction of the plasma oxidized form of CoQ10[7, 16, 17]. Thus, the reduced ratio reflects the ability to convert the plasma oxidized form of CoQ10 to plasma QH, which indicates anti-oxidation and is present in abundance in the body.

Senescence grading scores, which were designed to evaluate senescence more objectively, were graded according to changes in hair luster, lesions around the eyes, curve of the spine, and so on[10]. Senescence grading scores are generally used as an acceptance criterion for the aging process. Higher scores mean that the aging process is accelerated. In the present study, the grade for senescence was determined taking our preceding study into account[10]. Weight was measured in a resting state at the age of 38 weeks using scales for animals (KN type manufactured by Natsume Seisakusho Co., Ltd., Tokyo, Japan). The amount of QH consumed was based on a previous study[13]. QH (30% stabilized powder) was mixed in the animals' water bottles and consumed daily.

Running time, the d-ROM test, the BAP test, and the senescence score were measured three times: at the start of the study (age 39 weeks; baseline), after consumption of QH for seven months (age 63 weeks; 7 mo), and after consumption of QH for 10 months (age 76 weeks; 10 mo). Plasma QH values and plasma Q10 values were measured twice, at baseline and at 10 mo.

Numerical values are presented as average values ± standard deviation. SPSS (IBM, Tokyo, Japan, Ver 19.0 for Windows) was used for statistical analyses, and the Wilcoxon signed-rank test was used to test the significance of the differences. This study was conducted with the approval of the Animal Research Committee of Saitama Prefectural University (approval number: 39).

RESULTS

The running time, senescence score, and weight were compared between the two groups. At 10 mo, only the senescence score was significantly lower in Group A, and thus inhibition of senescence was seen ($p<0.05$) (Table 1). The mean rate of change in running time from baseline to 10 mo was −62.6±20.6% in Group A and −77.7±13.8% in Group B (difference in mean percentage change between the groups: 15.1%). The d-ROM test value, BAP test value, and B/R ratio were compared between the two groups. At 10 mo, the d-ROM test value was significantly higher in Group B, and the BAP/d-ROM ratio was significantly lower (both $p<0.05$) (Table 1). For the BAP test value and the B/R ratio at 7 mo, a significant decrease was seen in Group A (both $p<0.05$), and thus, variation was seen.

Comparisons of the plasma QH values, plasma oxidized form of CoQ10 value, and the reduced ratios between the two groups showed significant increases in the plasma QH value and reduced ratio at 10 mo in Group A (both $p<0.001$) (Table 2). In Group B, a significant increase only in the plasma Q10 value was seen at 10 mo compared with baseline ($p<0.05$).

DISCUSSION

Ubiquinol (QH) has engendered interest as a supplement ingredient that has both an energy production activating effect and an antioxidant action. Its physiological effects have been verified in various investigations[12, 17–19]; however, its

Table 2. Changes in oxidative stress

		Baseline	10 months of QH consumption	n
		(39 weeks old)	(76 weeks old):10 mo	
Plasma QH (μg/mL)	Group A	0.035±0.024	0.220±0.093†	7
	Group B	0.033±0.023	0.041±0.017***	8
Plasma Q10 (μg/mL)	Group A	0.025±0.005	0.065±0.015†	7
	Group B	0.030±0.014	0.048±0.015†	8
Reduced ratio (%)	Group A	55.114±7.900	75.339± 6.200†	7
	Group B	50.777±11.600	46.227±10.300***	8

Group A, high amount of QH (300 mg/kg); Group B, low amount of QH (30 mg/kg); QH, ubiquinol (reduced form of coenzyme Q10, H2CoQ10); Plasma QH, plasma concentration of QH; plasma Q10, plasma concentration of ubiquinone; reduced ratio, calculated from plasma QH and plasma Q10; Values are means±SD.
***$p<0.001$ compared with the value in the intergroup comparison by the Wilcoxon signed-rank test.
$^{\dagger}p<0.05$ compared with the value in the baseline by the Wilcoxon signed-rank test.

effects on exercise capacity and the oxidative stress defense system when consumed in different amounts over a long period remain unknown. The importance of this study lies in the fact that it was a basic study to investigate measures to raise exercise capacity, taking into account the oxidative stress defense system.

CoQ10 shows an antifatigue effect and has been widely used by athletes to increase physical strength[19]. When running time was compared in the two groups in this study at 10 mo, no significant difference was seen; however, the mean percentage change from baseline to 10 mo was a decrease of 62.6% in Group A, compared with a decrease of 77.7% in Group B. Thus, a difference of 15.1% was seen between the groups. This suggests that high-dose consumption of QH inhibits decreases in running time, and a relationship is thought to exist between dosage and maintenance of physiological effects associated with running time. These physiological effects related to running showed similar tendencies as in a previous study[20] with consumption of the oxidized form of CoQ10. In other words, not only does cardiac muscle metabolism increase with mitochondrial activation (increase in cardiac output), but oxygen demand to active muscle is also adequately compensated by actions such as decreased peripheral vascular resistance (increased oxygen availability in the periphery), and the effect is thought to be maintained.

In general exercise, oxygen uptake in the body occurs with the purpose of continuous ATP production, and so the generation of oxidative stress increases with prolonged running time (or a decrease is inhibited)[21]. It has also been reported that exercise, through promotion of oxidative stress, may increase the expression of enzymes and proteins in the body's defense systems, including antioxidant enzymes, and may inhibit expression of inflammation-related enzymes[22]. Such activation of antioxidant enzymes has been indicated to have a compensatory effect against increased generation of oxidative stress[23]. The body has defense mechanisms using the elimination action of vitamin C[24] and the CoQ10 redox cycle[19, 25] against continuous exposure to oxidative stress. The CoQ10 redox cycle is a mechanism that maintains QH in the body by reductase, and it is an important defense mechanism that reduces the oxidative stress produced by exercise. Despite the suggestion in this study that the decrease in running time at 10 mo was inhibited in Group A, a significant decrease was seen in the d-ROM test value. This suggests the possibility that exercise-induced oxidative stress was mitigated in Group A, and that the CoQ10 redox cycle defense mechanism was activated. It therefore stands to reason that the increase in exercise-induced oxidative stress is inhibited by activation of biological defense systems. In this study, the senescence score at 10 mo was significantly lower in Group A than in Group B, and the d-ROM test value and B/R ratio were significantly lower. This suggests an association between the senescence score and the d-ROM test vaiue and B/R ratio. Generally, the main factors related to the effects of senescence on the body are deterioration of the elimination function with respect to continuous oxidative stress and promotion of senescence by the resulting augmentation of oxidative stress[3].

Consequently, the significantly decreased senescence score (senescence inhibition effect) is thought to result from a decrease in oxidative stress from the antioxidant action of QH itself[7], and maintenance of the antioxidant system with the production of ATP[26]. Group A is therefore thought to have maintained the oxidative stress elimination function and latent antioxidant function better than Group B, with delayed senescence as a result.

Group A showed a significant increase in the plasma QH value and the reduced ratio at 10 mo compared with Group B. The general energy state is thus thought to have improved due to activation of energy production in Group A, leading to the improvement of the reduced ratio during exposure to exercise-induced oxidative stress. In Group B, a significant increase was seen only in ubiquinone at 10 mo compared with baseline. The plasma QH value is therefore thought to indicate oxidation from exposure to exercise-induced oxidative stress. Dose-dependence was demonstrated in the above effects from QH consumption. Investigations of QH levels in organs and other factors will be needed in the future.

Group A showed a significant decrease in the B/R ratio at 7 mo, but variation was seen. Considering that reduction of ferric ion is evaluated using plasma in the BAP test, it is possible that the level of reduction reflects the effects from QH consumption together with a general improvement in the antioxidative state (for example, effects from vitamin C or other antioxidants). Comprehensive evaluations of antioxidant activity based on investigations of plasma vitamin C levels and other factors will be needed in the future.

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