Effects of Quercetin Supplementation on Markers of Muscle Damage and Inflammation After Eccentric Exercise

Kevin S. O’Fallon, Diksha Kaushik, Bozena Michniak-Kohn, C. Patrick Dunne, Edward J. Zambraski, and Priscilla M. Clarkson

The flavonoid quercetin is purported to have potent antioxidant and anti-inflammatory properties. This study examined if quercetin supplementation attenuates indicators of exercise-induced muscle damage in a double-blind laboratory study. Thirty healthy subjects were randomized to quercetin (QU) or placebo (PL) supplementation and performed 2 separate sessions of 24 eccentric contractions of the elbow flexors. Muscle strength, soreness, resting arm angle, upper arm swelling, serum creatine kinase (CK) activity, plasma quercetin (PQ), interleukin-6 (IL-6), and C-reactive protein (CRP) were assessed before and for 5 d after exercise. Subjects then ingested nutrition bars containing 1,000 mg/d QU or PL for 7 d before and 5 d after the second exercise session, using the opposite arm. PQ reached 202 ± 52 ng/ml after 7 d of supplementation and remained elevated during the 5-d postexercise recovery period (p <.05). Subjects experienced strength loss (peak = 47%), muscle soreness (peak = 39 ± 6 mm), reduced arm angle (–7° ± 1°), CK elevations (peak = 3,307 ± 1,481 U/L), and arm swelling (peak = 11 ± 2 mm; p <.0001), indicating muscle damage and inflammation; however, differences between treatments were not detected. Eccentric exercise did not alter plasma IL-6 (peak = 1.9 pg/ml) or CRP (peak = 1.6 mg/L) relative to baseline or by treatment. QU supplementation had no effect on markers of muscle damage or inflammation after eccentric exercise of the elbow flexors.

Keywords: flavonoid, muscle soreness, DOMS, strength loss

Eccentrically biased exercise is well known to cause damage to skeletal-muscle fibers, characterized indirectly by decrements in strength, development of muscle soreness, swelling, and increased blood levels of muscle-specific proteins such as creatine kinase (CK) in the days after the exercise bout (Clarkson, 1992). Moreover, exercise-induced muscle damage (EIMD) initiates an inflammatory response associated with secondary muscle damage and remodeling (Clarkson & Hubal, 2002).

The inflammatory response to EIMD occurs in two primary phases. During the acute phase, both neutrophils and phagocytic macrophages can release reactive oxygen and nitrogen species and remove debris by phagocytosis (Tidball & Villalta, 2010). In addition, cytokines and byproducts of reactive oxygen and nitrogen species from the injured muscle are released into the blood, contributing to low-grade systemic inflammation and oxidative stress, elevating blood C-reactive protein (CRP) levels (Hirose et al., 2004), and altering glutathione redox status (Goldfarb, Bloomer, & McKenzie, 2005). Together, the proinflammatory and pro-oxidant processes can induce secondary damage to the injured tissue, prolonging repair and regenerative processes during the subsequent chronic inflammatory phase, characterized by restoration of muscle strength and resolution of inflammation (Peake, Suzuki, & Coombes, 2007; Smith, Kruger, Smith, & Myburgh, 2008).

Supplementation with antioxidant and anti-inflammatory nutrients can, in some cases, attenuate oxidative stress (Goldfarb et al., 2005; Goldfarb, Garten, Cho, Chee, & Chambers, 2011), inflammation (Phillips, Childs, Dreon, Phinney, & Leeuwenburgh, 2003), and muscle soreness (Bryer & Goldfarb, 2006) and improve strength recovery (Connolly, McHugh, Padilla-Zakour, Carlson, Y Sayers, 2006; Trombold, Barnes, Critchley, & Coyle, 2010) after eccentric exercise. Quercetin is a flavonol-type polyphenol that has been extensively studied for its antioxidant and anti-inflammatory properties (Abbey & Rankin, 2011; Nieman et al., 2009; Overman, Chuang, & McIntosh, 2011). Recently, Overman et al. reported that quercetin attenuated expression of inflammatory cytokine TNF-α, IFN-γ, IL-6, and IL-1β transcripts in cultured human macrophages, which are known contributors to secondary muscle damage (Tidball & Villalta, 2010). Those data suggest that polyphenols (i.e., quercetin) can modulate acute-phase inflammatory mediators. Phillips et al. showed that consumption of
a mixed dietary supplement containing 300 mg mixed tocopherols, 800 mg docosohexaenoate, and 300 mg polyphenols (100 mg hesperatin and 200 mg quercetin) for 14 days before eccentric exercise of the elbow flexors significantly attenuated changes in IL-6 and CRP levels at 3 days postexercise relative to placebo. Therefore, we tested the hypothesis that a mixed supplement containing 1,000 mg/d quercetin would attenuate strength loss and inflammation after strenuous eccentric exercise of the elbow flexors in young, healthy men and women.

Methods

Subjects

Thirty subjects age 18–25 years were recruited from the University of Massachusetts Amherst campus and the local community. After subjects provided written consent for participation (Visit 1), testing and analyses of blood measures (except quercetin and CK) were conducted. Subjects were sedentary to recreationally active, naïve to resistance training and resistance-type activities of the upper extremities for 6 months before participation, negative (by self-report) for family history of and current musculoskeletal or metabolic impairments, and not taking dietary supplements. Subjects refrained from resistance-type activities and use of dietary supplements and over-the-counter and prescription anti-inflammatory medications. At Visit 1, they were provided a list of quercetin-containing foods and agreed to refrain from excessive consumption of them (Chun, Chung, & Song, 2007) for 3 days before and during the study.

Study Design

The study was conducted in a randomized, double-blind, placebo-controlled manner (Figure 1). Subjects reported to the laboratory in a fasted state (≥10 hr) and rested for 10–15 min before data collection each day. They were randomly assigned in a permuted block design to perform exercise Bout 1 with either the nondominant or the dominant arm and take a quercetin- or placebo-containing supplement. Arm dominance was determined by subject self-report, and subjects were balanced for arm dominance as evenly as possible within groups. Fifteen subjects received placebo, and 15 subjects received the quercetin-fortified supplement. Subjects performed two bouts of 24 maximal eccentric contractions of the elbow flexors using a modified preacher-curl bench as reported previously (Nosaka & Clarkson, 1996). Bout 1 was performed in Phase 1, and Bout 2 was performed with the contralateral arm in Phase 3. During supplementation, subjects received either 1,000 mg/d quercetin aglycone (Merck, SA Brazil) via First Strike nutrition bars (Natick Soldier Center, Natick MA) or placebo First Strike bars. Each First Strike bar also contained 10 mg vitamin C and 7 mg total tocopherols, which resulted in daily doses of 20 mg vitamin C and 14 mg tocopherols. Six days after Bout 1 (i.e., 24 hr after Visit 8), subjects ingested one bar, twice daily at 12-hr intervals, for 7 consecutive days. The dosage regimen was based on our previous human

Figure 1 — Study design and timeline. The study consisted of 15 visits (V1–15) over 3 phases. Phase 1: performed eccentric contractions (EC) with 1 arm (V3), measures taken at baseline (V2–3), immediately postexercise (V3), and every 24 hr for 120 hr thereafter (V4–8). Phase 2: ingested quercetin or placebo bars at ~8 a.m. and ~8 p.m. each day for 7 days. Phase 3: continued supplementation (V9–15), EC of the contralateral arm (V10), measures taken at baseline (V9–10), immediately postexercise (V10), and every 24 hr for 120 hr thereafter (V11–15). Criterion measures: isometric and isokinetic strength at 60°/s and 180°/s (Strength), muscle soreness (Soreness), resting arm angle (RAA), arm circumference (AC). Biological markers: plasma quercetin (PQ), interleukin-6 (IL-6), C-reactive protein (CRP), and serum creatine kinase (CK).
pharmacokinetic studies (Kaushik, O’Fallon, Clarkson, & Michniak-Kohn, 2010) in which plasma quercetin (PQ) was undetectable at baseline via high-performance liquid chromatography (minimum limit of detection of 3 ng/ml), followed by significant \( (p < .05) \) peak increases (620.7 ng/ml) in PQ after participants (\( N = 18 \)) ingested quercetin-fortified First Strike bars. Thus, quercetin levels are expected to be negligible in the blood, unless quercetin is ingested via supplementation. Therefore, due to the prohibitive cost of analysis, PQ was not determined from subjects in the placebo group in the current study, which we highlight as a limitation. Compliance was verified by the investigator by counting empty supplement wrappers when subjects returned for Phase 3. Subjects ingested the quercetin or placebo supplement immediately after all measures were collected on each visit and were provided a second bar to ingest 12 hr later.

**Experimental Measures**

Isometric and isokinetic strength were assessed on an isokinetic dynamometer (Biodex System 3, Biodex, Shirley, NY). Subjects were seated on the Biodex with the elbow fixed at 90° flexion, and their body position, relative to the lever arm, was documented and maintained throughout the study. Three isometric strength trials (3 s/trial) with 1-min rests between trials, 12 consecutive isokinetic contractions at 60°/s and at 180°/s, muscle soreness, relaxed arm angle, and upper arm circumference were assessed daily.

Blood samples were obtained after subjects rested in the laboratory for 10–15 min and fasted overnight for ≥10 hr. Samples for PQ, CRP, and IL-6 were collected in K2 EDTA Vacutainers (BD Biosciences, USA) and centrifuged at 3,000 \( g \) at room temperature (quercetin) or at 4 °C (CRP and IL-6). Samples for CK enzyme activity were collected in serum Vacutainers (BD Biosciences), clotted for 15 min, and centrifuged at 3,000 \( g \) at room temperature. Aliquots were stored at –80 °C for subsequent analyses.

Analyses of PQ were performed at Rutgers University via high-performance liquid chromatography, with a minimum detectable [PQ] of 3 ng/ml. CRP was assessed using an enzyme-linked immunosorbent assay (ELISA; R&D Systems Inc., Minneapolis, MN) with a minimum detectable [CRP] of 0.010 ng/ml, as per manufacturer’s protocol. IL-6 was assessed via high-sensitivity ELISA (R&D Systems Inc.) with a minimum detectable [IL-6] of 0.039 pg/ml, as per manufacturer’s protocol. CK activity was assessed at Holyoke Hospital, Holyoke, MA.

**Statistical Analyses**

Data were analyzed using the Statistical Analysis Software (SAS) package, (V9.2; SAS Institute, Cary, NC). Reliability of preexercise measurements was assessed using an intraclass \( R \) analysis. For isometric and isokinetic strength, arm angle, and arm circumference, the \( R \) values were .94, .95, .93, and .94, respectively. A repeated-measures ANOVA with a grouping factor (placebo vs. quercetin) was used to compare measures before and after supplementation (Phase 1 vs. Phase 3). Arm circumference and CK data were not normally distributed and were log-transformed before analyses. Significance was set at \( p < .05 \).

**Results**

**Subject Characteristics**

Table 1 presents the physical characteristics for all subjects (\( N = 30 \)), partitioned by supplement group and sex. There were no significant differences between the placebo and quercetin groups. All subjects completed both exercise sessions and were compliant with the inclusion and exclusion criteria and supplementation requirements.

**Isometric and Isokinetic Strength**

Eccentric exercise induced significant \( (p < .01) \) isometric strength loss (Figure 2) immediately postexercise that returned toward baseline values over the next 120 hr. A significant \( (p < .01) \) Phase \( \times \) Time interaction was observed, with no significant Group \( \times \) Phase or Group \( \times \) Time interactions, indicating that the placebo and quercetin groups responded similarly. Isokinetic strength at 60°/s (Figure 3) and 180°/s (data not shown) was significantly \( (p < .01) \) decreased immediately postexercise and returned toward baseline values over the next 120 hr. However, no significant main effects of group or phase or interactions were observed, indicating that quercetin did not attenuate muscle-strength loss or facilitate recovery of baseline strength.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject Characteristics (( N = 30 )), ( M \pm SD )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristic</strong></td>
<td><strong>Placebo (( n = 15 ))</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Men (( n = 8 ))</strong></td>
</tr>
<tr>
<td>Age (years)</td>
<td>19.5 ± 1.1</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.78 ± 0.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.0 ± 17.2</td>
</tr>
<tr>
<td>Body-mass index (kg/m(^2))</td>
<td>24.0 ± 5.1</td>
</tr>
</tbody>
</table>
Muscle Soreness

Soreness was assessed using a 100-mm visual analog scale that indicates no soreness on the far left (0 mm) of the scale and unbearable pain on the far right (100 mm; Figure 4). Subjects made a single vertical mark on the scale that corresponded to their soreness level. A significant ($p < .01$) effect of time was observed, indicating a significant increase in muscle soreness that peaked within ~48 hr postexercise and returned toward baseline values by 120 hr. There were no significant main effects of group or phase, nor were any significant interactions observed, indicating that quercetin supplementation did not attenuate exercise-induced muscle soreness.

Swelling

Eccentric exercise produced a significant ($p < .01$) increase in upper arm circumference, indicating local muscle swelling within 24 hr after the exercise that peaked at ~120 hr (Figure 5). No significant main effects of group or phase or interactions were observed, indicating a similar response between the placebo and quercetin groups. There appeared to be a trend ($p = 0.14$) toward a significant attenuation in swelling (Bout 1 vs. Bout 2) in the quercetin group at ~120 hr (Figure 5[B]), which may suggest an effect of quercetin on swelling at this late time point. However, this trend was not statistically significant.

Resting Arm Angle

A significant ($p < .01$) decrease in arm angle was observed immediately postexercise, which returned to baseline at 120 hr. However, there were no significant main effects of group or interactions observed, indicating that the placebo and quercetin groups responded similarly (data not shown).

PQ

The mean PQ levels ranged from ~15 to 17 ng/ml during Phase 1 and significantly ($p < .05$) peaked at ~202 ± 52 ng/ml among subjects in the quercetin group after 7 days of supplementation (Phase 3, Visit 9) with quercetin-fortified (1,000 mg/d) First Strike bars (Table 2). During Phase 3, mean PQ remained significantly
Serum CK activity increased significantly \( (p < .05) \) within 24 hr and remained elevated at 120 hr postexercise (Figure 6). There were no significant main effects of group or phase on CK, nor were any significant interactions observed among the main effects, indicating no differences within or between placebo and quercetin groups.

**Serum CK**

Serum CK activity increased significantly \( (p < .05) \) within 24 hr and remained elevated at 120 hr postexercise (Figure 6). There were no significant main effects of group or phase on CK, nor were any significant interactions observed among the main effects, indicating no differences within or between placebo and quercetin groups.

**PQ, IL-6, and Serum CRP**

Table 2 presents the data for IL-6 in pg/ml and CRP in mg/L for the placebo and quercetin groups. No significant group, phase, or time effects on these markers of systemic inflammation were observed, nor any significant interactions. Neither the exercise protocol nor
Quercetin and Markers of Muscle Function or Inflammation

435

Quercetin supplementation altered blood IL-6 or CRP concentrations.

Discussion

The aim of this study was to assess the effects of quercetin supplementation on markers of muscle damage and systemic inflammation after strenuous eccentric exercise of the elbow flexors. Each exercise bout induced significant decrements in isometric and isokinetic strength, development of muscle soreness, elevation in serum CK, and development of local muscle swelling, patterns typical to previous investigations of EIMD (Miles et al., 2008; Nosaka & Clarkson, 1996; Trombold et al., 2010). However, we demonstrated no effect of quercetin supplementation on the aforementioned markers of EIMD and no effect of quercetin or eccentric exercise on biological markers of systemic inflammation (IL-6 and CRP). Our findings support the recent report by Goldfarb et al. (2011) that a nutritional supplement containing vitamins C and E and polyphenols does not attenuate changes in muscle force or function. Contrary to the report by Phillips et al. (2003), we found no significant increase in IL-6 and CRP, relative to baseline, in blood of fasted participants collected every 24 hr for 5 days postexercise. Although some studies have reported changes in IL-6 and CRP at similar time points after eccentric exercise (Paulsen et al., 2005; Phillips et al., 2003), others have not (Miles et al., 2008; Miles, Pearson, Andring, Kidd, & Volpe, 2007; Nosaka & Clarkson, 1996; Trombold et al., 2010). For example, Miles et al. (2008) found that plasma IL-6 significantly ($p < .003$) peaked at ~8 hr after eccentric exercise and was not significantly different from baseline at 24 through 120 hr postexercise, and no significant change in CRP was evident at 24 through 120 hr postexercise, relative to baseline. Those findings demonstrate that eccentric exercise of the elbow flexors induces a small increase in plasma IL-6 at ~8 hr that returns to baseline by 24 hr postexercise. Our data corroborate previous reports (Miles et al., 2008; Nosaka & Clarkson, 1996; Trombold et al., 2010) that eccentric exercise of the elbow flexors, performed at a volume and intensity comparable to that in the current study, does not produce sustained increases in systemic IL-6 and CRP levels lasting up to and beyond 24 hr postexercise (i.e., assessed at 48, 72, 96, and 120 hr postexercise).

Seven days of quercetin supplementation (1,000 mg/d) significantly elevated PQ concentrations (~202 ± 52 ng/ml) in blood samples collected from fasted (~210 hr) subjects, indicating that circulating PQ was detectable at appreciable concentrations at ~10–12 hr postsupplementation. These findings support recent work by Nieman et al. (2009) in which fasted PQ levels were elevated (~250 ng/ml) after 14 days of quercetin supplementation with 1,000 mg/d. Although our dosing regimen effectively maintained elevated PQ levels during supplementation, the supplement was not effective at attenuating changes in markers of EIMD. One possible explanation for these findings is that the biological activity of quercetin found in food is diminished during small-intestinal and hepatic metabolism (Kroon et al., 2004), resulting in decreased potency after absorption into the blood compartment. In the small intestine, quercetin is conjugated to sugar moieties to form quercetin-glucuronides and can be further glucuronidated, sulfated, or methylated in the liver (O’Leary et al., 2003). The metabolism of quercetin is believed to substantially lower its bioactivity in vivo. For example, Day, Bao, Morgan, and Williamson (2000) suggested that the antioxidant activity of conjugated quercetin is approximately 50% that of the parent quercetin aglycone, which is the most biologically active form. Moreover, quercetin aglycone is less bioavailable in humans than its metabolites such as quercetin-3′-O-sulfate and quercetin-3-glucuronide (Hong & Mitchell, 2006). Although our data showed that PQ was significantly elevated after oral ingestion, it is possible that the biological activities of the quercetin metabolites present in the blood after oral ingestion are not sufficient to exert protective effects against the negative consequences of eccentrically biased exercise.

Here we used an eccentric-exercise model of the elbow flexors to show that quercetin supplementation had no effect on any commonly used markers of muscle.
damage and recovery. Although our protocol did not detect an effect of quercetin, two recent studies found that polyphenol supplementation significantly improved muscle-strength recovery after eccentric actions of the elbow flexors (Connolly et al., 2006; Trombold et al., 2010), demonstrating that the elbow-flexor model is appropriate for detecting the effects of nutritional interventions on recovery of muscle strength after EIMD. Like Connolly et al., we observed comparable decrements in muscle strength immediately postexercise (–39% vs. –29%) and peak muscle soreness at 48 hr postexercise (+41% vs. +45%). Thus, we believe that our model was appropriate to detect an effect of quercetin on recovery of muscle strength had it been present.

In young, healthy individuals, quercetin is purported to have mild effects on innate immunity, manifested as a reduced rate of incidence of exercise-induced respiratory illness (Nieman, Henson, Gross, et al., 2007) and altered levels of inflammatory mediators after strenuous aerobic exercise (Nieman, Henson, Davis, et al., 2007; Nieman et al., 2009). These findings suggest that quercetin may, in some cases, alter the systemic inflammatory response to strenuous aerobic exercise; however, they do not support the notion that quercetin itself improves prolonged aerobic-exercise performance. In addition, Abbey and Rankin (2011) recently showed no effect of quercetin on repeated-sprint performance in college-age athletes, which suggests that quercetin also has no effect on physical performance of anaerobic exercise. Therefore, in the context of exercise performance in healthy humans, quercetin supplementation does not appear to enhance performance of aerobic exercise or attenuate decrements in muscle function in response to eccentrically biased resistance exercise, as we have shown here.

**Conclusion**

Our exercise protocol induced prolonged decrements in isometric and isokinetic peak torque, soreness, CK release into the blood compartment, and muscle swelling. Ingestion of 1,000 mg/day of quercetin delivered in fortified nutrition bars for 7 days before and for 5 days after an acute bout of eccentric contractions of the elbow flexors significantly increased PQ levels. However, no effect of quercetin was observed on any indices of muscle damage or inflammation. Our findings demonstrate that although quercetin is safe for human consumption, it did not attenuate the negative consequences of strenuous eccentrically biased exercise in this study.

**Acknowledgments**

This study was funded by the U.S. Army, contract # W911QY-07-C-0001. The authors would like to thank Brittany Rahmberg, James Webb, Trent Ainsworth, Himanshu Shah, and Ahmed Sandakli for their assistance in the laboratory and Dr. Christer Malm for his critical review during the manuscript preparation. None of the authors claim a conflict of interest.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the U.S. Army or the Department of Defense. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations.

**References**


