Quercetin Enhances Endurance Capacity *via* Antioxidant Activity and Size of Muscle Fibre Type 1

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Abstract: This study aimed to examine effects of quercetin on 1) endurance capacity 2) malondialdehyde (MDA) and superoxide dismutase (SOD) activity in skeletal muscle and 3) muscle fibre density and size in mice after an intense exercise. There were 5 groups: control, vitamin C (250 mg/kg body weight), quercetin 150, 300, and 450 mg/kg body weight respectively once a day for 28 days. Endurance capacity was measured by exhaustive swimming exercise test which was done 24-h after swimming at high intensity. Then muscles were analyzed for MDA, SOD activity, and muscle fibre density and size. After the 28-day treatment, endurance time in vitamin C and quercetin treated groups at dose of 150 mg/kg body weight were longer than the vehicle group ($p<0.05$). MDA concentration in extensor *digitorum longus* muscle was lower than vehicle group in vitamin C, Q150 and Q450 groups and in soleus muscle the highest was in Q300 group. SOD activity in soleus muscle in Q150 group was higher than vitamin C and Q450 groups. Significant increase in cell diameter of soleus muscle was found ($p<0.05$). Current findings suggest that ingestion of quercetin can improve endurance capacity, due probably to increased antioxidant activity and size of muscle fibre type 1.

Keywords: Quercetin, oxidative stress, time to exhaustion, slow twitch muscle fibre.

INTRODUCTION

Muscular rate of consumption of oxygen increases with strenuous exercises which in turn in increases level of free radical generation in the exercising muscles [1-3]. These free radical-induced reactions gradually destroy cell membrane structure, impede cell function and cause changes in gene expression. Also, muscular damage induced by free radical activity causes inflammation, early fatigue and decrease in muscle endurance.

Human body naturally produces antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase; however, during strenuous exercise, the antioxidant defense systems in the body are overwhelmed by the increased level of free radical generations. Therefore, certain nutrients from diet can help to augment the body's defense systems against free radicals. Proper antioxidant supplementation under conditions of strenuous exercise may enhance performance by preventing the exercise-induced tissue damage and increasing the recovery process [4]. On the other hand, the actual individual intakes of antioxidant nutrients are often below the recommended intakes, thus dietary habits that ensure adequate supply of antioxidants are crucial. Also, athletes who are involved in sports either recreational or professionally always seek improvement in their performance by using ergogenic aids effectively and legally. The null findings indicate that metabolic and physical performance consequences of quercetin supplementation observed in mice should not be generalized to humans [5].

Recently, quercetin (3,4,5,7-pentahydroxylflavone; molecular mass 302.236 g mol/1) caught the attention of sports scientists because it has shown several physiological benefits including cardioprotective, anticarcinogenic, antioxidant, antiapoptotic, and ergogenic properties [6-10]. Athletes may gain advantages from using quercetin to improve their muscular endurance. In fact, some studies have shown the beneficial effect of quercetin supplementation on endurance performance [11-14], but other studies have

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found no effect of quercetin on endurance capacity [15-18]. Moreover, it is noted that only one study examined the anti-oxidative effect of quercetin feeding on endurance performance [17]. This previous study did not find antioxidant capacity or oxidative damage after the ingestion of oral quercetin (250 mg, 4 times/day, 1,000 mg/day total) before and during an ultra-marathon challenge. Because endurance capacity is determined not only by cardiorespiratory function but also by metabolic profile of muscle, it should be assessed with muscle metabolic profile of different muscle fibre types. However, there is no study on the effect of quercetin on combined endurance capacity with metabolic profile and size of muscle fibre. We hypothesized that quercetin supplementation increased endurance capacity by enhancing activity of antioxidant enzyme and size of muscle fibre type 1. Therefore, this study aimed to examine the effects of a short term supplementation of quercetin on endurance capacity, oxidative stress and muscle fibre density and size in mice.

METHODS

Animal Experiment

Seven week-old male Imprinting Control Region (ICR) mice were maintained under constant environmental conditions (temperature: 21-24^{o}C, relative humidity: 35-65%, 12-h light/12-h dark cycle). All procedures with the animals were performed with approval of the Institutional Animal Care and Use Committee from Faculty of Medicine, Khon Kaen University (AKKU 42/2553). They were randomly divided into 5 groups with 8 mice per cage. Mice in group 1 (vehicle group) were treated with propylene glycol as a control, mice in group 2 were treated with vitamin C (250 mg/kg BW) as a treatment control and mice in group 3, 4 and 5 were treated with quercetin 150, 300 and 450 mg/kg BW respectively. Quercetin was administered by gavage to the mice once a day. After 28-day treatment the mice were sacrificed under ether anesthesia.

Endurance Capacity Test

In order to evaluate endurance capacity, the mice performed swimming endurance test after training by swimming for 14 days. In this test, the mice were forced to swim in a vertical plastic cylinder (diameter 21 cm, height 50 cm) containing 25 cm of water maintained at 25 ± 1˚C until their legs stop more than 10 seconds [19]. Then at day 1, 7, 14, 21 and 28, they performed a heavy exercise and at day 2, 8, 15, 22 and 29, they performed endurance capacity test by recording endurance time. The endurance capacity test was performed according to the model of Ikeuchi *et al*. [20]. Weight resistance of 5% BW was fixed at the tails of mice during exhaust swimming exercise test so that mice could not float on water surface but they tried to swim up to water surface for breathing within 5 seconds. At the end of the swimming test, the animal was removed from the cylinder, dried by a towel, and returned to its home cage. The water was changed after each rat swimming. The endurance time was recorded at day 2, 8, 15, 22 and 29.

Preparation of Tissue Homogenates

Afterwards, extensor digitarum longus (EDL), soleus and gastrocnemius muscles were obtained from mice legs to determine lipid peroxidation (MDA) and antioxidant (SOD). In addition, belly parts of these muscles were obtained to determine muscle fibre density and size. After the last administration of substances, all animals were anesthetized with intraperitoneal injection of pentobarbital sodium at dose of 50 mg/kg BW. Muscle tissues were isolated and kept cool in ice buckets. Then the tissues were homogenized in 4 volume of 1.15% KCl with glass Potter-Elvehjim homogenizer [21].

Determination of Malondialdehyde (MDA) Concentration and Superoxide Dismutase (SOD) Activity

EDL, soleus and gastrocnemius muscles were obtained from mice legs to determine MDA and SOD. The quantitative measurement of MDA in the muscle tissues was measured according to the method of Liu *et al*. [22]. The amount of MDA formed, a lipid peroxidation product, is quantified by the reaction with thiobarbituric acid (TBA). Reagents acetic acid 1.5 ml (20%) pH 3.5, 1.5 ml thiobarbituric acid (0.8%) and 0.2 ml sodium dodecyl sulphate (8.1%) were added to 0.1 ml of processed tissue samples, and heated at 100°C for 60 min. Mixture was cooled under tap water and 5 ml of n-butanol-pyridine (15:1), 1 ml of distilled water were added and vortexed vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was separated and absorbance was measured at 532 nm using a spectrophotometer. The results of MDA concentration were expressed as nmol/mg protein. The SOD activity was determined by the method of Sun *et al*. [23]. All tissues homogenates were evaluated for SOD spectrophotometrically.

Hematoxylin & Eosin (H&E) Staining Muscle Cell

EDL, soleus and gastrocnemius muscles were obtained to determine muscle fibre density and size.

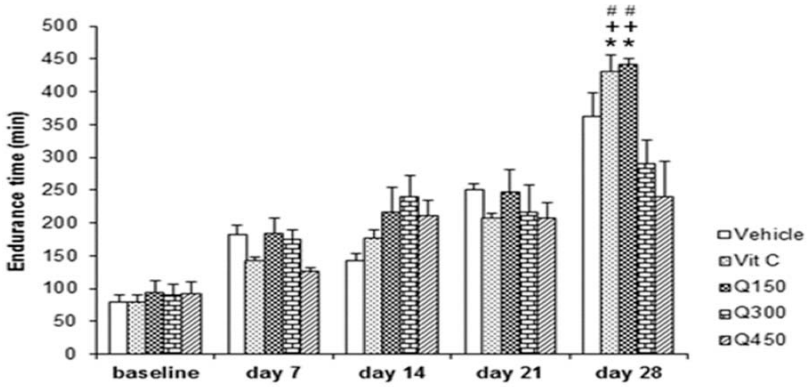

Figure 1: Comparison of endurance time (min) among the groups at the same time (baseline, day 7, day 14, day 21 and day 28). Values are expressed as mean ± SD; n=8/group. Vit C, vitamin C; Q150, quercetin 150 mg/kg BW group; Q300, quercetin 300 mg/kg BW group; Q450, quercetin 450 mg/kg BW group.

*Significantly different from vehicle group ($p<0.05$), + significantly different from quercetin 300 mg/kg BW group ($p<0.05$), # significantly different from quercetin 450 mg/kg BW group ($p<0.05$).

The animals were perfused transcardially with 0.9% normal saline. These muscles were collected and immersed sequentially for 24 h each in 10% formalin. Tissues were embedded in paraffin and cut by microtome at 10 µm thick and stained with hematoxylin and eosin. Histological analysis was performed by light microscopic examination to determine muscle fibre density and size by using the program of Image Pro-plus 5.1.

Statistical Analysis

All data were analyzed by one-way ANOVA and unpaired *t*-test using SPSS v 12. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Mice in vitamin C and quercetin 150 mg/kg BW groups had significantly longer exhaustive time than those in the vehicle group (Figure **1**).

MDA concentration in EDL muscle was the lowest in Q150 group and in soleus muscle the highest was in Q300 group (Table **1**). SOD activity in soleus muscle in Q150 group was higher than vitamin C and Q450 groups. There were no effects of quercetin supplementation on MDA concentration and SOD activity in gastrocnemius muscle (Table **1**).

There were no effects of quercetin supplementation on cell density of EDL, soleus and gastrocnemius muscles (Table **2**) and on the diameter of EDL and gastrocnemius muscles (Table **2**). However, the diameter of soleus muscle in quercetin 150 mg/kg BW group was significantly larger than that in vehicle group ($p < 0.05$).

DISCUSSION

The novel finding of this study was that the 4-week quercetin supplementation enhanced endurance capacity with increased in antioxidant activity and size of muscle fibre type 1.

Table 1: Malondialdehyde (MDA) Concentration and Superoxide Dismutase (SOD) Activity in Extensor *Digitorum longus* (EDL), Soleus and Gastrocnemius Muscles After the 4-Week Ingestion of Quercetin

Group	MDA concentration (µmol/mg protein)			SOD activity (µmol/mg protein)		
	EDL	Soleus	Gastrocnemius	EDL	Soleus	Gastrocnemius
Vehicle	0.40±0.02	0.08±0.01$^{\#}$	0.41±0.10	1.59±0.11	1.25±0.12	1.7±0.39
Vit C	0.13±0.02*	0.11±0.02$^{\#}$	0.5±0.09	1.72±0.25	0.61±0.14$^{\alpha}$	2.02±0.21
Q 150	0.09±0.01* $^{\#}$	0.05±0.01$^{\#}$	0.39±0.04	1.07±0.31	1.73±0.22	2.21±0.62
Q 300	0.36±0.04	0.42±0.05	0.58±0.14	1.77±0.42	0.86±0.12	2.35±0.19
Q 450	0.18±0.04*	0.18±0.01$^{\#}$	0.42±0.08	1.85±0.39	0.32±0.07$^{\alpha}$	1.03±0.19

Values are expressed as mean ± SD; n=8/group.
Vit C, vitamin C group; Q150, quercetin 150 mg/kg BW group; Q300, quercetin 300 mg/kg BW group; Q450, quercetin 450 mg/kg BW group.
*Significantly different from vehicle group ($p < 0.05$), $^{\alpha}$ significantly different from quercetin 150 mg/kg BW group ($p < 0.05$), $^{\#}$ significantly different from quercetin 300 mg/kg BW group ($p < 0.05$).

Table 2: Cell Density and Size of Extensor *Digitorum longus* (EDL), Soleus and Gastrocnemius Muscles After the 4-Week Ingestion of Quercetin Among Groups

	Cell density (cells/μm^2)			Diameter (μm)		
	EDL	soleus	gastrocnemius	EDL	soleus	gastrocnemius
Vehicle	84±8	106±20	76±18	35.9±2.3	36.8±0.9	42.6±2.2
Vit C	87±18	85±18	82±15	35.1±1.7	36.8±3.2	43.3±2.3
Q 150	81±8	91±35	68±14	36.3±3.2	43.6±7.3*	45.2±1.8
Q 300	93±14	91±19	65±12	35.3±3.2	40.6±4.1	43.4±1.2
Q 450	97±5	75±22	72±9	38.8±1.9	38.2±1.9	43.5±1.5

Values are expressed as mean ± SD. (n=8/group).
Vit C, vitamin C group; Q150, quercetin 150 mg/kg BW group; Q300, quercetin 300 mg/kg BW group; Q450, quercetin 450 mg/kg BW group.
*Significantly different from vehicle group ($p < 0.05$).

Similar to the result of our study, a previous animal study also showed that ingestion of quercetin for 7 days in mice increased running distance by 36-37% compared with the placebo group [10]. Yu *et al*. [24] reported that the swimming time was increased by 7.7%, 34.5%, and 61.5% after ingestion of rutin, catechin and isoquercetin, respectively.

Also, studies in human showed improved endurance performance after ingestion of 1 g of quercetin for 7-42 days in trained and untrained subjects [11, 13, 14]. On the other hands, other studies did not find any effect of quercetin on endurance performance in human [5, 15, 18, 25-27]. This may be due to low dose of quercetin ingestion, different performance test or different condition of subjects [28].

Possible mechanisms for the increased endurance capacity in this study may be attributed to improved antioxidant activity and size of muscle fibre type 1. In this study, quercetin is likely to attenuate exercise-induced elevation in reactive oxygen species (ROS) in a muscle fibre type 1 by improving antioxidant activity (indicated by SOD activity). This may result in decreased ROS-caused cytotoxicity, injury, and inflammation in soleus muscle. This contributed to enhanced endurance capacity after prior intense exercise in this study. Moreover, the supplementation of quercetin may increase mRNA of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) which related to mitochondrial biogenesis [29]. It has been reported that mRNA of PGC-1α is very highly expressed in type-1-rich muscle; soleus muscle [30], but is low in type-2-rich muscles; EDL, quadriceps, tibialis anterior and gastrocnemius. This may result in enlarged soleus muscle leading to improved endurance time in this study [10]. According to the unaltered cell density and diameter of EDL and gastrocnemius muscles after the supplementation of quercetin, this may confirm that quercetin may not work on the muscle fibre type **2** (EDL and gastrocnemius muscle).

In conclusion, a short-term ingestion of quercetin can improve endurance capacity with increases in the antioxidant activity and the size of muscle fibre type 1 during recovery from a prior intense exercise. This may be advantageous for athletes who need to play a subsequent sport match after one day break.

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APPENDIX

EDL = extensor digitorum longus

MDA = malondialdehyde

Q150 = quercetin 150 mg/kg BW group

Q300 = quercetin 300 mg/kg BW group

Q450 = quercetin 450 mg/kg BW group

SOD = superoxide dismutase

Vit C = vitamin C

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