

Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance

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Submitted 13 November 2008; accepted in final form 6 February 2009

Davis JM, Murphy EA, Carmichael MD, Davis B. Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance. *Am J Physiol Regul Integr Comp Physiol* 296: R1071–R1077, 2009. First published February 11, 2009; doi:10.1152/ajpregu.90925.2008.—Quercetin is one of a broad group of natural polyphenolic flavonoid substances that are being investigated for their widespread health benefits. These benefits have generally been ascribed to its combination of antioxidant and anti-inflammatory activity, but recent *in vitro* evidence suggests that improved mitochondrial biogenesis could play an important role. In addition, the *in vivo* effects of quercetin on mitochondrial biogenesis exercise tolerance are unknown. We examined the effects of 7 days of quercetin feedings in mice on markers of mitochondrial biogenesis in skeletal muscle and brain, and on endurance exercise tolerance. Mice were randomly assigned to one of the following three treatment groups: placebo, 12.5 mg/kg quercetin, or 25 mg/kg quercetin. Following 7 days of treatment, mice were killed, and soleus muscle and brain were analyzed for mRNA expression of peroxisome proliferator-activated receptor- γ coactivator (PGC-1 α) and sirtuin 1 (SIRT1), and mitochondrial DNA (mtDNA) and cytochrome *c*. Additional mice underwent a treadmill performance run to fatigue or were placed in voluntary activity wheel cages, and their voluntary activity (distance, time, and peak speed) was recorded. Quercetin increased mRNA expression of PGC-1 α and SIRT1 ($P < 0.05$), mtDNA ($P < 0.05$) and cytochrome *c* concentration ($P < 0.05$). These changes in markers of mitochondrial biogenesis were associated with an increase in both maximal endurance capacity ($P < 0.05$) and voluntary wheel-running activity ($P < 0.05$). These benefits of quercetin on fitness without exercise training may have important implications for enhancement of athletic and military performance and may also extend to prevention and/or treatment of chronic diseases.

flavonoid; mitochondria; endurance capacity

MITOCHONDRIAL DYSFUNCTION in peripheral tissues and brain plays an important role in the etiology of many diseases, including neurodegenerative disorders, cancer, diabetes, and cardiovascular myopathies, as well as the aging process and poor exercise tolerance (3, 28, 32). Although poor exercise tolerance is clearly a problem for athletes and military personnel, it is also a risk factor for development of these disorders (28). Exercise training is generally thought to be the best strategy to increase muscle mitochondria number and function, although little is known about the effect of exercise on brain mitochondria (20, 27). Given the difficulty in maintaining a regular exercise program, other strategies involving nutrition and drugs have received increasing attention. Among the most effective are caloric restriction, natural flavonoids like resveratrol, and drugs that have been shown

to increase mitochondrial biogenesis via an increase in the transcriptional coactivators sirtuin 1 (SIRT1) and peroxisome proliferator-activated receptor- γ coactivator (PGC-1 α) (13, 14, 17, 23). PGC-1 α is considered the “master regulator” of mitochondrial biogenesis; SIRT1 physically interacts with and deacetylates PGC-1 α and consequently increases PGC-1 α activity (24, 25). However, strategies involving caloric restriction, natural flavonoids, or drugs have not focused on effects on exercise tolerance or on brain mitochondria, and perhaps more importantly, they involve long-term interventions, megadoses, and/or drugs with uncertain side effects.

Quercetin, a natural polyphenolic flavonoid, is present in a wide variety of food plants, including red onions, apples, and berries (10), and has been shown in combination with other antioxidants and caffeine to improve endurance time-trial performance on a bicycle ergometer when fed for 6 wk in humans (15). However, the biological mechanisms of this observation have not been studied, and there is no evidence of an effect of quercetin on mitochondrial biogenesis. Given the similarity in the structure of quercetin to resveratrol and other flavonoid derivatives that have been shown to increase mitochondrial biogenesis (13, 17, 23) and *in vitro* evidence of an effect of quercetin on the energetics of isolated mitochondria (8, 23, 29), we hypothesized that quercetin would increase mitochondrial biogenesis in muscle and that this would be associated with an increase in exercise tolerance. We also evaluated effects of quercetin on brain mitochondrial biogenesis to explore the possible and often ignored role of the central nervous system (CNS) in exercise behavior (e.g., CNS fatigue).

The purpose of this study was to evaluate the role of short-term supplementation of quercetin at a dose that is both safe and practical for use as a dietary supplement on mitochondrial biogenesis in brain and soleus muscle, and endurance exercise tolerance. The soleus muscle was chosen because of its obvious relevance to endurance exercise capacity. We used an experimental mouse model to examine the effects of 7 days of quercetin feedings on markers of mitochondrial biogenesis, including gene expression of PGC-1 α and SIRT1, mitochondrial DNA (mtDNA) and cytochrome *c* enzyme concentration. In addition, we examined the effects of quercetin feedings on exercise tolerance using regimens designed to test both voluntary and involuntary running performance, which are influenced disproportionately by central and peripheral factors, respectively.

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METHODS

Animals. All mice were cared for in the animal facility at the University of South Carolina. The University's Institutional Animal Care and Use Committee approved all aspects of this experimental protocol. Male ICR mice, 8 wk of age, were purchased from Harlan Sprague-Dawley Laboratories and were acclimated to our facility for a period of at least 3 days before any experimental intervention. Mice were housed individually in regular cages or cages that contained a running wheel (Mini-Mitter, Bend, Oregon). Mice were maintained on a 12:12-h light-dark cycle in a low-stress environment (22°C, 50% humidity and low noise) and given food (Purina Chow) and water ad libitum. All experimental trials were performed at the beginning of the dark cycle (19:00 h).

Quercetin treatment. Mice were fed either quercetin (QU995; Quercegen Pharma, Newton, MA) at one of two doses (12.5 or 25 mg/kg) mixed with orange-flavored Tang (200 μ l; Kraft Foods, Northfield, IL) or placebo (Tang only) daily via gavage for 7 days before tissue collection. Tang masks the taste of quercetin very well and contains vitamins (especially B₃ and C) that have been shown to increase the bioavailability of quercetin (personal communication, Quercegen Pharma). Mice were briefly anesthetized using isoflurane during the gavage procedure. The feeding regime was altered for the activity wheel experiment; quercetin (25 mg/kg) or placebo was administered in the form of a 0.25-g highly palatable bacon-flavored rodent food pellet (Bio Serv, Frenchtown, NJ) for a period of 7 days. This was done to prevent interruption of the 24-h running patterns; previous work in our laboratory has shown that the gavage procedure is too disruptive to the normal running behavior of mice. Animals typically (>90%) consume these pellets within 30 min with no differences between the placebo and quercetin pellet. This was confirmed in these animals during the acclimation period. Mice that did not ingest the food pellet within 30 min of dropping it in the cage were excluded (<5%).

mRNA expression. Soleus muscle and brain tissue ($n = 15$ /group) were homogenized under liquid nitrogen with a Polytron (PT3100; Kinematica, Lucerne, Switzerland), and total RNA was extracted using the guanidine thiocyanate method with TRIzol Reagent (Life Technologies, GIBCO-BRL, Carlsbad, CA). The extracted RNA (2.5 μ l of sample) was dissolved in diethylpyrocarbonate-treated water and quantified spectrophotometrically at 260-nm wavelength. Quality of RNA was determined using the 260:280 ratio; RNA with a ratio >1.6 was included in the analysis. RNA was reverse transcribed into cDNA in a 50- μ l reaction volume containing 19.25 μ l RNA (1.5 μ g) in RNase-free water, 5 μ l 10 $\times$ RT Buffer, 11 μ l 25 mM MgCl₂, 10 μ l deoxy-NTP mixture, 2.5 μ l random hexamers, 1 μ l RNase inhibitor, and 1.25 μ l multiscribe reverse transcriptase (50 U/ μ l). Reverse transcription was performed at 25°C for 10 min, 37°C for 60 min, and 95°C for 5 min, followed by quick chilling on ice. Samples were stored at -20°C until subsequent amplification.

Quantitative RT-PCR analysis was done as per the manufacturer's instructions (Applied Biosystems, Foster City, CA) using TaqMan Gene Expression Assays. DNA amplification was carried out in 12.5 μ l Taqman Universal PCR Master Mix (AmpliTaQ Gold DNA Polymerase, Passive Reference 1, buffer, dNTPs; AmpErase UNG), 1 μ l cDNA, 9 μ l RNase-free water, 1.25 μ l 18S primer (VIC) and 1.25 μ l primer (FAM) (for endogenous reference and target gene) in a final volume of 25 μ l/well. Samples were loaded in a MicroAmp 96-well reaction plate. Plates were run using an ABI Sequence Detection System. After 2 min at 50°C and 10 min at 95°C, plates were coamplified by 40 repeated cycles of which one cycle consisted of a 15-s denaturing step at 95°C and a 1-min annealing/extending step at 60°C. Data were analyzed by ABI software using the cycle threshold (C_T), which is the value calculated and based on the time (measured by PCR cycle number) at which the reporter fluorescent emission increases beyond a threshold level (based on the background fluores-

cence of the system), and it reflects the cycle number at which the cDNA amplification is first detected.

Quantification of mRNA expression of PGC-1 α and SIRT1 was calculated using the $\Delta\Delta C_T$ method. This method uses a single sample, the calibrator sample, for comparison of every unknown sample's gene expression. This method of analysis and quantification has been shown to give similar results as the standard curve method. Briefly, $\Delta C_T [C_T (\text{FAM}) - C_T (\text{VIC})]$ was calculated for each sample. The average ΔC_T for placebo-treated mice was used as the calibrator. $\Delta\Delta C_T [\Delta C_T (\text{calibrator}) - \Delta C_T (\text{sample})]$ was then calculated for each sample, and relative quantification was calculated as $2^{\Delta\Delta C_T}$. Initial exclusion criteria consisted of FAM C_T ≥ 40 and VIC C_T ≥ 23 (18, 19).

mtDNA content. DNA was isolated from muscle ($n = 15$ /group) using a QIAamp DNA mini kit (Qiagen, Valencia, CA). Briefly, the soleus muscle was lysed with proteinase K in the presence of a lysis buffer (Qiagen) at 56°C for 3 h. A second lysis (Qiagen) buffer was added to the sample followed by incubation for 10 min at 70°C. Ethanol was added to the sample before passing it through a spin column. The bound DNA was washed two times before being eluted using an elution buffer (10 mM Tris and 0.5 mM EDTA, pH 9.0) (Qiagen). DNA was isolated from brain ($n = 15$ /group) using DNAzol reagent (Life Technologies, GIBCO-BRL). Briefly, the brain tissue was homogenized with 1 ml of DNAzol under liquid nitrogen with a Polytron. Following a 10-min centrifugation, the supernatant was transferred to a new tube, and 500 μ l of ethanol were added. Following a brief incubation period (3–4 min), samples were centrifuged again, the supernatant was discarded, and the pellet was washed two times with 75% ethanol. The pellet was dried and resuspended in 400 μ l of 8 mM NaOH. Samples were incubated at 55°C for 10 min and centrifuged, and the supernatant was neutralized with HEPES buffer. DNA in brain and muscle was quantified spectrophotometrically at 260 nm wavelength. The DNA was subject to quantitative real-time PCR (100 ng/reaction) carried out with Universal PCR Master Mix (Applied Biosystems). Relative amounts of nuclear and mtDNA were determined by comparison of kinetics of amplification of β -actin [Endogenous Control (VIC)/MGB Probe, catalog no. 4352341E] (Applied Biosystems) and cytochrome *b* (forward: TATTCCTTCATGTCGGACGA; reverse: AAATGCTGTGGC-TATGACTG; probe: ACCTGAAACATTGGAGTACTTC-TACTG), respectively.

Cytochrome *c* concentration. Tissue (soleus muscle or brain) ($n = 8$ /group) was homogenized in PBS with 0.5% Triton X-100 using a Polytron, and protein was dissociated from the tissue using an ultrasonic cell disruptor (Fisher Scientific, Pittsburgh, PA). Sonication consisted of 10 s of cell disruption at setting number 10. Sonicated samples were diluted in PBS containing 0.5% Triton X. Following a 10-min incubation, the samples were centrifuged at 4°C and 14,000 revolutions/min for 10 min. Samples were analyzed for cytochrome *c* using an enzyme-linked immunosorbent assay (ELISA; R & D Systems, Minneapolis, MN). Briefly, standards or samples (diluted in calibrator diluent) were incubated for 2 h with an enzyme conjugate in a plate coated with an antibody specific for cytochrome *c*. Following the incubation period, the plate was washed, and substrate solution was added to the wells for a period of 30 min. The reaction was terminated using stop solution, and the plate was read on a microplate reader at 450 nm. Data were analyzed using a log regression equation. Total protein concentration was also determined using a BCA protein assay (Pierce, Rockford, IL). The concentration of cytochrome *c* was expressed as nanomoles per milligram total protein.

Treadmill performance (maximal endurance capacity). Endurance capacity was measured by treadmill running to fatigue following 7 days of either quercetin or placebo treatment ($n = 16$ /group). Mice were run on a motorized treadmill (2/lane) at a speed of 36 m/min and a grade of 8% until they reached fatigue. Fatigue was defined as the inability of the mouse to maintain the appropriate pace despite continuous hand prodding for 1 min, at which time the mouse was

removed from the treadmill and its run time recorded. Electric shock was never used in these experiments, since mice readily respond to a gentle tap of the tail or hindquarters encouraging them to maintain pace with the treadmill. Mice rarely required this type of continual prodding until they approached the point of fatigue.

Wheel-running performance (voluntary physical activity). Mice were used to assess wheel-running performance following 7 days of either quercetin or placebo treatment. Mice ($n = 18/\text{group}$) were acclimated to cages containing a running wheel (Mini-Mitter) that offered continuous access for 10 days before quercetin or placebo treatment. Typically, running behavior increases over the first 7 days after introducing mice to their novel wheel cages, after which it reaches a plateau and remains relatively constant. Baseline running activity was collected over 3 days (days 8–10) following acclimation. After baseline collection, mice were administered either quercetin or placebo treatment daily for seven consecutive days. Voluntary wheel-running activity was measured automatically during the treatment period as well as for 7 days following the treatment period (14 consecutive days) via computer using Vital View physiological and behavioral monitoring software (Mini-Mitter). However, only data collected during the “active” dark period (19:00–07:00 h) were analyzed. Earlier experiments in our laboratory have shown that voluntary wheel-running activity is minimal during the “inactive” light period (07:00–19:00 h) (~300 vs. 6,000 m during the active dark period from 19:00 to 07:00 h). Voluntary activity was quantitated for total distance (Distance), time on wheel (Time), and peak speed (P-Speed) as calculated using the following equations: Distance =

(no. of wheel rotations during a 2-min interval) $\times$ [circumference of the running wheel (0.7581 m)]; Time = [(no. of 2-min intervals where wheel rotations were >0) $\times$ 2]; P-Speed = (95th percentile of rotations during a given time interval) $\times$ [circumference of wheel (0.7581 m)/2] (21). Data are presented as the average of all 2-min interval collected over the 12-h active dark cycle and expressed as a percentage of baseline (average of data collected for 3 days before treatment) (6).

Statistical analysis. Results were analyzed using SigmaStat (version 3.1.1, SigmaStat; SPSS, Chicago, IL) with one-way ANOVA with Student-Newman-Keul’s post hoc comparisons. Sample size was determined based on previous experiments in our laboratory involving similar nutritional interventions. Statistical significance is shown as $P < 0.05$.

RESULTS

mRNA expression of PGC-1 α and SIRT1. Quercetin feeding for 7 days resulted in an increase in PGC-1 α (Fig. 1A) and SIRT1 mRNA (Fig. 1B) in slow-twitch muscle and in the brain ($P < 0.05$). We found approximately a 100% increase in PGC-1 α gene expression in the soleus muscle with both doses of quercetin and a 50 and 100% increase in the brain following 12.5 and 25 mg/kg quercetin, respectively (Fig. 1A). SIRT1 expression increased almost 200% in the muscle with both

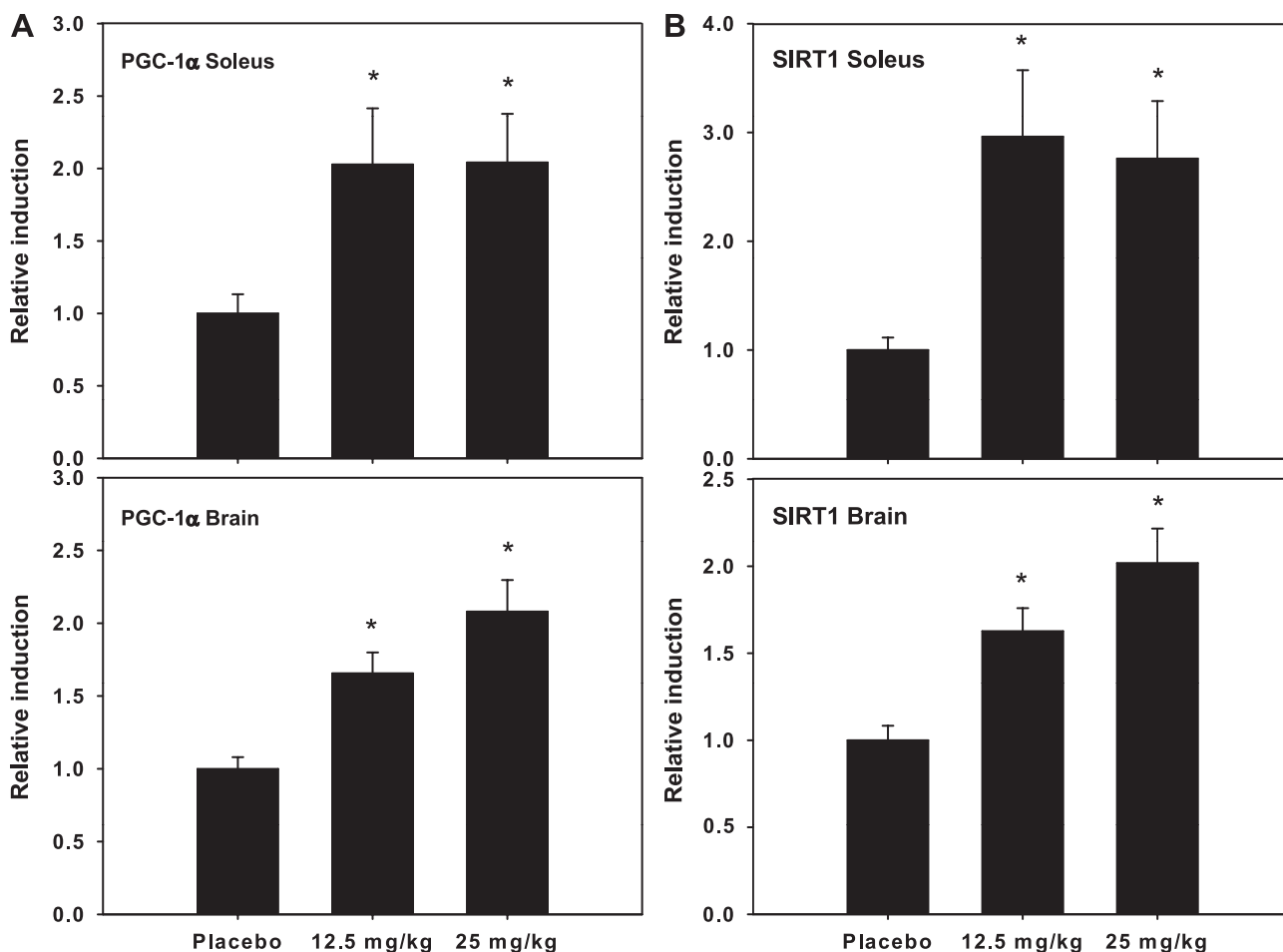


Fig. 1. Short-term feedings of the dietary flavonoid quercetin increase expression of genes associated with mitochondrial biogenesis. Peroxisome proliferator-activated receptor- γ coactivator (PGC-1 α) and sirtuin 1 (SIRT1) expression were measured in skeletal muscle and brain using RT-PCR following 7 days of quercetin feedings ($n = 15/\text{group}$). Values are means $\pm$ SE. *Significantly different from placebo ($P < 0.05$).

doses of quercetin and by 50 and 100% in the brain following 12.5 and 25 mg/kg quercetin, respectively (Fig. 1B).

mtDNA content. The relative amount of soleus muscle and brain mtDNA was determined using RT-PCR. Using cytochrome b as the target gene for mtDNA and β -actin as the internal control for nuclear DNA, mitochondrial biogenesis was determined by an increase in copy number of mtDNA per diploid nuclear genome. mtDNA was approximately doubled in the muscle and brain following 7 days of feedings with the 25 mg/kg dose of quercetin ($P < 0.05$), but there was no change with the 12.5 mg/kg dose (Fig. 2).

Cytochrome c concentration. For cytochrome c enzyme concentration, slow-twitch soleus muscle and brain were analyzed using ELISA techniques. These findings indicate that quercetin increases cytochrome c concentration in both muscle and brain following 7 days of feedings (Fig. 3) ($P < 0.05$). Cytochrome c concentration was increased in the muscle by 18 and 32% in the 12.5 and 25 mg/kg groups, respectively. Similarly, cytochrome c concentration was increased in the brain by 17% (12.5 mg/kg) and 21% (25 mg/kg).

Treadmill performance (maximal endurance capacity). To test the effects of quercetin on maximal running capacity, mice

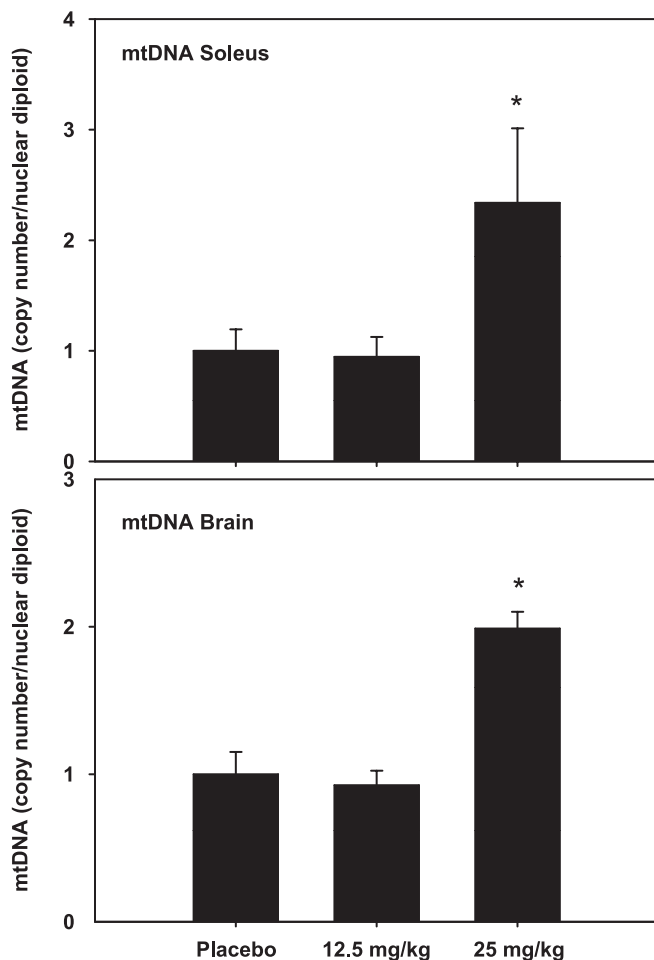


Fig. 2. Quercetin feedings increase mitochondrial DNA (mtDNA) content in muscle and brain. Quercetin was administered at 12.5 or 25 mg/kg for 7 consecutive days. The relative amount of soleus muscle and brain mtDNA was determined using RT-PCR ($n = 15/\text{group}$). Values are means $\pm$ SE. *Significantly different from placebo ($P < 0.05$).

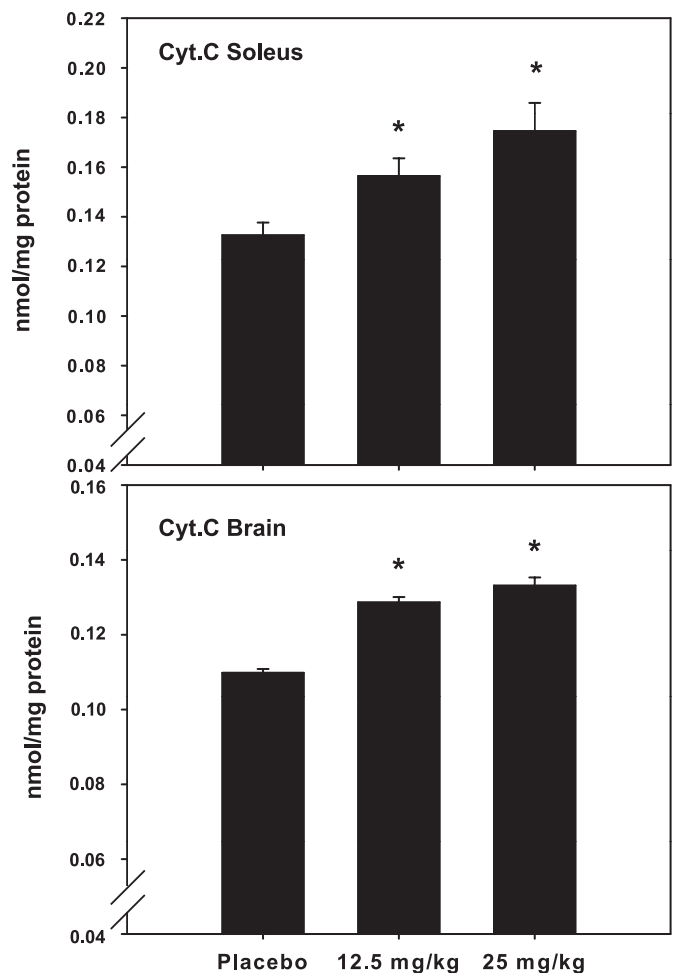


Fig. 3. Quercetin increases cytochrome c enzyme concentration in muscle and brain. Quercetin was administered at 12.5 or 25 mg/kg for 7 consecutive days, and cytochrome c enzyme concentration was determined using enzyme-linked immunosorbent assay ($n = 8/\text{group}$). Values are means $\pm$ SE. *Significantly different from placebo ($P < 0.05$).

were fed quercetin (12.5 or 25 mg/kg) or placebo for 7 days before the run to fatigue; for these experiments, the treadmill was operated at a fixed speed and grade (36 m/min; 8% grade). Short-term feedings of both doses of quercetin were associated with increased exercise capacity (Fig. 4) ($P < 0.05$). The 12.5 mg/kg dose increased run time by 36% and the 25 mg/kg dose by 37%.

Wheel-running performance (voluntary physical activity). In the voluntary wheel-running test, mice were fed placebo or quercetin at 25 mg/kg for 7 days in the form of a palatable rodent food pellet (bacon flavor, 250 mg). This was done because the gavage procedure was determined to be too disruptive to the normal activity or running behavior of mice. Voluntary physical activity (moment-to-moment activity including distance run, time on the wheel, and peak speed) was measured 24 h/day throughout the 7-day treatment period and for 7 days following the treatment (days 8–14). Distance run, time on the wheel, and peak speed were analyzed. Quercetin increased distance on days 6–14 ($P < 0.05$), time on the wheel on days 7 and 14 ($P < 0.05$), and peak speed on days 2 and 3 ($P < 0.05$) (Fig. 5).

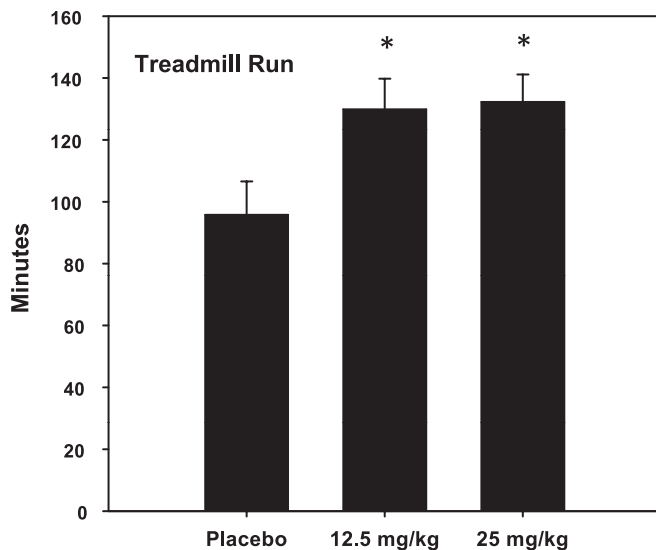


Fig. 4. Short-term quercetin feeding increases maximal endurance capacity. Mice were fed quercetin (12.5 or 25 mg/kg) or placebo via gavage for a period of 7 days after which mice were run to fatigue on a treadmill, and run time was recorded ($n = 16/\text{group}$). Both doses of quercetin increased run time to fatigue. Values are means $\pm$ SE. *Significantly different from placebo ($P < 0.05$).

DISCUSSION

Quercetin is one of a broad group of natural polyphenolic flavonoid substances that are being investigated for their widespread health benefits. These benefits have generally been ascribed to its combination of antioxidant and anti-inflammatory activity, but recent *in vitro* evidence suggests that increased mitochondrial biogenesis could play an important role. However, the effects of quercetin on mitochondrial biogenesis and exercise tolerance are unknown. This study examined the effects of short-term quercetin feedings on markers of mitochondrial biogenesis, including expression of PGC-1 α and SIRT1, mtDNA and cytochrome *c* concentration in both skeletal muscle and brain. The data indicate that short-term feedings of the dietary flavonoid quercetin can increase mRNA expression of PGC-1 α and SIRT1, and mtDNA and cytochrome *c* in both skeletal muscle and brain. Furthermore, we determined if these changes in mitochondrial biogenesis were associated with an increase in maximal endurance capacity and voluntary wheel-running activity; both were increased following 7 days of quercetin feedings.

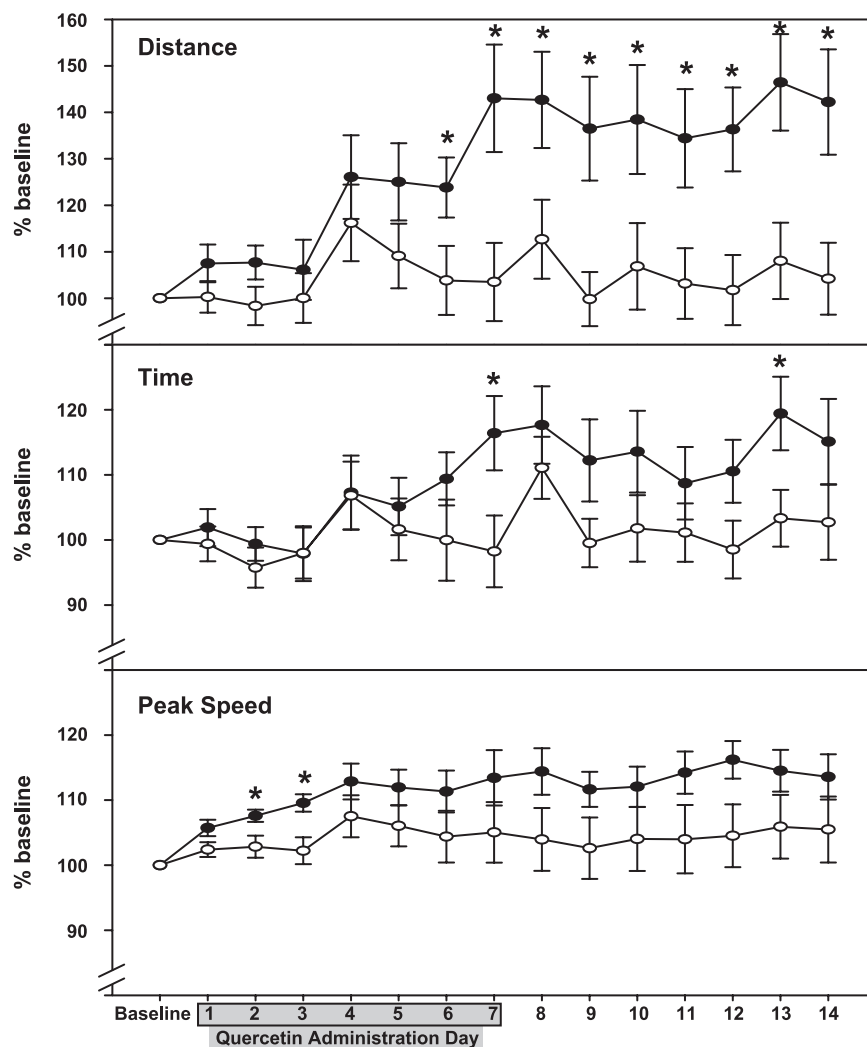
PGC-1 α has been reported to play an important role in stimulating mitochondrial biogenesis following physiological demands and nutritional inputs, such as exercise or the dietary flavonoid resveratrol (4, 13, 33). Unlike most known transcriptional coactivators, PGC-1 α expression is enriched in tissues with high-capacity mitochondrial systems, drives the formation of slow-twitch muscle fibers, and is a critical regulator of skeletal muscle fuel stores, all of which are essential to endurance exercise capacity (4, 12, 22, 33). However, although often ignored, the brain also plays a primary role in exercise tolerance. Cerebral metabolism has important consequences on motivation, mood (e.g., vigor, fatigue, anxiety, depression), and central motor drive from the cortex (20, 27, 31), and increased brain mitochondrial activity could certainly enhance cerebral metabolism. PGC-1 α expression is linked to the demand for mitochondrial ATP production and intracellular cal-

cium levels (12, 25), both of which are known to increase under physiologically demanding conditions such as exercise and energy deprivation (12). PGC-1 α activates mitochondrial biogenesis and increases oxidative phosphorylation by facilitating transcription, translation, and replication (22). As a result, peak oxygen uptake increases and fatigue is delayed during prolonged exercise (4). SIRT1 functions together with PGC-1 α to promote mitochondrial biogenesis (24); SIRT1 interacts with and deacetylates PGC-1 α at multiple lysine sites, increasing PGC-1 α activity. This information has led to great interest in developing drugs to target the SIRT1-PGC-1 α coactivator complex or related signaling pathways in the muscle that would mimic or potentiate the effects of exercise to treat metabolic diseases (17, 24). However, there have been no studies that focus on this effect in the brain. Our data indicate that PGC-1 α and SIRT1 expression are increased significantly in both skeletal muscle and brain following just 7 days of quercetin feedings. We did not measure protein concentration of PGC-1 α or SIRT1; however, changes in mRNA of these factors generally reflect changes in protein and activity (13).

This increase in SIRT1 and PGC-1 α gene expression is generally associated with an increase in mitochondrial biogenesis, but the specific effect of quercetin on mitochondrial biogenesis has not been determined. Here we show the effects of quercetin feedings on markers of mitochondrial biogenesis. An increase in mtDNA is determined by an increase in relative copy number of mtDNA per diploid nuclear genome. Cytochrome *c* is a component of the electron transport chain, encoded by nuclear genes. Increases in cytochrome *c* concentration typically occur in conjunction with similar increases in other mitochondrial enzymes of the electron transport chain, and enzymes in the tricarboxylic acid cycle and the β -oxidation pathway that lead to an overall increase in mitochondrial capacity. Although increases in muscle mtDNA and mitochondrial enzymes have well-established benefits on exercise tolerance (4, 12), much less is known about the impact of these changes in the brain (3, 20, 27, 31). The absence of an increase in mtDNA content with the 12.5 mg/kg dose of quercetin may be explained by the short feeding duration. Indeed, increases in mitochondrial enzymes have been shown to occur more quickly and with less stimuli than increases in mitochondrial replication (16). This rapid induction of cytochrome *c* is consistent with other reports of exercise-induced increases in mitochondrial enzymes within 2–7 days in rats and humans, and other flavonoid derivatives *in vitro* (23, 26).

An increase in muscle mitochondrial biogenesis is perhaps the most important factor responsible for increased endurance exercise tolerance in response to exercise training. The typical doubling of muscle mitochondria that occurs with exercise training is largely responsible for increased oxygen utilization, shifts in substrate utilization toward increased oxidation of fat relative to carbohydrate, and increased lactate threshold, which are primary limiting factors to endurance performance (4, 11). $\dot{V}O_{2\text{max}}$ is also influenced by muscle mitochondrial oxidative capacity, but, relative to endurance capacity, it is limited to a greater extent by oxygen delivery by the cardiovascular system (2). Therefore, we determined whether the quercetin-induced increases in mitochondrial biogenesis were associated with an increase in endurance exercise tolerance. We employed two different paradigms of exercise (treadmill running and voluntary wheel running). Although both are commonly used exer-

Fig. 5. Quercetin feedings increase voluntary wheel-running activity in mice. Mice were fed quercetin (25 mg/kg) or placebo in the form of a palatable rodent pellet daily for 7 days ($n = 18/\text{group}$). Voluntary activity was monitored throughout the 7 days of quercetin feedings and for 7 days postquercetin feedings. Distance run, time on the wheel, and peak speed were analyzed. Quercetin increased distance on days 6–14 ($P < 0.05$), time on the wheel on days 7 and 14 ($P < 0.05$), and peak speed on days 2 and 3 ($P < 0.05$). Values are means $\pm$ SE. *Significantly different from placebo ($P < 0.05$).



cise models, the stimuli are very different. During treadmill running, mice run at a given intensity until they can no longer maintain the pace necessary to keep up with the moving belt even in the presence of gentle hand prodding or electrical shock. This fatigue is thought to arise primarily from limitations in the periphery (e.g., cardiovascular system and muscle). Voluntary wheel-running behavior, almost by definition, is more centrally influenced. With wheel running, mice run for frequent short periods of varying distances at different intensities based on their own volition, a situation more similar to that experienced in an unstructured, free-living environment. Treadmill running is a better indicator of a mouse's maximal running capacity as opposed to wheel running, which is heavily influenced by behavioral factors. Both behaviors are clearly influenced by an increase in both muscle and brain mitochondria, although the brain is seldom mentioned in this context (5). Quercetin feedings increased voluntary activity during the feeding period as well as during the 7-day period afterward. This prolonged response is probably due to the combined effects of quercetin and exercise itself on mitochondrial biogenesis, since the quercetin group was running significantly more than the placebo group over that period of time. The plasma half-life of quercetin is 6–12 h, which argues against a

slow washout of quercetin (10). Distance run on the wheel was increased by ~35% by day 6 in the quercetin group over the placebo group, which was due in part to increases in both time on the wheel and increased peak speed. We interpret this to be at least partially the result of an increase in mitochondrial biogenesis in both muscle and brain. The motivation/willingness to engage in physical activity is driven more by CNS factors, although muscle-specific increases in oxidative metabolism would also contribute by reducing muscle fatigue. Alternatively, the quercetin-induced increase in maximum speed that was found on days 2–3 is not likely explained by any significant change in mitochondrial capacity in this short time frame. Quercetin, like caffeine, has been shown to be an adenosine A_1 receptor antagonist in vitro (1), which is at least partially responsible for the psychostimulant and ergogenic effects of caffeine (1, 5, 7, 9). Therefore, in addition to its effects on mitochondrial biogenesis, quercetin may enhance exercise tolerance through its activity as an adenosine A_1 receptor antagonist in the brain.

In summary, short-term feedings of relatively low doses of the naturally occurring dietary flavonoid quercetin can enhance mitochondrial biogenesis in muscle and brain that was associated with an increase in both maximal endurance running

capacity and active involvement in prolonged exercise activity. Of particular importance is the effect in the brain that is often ignored in nutrition studies and those involving exercise tolerance. We believe this increase in exercise tolerance to be at least partly due to the result of an increased oxidative metabolism in both muscle and brain, but there may also be an added benefit of quercetin as an adenosine A₁ receptor antagonist in the brain. This is evidenced by the increase in running performance in two very different models of exercise that are influenced disproportionately by muscle and brain factors. Maximal exercise capacity was improved in an environment where mice were "forced" to run at a constant rate until exhaustion. Alternatively, the increase in voluntary running behavior generally reflects increased willingness/motivation to be active. The practical importance of this discovery lies in the fact that, unlike other flavonoids, like resveratrol, being studied for their benefits to health and performance, the plant source of quercetin is relatively inexpensive to grow and harvest, and the purification of quercetin is straightforward. It has also been shown to be safe and effective at relatively low dosages (e.g., 500–1000 mg/day) (10, 30). If these results translate clinically, these benefits of quercetin may have important implications for enhancement of athletic and military performance. It is also intriguing to consider the possible relevance of these benefits of quercetin on various chronic diseases like cardiovascular, metabolic (e.g., type 2 diabetes), and neurodegenerative diseases in which physical inactivity and mitochondrial dysfunction are hallmarks.

GRANTS

This work was supported by grants from the Department of Defense Combat Feeding Program and the Defense Advanced Research Projects Agency (DAAD16-02-C-0015).

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