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Anti-Fatigue Effects of Oral Supplementation with Reduced Coenzyme Q10 in Adult and Elderly Dogs

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Abstract: Elderly beagles were subjected to periods of constant exercise loads on a treadmill following administration of a reduced form coenzyme Q10 (ubiquinol) for 30 days. The anti-fatigue effect of ubiquinol on the capacity for exercise was evaluated. When ATP consumption is elevated during exercise, it is thought that ubiquinol contributes to supplying energy by promoting ATP biosynthesis. Here, we demonstrate that administration of ubiquinol suppressed serum IL-6-induced catabolism and TGF-β-associated fatigue perception. Furthermore, induction of *sirt1* gene expression and the associated activation gluconeogenesis was not induced. Lastly, PPAR-α and PPAR-γ which regulate mitochondrial composition and the acceleration of energy production by mitochondrial hyperfunction were not influenced by ubiquinol administration.

Key words: Ubiquinol, anti-fatigue, dogs, exercise, energy

INTRODUCTION

Coenzyme Q10 (CoQ10) is an electron transporter located in the inner mitochondrial membrane and prokaryotic cell membranes. It is an intermediate electron transport molecule in respiratory chain complex III (cytochrome bc1 complex), receiving electrons from complex I (NADH dehydrogenase) and complex II (succinate dehydrogenase) of the electron transport chain (McPhail and Cunningham, 1975). CoQ10 is located in phospholipid bilayers (biomembranes) and is highly hydrophobic as a result of its structure containing a quinone derivative with a relatively long isoprenoid side chain. CoQ10 exists in reduced (ubiquinol) and oxidized (ubiquinone) forms with the majority of *in vivo* CoQ10 being present in the reduced form (Aberg *et al*., 1992). Ubiquinol exhibits antioxidant activity due to its ability to directly scavenge reactive oxygen species or by regenerating other antioxidants such as vitamin E (Stocker, 1994). Due to the unique antioxidant properties of ubiquinol in the absence of ubiquinol vitamin E radical oxidizes lipid and because it cannot be removed (Mohr *et al*., 1992). Biosynthesis of CoQ10 takes place in the liver with the quinone nucleus and isoprenoid side chain synthesized from tyrosine and mevalonic acid derived from acetyl CoA, respectively (Fears, 1981). Meanwhile, it is known that CoQ10 levels decline with advancing age *in vivo* (Kalen *et al*., 1989). By supplementation with ubiquinol, aging in the senescence-accelerated mouse and aging-associated hearing deterioration in mice are suppressed (Yan *et al*., 2006). In addition, it is reported that endogenous CoQ10 is decreased in various diseases (Kalen *et al*., 1989) and post-operative hospitalization is reduced with CoQ10 supplementation (Rosenfeldt *et al*., 2005). With respect to oral supplementation of CoQ10, it is reported that the gastrointestinal absorptivity of ubiquinol is higher than the oxidized form (Mae *et al*., 2001; Kitano *et al*., 2008). Ubiquinol supplementation is expected to be beneficial due to its absorbency and functionality in exerting effects after conversion of the absorbed oxidized form into ubiquinol in the body.

The lifespan of companion animals such as dogs and cats has recently been extended due to developments in veterinary medicine as well as nutritional and functional improvements in pet foods, remediation of breeding environments and management of breeding programs. However, prolonged lifespans are accompanied by increased risk of developing various diseases including lifestyle-related diseases similar to those observed in

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humans. Therefore, improving the Quality of Life (QOL) in aging companion animals is beneficial for both animals and owners.

In this study, relatively elderly beagles were subjected to periods of constant exercise loads on a treadmill following administration of ubiquinol for 30 days. The anti-fatigue effects of ubiquinol were evaluated by assessing serum levels of various fatigue markers as well as the expression of genes related to energy metabolism and stress tolerance in the liver.

MATERIALS AND METHODS

All animal experiments in the present study were conducted according to the guidelines of the Committee for Animal Experimentation at Azabu University (Authorization No. 120510).

Animals and treatments: The studies were conducted on 5-9 years old beagle dogs (4 male and 4 female; 10.0-13.5 kg body weight). The animals were housed in separate cages (width 47×depth 75×height 56 cm) and maintained on a commercial diet (LabDiet 5006; Japan SLC Inc., Shizuoka, Japan) and tap water *ad libitum* with a temperature of 22±3°C, relative humidity of 55±10% and a 12 h light: 12 h dark schedule. The subjects were divided into two groups (four dogs per group) and allocated to the reduced coenzyme Q10 (ubiquinol) administration group or placebo control group. Two Softgel capsules containing 50 mg ubiquinol per tablet (Kaneka Corporation, Osaka, Japan) or two Softgel capsules containing no ubiquinol were administrated orally in the afternoon meal (16:00 h) for 30 days.

Treadmill exercise tolerance test and sampling: On the 15th and 30th day of administration, all dogs were exercised and blood was drawn from the cephalic vein immediately before and following exercise and 60 min after exercise. All dogs underwent treadmill training every other day for ~1 month prior to initiation of the study. The treadmill used in this study was a PerRun (PR720F, IWATE International Developing Co., Ltd. TaiChung, Taiwan). The procedure for each walk was performed was identical for all dogs. Each dog was restrained with a standard harness that was loosely attached to the treadmill with a leash. After increasing the treadmill belt speed until a steady walk was obtained, recording of the dog walking at a treadmill belt speed of 100 m min^{-1} was obtained and walking gait data were recorded for 30 min at the defined speed. Liver tissues were harvested by biopsy following 3 h of post-exercise behavioral observation recording on the 30th day of administration. The liver tissue biopsies were performed under inhalational anesthesia with isoflurane following the induction of anesthesia with the intravenous propofol. Using an ultrasonographic guide, the biopsy needle was inserted from the abdomen and liver tissue was obtained.

Serum cytokine and hormone analysis: Serum samples were frozen at -80°C until cytokine and hormone analysis. Cytokines were measured using commercial ELISA kits (IL-6 and TGF-β1: R&D Systems, Inc., Minneapolis, MN).

RNA isolation and Reverse Transcription Polymerase Chain Reaction (RT-PCR): Total RNA was extracted from the biopsied liver tissues with a high pure RNA tissue kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. First-strand cDNA was synthesized from 500 ng of total RNA using reverse transcriptase (PrimeScript II RTase, Takara Bio Inc., Shiga, Japan) and an oligo dT primer. PCR amplification from reverse-transcribed cDNA was carried out with primers specifically designed for the target genes (Table 1). GAPDH was used as an internal control to normalize template concentrations. Briefly, the PCR reactions were performed by adding 1 μL of reverse-transcribed cDNA to a 50 μL reaction mixture containing each dNTP at 2.5 mM, 1.25 units of Taq polymerase (ExTaq; Takara Bio Inc.) and each primer at 0.2 μM. Thermal cycling was performed with a thermal cycler (PC812; Astec Co., Ltd. Fukuoka, Japan) according to the conditions shown Table 1. The PCR product concentrations were proportional to the starting cDNA concentrations with the above cycle profile for each gene. PCR products were detected on a 1.5% agarose gel and stained with ethidium bromide (Nacalai Tesque). Quantitative analysis of gene expression levels was performed by scanning gels stained with ethidium bromide with a gel imager (Gel Scene; Astec) and subsequent analysis with Image J Software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis: Data are expressed as means±SD. Differences among groups of rats were assessed using t-tests. Following analysis of variance with the F-test, the data showing equality of variance was examined with the student's t-test while the data with unequal variance was examined using Welch's t-test. $p<0.05$ were regarded as statistically significant.

Table 1: Oligonucleotide sequences used for PCR amplification

Genes	Primer sequences	Anealing temperature	Amplicon size (bp)
Sirt1	F: 5'-TGCATTGCTTCTTTCCCACA-3'	64	152
	R: 5'-AGTGGCCTGTTGCTCTCCTC-3'		
PPAR-α	F: 5'-GCTCAAGTAGGGTCACCGTG-3'	62	104
	R: 5'-GGCAGTTCGGAGGTAAAGG-3'		
PPAR-γ	F: 5'-CCCTCTTCCATGCTGTTATGG-3'	64	182
	R: 5'-GTCATCCATTACGGACAGATCC-3'		
Glucose-6-Phosphatase (*G6P*)	F: 5'-TGGACAGCGTCCATACTGGTG-3'	64	130
	R: 5'-TGTACCCATGGCATGACCAGA-3'		
Hepatocyte Nuclear Factor 4 (*HNF4*)	F: 5'-AACGTGCAGGTGTTGACGATG-3'	64	98
	R: 5'-ATGGCACACAGAGCGCTGAC-3'		
Forkhead box 01 (*FOX01*)	F: 5'-GCATGTTTATTGAGCGCTTGGA-3'	64	148
	R: 5'-ACCCAGCTATGTGTCGTTGTCTTG-3'		
Glyceraldehyde-3-Phosphate Dehydrogenage (*GAPDH*)	F: 5'-GCCCTCAATGACCACTTTGT-3'	60	101
	R: 5'-TCCTTGGAGGCCATGTAGAC-3'		

RESULTS AND DISCUSSION

Changes in serum cytokine levels by ubiquinol administration: Figure 1 shows the serum levels of IL-6 and TGF-β observed before and after the Treadmill Exercise Tolerance test following 15 or 30 days of ubiquinol administration. There were no significant alterations in the levels of either serum cytokine before examination and pre-exercise in the ubiquinol administered group. Therefore, ubiquinol did not influence resting serum levels of either cytokine. Serum IL-6 levels increased in the placebo group 60 min post-exercise whereas elevated IL-6 levels were not observed 60 min post-exercise in the ubiquinol-treated group, regardless of the administration period. Serum TGF-β levels showed a tendency to increase post-exercise with considerable individual variability in the placebo group. However, serum TGF-β levels in the ubiquinol administration group remained unaltered.

Change in gene expression in the liver: Figure 2 shows the expression levels of stress-tolerance, glycolysis and gluconeogenesis genes in the liver after the 3 h treadmill exercise tolerance test following 30 days of administration of ubiquinol. Of the genes that participate in stress resistance, the expression of sirtuin (sirt) 1 showed a tendency to decrease ($p = 0.054$) while Peroxisomal Proliferator-Activated Receptor (PPAR)-α and PPAR-γ were not different from the placebo group. The expression of Glucose-6-Phosphatase (G6P; the rate-limiting enzyme in the gluconeogenesis pathway), Hepatocyte Nuclear Factor (HNF) 4 and Forkhead box (FOX) 01 which promotes transcription of G6P were not significantly altered by ubiquinol administration.

In this study, relatively elderly beagles were subjected to periods of constant exercise loads on a treadmill following administration of ubiquinol for 30 days. The anti-fatigue effects of ubiquinol were evaluated by assessing serum levels of various fatigue markers as well as the expression of genes related to energy metabolism and stress tolerance in the liver. It was found that the exercise-induced elevation in serum levels (60 min post-exercise) of IL-6 and TGF-β were suppressed

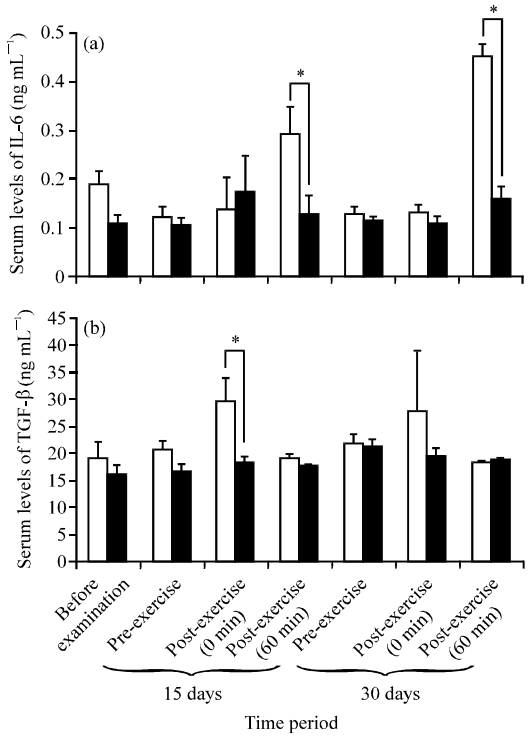

Fig. 1: Alterations in serum levels of IL-6 and TGF-β. Serum levels of: a) IL-6 and b) TGF-β were investigated as sensitive markers of fatigue levels. Data are expressed as means±SD of four individual animals. Open and closed bars represent placebo and ubiquinol treatment, respectively. Data that differ significantly ($p<0.05$) are indicated by an asterisk at the top of the bar

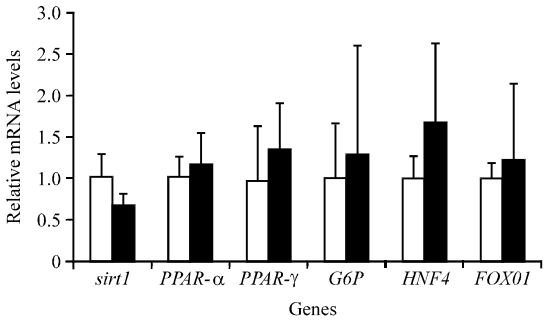

Fig. 2: Alterations in mRNA levels of fatigue related genes in the liver. Relative levels of each gene were calculated as a percentage relative to levels of an appropriate control gene, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH). Data are expressed as means±SD of four individual animals. Open and closed bars represent placebo and ubiquinol treatment, respectively

by ubiquinol administration while pre and post-exercise levels were not different from pre-exercise levels. Furthermore, administration of ubiquinol suppressed *sirt1* gene expression which is induced by energy depletion following exercise and did not influence gene expression of PPAR-α and PPAR-γ which regulate mitochondrial composition and the acceleration of energy production by mitochondrial hyperfunction.

IL-6 is known to be secreted following muscular contraction and increases insulin secretion via Glucagon Like Peptide (GLP)-1 (Pedersen and Febbraio, 2008; Steensberg *et al*., 2000; Ellingsgaard *et al*., 2011). Exercise-induced IL-6 secretion promotes glucose mobilization by glycogenolysis and fatty acid mobilization by lipolysis in an effort to compensate for energy depletion (Ellingsgaard *et al*., 2011). Therefore, in the placebo group, it is thought that exercise-induced energy depletion precipitated a compensatory increase in IL-6 secretion. In cultured cardiomyocytes, it has been reported that CoQ10 promotes ATP biosynthesis (Langsjoen and Langsjoen, 2008). When ATP consumption is high (during exercise and other conditions), it is thought that CoQ10 contributes to supplying energy by promoting ATP biosynthesis. Thus, in the ubiquinol administration group, serum levels of IL-6 would not change following exercise because extreme energy depletion is not achieved.

It is reported that TGF-β participates in the generation of feelings of fatigue following exercise. Brain levels of TGF-β are increased by exercise in the rat (Inoue *et al*., 1999) whereas spontaneous behavior is decreased by TGF-β administration in the rat brain at rest (Arai *et al*., 2002). Thus, TGF-β is understood to be one of the substances that communicate feelings of fatigue in the central nervous system. In the placebo group, elevated serum levels of TGF-β following exercise were observed. However, in the ubiquinol-treated group, serum levels of TGF-β were largely unchanged before and after exercise. Thus, the perception of fatigue resulting from exercise was possibly reduced in the ubiquinol-treated group.

When cellular ATP levels are decreased by exercise, NAD^+ levels and AMPK activity increase (Canto *et al*., 2009; Christopher *et al*., 2003). Increased levels of NAD^+ activate *sirt1* gene expression and activated AMPK induces gene expression of PGC-1α (Canto *et al*., 2009). SIRT1 induces gluconeogenesis gene by deacetylation of PCG-1α (Canto *et al*., 2009), thereby promoting reuse of lactate and synthesis of the ATP. Although, the exercise conditions in this study were likely to decrease ATP levels, the expression of the *sirt1* gene in the ubiquinol-treated group was low, compared to the control group. There was no change in the expression of the *G6P* gene which is the rate-limiting enzyme in the glycolytic pathway in both groups. Furthermore, significant differences were not observed in the expression of the *HNF4* and *FOX01* genes which act as transcription factors driving *G6P* gene expression (Hirota *et al*., 2008). Because the exercise-induced depletion of ATP was immediately replenished by the high efficiency ATP production induced by ubiquinol administration, it is thought that the decreased gene expression of sirt1 resulted from the prevention of elevated NAD^+ levels.

It is reported that PPAR-α functions to reduce mitochondrial gene expression or to inhibit energy production by forming a complex with SIRT1 (Oka *et al*., 2011). In addition, PPAR-γ requires heterodimer formation with Retinoid X Receptor (RXR) to act as a transcription activator and acts as a transcription factor for gluconeogenesis (Chandra *et al*., 2008). In this study, the gene expression of PPAR-α and PPAR-γ were not altered by ubiquinol treatment. However, PPAR-RXR complex formation is promoted by PGC-1α (Lowell and Spiegelman, 2000) and the expression of the *PGC-1α* gene would be low or not activated, owing to the low expression of sirt1 mentioned above. Because PPAR-γ was not required, it is thought that the unaltered *PPAR* gene expression observed here is consistent with expectations. However, because ubiquinol administration decreased *sirt1* gene expression but did not change *PPAR-α* gene expression, it is thought that the inhibition of mitochondrial activation by the SIRT1-PPAR-α complex (Oka *et al*., 2011) was diminished in this study. These conditions will enable ubiquinol-induced increases in ATP production efficiently in the electron transport chain.

Animals are routinely exposed to a variety of stresses, much like Homo sapiens. Animals can avoid stress if they are in a healthy state. However, reduction and avoidance of stress may be difficult in situations such as advanced age or decreased QOL. These situations may lead to susceptibility to various kinds of illness, aggravation of symptoms and problematic behavior (Beerda *et al*., 1997, 1998). Ubiquinol is a material capable of scavenging oxygen radicals generated as a co-product of the oxygen-dependent energy production process as well as promoting ATP production. In addition, the reduced form (ubiquinol) which exists *in vivo* is superior to the oxidized form in oral absorbability and can be expected to be a more effective oral therapeutic agent. There is a report that congestive heart failure symptoms were improved by administration of ubiquinol in a patient that had previously failed to exhibit improvements following continued administration of oxidized coenzyme Q10 (Langsjoen and Langsjoen, 2008). In the previous study, researchers confirmed that administration of ubiquinol induces increased active mass and mental sanity in elderly people.

CONCLUSION

In this study, we evaluated the stress reduction effects of ubiquinol administration using an experimental model involving exercise on a treadmill in adult to elderly dogs. As a result, it is suggested that exercise-induced stress was reduced because continuous ubiquinol treatment facilitated internal energy supply. This stress-reduction effect contributes to maintenance of the daily QOL in elderly animals as well as effective treatment by improving perioperative and postoperative QOL. Furthermore, it is thought that the effects of ubiquinol can greatly contribute to veterinary medical care.

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