Effect of Quercetin Supplementation on Repeated-Sprint Performance, Xanthine Oxidase Activity, and Inflammation

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Maintenance of repeated-sprint performance is a goal during team-sport competition such as soccer. Quercetin has been shown to be an adenosine-receptor antagonist and may reduce oxidative stress via inhibition of the enzyme xanthine oxidase (XO). The purpose of the study was to determine the effect of quercetin consumption on performance of repeated sprints and, secondarily, the XO and inflammatory-marker response induced by repeated-sprint exercise. Fifteen recreationally active, young adult men completed 2 repeated-sprint tests (RST), 12 × 30-m maximal-effort sprints (S1–S12), each after 1 wk supplementation with a placebo, a 6% carbohydrate commercial sports drink, or that drink with 500 mg of quercetin-3-glucoside, consumed twice a day (1,000 mg/d). Blood samples were collected before supplementation (B0), at baseline before each RST (B1), immediately after RST (B2), and 1 hr after RST (B3). Mean sprint time increased progressively and was significantly higher by S9 for both treatments (5.9%); however, there were no significant differences between treatments. Percent fatigue decrement (%FD) for placebo (3.8% ± 2.3%) was significantly less than with quercetin (5.1% ± 2.7%). Changes in blood XO, IL-6, and uric acid from B1 to B2 were +47%, +77%, and +25%, respectively, with no difference by treatment. In conclusion, repeated-sprint performance was not improved by quercetin supplementation and was worse than with placebo when expressed as %FD. Quercetin did not attenuate indicators of XO activity or IL-6, a marker of the inflammatory response after sprint exercise.

Keywords: team sport, oxidative stress, IL-6, uric acid

Identification of nutritional interventions that can improve initial sprint speed or delay fatigue during repeated sprinting could be valuable for athletes performing in team sports such as soccer or basketball. Researchers have explored the ergogenic value of compounds including creatine, bicarbonate, or caffeine for repeated sprinting (Bishop & Claudius, 2005; Glaister, Howatson, Abraham, et al., 2008; Rockwell, Rankin, & Toderico, 2001).

Research on the potential value of quercetin for physical performance has focused primarily on prolonged aerobic exercise because there is evidence it may stimulate mitochondrial biogenesis (Davis, Murphy, Carmichael, & Davis, 2009; MacRae & Mefferd, 2006). For example, one study reported a 13.2% longer cycle time to fatigue in untrained humans who consumed 1,000 mg of quercetin for 7 days (Davis, Carlstedt, Chen, Carmichael, & Murphy, 2010).

No studies have been published on the potential benefit of quercetin for brief, anaerobic exercise in spite of the evidence from rodent studies that unconjugated quercetin causes adenosine-receptor antagonism and therefore may stimulate the central nervous system in a manner similar to caffeine (Davis, Murphy, & Carmichael, 2009). In addition, there is in vitro (Van Hoorn et al., 2002) and in vivo (Mo et al., 2007) evidence that quercetin, a flavonol found in apples, onions, and other plant foods, is an inhibitor of xanthine oxidase (XO), one source of production of reactive oxygen species (ROS) during exercise.

XO is likely a more important source of ROS during sprinting than for endurance exercise. Temporary hypoxia, as well as reduction in ATP, can increase hypoxanthine production, accumulation, and spillover into the blood to serve as substrate for hepatic production of uric acid. The increased conversion of xanthine dehydrogenase to XO increases production of superoxide (Bloomer & Goldfarb, 2004; Vollaard, Shearman, & Cooper, 2005). Previous experimental evidence demonstrated that repeated sprinting causes a rise in uric acid, considered evidence of activation of the XO pathway (Stathis, Zhao, Carey, & Snow, 1999). ROS may damage compounds including cell membranes, signaling proteins, and DNA; affect calcium release and uptake by the sarcoplasmic reticulum; and, although controversial, affect cellular processes that contribute to fatigue (Reid, 2008; Vollaard et al., 2005).

If there is inadequate removal of ROS via antioxidants, oxidative stress (OS) can cause increased production of inflammatory factors (i.e., cytokines) via activation and translocation of the transcriptional factor NF-κB (Kramer & Goodyear, 2007). Evidence that this occurs in response to sprinting includes observation of activation of NF-κB (Cuevas et al., 2005), as well as induction of an acute monocytosis and increase in IL-6 (Meyer, et al., 2002) and in vivo (Mo et al., 2007) evidence that quercetin, a flavonol found in apples, onions, and other plant foods, is an inhibitor of xanthine oxidase (XO), one source of production of reactive oxygen species (ROS) during exercise.

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Gabriel, Ratz, Muller, & Kindermann, 2001) after single or repeated sprints. Thus, the link between sprinting and an inflammatory response may be OS, resulting at least partly from XO activation.

Dietary factors including some phytochemicals may inhibit XO and thus potentially influence OS and the inflammatory response to repeated sprints. Although published studies have demonstrated no effect of quercetin supplement consumption on markers of OS or inflammation after a prolonged aerobic-exercise bout (Nieman, Henson, Davis, Murphy, et al., 2007; Nieman, Henson, Davis, Dumke, et al., 2007; Quindry et al., 2008), XO is less likely to play a role for that type of activity (Vollaard et al., 2005). Thus, these studies do not evaluate performance of an activity most likely to be influenced by quercetin ingestion via an inhibition of XO activity.

Collectively, there is evidence to theorize that quercetin may improve performance of repeated sprints. In addition, if quercetin ingestion inhibits XO, it could attenuate the OS and inflammatory response. The objective of the study was to determine the value of quercetin consumption in performance of repeated sprints and its ability to affect XO activity and a marker of the inflammatory response to this exercise.

**Methods**

**Subjects**

After approval of the study by the institutional review board, 15 healthy, male, team-sport-trained athletes (e.g., soccer and basketball), age 18–30 years were recruited (Table 1). All subjects provided written informed consent before participation. Exclusion criteria included history of inflammatory conditions (e.g., inflammatory bowel disease, arthritis), liver dysfunction, or recent or chronic musculoskeletal injuries or illness. Current smokers and those taking antioxidant supplements were also excluded. Subjects were asked to maintain their weight and refrain from dietary supplements for the duration of the study.

**Experimental Procedures**

After an overnight fast, subjects’ baseline measurements (body mass and height) and an initial blood draw (B0) were completed at the testing facility. Subjects underwent a familiarization trial of the repeated-sprint test (RST) to acquaint them with the expectations of the test and to diminish any learning effect.

Under double-blind procedures, subjects were randomly assigned to receive either a placebo (P) or quercetin (Q) supplement for the first 7-day intervention period followed by a 1-week washout period and crossed over for the alternate intervention for the last 7 days. The two treatments (P and Q) were provided in individual serving-size packets of dry powder that the subjects mixed with 591 ml of water in a provided bottle to be consumed twice per day (i.e., morning and evening) for 7 days. Q was a 6% carbohydrate, commercially available sports drink (Gatorade, Chicago, IL) with 500 mg of quercetin-3-glucoside per serving (1,000 mg/day; Nieman, Henson, Davis, Dumke, et al., 2007); P was the same beverage without quercetin.

Subjects were instructed to follow a diet moderate in carbohydrate (55% total kcal) and kept a 1-day food and activity record before baseline measurements and each RST. Food records were analyzed later using Nutritionist Pro software (Axxya Systems, Stafford, TX). After 1 week of supplementation, the subjects arrived at the laboratory, having eaten their last meal no later than 10:00 the previous evening, and drinking only water from that time until testing. They had been instructed not to participate in any strenuous exercise for the previous 24 hr. On arrival, subjects were queried on their physical activity and diet from the previous 2 days, as well as their general health and symptoms of infection (to exclude anyone with acute infection).

After collection of initial blood samples (B1), the assigned beverage was ingested. The subjects then rested for 15 min, walked to the testing location, and began a 15-min standardized warm-up of jogging, static and dynamic stretching, and practice sprints. Based on similar studies (Glaister et al., 2007), subjects performed 12 × 30-m sprints (S1–S12) every 35 s. They were verbally encouraged to give a maximum effort for each sprint and were not given any feedback as to their times to avoid pacing. Dual-beam electronic timing gates (SpeedLight, Swift Sports, Australia) recorded completion times. For the recovery period, subjects decelerated, walked back to the start, and waited until the next sprint. Immediately after S12, subjects were queried as to their rating of perceived exertion using a 0–10 scale. Within 2–3 min, a second blood sample (B2) was taken. A final blood sample was drawn 1 hr posttest (B3); only water was ingested between B2 and B3.

All blood samples were drawn via venipuncture and collected in one heparin-coated and one serum-separator tube. The latter was allowed to stand for 30 min before centrifugation at 3,000 g for 10 min at 4 °C. Aliquots of serum and plasma were stored at −80 °C until analysis. Plasma was analyzed for XO activity using an Amplex Red xanthine/xanthine oxidase assay kit (Molecular Probes, Eugene, OR). Serum IL-6 was measured using high-sensitivity ELISA procedures (R&D Systems, Minneapolis, MN), and serum uric acid

**Table 1 Subject Characteristics (N = 15)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>M (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.3 (2.6)</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>81.7 (10.9)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>179.2 (5.9)</td>
</tr>
</tbody>
</table>
was analyzed with a calorimetric QuantiChrom assay kit (BioAssay Systems, Hayward, CA). All samples were run in duplicate.

Statistical analyses were performed using SAS software for Windows (version 9.2; Cary, NC). Group data were expressed as $M \pm SD$, and a $p$ value of <.05 was considered significant. Baseline characteristics of subjects were analyzed using descriptive statistics. Because some research groups recommend use of average sprint time (Rampinini et al., 2007) but others %FD (Glaister, Howatson, Pattison, & McInnes, 2008), and one report determined a significant improvement in the fastest sprint of an RST with a nutritional intervention (caffeine; Glaister, Howatson, Abraham, et al., 2008), we chose to examine performance four ways: analysis of variance (ANOVA) with repeated measures with time points S1–S12 and two treatments (Q, P), paired $t$ test of calculated mean sprint time over the 12 sprints between treatments, paired $t$ test of the fastest sprint in the RST, and paired $t$ test of %FD as calculated, described, and validated by Glaister, Howatson, Pattison, and McInnes (2008).

To evaluate any effect of the treatment on fasted, resting blood factors, all blood measures were compared between B0 and B1 with one-way repeated-measures ANOVA. Response of blood measures to exercise was analyzed by expressing B2 and B3 as a percentage of B1 (fasted, resting) and analyzing using ANOVA with repeated measures.

## Results

There was no difference in total daily energy consumption or percent macronutrient intake between baseline (11.5 ± 3.1 MJ; 54.2% ± 11.2% carbohydrate, 18.4% ± 3.4% protein, 32.7% ± 11.5% fat), Q (11.2 ± 4.9 MJ; 45.6% ± 11.3% carbohydrate, 22.6% ± 7.3% protein, 32.6% ± 10.8% fat), and P (12.1 ± 3.4 MJ; 50.0% ± 9.2% carbohydrate, 19.2% ± 4.9% protein, 32.0% ± 6.1% fat) for the 24 hr before testing.

Sprint time (Figure 1) increased with each sprint and was significantly higher (5.9%) by S9 for both treatments, with no significant difference between groups or group-by-time interaction. Fastest sprint time was lower numerically but not statistically for Q than P (4.62 ± 0.22 and 4.68 ± 0.26 s, $p = .132$). Average sprint times were the same for both treatments (4.85 ± 0.24). For %FD, P (3.8% ± 2.3%) was significantly less ($p = .017$) than Q (5.1% ± 2.7%). Mean rating of perceived exertion was not statistically different between Q (7.5 ± 1.6) and P (7.3 ± 1.3).

Mean values for all blood measures are reported in Table 2. No significant differences between treatments were observed between B0 and B1 for any blood measure. Time effects but no group effects or interactions were observed for exercise response of all blood measures. Serum XO, IL-6, and uric acid increased immediately after the repeated sprints (47%, 77%, and 25%,

![Figure 1](image-url) — Mean sprint times (±SD) from a repeated-sprint test for quercetin (Q) and placebo (P). *$p < .05$ from S1. †$p < .05$ from S2. ‡$p < .05$ from S3. #$p < .05$ from S4.
Table 2  Blood-Marker Concentrations Relative to Pretest Values, M (SD)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pretest</th>
<th>Posttest</th>
<th>1 hr posttest</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>quercetin</td>
<td>1.00</td>
<td>1.83 (0.54)*</td>
<td>1.38 (0.51)*†</td>
</tr>
<tr>
<td>placebo</td>
<td>1.00</td>
<td>1.72 (0.45)*</td>
<td>1.58 (0.57)*†</td>
</tr>
<tr>
<td>XO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>quercetin</td>
<td>1.00</td>
<td>1.52 (0.54)*</td>
<td>0.78 (0.16)*†</td>
</tr>
<tr>
<td>placebo</td>
<td>1.00</td>
<td>1.41 (0.32)*</td>
<td>0.81 (0.14)*†</td>
</tr>
<tr>
<td>Uric acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>quercetin</td>
<td>1.00</td>
<td>1.25 (0.10)*</td>
<td>1.19 (0.14)*†</td>
</tr>
<tr>
<td>placebo</td>
<td>1.00</td>
<td>1.25 (0.09)*</td>
<td>1.18 (0.10)*†</td>
</tr>
</tbody>
</table>

*Absolute pretest concentrations for quercetin and placebo for IL-6 (0.83 and 0.71 pg/ml), xanthine oxidase (XO; 15.1 and 12.5 mU/ml), and uric acid (8.9 and 8.4 mg/dl).

*Significantly different from pretest (p < .05). †Significantly different from posttest (p < .05).

respectively) and significantly declined but remained elevated above resting levels 1 hr after the exercise bout.

Discussion

The results of this study did not support the hypothesis that quercetin consumption would improve repeated sprinting performance. In addition, the secondary hypotheses that quercetin would inhibit XO activity during repeated sprinting and thus reduce the rise in IL-6, a marker of the inflammatory response, was not upheld.

Using a protocol similar to ours, Glaister, Howatson, Abraham, et al. (2008) concluded that another dietary component known as an adenosine-receptor antagonist, caffeine, was ergogenic for repeated sprinting. They reported increased %FD concurrent with an improvement in fastest sprint time after caffeine supplementation. In other words, the caffeine-supplemented subjects began sprinting at a faster speed but were not able to sustain it over time. In our study, the fastest sprint times were numerically but not statistically (p = .132) faster with quercetin. It is possible that with a higher number of subjects and statistical power that quercetin may have had an effect similar to that observed for caffeine, leading to a faster initial sprint pace and increased %FD. This hypothesis is only suggested by our results and requires further experimentation to confirm.

Previous research (Stathis, Carey, & Snow, 2005), as well as this study, report that repeated sprints increase activation of XO (Stathis et al., 2005). Inhibition of XO using allopurinol reduced the increase in OS and markers of muscle damage in exercising rodents (Judge & Dodd, 2004; Viña et al., 2000) and endurance runners and cyclists (Gómez-Cabrera, Pallardó, Sastre, Viña, & García-del-Moral, 2003), demonstrating the contribution of this enzyme to OS and muscle damage from exercise.

The potential value of inhibition of XO on muscle performance is supported by the evidence that a single day of administration of allopurinol, in patients with chronic obstructive pulmonary disease, improved maintenance of repeated knee-extension endurance in the second of two bouts. This suggests that inhibition of XO and subsequent reduction in ROS can improve muscle performance when oxygen is limiting (Delamare et al., 2008).

The rise in XO activity and uric acid levels in our study suggests that the RST increased OS. Although we did not directly assess other markers of OS, others have confirmed an increase in protein carbonyls after sprint exercise (Bloomer, Fry, Falvo, & Moore, 2007). An increase in OS can activate the transcriptional factor NFκB, which increases gene expression of cytokines and an inflammatory response (Cuevas et al., 2005). The significant increase in IL-6 levels we observed is consistent with increased OS, activation of NF-κB, and increased translation of cytokines as a result of repeated sprinting. Measurement of additional markers of OS and inflammation would be necessary to fully characterize the effects of quercetin ingestion on these responses.

The lack of an effect of quercetin on these responses may be because quercetin is converted to various metabolites that have varying ability to inhibit XO after digestion and does not appear in the blood as free quercetin (Manach, Williamson, Morand, Scalbert, & Remesy, 2005). Previous research has shown that the predominant metabolites present in blood after ingestion of a high-quercetin food (onions) were quercetin-3-glucuronide and 3′-methylquercetin-3-glucuronide, forms that do not retain XO-inhibition activity (Day et al., 2001). We did not measure the quercetin metabolites in the blood, but it is possible that the explanation for the lack of effect on inflammation is that the primary metabolites in the blood were not those that inhibit XO. Additional research that includes measurement of these metabolites after ingestion of this form of quercetin is required to confirm this hypothesis.

Our study illustrates the difficulty in translating in vitro research to in vivo effects. Only one in vivo study reported significant inhibition of XO with a high dose of quercetin (100 mg/kg), administered for 3 days in hyperuricemic mice (Mo et al., 2007). Our findings support those of other studies using human subjects that did not find an in vivo effect of quercetin on exercise-induced inflammatory or OS markers (McAnulty et al., 2008; Nieman, Henson, Davis, Dumke, et al., 2007; Quindry et al., 2008).

One way to get around the issue of digestion and conversion to metabolites is to add quercetin directly to collected human blood. Boots, Wilms, et al. (2008) observed that exposure of human red blood cells to quercetin had an anti-inflammatory effect in that there was a dose-dependent inhibition of production of an inflammatory marker, TNFα (but not IL-10), after exposure to lipopolysaccharide. However, no anti-inflammatory effect was evident ex vivo after 4 weeks of ingestion of a blueberry–apple juice mixture with 97 mg/L quercetin. This suggests that directly applied quercetin can inhibit...
human XO in blood cells but that ingestion eliminates this effect, likely because the postdigestion form of quercetin is no longer an active inhibitor of XO. Currently there is little in vivo support for the observed in vitro effects of quercetin on XO inhibition.

We do not believe that dose or form of the supplement was a limitation because we used a highly absorbable form of quercetin, quercetin-3-glucoside (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004), and based the dose and timing of quercetin on studies by others (MacRae & Mefferd, 2006; Nieman, Henson, Davis, Murphy, et al., 2007). In another study, quercetin ingestion in the doses we used caused rapid increases in plasma quercetin within 30 min, which reached a peak at approximately 2 hr and began to decline around 6 hr (Davis, Murphy, & Carmichael, 2009). This suggests that plasma quercetin or its metabolites were likely elevated at least half of each day for our subjects and almost certainly during the RST. Because some have claimed that the bioavailability of quercetin is enhanced when it is provided in combination with other antioxidants such as vitamin C (Boots, Haenen, & Bast, 2008), it is possible that lack of antioxidants limited bioavailability of the beverage we used.

In future studies, measurement of quercetin metabolites after ingestion of supplements would add to our understanding of the potential differential effects of quercetin forms and doses on the metabolites known to potentially inhibit XO.

**Conclusion**

Repeated sprinting exercise, characteristic of team sports such as soccer, caused fatigue, acute activation of XO, and increases in the inflammatory response as measured by serum IL-6. Supplementation with quercetin did not improve performance and led to greater %FD than placebo. Our results add support to previous studies that reported that quercetin supplementation does not affect exercise-induced OS and inflammatory response in vivo.

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**References**


