Evaluation of anti-fatigue and immunomodulating effects of quercetin in strenuous exercise mice

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Abstract. The purpose of the present study was to investigate the anti-fatigue and immunomodulating effects of quercetin in strenuous exercise mice. Mice were given orally either corn oil or quercetin (20, 40 and 60 mg/kg body weight suspended in corn oil) by gavage once a day for 28 day. All mice were sacrificed after rotarod test and the major biochemical parameters were analyzed in serum and liver. The results indicated that quercetin possessed anti-fatigue effects by prolonging retention times, decreasing levels of blood lactate and serum urea nitrogen, and increasing levels of blood glucose, tissue glycogen and serum glucagon. Furthermore, quercetin could improve the immune function of fatigue mice by decreasing tumor necrosis factor-α levels, and elevated interleukin-10 levels. Quercetin possessed anti-fatigue effects may be related to its immunomodulating effects.

1. Introduction

Flavonoids, a large group of polyphenolic derivatives of benzo-c-pyrone, are widely distributed in the plant community and have a variety of pharmacological and biological activities. According to the different structure, flavonoids can be classified as flavones, flavonols, flavanones, isoflavones, chalcone, dihydrochalcone, orange ketones, flavans, flavanols, and so on [1]. Quercetin belongs to flavonols group, which consists of 3 rings and 5 hydroxyl groups, and exists as a variety of glycosides or in aglycone form [2]. Quercetin is widely found in the flowers, leaves and fruits of plants. Many food sources, including leeks, tomatoes, broccoli, apples, green tea, black tea, black grapes, blueberries and buckwheat, are rich in quercetin [3]. In addition, there are more than 100 kinds of Chinese medicinal plants containing quercetin, such as *Dendranthema morifolium* (Ramat.)Tzvel, *Plantago asiatica* L, *Gynostemma pentaphyllum* (Thunb.) Makino, *Forsythia suspensa* (Thunb.) Vah, *Houttuynia cordata* Thunb, *Phyllanthus emblica* Linn. etc. Modern pharmacological studies have confirmed that quercetin has multiple biological activities, including anti-oxidation, anti-cancer, anti-thrombosis, anti-diabetes, anti-ulcer, anti-inflammatory, anti-allergy, anti-virus, anti-ageing, anti-apoptosis, cardioprotection, cataract prevention, and neuroprotective [4-5]. Nevertheless, few studies have examined the effects of quercetin on physical fatigue. Thence, the purpose of the current study was to investigate the anti-fatigue and immunomodulating effects of quercetin in exhaustive exercise mice using rotarod test, which will provide a new experimental basis for quercetin as a nutritional supplement.

2. Materials and methods

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2.1. Chemicals and reagents

Quercetin (> 98% purity) was obtained from Yiji Biomedical Co., Ltd (Shanghai, China). The assay kits for the detection of blood glucose, blood lactate (BLA), and serum urea nitrogen (SUN) were obtained from Jiancheng Biological Reagents Co. (Nanjing, China). The assay kit for the detection of serum glucagon was obtained from Meilian Biological Technology Co., Ltd (Shanghai, China). The assay kits for the detection of tissue glycogen were obtained from Leigen Biotechnology Co., Ltd. (Beijing, China). The assay kits for the detection of interleukin (IL)-10 and tumor necrosis factor-α (TNF-α) were obtained from Yuanye Bioengineering Co., Ltd (Shanghai, China). All other chemicals and reagents (analytical grade) were obtained from commercial channels.

2.2. Animals and treatment

Male Kun-Ming mice, weighing approximately 20 ± 2 g, were obtained from the Drug Safety Evaluation Research Center of Hunan Province (Changsha, China). The animals were fed with standard rodent feed and had free access to drinking water, and housed in a feeding room maintained at 23 ± 2 °C with a 12–12-h light–dark cycle. The experimental animals were processed in strict accordance with the Measures for the Administration of Laboratory Animals in Central South University (Changsha, China). The approval certificate of this experiment was obtained from the Ethics Committee of Central South University. After 7 days of adaptive feeding, the mice were randomly distributed into the following four groups (10 mice per group): control group (C), quercetin treatment at low dose group (LQu), quercetin treatment at medium dose group (MQu), and quercetin treatment at high dose group (HQu). The quercetin treatment groups were administered with various doses of quercetin (20, 40 and 60 mg/kg body weight suspended in 1.0 mL of corn oil). Meanwhile, the C group was administered with 1.0 mL of corn oil. The mice were treated using gavage by oral administration, once a day for 28 days.

2.3. Rotarod test

The mice of all groups were subjected to adapt to the automated rotarod instrument (ZH-YLS-4C, Anhui Zhenghua Biological Instrument Equipment Co., Ltd, Huaibei, China) for two days at a speed of 18 m/min for 10 min before the rotarod test. On the last day of the experiment, 30 min after the last administration, the mice in all the groups were made to carry out the rotarod test at a speed of 36 m/min until exhausted. Exhaustion is defined as the mouse falling from the rotarod, and the retention times of mice on the rotarod were recorded.

2.4. Biochemical analysis

After the rotarod test, the mice were sacrificed by decapitation under anaesthesia (ether). Blood was collected for the BLA and glucose analysis, and then serum was separated by centrifugation (3,000 × g, 25 °C, 10 min) for the SUN, glucagon, TNF-α, and IL-10 analysis. After the blood was collected, the liver and gastrocnemius muscle were immediately resected and washed in ice-cold saline solution. The samples were then frozen in liquid nitrogen and kept at -80 °C for the tissue glycogen analysis. The above biochemical indicators were analyzed by using commercial kits and were carried out according to the manufacturer's recommended method.

2.5. Statistical analysis

Means ± SD was use for data express. Statistical analysis was performed using data analysis software (SPSS 17.0) by one-way analysis of variance (ANOVA) and then Dunnett's test. $P<0.05$ considered to be significant.

3. Results and discussion

3.1. Effects of quercetin on retention times of mice

Exercise endurance is the most direct and objective expression of fatigue resistance, and the degree of fatigue can be determined by the length of retention times [6]. As shown in Figure 1, the retention

times in the LQu, MQu and HQu groups were significantly longer than those of the C group (P<0.05). These results indicated that quercetin could enhance exercise endurance and possessed anti-fatigue effects.

3.2. Effects of quercetin on BLA and SUN of mice

BLA is a carbohydrate glycolytic product under anaerobic conditions, and it is well known that strenuous exercise increases the energy consumption in the body, and therefore enhances the production of lactate. Increased lactate levels further reduce pH value, which can induce a variety of biochemical and physiological side effects, leading to fatigue [7]. Urea nitrogen is a metabolite of protein. During strenuous exercise, protein and amino acid catabolism would be involved in energy supply when the body can't get enough energy through the sugar and fat metabolism, and produce urea nitrogen. There is an inverse correlation between the SUN level in vivo and the exercise tolerance [8]. Therefore, BLA and SUN are important blood biochemical parameter associated with fatigue. As shown in Figure 2, the BLA and SUN levels in the LQu, MQu and HQu groups were significantly lower than those of the C group (P<0.05). These results indicated that quercetin could effectively delay the production of BLA or remove the accumulation of BLA, reduce protein metabolism by decreasing SUN levels, and ultimately alleviates fatigue.

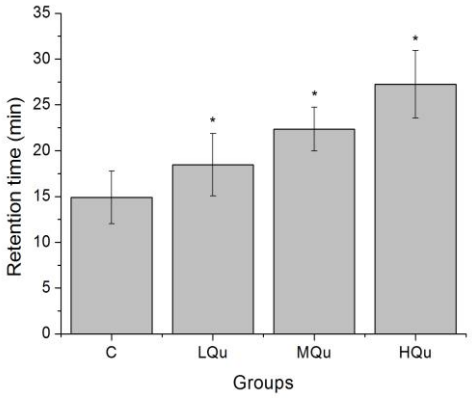

Figure 1. Effects of quercetin on retention times of mice. Data were expressed as means ± SD. *P<0.05, compared with C group.

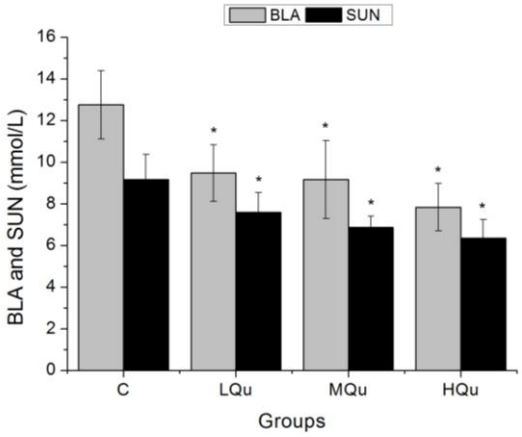

Figure 2. Effects of quercetin on BLA and SUN of mice. Data were expressed as means ± SD. *P<0.05, compared with C group.

3.3. Effects of quercetin on blood glucose and tissue glycogen of mice

Sugar oxidation has the advantages of low oxygen consumption and large output power, which is the main source of energy required for the anaerobic exercise. It is important to keep blood glucose values within the physiological range, because it is expeditiously available and can be directly oxidized to supply ATP in the blood [9]. During strenuous exercise, blood glucose is quickly depleted, if not promptly supplied, resulting in hypoglycemia, which could inhibit the central nervous system function, thereby reducing exercise endurance. So, blood glucose levels can be used to indicate the rate and extent of fatigue development [10]. Besides, energy for exercise is originally derived from the decomposition of glycogen, and glycogen stored in the liver and muscles as an energy reserve and balancing blood glucose values, which is another important factor related to exercise endurance [11]. As shown in Figure 3, the levels of blood glucose and liver glycogen in the LQu, MQu and HQu groups, as well as muscle glycogen levels in the MQu and HQu group were significantly higher than those of the C group (P<0.05). These results indicated that quercetin made mice resistant to fatigue by means of maintaining blood glucose values within the physiological range and increasing the reserve of liver and muscle glycogen.

3.4. Effects of quercetin on serum glucagon of mice

Glucagon is a peptide hormone, secreted from the islet α-cells. Glucagon plays an important role in glucose homeostasis by regulating liver glucose output in both normo- and hypoglycemic conditions [12]. Glucago can activate the liver phosphorylase, thereby promoting the rapid decomposition of glycogen into glucose. In addition, glucago can rapidly promote gluconeogenesis, including lactic acid, glycerol and sugar amino acid non-sugar substances into glucose, so as to maintain the glucose homeostasis [13]. As shown in Figure 4, the serum glucagon level in the LQu, MQu and HQu groups were significantly higher than those of the C group ($P<0.05$). These results indicated that quercetin could regulate the secretion of glucagon, which could rapidly promote gluconeogenesis, saving the consumption of liver glycogen during strenuous exercise. This might be another mechanism of its anti-fatigue effects.

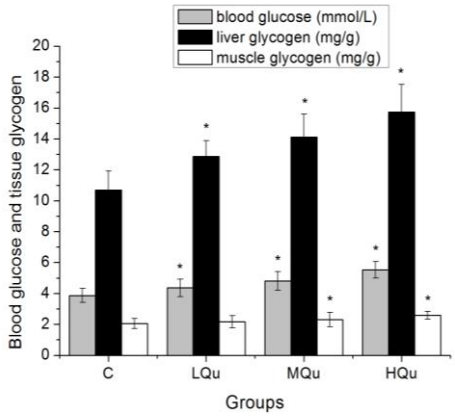

Figure 3. Effects of quercetin on blood glucose and tissue glycogen of mice. Data were expressed as means ± SD. $^{*}P<0.05$, compared with C group.

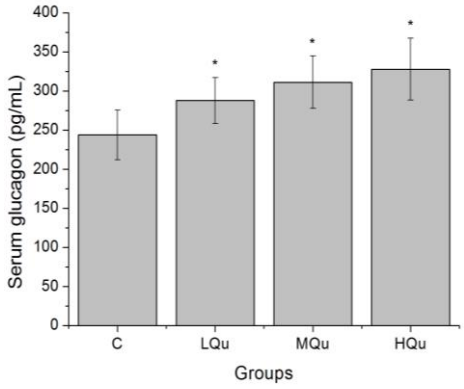

Figure 4. Effects of quercetin on serum glucagon of mice. Data were expressed as means ± SD. $^{*}P<0.05$, compared with C group.

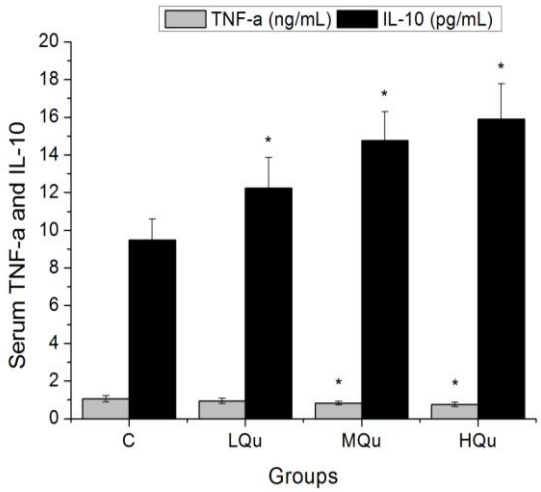

Figure 5. Effects of quercetin on serum TNF-α and IL-10 of mice. Data were expressed as means ± SD. $^{*}P<0.05$, compared with C group.

3.5. Effects of quercetin on serum TNF-α and IL-10 of mice

Fatigue is also related to production of inflammatory cytokines, immune dysfunction, and cytokine imbalances [14]. Several studies have indicated that the causative role of proinflammatory cytokines in fatigue severity, evidenced by elevated production of inflammatory cytokines including TNF-a, IL-1, and IL-6 [15] .TNF-α generates ROS at the mitochondrial inner membrane, which may easily result in the progressive destruction of the mtDNA [14]. IL-10 is an important immunoregulatory cytokine that is produced by numerous cell types. It is an important inhibitor of many aspects of the inflammatory response [16]. As shown in Figure 5, the serum TNF-α levels in the MQu and HQu groups were significantly lower than those of the C group ($P<0.05$). The serum IL-10 levels in the LQu, MQu and HQu groups were significantly higher than those of the C group ($p<0.05$). These results indicated that quercetin could improve the immune function of fatigue mice by decreasing pro-inflammatory cytokines (TNF-α) levels, and elevated anti-inflammatory cytokines (IL-10) levels. quercetin possessed anti-fatigue effects may be related to its immunomodulating effects.

4. Conclusions

In conclusion, the present study indicated that quercetin possessed anti-fatigue effects by prolonging retention times, decreasing levels of BLA and SUN, and increasing levels of blood glucose, tissue glycogen and serum glucagon. Furthermore, quercetin could improve the immune function of fatigue mice by decreasing pro-inflammatory cytokines (TNF-α) levels, and elevated anti-inflammatory cytokines (IL-10) levels. Quercetin possessed anti-fatigue effects may be related to its immunomodulating effects. Further work is also needed to clarify the detailed molecular mechanism for the anti-fatigue and immunomodulating effects of quercetin.

References

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