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## Quercetin's Influence on Exercise Performance and Muscle Mitochondrial Biogenesis

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### ABSTRACT

**Purpose:** To determine the influence of 2 wk of quercetin (Q; 1000 mg·d<sup>-1</sup>) compared with placebo (P) supplementation on exercise performance and skeletal muscle mitochondrial biogenesis in untrained, young adult males (N = 26, age = 20.2 ± 0.4 yr, V·O<sub>2max</sub> = 46.3 ± 1.2 mL·kg<sup>-1</sup>·min<sup>-1</sup>).

**Methods:** Using a randomized, crossover design with a 2-wk washout period, subjects provided blood and muscle biopsy samples presupplementation and postsupplementation periods and were given 12-min time trials on 15% graded treadmills after 60 min of moderate exercise preloads at 60% V·O<sub>2max</sub>.

**Results:** Plasma Q levels rose significantly in Q versus P during the 2-wk supplementation period (interaction P value <0.001). During the 12-min trial, the net change in distance achieved was significantly greater during Q (2.9%) compared with P (-1.2%; 29.5 ± 11.5 vs -11.9 ± 16.0 m, respectively, P = 0.038). Skeletal muscle messenger RNA expression tended to increase (range = 16-25%) during Q versus P for sirtuin 1 (interaction effect, P = 0.152), peroxisome proliferator-activated receptor [gamma] coactivator-1[alpha] (P = 0.192), cytochrome c oxidase (P = 0.081), and citrate synthase (P = 0.166). Muscle mitochondrial DNA (relative copy number per diploid nuclear genome) increased 140 ± 154 (4.1%) with Q compared with -225 ± 157 (6.0% decrease) with P (P = 0.098).

**Conclusions:** In summary, 1000 mg·d<sup>-1</sup> Q versus P for 2 wk by untrained males was associated with a small but significant improvement in 12-min treadmill time trial performance and modest but insignificant increases in the relative copy number of mitochondrial DNA and messenger RNA levels of four genes related to mitochondrial biogenesis.

Polyphenolic compounds, or polyphenols, are polyhydroxylated phytochemicals, of which the two main classes include flavonoids and phenolic acids. Flavonoids are a large family of secondary plant phenolic of low molecular weight, derivatives of benzo-[gamma]-pyrone, and present in most edible fruits and vegetables (2,12,23). Flavonoid consumption has been associated with a lower incidence of heart disease and certain types of cancers, and mechanisms may include flavonoid-related antioxidant, anti-inflammatory, and gene regulatory effects (12,23). The influence of flavonoid supplementation on exercise-induced physiologic stress and performance has been a focus of our research team during the past several years (31-33).

Cardiorespiratory endurance exercise increases active skeletal muscle mitochondrial density by 20%-100%, depending on the initial fitness level and duration, frequency, and intensity of training (4,13,15). Exercise-induced mitochondrial biogenesis is mediated by the increase in intracellular calcium levels during muscle fiber contraction and is a multifaceted process involving the coordinated expression of mitochondrial and nuclear genes (7,11,14). In particular, the transcriptional coactivator peroxisome proliferator-activated receptor [gamma] coactivator-1[alpha] (PGC-1[alpha]) plays a key role in regulating skeletal muscle mitochondrial adaptive responses to regular endurance exercise (7). Recent evidence in animal models indicates that some of the adaptations in muscle phenotype elicited by exercise can be mimicked by energy restriction, genetic manipulation, drug treatment, and certain types of polyphenols (3,6,20,28,29,34). Few human studies, however, have been conducted to determine whether nonexercise methods can increase mitochondrial density in skeletal muscle.

Soy isoflavone derivatives (34), resveratrol (20), and tea catechins including epigallocatechin gallate (EGCG) (25,28) are associated with induction of genes for mitochondrial biogenesis and oxidative phosphorylation. Treatment of mice with large quantities of resveratrol (200-400 mg·kg<sup>-1</sup>·d<sup>-1</sup>) for 15 wk induced PGC-1[alpha] with activation from sirtuin 1 (SIRT1), resulting in a significant increase in mitochondrial density and endurance running capacity (20). Tea catechin intake improved physical performance and mitochondrial function in the skeletal muscle of aged mice, especially when combined with exercise (27,28). Cell culture studies show that isoflavone derivatives induce the activation of PGC-1[alpha], SIRT1, and mitochondrial biogenesis (34). Other studies show that EGCG accumulates in mitochondria and exerts anti-apoptotic effects (37) and that specific flavonoids induce a mild uncoupling of mitochondrial oxidative phosphorylation and inhibit mitochondrial membrane lipid peroxidation (8,9,19,36). Recent evidence indicates that quercetin supplementation induces a strong increase in mitochondrial biogenesis and endurance performance in mice (6).

Quercetin is a flavonol that constitutes the aglycone of the plant glycosides rutin and quercetrin. Of all flavonoids, quercetin is among the most widespread, with broad spectrum bioactive effects (5,12,23). Two reports support the safety of quercetin as a nutritional supplement (12,40). Human subjects can absorb significant amounts of quercetin, with a reported half-life ranging from 3.5 to 28 h (23,26). A few recent studies have investigated quercetin's effects on endurance performance and mitochondrial biogenesis. One study of 11

elite male cyclists reported a 1.7% 30-km time trial performance enhancement above placebo after 6 wk of quercetin supplementation (24). Another study using sedentary mice showed that 7-d quercetin feeding (both 12.5 and 25 mg·kg<sup>-1</sup>) increased soleus muscle PGC-1[alpha] and SIRT1 messenger RNA (mRNA) levels two- to threefold, cytochrome c concentration by 18%-32%, and treadmill run time to fatigue by 37% (6). A study of 40 trained cyclists randomized to 1000 mg·d<sup>-1</sup> quercetin or placebo for 3 wk failed, however, to show any group differences in skeletal muscle mRNA expression for PGC-1[alpha] or SIRT1 (31,32). Another study of 39 trained cyclists also showed no effect of 1000 mg of quercetin a day compared with placebo on mRNA expression for mitochondrial biogenesis or cycling time trial performance (33).

Quercetin supplementation may have a larger effect on mitochondrial biogenesis and endurance performance in untrained compared with trained subjects due to differences in prestudy muscle mitochondrial density. The purpose of this study was to determine the influence of 2 wk of quercetin supplementation (1000 mg·d<sup>-1</sup>) compared with placebo ingestion on skeletal muscle mitochondrial biogenesis in untrained males and the ability to perform during a 12-min time trial on a graded treadmill after a 60-min moderate exercise preload.

## METHODS

**Subjects.** Thirty young male adults between the ages of 18 to 28 yr were recruited. Selection criteria included the following: body mass index of less than 27 kg·m<sup>-2</sup>; healthy, with no known diseases or disease risk factors; not on any type of medications or supplements, particularly herbal supplements; physically sedentary or less than two aerobic exercise sessions a week of less than 20-min duration during the previous 6 months; not physically active during work; moderate consumer of caffeine (<250 mg·d<sup>-1</sup>); and nonsmoker. Written informed consent was obtained from each subject, and the experimental procedures were approved by the institutional review board of Appalachian State University.

**Research design.** This study used a double-blinded, placebo-controlled, randomized, counterbalanced, crossover design, with subjects acting as their own controls. Subjects provided blood and muscle samples before and after the 2-wk supplementation with quercetin and placebo beverages, with a 2-wk washout period before crossover. For the entire 6-wk study, subjects avoided all types of anti-inflammatory medications and herbal or nutrient supplements.

Subjects first reported to the Human Performance Laboratory (HPL) for orientation, body composition testing (three-site skinfold test), and measurement of peak oxygen consumption ( $\dot{V}\text{O}_{2\text{max}}$ ) during a graded treadmill exercise test using the Cosmed FitMate metabolic system (Cosmed, Rome, Italy). Subjects also practiced a 12-min time trial on a 15% graded treadmill, trying to cover the greatest distance possible with self-selection of the speed setting. Subjects were told that the 12-min time trial would follow a 60-min treadmill exercise preload at 60%  $\dot{V}\text{O}_{2\text{max}}$  with a 10% treadmill grade. This type of exercise test has been shown to have high reliability in trained subjects on cycle ergometers and treadmills as compared with time-to-

exhaustion tests (18,35). We used graded treadmill walking for our untrained subjects because we reasoned they were more accustomed to treadmill walking than cycle ergometer exercise, and we chose a time trial duration of 12 min to ensure completion of the protocol. These protocol adaptations were based on prestudy trials in our laboratory, but a validation study for this exact protocol has not been published. The coefficient of variation for the subjects in this study completing all four exercise time trials was 2.65%.

After baseline testing, subjects returned to the HPL at 2:30 p.m. for the first of four test sessions (with all test sessions following exactly the same time schedule). A blood sample was collected (for plasma quercetin analysis) followed by a muscle biopsy sample from the vastus lateralis, with the incision closed with steri-strips. At 3:00 p.m., subjects exercised for 60 min at 60%  $V\cdot O_{2max}$  on 10% graded treadmills immediately before the 12-min time trial on 15% graded treadmills with verbal urging by the HPL staff to cover the greatest distance possible. Metabolic measurements were continuously monitored using the Cosmed FitMate metabolic system with a face mask and recorded every 15 min during the 60-min preload bout and every minute during the 12-min time trial. If the HR during the 12-min trial dipped less than 93% of their  $HR_{max}$ , subjects were questioned as to whether or not they were pushing as hard as they could and verbally urged to make a full effort.

For 3 d before each test session, subjects avoided all foods and beverages containing caffeine and chose foods from a list used in previous studies to ensure a moderate carbohydrate intake (30-32). At 11:30 a.m. to 12:15 p.m. before each test session, subjects ingested a standardized liquid meal (Boost Plus; Mead Johnson Nutritionals, Evansville, IN) at an energy level of 42 kJ·kg<sup>-1</sup> (10 kcal·kg<sup>-1</sup>). Boost Plus is a nutritionally complete, high-energy oral supplement with an energy density of 6.4 kJ·mL<sup>-1</sup> (1.52 kcal·mL<sup>-1</sup>) and 16% of energy as protein, 34% as fat, and 50% as carbohydrate. In quantities of 1000 mL, Boost Plus exceeds daily value recommendations for all major vitamins and minerals. No other food and beverage (other than water) was consumed from this meal until the end of the test session.

After the first test session, subjects were randomized to placebo or quercetin supplementation for 2 wk. When returning to the HPL 2 wk later, subjects ingested half of their quercetin or placebo supplement beverage at 8:00 a.m. and the other half at 1:00 p.m. (thus 2 h before the start of the treadmill performance test).

**Supplement.** Subjects ingested quercetin or placebo beverages for 2 wk before and after the 2-wk washout period. The supplement beverages were coded and prepared in 32-oz bottles using a sugarless PowerAde base by Coca-Cola (Atlanta, GA). Pure food-grade quercetin, QU995, was supplied by Quercegen Pharma (Newton, MA). The quercetin and the placebo beverages were similar in taste and appearance, and a poststudy survey revealed no significant ability of the subjects to detect the type of beverage being consumed. Subjects consumed 16 oz of the supplement beverage at 8:00 a.m. and then again at 1:00 p.m. each day.

**Blood samples and plasma quercetin.** Blood samples were drawn from an antecubital vein into sodium-heparinized tubes with subjects in the seated position. Plasma was aliquoted and then stored at -80°C before plasma quercetin analysis. Total plasma quercetin (quercetin and its

primary conjugates) was measured after solid-phase extraction via reversed-phase HPLC with ultraviolet detection as previously described (31-33). Quercetin conjugates were hydrolyzed by incubating 500  $\mu$ L of plasma aliquots with 10  $\mu$ L of 10% dl-dithiothreitol solution, 50  $\mu$ L of 0.58 M acetic acid, 50  $\mu$ L of a mixture of [ $\beta$ ]-glucuronidase/arylsulfatase, and crude extract from *Helix pomatia* (Roche Diagnostics Corp., Indianapolis, IN) for 2 h at 37°C. Chromatographic analysis was performed using the Ultimate 3000 HPLC-PDA system (Dionex Corp., Sunnyvale, CA), with a Gemini C18 column (Phenomenex, Torrance, CA).

**Muscle biopsies.** Muscle biopsies were obtained from the vastus lateralis before and after each 2 wk of supplementation. The postsupplementation biopsy was obtained approximately 2 cm proximal from the presupplementation biopsy site. Muscle samples were collected from the opposite leg for the second supplementation period, with leg order randomly determined. Local anesthesia (2% xylocaine) was injected subcutaneously and intramuscularly. After a small incision (~0.5 cm), a muscle biopsy sample (~100 mg) was obtained using the percutaneous needle biopsy procedure modified to include suction (30). Muscle was trimmed of connective tissue and fat and immediately frozen in liquid nitrogen. Samples were stored at -80°C until subsequent analysis.

**Muscle RNA isolation and complementary DNA synthesis.** Skeletal muscle was homogenized under liquid nitrogen with a micropestle, and total RNA was extracted using the guanidine thiocyanate method with TRIzol (Invitrogen, Carlsbad, CA). The extracted RNA was dissolved in diethylpyrocarbonate-treated water and quantified spectrophotometrically at 260 nm. Intact RNA was confirmed by denaturing agarose gel (1%) electrophoresis. RNA was reverse transcribed using the high capacity complementary DNA (cDNA) reverse transcription kit (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol.

**Quantitative real-time polymerase chain reaction analysis.** Briefly, 25 ng of cDNA was used in each reaction (TaqMan Gene Expression Assays; Applied Biosystems) as per manufacturer's instructions. Relative expression levels of citrate synthase, cytochrome c oxidase I, SIRT1, PGC-1[ $\alpha$ ], and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were determined with predesigned, preoptimized gene-specific probe sets provided by Applied Biosystems (TaqMan Gene Expression Assays). Samples were loaded in a MicroAmp 96-well reaction plate and run in triplicate. Quantitative real-time polymerase chain reaction was performed on an ABI 7300 sequence detector. The data were analyzed using Applied Biosystems 7300 sequence detection software version 1.3.1. Relative mRNA levels were determined using the  $^{[\Delta][\Delta]}$ Ct method, with GAPDH serving as the as the endogenous control (4,21).

**Mitochondrial DNA quantification.** A portion of each skeletal muscle biopsy (~20 mg) was homogenized with a Polytron homogenizer, and total DNA (mitochondrial and nuclear) was extracted using a QIAamp DNA minikit (QIAGEN, Valencia, CA). The concentration of each sample was determined spectrophotometrically at 260 nm. Mitochondrial DNA (mtDNA) content was determined via quantitative real-time polymerase chain reaction using 10 ng of total DNA per reaction. The 12S ribosomal RNA (rRNA) and the [ $\beta$ ]-microglobulin were used as the mtDNA and nuclear DNA (nDNA) targets, respectively. mtDNA levels were measured with a

predesigned, preoptimized 12S rRNA-specific probe set provided by Applied Biosystems (TaqMan Gene Expression Assays). The primers and the probe used to quantify nDNA, [beta]2-microglobulin, were previously published by Bai and Wong (1). All samples were assayed in duplicate. The 12S rRNA and the [beta]2-microglobulin primer and probe sets resulted in reaction efficiencies of 98.8% and 98.5%, respectively; reaction efficiencies were sufficiently similar to allow quantification of mtDNA (12S rRNA) relative to the nDNA target ([beta]2-microglobulin). The threshold cycle values for 12S rRNA and [beta]2-microglobulin were used to calculate the relative copy (Rc) number of mtDNA per diploid nuclear genome according to Szuhai et al. (39):  $Rc = 2^{[\Delta Ct]}$  and  $[\Delta Ct] = Ct_{[\beta]2\text{-microglobulin}} - Ct_{12S\text{ rRNA}}$ .

**Statistical analysis.** Data are expressed as mean  $\pm$  SE and were analyzed using a 2 (condition)  $\times$  2 (time) repeated-measures ANOVA, within-subjects design. Presupplement to postsupplement changes within conditions were compared between trials using paired t-tests, with significance set at  $P \leq 0.05$ . For skeletal muscle mRNA, presupplementation levels for each 2-wk period were set at 1, with 2-wk fold changes calculated from the presupplementation levels. Because of the lack of variance when presupplementation levels were set at 1, we compared 2-wk fold changes between conditions using paired t-tests. A one-factor repeated-measures ANOVA was used to assess the GAPDH data.

## RESULTS

Subject characteristics for the 26 subjects completing all phases of the study are summarized in Table 1 and indicate typical aerobic fitness and body composition levels for untrained, young adult males. Plasma quercetin levels rose significantly during the 2-wk quercetin compared with placebo supplementation period (interaction  $P$  value  $< 0.001$ ), and the 2-wk washout period was sufficient to bring plasma quercetin down to presupplementation levels (Fig. 1).

TABLE 1. Subject characteristics (N = 26).

| Variable   | Mean $\pm$ SE   |
|--|-----------------|
| Age (yr)   | 20.2 $\pm$ 0.4  |
| Height (m)   | 1.77 $\pm$ 0.01 |
| Body mass (kg)   | 74.8 $\pm$ 1.6  |
| Body composition (% fat)                                     | 16.1 $\pm$ 0.9  |
| Peak aerobic power (mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) | 46.3 $\pm$ 1.2  |
| HR <sub>max</sub> (beats·min <sup>-1</sup> )                 | 197 $\pm$ 1.4   |

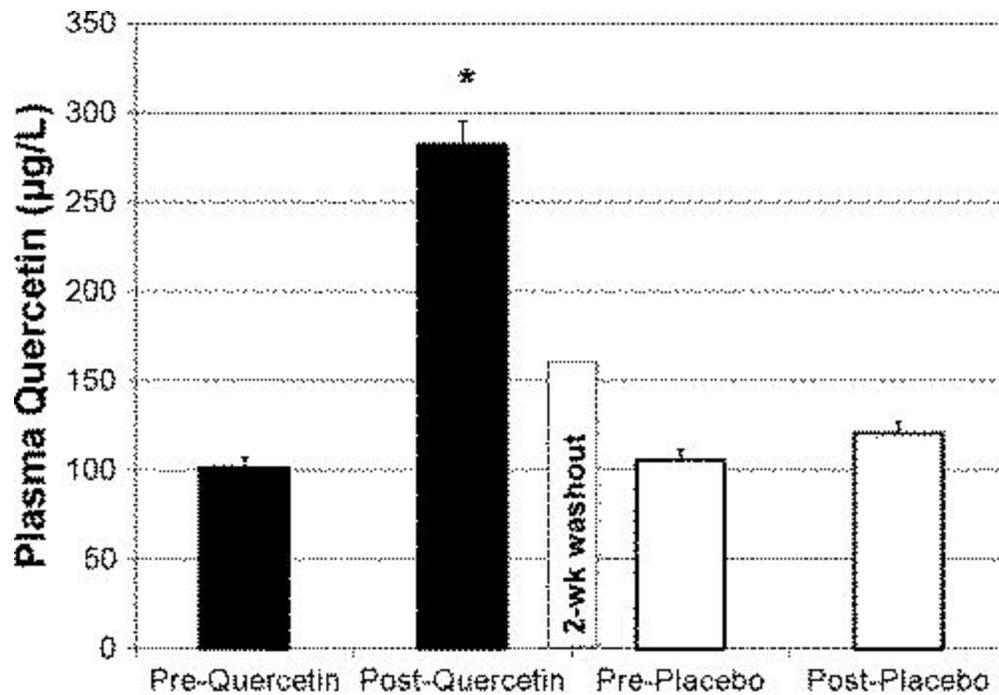


FIGURE 1. Plasma quercetin levels presupplementation and postsupplementation with quercetin (1000 mg·d<sup>-1</sup>) or placebo in 26 untrained males. \*Interaction effect, P < 0.001.

During the 60-min exercise preload, no significant differences in exercise workload were measured between quercetin and placebo trials (Table 2). For all four test sessions combined, subjects averaged 62.5% ± 1.5% peak oxygen consumption ( $V\cdot O_{2max}$ ) and 77.1% ± 0.8% HR<sub>max</sub>. The net change (presupplementation to postsupplementation change) in RPE tended to be lower in the quercetin compared with placebo trial (Table 2).

TABLE 2. Exercise performance data.

| Variable  | Quercetin     |                | Placebo       |                | Interaction P Value |
|---|---------------|----------------|---------------|----------------|---------------------|
|   | Presupplement | Postsupplement | Presupplement | Postsupplement |                     |
| <b>A. 60-min exercise preload data</b>                        |               |                |               |                |                     |
| VO <sub>2</sub> (mL·kg <sup>-1</sup> ·min <sup>-1</sup> )     | 29.1 ± 1.0    | 27.8 ± 1.2     | 29.1 ± 1.0    | 29.1 ± 1.0     | 0.361               |
| HR (beats·min <sup>-1</sup> )                                 | 152 ± 2.0     | 151 ± 1.9      | 152 ± 1.7     | 151 ± 2.2      | 0.967               |
| Ventilation (L·min <sup>-1</sup> )                            | 58.8 ± 2.2    | 58.3 ± 2.1     | 59.9 ± 2.2    | 59.0 ± 1.9     | 0.768               |
| Respiratory rate (breaths·min <sup>-1</sup> )                 | 31.8 ± 1.3    | 31.2 ± 1.2     | 31.4 ± 1.4    | 31.4 ± 1.3     | 0.397               |
| RPE   | 12.3 ± 0.4    | 11.8 ± 0.5     | 11.9 ± 0.4    | 12.4 ± 0.5     | 0.071               |
| <b>B. 12-min time trial, after 60 min of exercise preload</b> |               |                |               |                |                     |
| VO <sub>2</sub> (mL·kg <sup>-1</sup> ·min <sup>-1</sup> )     | 39.8 ± 1.1    | 40.5 ± 1.6     | 39.8 ± 1.1    | 40.9 ± 1.6     | 0.863               |
| HR (beats·min <sup>-1</sup> )                                 | 183 ± 1.6     | 180 ± 2.2      | 184 ± 1.6     | 184 ± 1.7      | 0.290               |
| Ventilation (L·min <sup>-1</sup> )                            | 95.1 ± 3.4    | 99.1 ± 3.7*    | 100.6 ± 3.7   | 95.6 ± 2.9     | 0.031               |
| Respiratory rate (breaths·min <sup>-1</sup> )                 | 42.9 ± 2.5    | 41.5 ± 1.2     | 42.8 ± 1.2    | 40.5 ± 1.1     | 0.691               |
| RPE   | 16.4 ± 0.3    | 16.3 ± 0.3     | 16.6 ± 0.3    | 16.7 ± 0.3     | 0.595               |
| Distance (m)  | 984 ± 19.7    | 1013 ± 20.9*   | 1002 ± 24.4   | 990 ± 20.5     | 0.038               |

Values are presented as mean ± SD.

\* P < 0.05, change before and after quercetin supplement compared with placebo.

During the 12-min trial (15% treadmill grade, self-selected speed), the net change in distance achieved was significantly greater during the quercetin compared with placebo condition (interaction effect, P = 0.038; Fig. 2 and Table 2). Oxygen consumption and HR did not differ

during the quercetin and the placebo 12-min trials and averaged  $87.1\% \pm 0.7\%$  of  $V\cdot O_{2max}$  and  $93.0\% \pm 0.5\%$   $HR_{max}$  (Table 2). Respiratory rate and RPE did not differ between the quercetin and the placebo 12-min trials, with a small but significant net increase in ventilation during the quercetin compared with placebo trials.

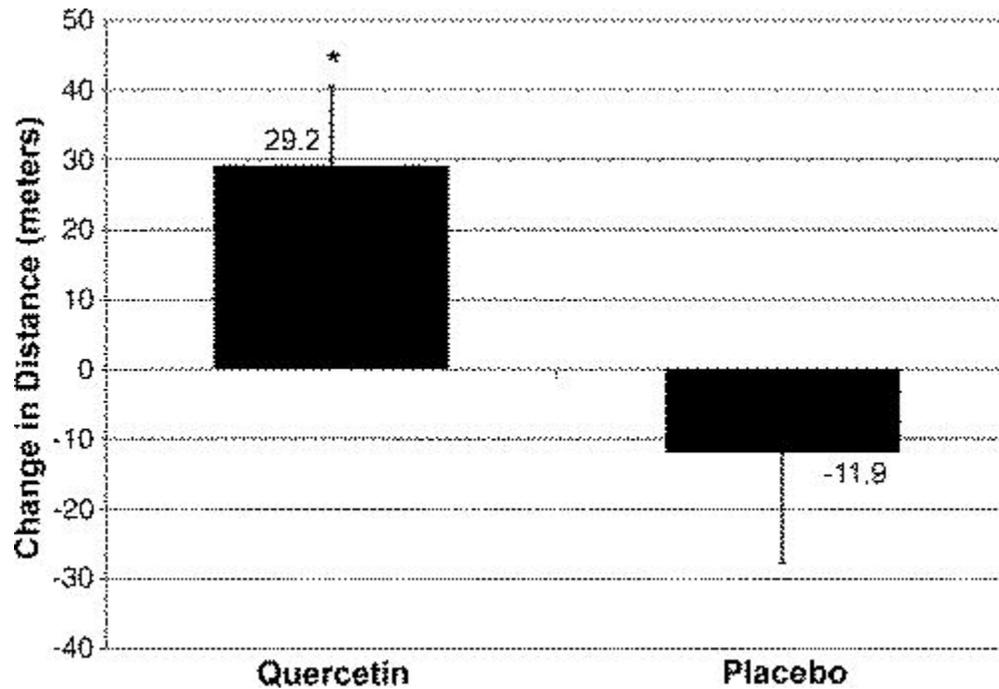


FIGURE 2. Presupplementation to postsupplementation change in distance covered during a 12-min treadmill time trial after a 60-min preload at 60%  $V\cdot O_{2max}$  in quercetin and placebo trials. \*Interaction effect,  $P = 0.038$ .

Skeletal muscle mRNA levels (Fig. 3) tended to increase after 2 wk of supplementation with quercetin compared with placebo for SIRT1 (interaction  $P$  value = 0.152), PGC-1[ $\alpha$ ] ( $P = 0.192$ ), cytochrome c oxidase ( $P = 0.081$ ), and citrate synthase ( $P = 0.166$ ). A one-way ANOVA of the GAPDH data revealed no change across time points ( $P = 0.689$ ), indicating that GAPDH was a valid normalizer for this study.

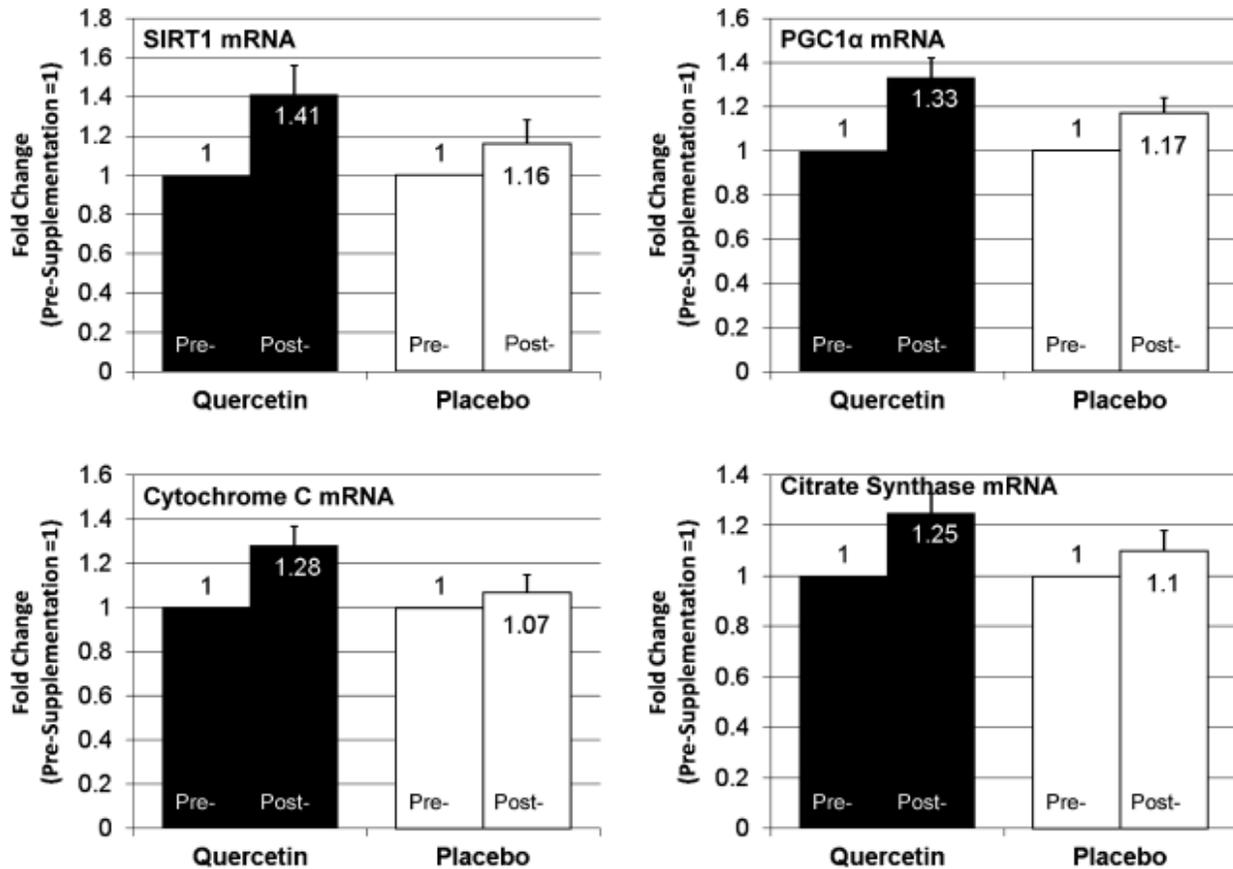


FIGURE 3. Skeletal muscle mRNA levels for quercetin compared with placebo for SIRT1 (interaction P value = 0.152), PGC-1[ $\alpha$ ] (P = 0.192), cytochrome c oxidase (P = 0.081), and citrate synthase (P = 0.166).

Skeletal muscle mtDNA relative copy number increased from  $3399 \pm 251$  to  $3538 \pm 266$  in the quercetin trial (4.1%) in contrast to a decrease from  $3762 \pm 217$  to  $3538 \pm 246$  (-6.0%) in the placebo trial (interaction effect, P = 0.098; Fig. 4). Presupplementation to postsupplementation levels of the nDNA target, [beta]2-microglobulin, did not differ between quercetin and placebo trials (interaction effect, P = 0.444).

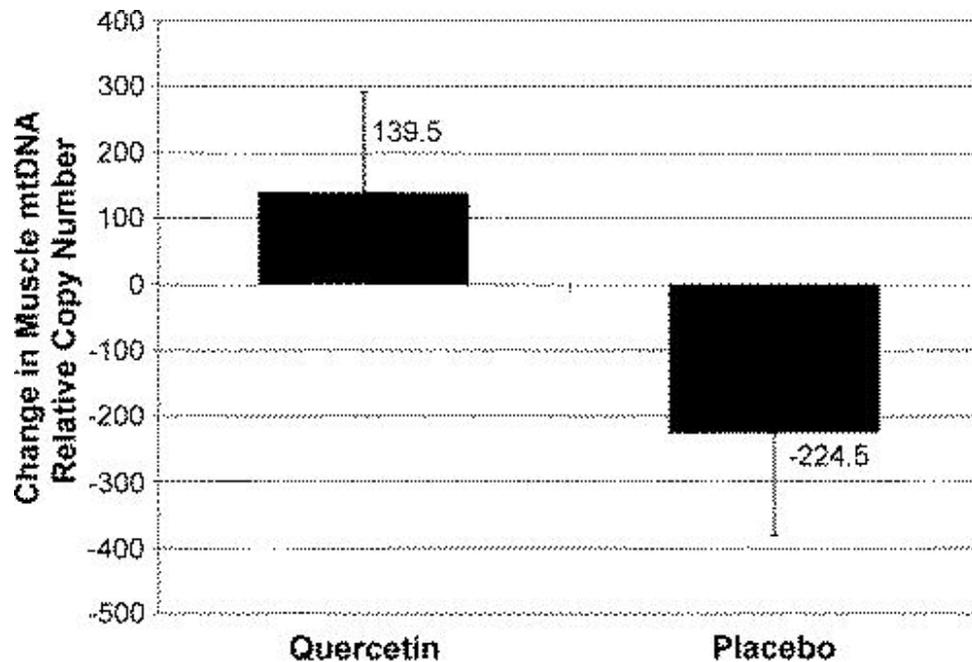


FIGURE 4. Presupplementation to postsupplementation change in the relative copy number of muscle mitochondrial DNA in quercetin and placebo trials (interaction P value = 0.098).

## DISCUSSION

Using a randomized, crossover design with 26 untrained males, a small but significant performance effect was measured with 2-wk quercetin (2.9%) compared with placebo (-1.2%) supplementation at the level of 1000 mg·d<sup>-1</sup>. Nonsignificant increases in mRNA expression of four genes related to skeletal muscle mitochondrial biogenesis and in mtDNA copy number were also measured after quercetin supplementation.

This performance benefit in untrained males contrasts with what we have previously reported in trained cyclists who experienced no quercetin-related improvement when performing 5-, 10-, and 20-km time trials at the end of three 3-h cycling bouts (33). Given these results in trained subjects, we reasoned that quercetin supplementation would be more likely to improve endurance performance and augment skeletal muscle mitochondrial biogenesis in untrained subjects (33). This hypothesis was also based in part on a study with ICR male mice showing an increase in soleus muscle PGC-1[α] (~100%) and SIRT1 (~200%) mRNA, cytochrome c concentration (18%-32%), and treadmill running time until fatigue (~37%) after a 7-d period of quercetin feeding at 12.5 and 25 mg·kg<sup>-1</sup> (6). Mouse soleus muscle mtDNA was approximately doubled after 1 wk with the 25-mg·kg<sup>-1</sup> dose of quercetin but not with the 12.5-mg·kg<sup>-1</sup> dose (6). The mice were housed individually in regular cages and were not trained through forced treadmill running. In a separate experiment, mice fed with quercetin and given access to running wheels increased running distance by 35% after 6 d compared with the placebo group (6).

The magnitude of quercetin-induced changes in muscle PGC-1[alpha] and SIRT1 mRNA and endurance performance in these mice was much higher than we found in our untrained human subjects. There are several potential reasons, including the applicability of findings from the mouse model to humans in quercetin and flavonoid-related research. For example, quercetin exerts antioxidant effects in the mouse (38), but thus far this effect has not been shown in humans except when combined with other flavonoids such as EGCG (10,33). Other considerations relate to supplementation issues such as the length of time quercetin was ingested and the type and amount of quercetin used.

We chose a 2-wk period for both quercetin supplementation and washout, and this was based on previous human and animal studies and quercetin's relatively short half-life (6,23,33). Given the metabolic and lifespan differences of mice and humans, future research is needed to determine whether a longer supplementation period (e.g., 4-6 wk) is preferable in humans to measure potential quercetin effects on mitochondrial biogenesis. For example, although data are lacking, mice and humans may differ in the extent of metabolic transformation that occurs during first-pass conjugation of quercetin in the liver. Species differences in quercetin conjugate profiles, disappearance rates, and tissue incorporation may influence the process of mitochondrial biogenesis. The 2-wk washout period seemed appropriate given our plasma quercetin findings, but tissue quercetin levels were not measured. Incorporation of quercetin into human muscle cells *in vivo* has not yet been determined, but one study in pigs showed that skeletal muscle did not accumulate quercetin even after 4 wk of supplementation (50 mg of quercetin per kilogram each day) (2). Pig skeletal muscle did incorporate a small amount of quercetin within 90 min of ingesting a 25-mg·kg<sup>-1</sup> dose. In our study, muscle mtDNA tended to decrease during the placebo condition and, although not statistically significant, could have been driven in part by the increase experienced in half the group receiving quercetin during the first 2 wk followed by an extended decrease for several weeks.

Another potential issue is the quercetin dose, set in this study at 1000 mg·d<sup>-1</sup>. Human subjects may require a higher quercetin dose to more consistently induce mitochondrial biogenesis. The aglycone form of quercetin was used in this study, and future research may determine that the more bioavailable isoquercetin, as found naturally in onions and apples, has greater bioactive effects, especially when combined with other flavonoids and food components (12,23,33).

Underlying mechanisms for PGC-1[alpha] activators such as soy isoflavone derivatives (34), resveratrol (20), EGCG (28), and quercetin (1,6) are still being explored and debated. Multiple factors regulate mitochondrial biogenesis through PGC-1[alpha], and these include modifications in energy homeostasis from energy restriction and exercise (3,11,16,17,22). Cell culture studies indicate that quercetin activates the adenosine monophosphate-activated protein kinase (AMPK) and mitogen-activated protein kinase (MAPK) signaling pathways, both of which are upstream to PGC-1[alpha] activation (7). Polyphenols such as quercetin may also increase SIRT1 deacetylase activity and LKB1 phosphorylation, providing another pathway for PGC-1[alpha] activation (16).

Most in vitro studies, however, have not used quercetin conjugates as found in human blood after oral ingestion (12,23), and this type of research is needed to improve understanding of potential pathways to PGC-1[alpha] and mitochondrial biogenesis activation. Our data indicate that 1000-mg·d<sup>-1</sup> quercetin ingestion for more than 2 wk has a mild but inconsistent effect on mtDNA copy number in human skeletal muscle, and this may be due in part to interindividual variation in genetic responses to quercetin conjugates. For each of the mRNA and mtDNA measures in this study, 5 to 8 out of 26 subjects were nonresponders to quercetin supplementation and had higher levels in the placebo condition. Individuals may also vary in other reported flavonoid effects on mitochondria, including regulation of apoptosis, uncoupling of oxidative phosphorylation, and attenuation of membrane lipid peroxidation (8,9,19,36,37).

In summary, quercetin supplementation at the level of 1 g·d<sup>-1</sup> for 2 wk by untrained males was associated with a small but significant improvement in 12-min treadmill time trial performance after a 1-h preload. Nonsignificant trends for quercetin-related increases in mRNA expression for PGC-1[alpha], SIRT1, citrate synthase, and cytochrome c oxidase (net range of 16%-25% above placebo) and in mtDNA copy number from skeletal muscle biopsies provide a partial explanation for the performance enhancement. These quercetin-related effects on performance and mitochondrial biogenesis in untrained humans were modest and far below those reported in mice (6), and future research should emphasize larger doses for longer supplementation periods with added food components and flavonoids such as EGCG and isoquercetin that may augment quercetin's bioactive effects on mitochondrial biogenesis. In addition, the synergistic effect of intensive exercise training with quercetin supplementation by untrained subjects should be tested, as modeled by Narkar et al. (29).

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Results of the present study do not constitute endorsement by the American College of Sports Medicine.

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