Dietary Antioxidant Supplementation Combined with Quercetin Improves Cycling Time Trial Performance

Holden S-H. MacRae and Kari M. Mefferd

We investigated whether 6 wk of antioxidant supplementation (AS) would enhance 30 km time trial (TT) cycling performance. Eleven elite male cyclists completed a randomized, double-blind, cross-over study to test the effects of twice daily AS containing essential vitamins plus quercetin (FRS), and AS minus quercetin (FRS-Q) versus a baseline TT (B). MANOVA analysis showed that time to complete the 30 km TT was improved by 3.1% on FRS compared to B ($P \leq 0.01$), and by 2% over the last 5 km ($P \leq 0.05$). Absolute and relative ($\%HR_{max}$) heart rates and percent $\text{VO}_2_{max}$ were not different between trials, but average and relative power ($\%$ peak power) was higher on FRS ($P \leq 0.01$). Rates of carbohydrate and fat oxidation were not different between trials. Thus, FRS supplementation significantly improved high-intensity cycling TT performance through enhancement of power output. Further study is needed to determine the potential mechanism(s) of the antioxidant efficacy.

Key Words: free radicals, endurance performance, vitamins

Strenuous exercise, such as that performed by athletes in training and competition, markedly increases oxygen consumption and the generation of intracellular free radicals, and can cause disturbance of pro-oxidant-antioxidant homeostasis. The increased generation of reactive oxygen species (ROS) during strenuous exercise challenges the cellular antioxidant defense system, resulting in a diminished reserve of antioxidants and an increased tissue susceptibility to oxidative damage (19). Although studies generally report decreased biomarkers of oxidative stress after antioxidant supplementation (11, 35), the efficacy of antioxidant supplementation in athletic performance remains ambiguous (for review, see reference 32). Although several animal experiments have shown that antioxidant supplementation can improve muscular performance (2, 29, 30), there is limited evidence that dietary supplementation with antioxidants will improve human exercise performance (32). Most dietary supplementation studies with antioxidants have used vitamins A, C, and E in the supplementation regimens. Dietary antioxidant supplementation may improve endurance exercise performance by minimizing damage to membranes and contractile and structural proteins in muscle (18, 41) thus reducing inflammation, altering skeletal muscle redox state, thereby limiting the acute fatiguing effects of

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exercise-induced ROS (34), or alter the mediating effect of ROS on signal transduction pathways (10).

Results of studies in which antioxidant supplements have been administered to determine their effects on exercise performance provide disparate results. The reviews of Kanter (20) and Dekkers et al. (9) suggest evidence for a reduction in markers of muscle damage with antioxidant supplementation, but with no beneficial effect of the supplementation on exercise performance. The review of Powers et al. (32) suggests the likely reasons for the conflicting results in human studies being the delivery of single or a limited number of antioxidants, limited information on the antioxidant dosage required for efficacy, variable durations of dietary antioxidant supplementation which makes interpretation of the research results difficult, and a wide range of types and intensities of exercise performed.

Given that the defense against oxidative stress is dependent on an orchestrated synergism between several antioxidants, it is likely that a combination of scavengers and inhibitors of ROS-producing enzymes is more effective in combating elevated ROS levels than any single antioxidant agent (38). The dose of antioxidants selected for the cocktail supplement used in this study was based on the dietary reference intake (DRI) recommendations of the Institute of Medicine (Food and Nutrition Board of the Institute of Medicine, 2002) and was well within the upper limits of the daily intakes recommended by that organization. One of the treatments also contained quercetin, one of the major flavonoids in some fruits and vegetables that has much stronger antioxidative and anticarcinogenic activities than vitamin C (14). Commonly-consumed fruits and vegetables, such as onions and apples, are the primary dietary sources of quercetin, containing the flavonol at levels as high as approximately 350 mg/kg fresh edible weight in onions (expressed as the aglycone), 110 mg/kg in kale, while French bean, apple, broccoli, and apricot exhibit concentrations in the range of 20 to 40 mg/kg (8). In the US, consumption of the flavonol glycosides, expressed as quercetin equivalents, from the habitual diet was estimated to be approximately 107 mg/d. However, in high-end consumers of fruits and vegetables (90th percentile), estimates are as high as 226 mg/d (42). Quercetin also inhibits protein kinases and DNA topoisomerases, and regulates gene expression (25), and the bioavailability of quercetin exceeds that of other major antioxidants (37).

The low ROS levels present under basal conditions are essential for normal muscle force production, and modest increases in ROS production enhances skeletal muscle contractile function in vitro (34). However, as ROS accumulate in the working muscle, they inhibit force production and contribute to acute muscle fatigue (34). Because the increase in ROS production may have deleterious effects on muscle function during strenuous exercise, and since these potential deleterious effects are amplified during high exercise volumes (15) and/or intensities (1, 33), the aim of this study was to determine whether 6 wk of antioxidant supplementation (AS) with a free radical scavenger cocktail (FRS) would enhance 30 km time trial (TT) cycling performance in elite male cyclists.

Methods

Experimental Volunteers

Following approval of this project by the Institutional Review Board of Pepperdine University, 12 elite male cyclists were recruited to participate in the study.
Inclusionary criteria were regular participation in competitive bicycle racing (at least one race per month during the competitive season), and at least 6 months of continuous training without interruption. The subjects were all in the early phase of the competitive racing season at entry into the study.

**Study Design**

This study employed a randomized, double-blind, crossover design to test the effects of 6 wk of a free radical scavenger cocktail containing quercetin (FRS) versus FRS minus quercetin (FRS-Q) on 29.82 km (rounded to 30 km for the remainder of this article) cycling time trial (TT) performance. The contents of the FRS and FRS-Q supplements are described below. The six laboratory visits are described below, and are summarized in Figure 1.

**Laboratory Test Session #1.** Subjects reported to the laboratory in the morning for assessment of body composition and aerobic cycling performance testing ($\text{VO}_{2\text{max}}$). Subjects were asked to refrain from any intense training in the 24 h prior to testing, but were allowed to perform their usual daily exercise/activity patterns. Body composition was determined via 8-site skinfold thickness assessment using Harpenden skinfold calipers (British Indicators, St. Albans, UK), and body composition was estimated using standard prediction equations (17).

Following a 10 to 15-min warm-up at a self-selected intensity not exceeding 125 watts (W), subjects underwent a progressive incremental cycling test (25 W/min step increment every 1 min, initial load 100 W) on an electrically braked cycle ergometer fitted with the subject’s own pedals, and adjusted to their preferred cycling position (LODE Excalibur Sport, Lode NV, Netherlands) for determination of $\text{VO}_{2\text{max}}$ and peak sustained power output (PPO). Test termination occurred at
volitional fatigue or when the subjects could no longer maintain a cadence of 55 rpm. 
$\text{VO}_2\text{max}$ was determined by directing expired air to a MedGraphics CardioO$_2$ system (Medical Graphics, St. Paul, MN) calibrated before each test with commercially prepared gas mixtures. If two of the following criteria were satisfied, then the test was considered to be maximal: 1) $\text{VO}_2$ increase of $\leq 100$ mL with increasing work rate; 2) heart rate within $\pm 10$ bpm of 220-age predicted maximum; or 3) RER value in excess of 1.15. Heart rates were recorded at 1-min intervals during the exercise test using a wireless transmitter system (Polar Electro Inc., Woodbury, NY). Peak sustained power output on the cycling test was calculated as described by Kuipers et al. (21).

Subjects were oriented to the Velotron Pro cycle ergometer system (Racermate, Inc., Seattle, WA) that was used for the 30 km TT testing following the $\text{VO}_2\text{max}$ test. Each subject rode a course that included rolling hills to orient them to the system, its’ interactive capabilities, and to orient the subjects to the gear shifting required on the simulated course. The Velotron system has a permanent (no-drift) factory calibration with an accuracy of $\pm 1.5\%$ across the entire load range (5 to 2000 W), and repeatability of $\pm 0.2\%$ or better which was confirmed on a weekly basis.

**Laboratory Test Session #2: Baseline TT Test.** The baseline TT test served as the control condition and took place no more than 2 wk after laboratory session #1. The subjects were instructed to maintain and not modify their usual training, racing, and dietary program, and to prepare for this laboratory visit as they would for a competitive cycling event. TT testing was conducted in the morning on a Tuesday, Wednesday, or Thursday, beginning at 7 AM and completed by noon, this day selection being made to permit adequate recovery from and for weekend racing/training. Each subject was weighed upon entry to the laboratory, and following completion of body composition testing, a brief description of their previous day’s training, prior weekend racing/training, dinner the previous evening, and breakfast on the day of testing was recorded. Subjects were instructed to adhere as closely as possible to this pre-TT regimen for all subsequent TTs.

Following a 10-min warm-up on the Velotron ergometer, the subjects were instructed to complete the 30 km TT course (Figure 1) in as short a time as possible. Each subject was able to view on a computer monitor a single rider (representing the test subject), his elapsed time and distance covered, as well as instantaneous, average, and maximum speed, cadence, heart rate, and power during the TT. Time and average values for each of these measures were recorded at 8 km, 18 km, 23 km, and for the last 5 km of the course where subjects were encouraged to ride the last 5 km (4.82 km) as quickly as possible to simulate a finishing “sprint.” Expired gas measurements, as determined in the aerobic capacity test ($\text{VO}_2\text{max}$), were administered for 2 min at 8 km, 18 km, and 23 km during the TT. Following each expired gas measurement, subjects were asked to rate their perceived level of exertion (RPE) using the Borg 6-20 category scale (5). Subjects were permitted to consume water ad libitum throughout the TT, were fan-cooled, and laboratory temperature was maintained at approximately 20 °C for each laboratory test session.

Whole body rates of carbohydrate (CHO) oxidation and fat oxidation (g/min) were calculated for the 8 km, 18 km, and 23 km time-points from the average VCO$_2$ and $\text{VO}_2$ by using non-protein RER values (31). In well-trained subjects similar to those employed in this investigation, indirect calorimetry is a valid method for
quantifying rates of substrate oxidation during strenuous exercise at 85% VO$_{2\text{max}}$ (36). These gas exchange measurements, together with the power output data, were used to determine economy during the TT. Economy (VO$_{2\text{submax}}$ at a given work rate) was determined at 8, 18, and 23 km for each condition (B, FRS, and FRS-Q) with linear regression used to describe the relationship between workrate and VO$_2$ (mean $R^2$ values for 8, 18, and 23 km: B $R^2 = 0.76$, FRS $R^2 = 0.87$, FRS-Q $R^2 = 0.86$). These equations were then used to determine the VO$_2$s for the range of power outputs observed during the TTs (225 W to 375 W) at 8, 18, and 23 km for each condition. Economy, used in this context, is practically useful in the evaluation of performance of endurance activities because the fractional utilization of VO$_{2\text{max}}$ is an important determinant of endurance performance (6).

A detailed copy of the subject’s TT performance was generated by downloading their TT data into a software program (Cycling Peaks version 1.1, www.cycling-peakssoftware.com), which was then e-mailed to each subject later that day. As such, each subject was familiar with his TT performance/s for each subsequent TT.

**Dietary Supplementation Protocol**

Subjects were randomized to FRS or FRS-Q supplements developed by New Sun Nutrition (Carpinteria, CA) and formulated and packaged by Wild Flavors, Inc. (Erlanger, KY). Subjects were required to consume the liquid FRS or FRS-Q supplement (300 mL, 2 x per day) for 6 wk, one drink in the morning with a meal, and one in the afternoon or evening with a meal. The antioxidant dose in this study was based on the elimination half-life (t ½) and bioavailability of three major antioxidants (37), vitamin C (estimated t ½ of 10 h), quercetin (t ½ of 12 to 19 h), and catechins (t ½ of 2 to 4 h). Each serving of FRS and FRS-Q contained green tea extract (300 mg), vitamin C (150 mg), vitamin E (50 mg), caffeine (45 mg), niacin (25 mg), taurine (9 mg), vitamin B-6 (2.5 mg), vitamin B-2 (2.1 mg), vitamin B-1 (1.9 mg), and vitamin B-12 (0.008 mg). In addition, the FRS treatment contained 300 mg of the flavonoid quercetin per serving. Both the FRS and FRS-Q drinks also contained 60 kJ glucose per serving, were orange in color and flavor, tasted the same, and visually only differed in the cap used to seal the bottle (i.e., a purple or white cap).

**Laboratory Test Sessions #3 and #5—Time-Trial (TT) and VO$_{2\text{max}}$ Performance Testing.** The protocol employed for the TT at baseline described above was repeated at 3 wk after initiation of dietary supplementation, and the VO$_{2\text{max}}$ test conducted at laboratory session #1 was repeated at 10 wk (i.e., after 4 wk of supplementation on FRS or FRS-Q following the cross-over). The rationale for conducting a 3 wk TT was to determine whether there was any learning effect on the Velotron, and the 10 wk VO$_{2\text{max}}$ test was conducted to assess whether the subject’s training/racing had any measurable effect on aerobic capacity or peak power output, since these variables are strong predictors of TT performance. Furthermore, any dietary supplement effects could also be evaluated at these time-points.

**Laboratory Test Sessions #4 and #6—Time-Trial (TT) Performance Testing.** The protocol employed for the baseline TT was repeated at 6 wk and 12 wk (i.e., after 6 wk of supplementation on either FRS or FRS-Q).
Data Analysis

The statistical analysis was conducted by an independent entity (Vital Research, Los Angeles, CA). Correlation matrices of similar variables were examined to determine whether multivariate analysis was needed. Moderate to high intercorrelations ($r = 0.3$ or higher) warranted multivariate analysis of variance (MANOVA), otherwise ANOVAs were used. Simple main effects analyses were conducted on all univariate analyses having $F$ ratios achieving $P \leq 0.05$. Where appropriate, the analyses were controlled for familywise error, and the Bonferroni correction factor was used to control for inflation error in carrying out multiple comparisons. Values are presented as means ± standard deviation, and the significance level of $P \leq 0.05$ was used.

Results

All 12 subjects completed the 6-wk FRS-Q supplementation protocol, but only 11 completed 6 wk of FRS supplementation before being laboratory tested (1 subject completed 4 wk of FRS supplementation due to out-of-country work commitments). As such, that subject was dropped from the statistical analysis for both FRS and FRS-Q conditions, and hence the data reported are for 11 elite male cyclists. Their characteristics are shown in Table 1.

Table 1  Subject Characteristics at Laboratory Session #1, Prior to Baseline Time Trial, and at 10 Weeks into Dietary Supplementation Protocol

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>10 wk</th>
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<tbody>
<tr>
<td>Mass (kg)</td>
<td>70.7 ± 7.3</td>
<td>71.4 ± 7.6</td>
</tr>
<tr>
<td>Heart rate$_{\text{max}}$ (beats/min)</td>
<td>186 ± 8.2</td>
<td>182 ± 9.3*</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$ (L/min)</td>
<td>4.56 ± 0.7</td>
<td>4.53 ± 0.5</td>
</tr>
<tr>
<td>(mL · kg$^{-1}$ · min$^{-1}$)</td>
<td>64.4 ± 6.7</td>
<td>63.5 ± 4.4</td>
</tr>
<tr>
<td>VE$_{\text{max}}$ (L/min)</td>
<td>164 ± 23.9</td>
<td>172 ± 24.5</td>
</tr>
<tr>
<td>Peak power (W)</td>
<td>429 ± 38.6</td>
<td>429 ± 29.3</td>
</tr>
<tr>
<td>(W/kg)</td>
<td>6.09 ± 0.4</td>
<td>6.04 ± 0.4</td>
</tr>
<tr>
<td>Sum of 8 skinfolds (mm)</td>
<td>63.9 ± 27.8</td>
<td>68.4 ± 25.5</td>
</tr>
<tr>
<td>% body fat</td>
<td>9.3 ± 4.8</td>
<td>9.9 ± 5.5</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation for 11 elite male cyclists; * significant difference ($P \leq 0.01$) between baseline and 10 wk.

Time Trial Performance

The primary outcome measure to assess whether antioxidant dietary supplementation (AS) was beneficial to cycling performance, was the time to complete a 30 km TT. The TT was performed at baseline, 3 wk, and after 6 wk of supplementation with FRS or FRS-Q as shown in Table 2 (baseline and 6-wk data). Average within-
subject variation for 30 km TT time across the four TTs expressed as a coefficient of variation (CV) was 1.5% (95% CI 1.09 to 1.91), and the CV for the final 5 km of the TT was 1.5% (95% CI 1.06 to 1.94). There were no significant learning/supplement effects detected at the 3 wk 30 km TT versus baseline (FRS = 50.95 ± 2.19 min, FRS-Q = 51.78 ± 2.64 min). Time to complete both the 30 km TT distance as well as the final 5 km of the course was significantly improved by 6 wk of FRS supplementation (Table 2, \( P \leq 0.01 \) and \( P \leq 0.05 \), respectively). Subjects completed the TT in a faster time when supplemented with FRS (Table 2) by generating higher absolute W, \( (P \leq 0.01) \) and relative \( (\%\text{PPO}_{\text{max}}, P \leq 0.05) \) power outputs, and consequently speed during the TT \( (P \leq 0.01) \), but with no significant difference in absolute heart rate or relative exercise intensity as expressed by \%HR_{\text{max}} \) or \%VO_{2\text{max}} \) (Table 2). TT performance time was highly correlated with average power output, i.e., 0.938 at baseline, 0.961 for FRS, and 0.923 for the FRS-Q condition.

Table 2  30 km Time-Trial Average Values for Cycling-Related Variables in 11 Elite Male Cyclists at Baseline, and After 6 Weeks of FRS and FRS-Q Supplementation

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>FRS</th>
<th>FRS-Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>52.30 ± 2.03</td>
<td>50.70 ± 2.22(^a)</td>
<td>51.53 ± 2.48</td>
</tr>
<tr>
<td>Final 5 km time (min)</td>
<td>7.44 ± 0.25</td>
<td>7.29 ± 0.34(^b)</td>
<td>7.37 ± 0.36</td>
</tr>
<tr>
<td>Speed (km/h)</td>
<td>34.24 ± 1.3</td>
<td>35.24 ± 1.4(^a)</td>
<td>34.76 ± 1.7</td>
</tr>
<tr>
<td>Power (W)</td>
<td>277 ± 27.3</td>
<td>303 ± 36.6(^a)</td>
<td>293 ± 32.8(^b)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>172 ± 11.9</td>
<td>170 ± 9.9</td>
<td>168 ± 10.9</td>
</tr>
<tr>
<td>Cadence (rev/min)</td>
<td>97 ± 7.9</td>
<td>90 ± 6.9(^a)</td>
<td>89 ± 8.6(^a)</td>
</tr>
<tr>
<td>Rating of perceived exertion</td>
<td>16.4 ± 0.6</td>
<td>17.5 ± 0.8(^b)</td>
<td>17.3 ± 0.6(^b)</td>
</tr>
<tr>
<td>% peak power output</td>
<td>65 ± 5.8</td>
<td>70 ± 3.2(^b)</td>
<td>68 ± 3.7</td>
</tr>
<tr>
<td>% heart rate(_{\text{max}})</td>
<td>92 ± 4.7</td>
<td>91 ± 2.4</td>
<td>90 ± 2.8</td>
</tr>
<tr>
<td>% VO_{2\text{max}}</td>
<td>78.6 ± 7.1</td>
<td>82.3 ± 5.5</td>
<td>81.7 ± 7.9</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation; \(^a(P \leq 0.01)\), \(^b(P \leq 0.05)\) indicates significant difference between baseline and treatment condition.

Substrate Use

CHO and fat oxidation rates were calculated from expired gas measurements made for 2 min at 8, 18, and 23 km at the time points indicated in Figure 1. No significant AS effect was seen for substrate oxidation at 8 km, 18 km, or 23 km (Table 3, \( P \geq 0.05 \)).

Cycling Performance and RPE

Economy was improved on both FRS-Q and FRS between 225 W and 375 W, with this improvement being greater on FRS (Figure 2). Time to each checkpoint (8, 18, and 23 km) was improved on FRS but not on FRS-Q (Table 3, \( P \leq 0.01 \)), a result of higher cumulative average speeds at each checkpoint (Table 3, \( P \leq 0.01 \)). RPEs
Table 3  Selected Performance Variables, and Rates of Carbohydrate and Fat Oxidation at Three Time-Points During a 30 Km Time Trial in 11 Elite Male Cyclists

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th></th>
<th></th>
<th></th>
<th>FRS</th>
<th></th>
<th></th>
<th></th>
<th>FRS-Q</th>
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<tbody>
<tr>
<td></td>
<td>8 km</td>
<td>18 km</td>
<td>23 km</td>
<td>8 km</td>
<td>18 km</td>
<td>23 km</td>
<td>8 km</td>
<td>18 km</td>
<td>23 km</td>
<td>8 km</td>
<td>18 km</td>
</tr>
<tr>
<td>Time</td>
<td>15.21 ± 0.7</td>
<td>34.29 ± 1.4</td>
<td>42.49 ± 2.1</td>
<td>14.74 ± 0.6a</td>
<td>33.18 ± 1.5a</td>
<td>40.64 ± 1.8a</td>
<td>14.99 ± 0.8</td>
<td>33.77 ± 1.8</td>
<td>41.33 ± 2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Speed</td>
<td>32.9 ± 1.3</td>
<td>32.3 ± 1.4</td>
<td>33.2 ± 1.4</td>
<td>33.8 ± 1.5b</td>
<td>34.4 ± 1.5a</td>
<td>34.5 ± 1.5a</td>
<td>33.3 ± 1.8</td>
<td>32.8 ± 2.1</td>
<td>33.9 ± 1.6</td>
<td>1.8 ± 0.5a</td>
<td>1.7 ± 1.1</td>
</tr>
<tr>
<td>RPE</td>
<td>15.1 ± 1.0</td>
<td>15.6 ± 0.7</td>
<td>16.2 ± 0.9</td>
<td>16.5 ± 1.2a</td>
<td>17.1 ± 0.7</td>
<td>17.2 ± 0.9</td>
<td>16.4 ± 1.1</td>
<td>17.0 ± 1.0</td>
<td>17.1 ± 1.1</td>
<td></td>
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<tr>
<td>CHO</td>
<td>3.64 ± 0.8</td>
<td>3.28 ± 0.9</td>
<td>3.29 ± 0.9</td>
<td>4.30 ± 1.1</td>
<td>3.87 ± 0.9</td>
<td>4.05 ± 0.9</td>
<td>4.20 ± 1.0</td>
<td>3.77 ± 1.0</td>
<td>3.96 ± 1.0</td>
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<tr>
<td>Fat</td>
<td>0.52 ± 0.2</td>
<td>0.60 ± 0.2</td>
<td>0.58 ± 0.2</td>
<td>0.35 ± 0.3</td>
<td>0.46 ± 0.3</td>
<td>0.39 ± 0.2</td>
<td>0.39 ± 0.2</td>
<td>0.46 ± 0.2</td>
<td>0.41 ± 0.2</td>
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Values are means ± standard deviation; * (p ≤ 0.01), † (p ≤ 0.05) indicates significant difference between baseline and treatment condition; treatment conditions after 6 wk of supplementation with FRS and FRS-Q.

Figure 2 — Economy in 11 elite male cyclists after 6 wk of FRS and FRS-Q supplementation at the range of power outputs observed during the 30 km TT.
at 8 and 18 km were higher during the FRS and FRS-Q treatments vs. baseline, but not different at 23 km (Table 3). The average RPEs for the 30 km TT were significantly higher for both the FRS and FRS-Q condition when compared with the baseline trial (Table 2, \( P \leq 0.05 \)). Mean RPE data were plotted against mean power output data at 8, 18, and 23 km, and the relationship between these variables was illustrated using linear regression (Figure 3). At any given RPE value, power output was higher during the FRS treatment.

![Figure 3](image_url)

**Figure 3** — Relationship between mean RPE and mean power output at 8, 18, and 23 km after 6 wk of FRS and FRS-Q supplementation, compared with baseline time trial in 11 elite male cyclists.

**Discussion**

We were particularly interested in whether a measurement of endurance exercise performance (30 km TT), conducted at high intensity, would be affected by antioxidant administration while the athletes participated in their typical training and racing schedule. The main finding of this study was that 6 wk of FRS supplementation significantly improved 30 km high-intensity cycling TT performance, and also improved the final 5 km performance compared to the baseline TT (Table 2). Furthermore, the consumption of a cocktail containing antioxidants (FRS-Q), did not significantly improve high-intensity cycling performance.

The 30 km hilly course required exercise at sustained high intensities, the subjects average exercise intensities being \( \sim 82\% \text{ VO}_{2\text{max}} \) and \( \sim 91\% \text{ HR}_{\text{max}} \) for each TT (Table 2). The magnitude of the practical effect of FRS on time to complete the 30 km TT can be represented using the effect size statistic (7). For the 30 km time in the FRS treatment, the effect size is 0.79, and for the final 5 km time this value is 0.60, indicating a large and moderate-to-large effect, respectively (0.22 FRS-Q for
30 km and 5 km; small effect). Another way to assess the practical significance of the improved TT performance when supplemented with FRS is to view this result in relation to beneficial exercise performance changes. It has been suggested that a 1% improvement in exercise performance during competition in elite athletes is significant (16). For example, the difference between 1st place and 5th place in the men’s 2004 Olympic 50 km road TT was 1%, and the difference between 1st and 9th place was 3%.

Therefore, the TT performance improvement of 3.1% over the 30 km course, and the 2% improvement in final 5 km time, indicate that FRS supplementation can be of significant practical advantage to individuals training for and competing in high-intensity endurance events, because these performance changes were realized without a modification of the cyclists’ diets, training, or racing regimens. The subjects in this study were not professional cyclists, but did meet laboratory measured criteria for elite endurance performance (12).

The FRS drink contained quercetin (300 mg per drink), a major flavonoid in some fruits and vegetables, which has much stronger antioxidative and anticarcinogenic activity than vitamin C (14), and also inhibits protein kinases, inhibits DNA topoisomerases, and regulates gene expression (25). The effects of quercetin only, or in combination with other antioxidants, on exercise performance in humans, is not known. Thus, this is the first study to our knowledge, in humans, that demonstrates an ergogenic effect of dietary AS including quercetin (600 mg/d) on endurance exercise performance. One potential limitation of this study is that there was not a quercetin treatment only condition. Although it is tempting to believe that a single antioxidant might improve exercise performance, it is more likely that a combination of scavengers and inhibitors of ROS-producing enzymes would be more effective in combating elevated ROS levels than any single antioxidant agent. The within-subject CV for the four TTs was 1.5% (95% CI 1.09 to 1.91). Thus, although the FRS-Q TT time was improved by 1.5% vs. baseline, this improvement was not significant and is unlikely to be beneficial (Table 2). Therefore, a quercetin only treatment is unlikely to be effective since the defense against oxidative stress depends on an orchestrated synergism between several antioxidants (38).

The improvement in TT performance for the FRS condition cannot be due to a “learning” effect or cycling-related fitness change. The 3-wk TT results were not different to baseline (see results), the subjects were exercising at similar intensities at each TT (HR, %HR max; %VO2 max; see Table 2), and there was no change in VO2 max or PPO after 10 wk of AS (see Table 1). Furthermore, the within-subject variation (CV) for TT time across the four TTs was 1.5%, much less than the measured 3.1% improvement in performance during the FRS condition, and there was no “ceiling effect” observed in TT performance outcome. The six subjects with the lowest VO2 max values (mean = 60.1 mL · kg⁻¹ · min⁻¹) improved their FRS TT time by 2.3%, and the five subjects with the highest VO2 max values (mean = 69.6 mL · kg⁻¹ · min⁻¹) improved by 4.0%. The FRS-Q condition exhibited similar findings, i.e., low VO2 max increased 0.9% and high VO2 max increased 2.2% vs. baseline TT (P ≥ 0.05).

Since ROS directly damage membranes and contractile and structural proteins in muscle (18, 41), effects which may be amplified during high exercise volumes (15) and/or intensities (1, 33), it is possible that less muscle damage and inflammation occurred during training and competition with the 6 wk of FRS supplemen-
Antioxidant Supplementation and Exercise Performance

This could have permitted maintenance of contractile function in the fast type glycolytic fibers that are recruited during high-intensity exercise, resulting in a higher cycling speed (Table 2). Fatigue and performance incompetence in heavy training and overtraining has been attributed to an “overtraining myopathy” in skeletal muscle (22). The typical observation in muscle subjected to this state is a depressed turnover of contractile proteins, particularly in fast type glycolytic fibers, with a concomitant increase in slow myosin (3). The higher sustained power outputs while supplemented with FRS may be in part due to protection of the subjects fast glycolytic muscle fibers. Vassilakopoulos et al. (43) showed that administration of antioxidants blunted the exercise-induced increase in plasma TNF-α, IL-1β, and IL-6 during 45 min of endurance exercise at 70% VO2max, and because ROS are general mediators of signal transduction pathways (10), it was concluded that oxidative stress is a strong stimulus for the exercise-induced increase in these inflammatory cytokines. Furthermore, acute administration of antioxidants containing polyphenols reduced plasma markers of protein oxidation by 23% (23) and 29% (24) during 90 min of exercise at 70% VO2max, suggesting a reduction in muscle damage. It is also possible that the higher power outputs observed during FRS supplementation were due to changes in skeletal muscle redox state. The AS with FRS could have shifted the redox status of the cyclists muscle’s toward a more reduced state, thus limiting the acute fatiguing effects of exercise-induced ROS production on skeletal muscle force production (34). The oxidative stress caused by fatiguing exercise shifts the redox status of muscle to a more oxidized state, thereby depressing force development. AS increases force production by returning cellular redox state toward optimal (34). Further research to quantify damage in skeletal muscle during high-intensity training and competition, and the effects of AS on skeletal muscle force production in humans are required.

TT performance could also have been improved with FRS through quercetin’s inhibition of catechol-O-methyltransferase (COMT; 26, 39). The last 4.82 km (5 km) of the TT course in this study represents 16% of the total TT distance. The ability to increase speed in the last 10 to 20% of a competitive event distinguishes the world’s best runners, which is in contrast to expected pacing strategies where athletes slow over the course of a competitive event, even though they try to maintain an even pace (27). Cycling speed was significantly higher over the last 5 km on FRS (Table 2), potentially due to additional recruitment of motor units, and/or potentiation of active motor units in the exercising limbs when the cyclists should have been most fatigued, i.e., via inhibition of COMT. This higher cycling speed could also have resulted from a superior capacity for oxygen utilization (Figure 2), or other adaptations (cellular, vascular, local hormonal) that reduced afferent sensory information to a supposed central brain controller that regulates motor unit recruitment during exercise (28). Evidence to support this contention comes from an animal study in which quercetin dependently reversed perphenazine as well as reserpine-induced catalepsy in rats through its COMT and MAO enzyme-inhibiting properties (39). Additional evidence for this supposition can be seen in Figure 3 where the cyclists were able to sustain higher average power outputs during the FRS treatment for any given RPE value, thereby providing support for the theory of a central brain governor that regulates exercise performance (28, 40).

The performance changes resulting from FRS supplementation cannot be explained by changes in estimated rates of CHO and fat oxidation since indirect
calorimetry measurements at the 8, 18, and 23 km distances were not significantly different between B and FRS or FRS-Q (Table 3). Our estimated rates of CHO and fat oxidation are similar to those previously measured during high-intensity cycling (13), and confirm that the choice of fuel by working muscle at the intensities at which most endurance athletes train and compete, is that of carbohydrate oxidation, independent of dietary manipulation and substrate availability (4, 44). However, an improved capacity for oxygen utilization for a given work rate, that is economy, was observed during both the FRS-Q and FRS condition at any given power output (Figure 2). The average power output across the three conditions was around 291 W. Using the equations in Figure 2, this would result in a VO$_2$ of 3803 mL/min for B, 3692 mL/min for FRS-Q (2.9% lower), and 3608 mL/min for FRS (5.1% lower). This enhancement in cycling economy was not reflected in higher power outputs or a significant improvement in TT time during FRS-Q, but could have contributed to the significant performance improvement seen during FRS supplementation (Table 2). The mechanism for this enhancement in cycling economy requires further investigation, particularly given that the fitness levels of the subjects were not altered by the supplementation regimens (Table 1).

One potential issue of concern with AS is that of pro-oxidation rather than anti-oxidation. We did not observe any side effects associated with AS, and no subject in the study reported any ill-effects consequent to the AS regimen. Had the AS induced pro-oxidation, then we would have expected to see a significant decrement in exercise performance during the 30 km TT, as well as in the subjects’ daily training and weekly racing. This did not happen. Thus, we conclude that the AS used in this 12-wk study did not produce any observable pro-oxidation effect.

In conclusion, the results of this study show that 6 wk of free radical scavenger (FRS) supplementation significantly improved high-intensity cycling time trial performance during the competitive road racing season, without modification of the cyclists’ diet, training, or competitive race schedules. This significant improvement in performance is likely to be of practical significance for endurance athletes who train and compete at high intensities. The performance improvement could have occurred through favorable alterations in skeletal muscle redox state, reductions in muscle inflammation and protection of skeletal muscle protein, and facilitation and/or potentiation of motor-unit recruitment. Additional studies are required to determine the mechanisms whereby the antioxidant efficacy occurred.

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