Exercise, but not quercetin, ameliorates inflammation, mitochondrial biogenesis, and lipid metabolism in skeletal muscle after strenuous exercise by high-fat diet mice

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(Received: 2014/01/29, Revised: 2014/02/14, Published online: 2014/02/26)

[Purpose] The purpose of this study was to investigate whether moderate exercise and quercetin intake with a low fat diet contribute to inflammatory cytokine production, mitochondrial biogenesis, and lipid metabolism in skeletal muscle after strenuous exercise by high-fat diet mice. [Methods] Male C57BL/6 mice were randomly divided into four groups: (1) High-fat for 12 weeks and low-fat diet control (C; n = 6); (2) high-fat diet for 12 weeks and low-fat diet with quercetin (Q; n = 4); (3) high-fat diet for 12 weeks and low-fat diet with exercise (E; n = 4); or (4) high-fat diet for 12 weeks and low-fat diet with exercise and quercetin (EQ; n = 5). Quercetin (10 mg/kg) was administered once per day, 5 day/week for 8 weeks. Exercise training was performed at moderate intensity for 8 weeks, 5 days/week for 30–60 min/day. Mice were subjected to a strenuous exercise bout of 60 min at a speed of 25 m/min (VO2 max 85%) conducted as an exercise-induced fatigue just before sacrifice. [Results] As results, body weights were significantly different among the groups. Exercise training significantly reduced inflammatory cytokines after strenuous exercise in skeletal muscle of high-fat diet mice. Exercise training increased Tfam mRNA in the soleus muscle after strenuous exercise. Exercise training significantly decreased lipogenesis markers in skeletal muscle of obese mice after strenuous exercise. Moderate exercise significantly increased lipolysis markers in the tibialis anterior muscle. [Conclusion] These findings suggest that exercise training reduced inflammatory cytokines after strenuous exercise in skeletal muscle of high-fat diet mice. Exercise training increased Tfam mRNA in the soleus muscle after strenuous exercise. Exercise training significantly decreased lipogenesis markers in skeletal muscle of obese mice after strenuous exercise. Moderate exercise significantly increased lipolysis markers in the tibialis anterior muscle. [Keyword] obese; exercise; quercetin; inflammatory cytokine; mitochondrial biogenesis; skeletal muscle

INTRODUCTION

Obesity is a heightened state of inflammation. The adipose tissue is an active secretory organ that produces and releases a variety of proinflammatory [e.g., interleukin-1beta (IL-1β)], leptin, tumor necrosis factor-alpha (TNF-α), IL-6] and anti-inflammatory proteins (e.g., IL-10, interleukin-1 receptor antagonist). TNF-α is also over-expressed in adipose and muscle tissues of obese humans, and when administered exogenously leads to insulin resistance [1-3].

Quercetin exhibits antioxidative properties that are much stronger than those of vitamin C as well as anti-inflammatory [4,5] and anti-pathogenic properties [6]. Phenolic compounds selectively interfere with cytokine production and function. For example, quercetin and catechins inhibit TNF-α and IL-6, and simultaneously induce IL-10 release, and thus evoke the anti-inflammatory effect [7].

Exercise can have both positive and negative effects on inflammatory and redox status. While moderate activity may enhance immune function over sedentary levels, excessive, prolonged and high-intensity exercise may impair immune function [8]. Strenuous exercise increases production of several pro-and anti-inflammatory cytokines, endogenous cytokine inhibitors and chemokines [9]. Plasma IL-6 has been consistently shown to increase following acute knee-extensor, cycling, and resistance exercise [10]. Additionally, muscles release inflammatory cytokine during acute exercise, and levels can increase significantly [11]. Obesity and type 2 diabetes have been associated with a high-fat diet (HFD) and reduced mitochondrial mass and function [12]. Furthermore, peroxisome

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proliferator-activated receptor gamma coactivator-1 (PGC-1α) is important in the development of diabetes mellitus [13] and a master regulator of oxidative phosphorylation gene expression and mitochondrial biogenesis [14].

Given the similarity in the structure of quercetin to resveratrol and other flavonoid derivatives that have been shown to increases PGC-1α leading to mitochondrial biogenesis, oxidative phosphorylation contributes to suppress lipid accumulation [15]. There is in vitro evidence of an effect of quercetin on the energetics of isolated mitochondria [16].

Low-intensity prolonged exercise training simultaneously increases the activity of skeletal muscle mitochondrial enzymes involved in the tricarboxylic acid cycle and fatty acid β-oxidation [17]. Previous studies have demonstrated that PGC-1α is expressed in several tissues, including skeletal muscle and brown adipose tissue. PGC-1α increases mitochondria biogenesis and fatty acid oxidative metabolism [18]. In rats, PGC-1α mRNA and protein levels increase after a single bout of exercise as well as after several days of training [19].

It is generally accepted that the majority of the pleiotropic effects of long-term HFD is accompanied with changes in gene expression profiles. Several genes that encode enzymes or signal mediators involved in lipid and glucose metabolism respond to long-term HFD. For example, acyl-CoA oxidase (ACOX) and uncoupling protein-2 genes are altered in livers of long-term HFD mice, accompanied by an increase in the mRNA level of sterol regulatory element binding protein-1 (SREBP-1), the major transcriptional regulator for lipogenic genes [20].

Chronic exercise improves the capacity to utilize fatty acids by a coordinated upregulation of proteins involved in sarcomemal uptake (fatty acid translocase), mitochondrial transport [carnitine palmitoyl transferase 1 (CPT1)] and β-oxidation (hydroxyacyl-coenzyme-A) of fatty acids [21]. Muscle AMP-activated protein kinase (AMPK) is stimulated during contraction, and may mediate multiple beneficial effects of exercise, specifically by increasing fatty acid oxidation and glucose uptake and promoting mitochondrial biogenesis. Malonyl-CoA is a potent allosteric inhibitor of CPT1, the rate-limiting enzyme that transfers long-chain acyl-CoA into mitochondria for β-oxidation [22].

Several studies have shown the effect of quercetin supplementation or exercise training separately. However, the synergetic effect of quercetin supplementation and exercise training has not been investigated after strenuous exercise as an oxidative stress. The aim of the present study was to investigate the effect of moderate exercise training and quercetin supplementation on inflammatory cytokine production, mitochondria biogenesis, and lipid metabolism after strenuous exercise in skeletal muscle of HFD mice.

METHODS

Animals care and diet

Male C57BL/6 mice (5-weeks-old) were purchased from Chungang Laboratory Animals (Seoul, Korea) and were housed in standard cages placed in a room at 22 ± 2.0°, 55 ± 10% relative humidity, and a 12 hour-light/12hour-dark cycle. All mice consumed a commercial diet and tap water ad libitum for 1 week.

Mice were randomly divided into four groups:(1) HFD for 12 weeks and low-fat diet for 8 weeks control (C; n = 6); (2) HFD for 12 weeks and low-fat diet for 8 weeks with quercetin (Q; n = 4); (3) HFD for 12 weeks and low-fat diet for 8 weeks with exercise (E; n = 4); or (4) HFD for 12 weeks and low-fat diet for 8 weeks with exercise and quercetin (EQ; n = 5). The mice were weighed every 2 weeks during the experimental period. Commercially available dried quercetin dihydrate (Sigma, St. Louis, MO, USA; ≥ 98% purity by high-performance liquid chromatography) was used and dissolved in 50% propylene glycol. The control (C) and exercise (E) groups were orally administrated 100 µl of propylene glycol as a vehicle. The quercetin (Q) and exercise with quercetin (EQ) groups were orally gavaged with quercetin (10 mg/kg body weight) dissolved in 50% propylene glycol. Each treatment was administered once per day, 5 days/week for 8 weeks. All treatments were performed 1 h after exercise.

<table>
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<th>Product</th>
<th>High fat Diet</th>
<th>Low fat Diet</th>
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<tr>
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</tr>
</tbody>
</table>

High-fat diet (#D12451, Orientbio Inc, Seoul, Korea), Low-fat diet (#D12450, Orientbio Inc)

Exercise protocol

Exercise training was performed on a motor treadmill at moderate intensity for 8 weeks, 5 days/week for 30-60 min/day during the dark cycle. This exercise intensity corresponds to 65-70% of maximal oxygen uptake. Just before