

**Quercetin can increase mRNA expression of PGC-1 $\alpha$  and SIRT1, and mtDNA and cytochrome *c* in both skeletal muscle and brain.**

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- $\gamma$  coactivator (PGC-1 $\alpha$ )** (13, 14, 17, 23). PGC-1 $\alpha$  is considered the “master regulator” of mitochondrial biogenesis; SIRT1 physically interacts with and deacetylates PGC-1 $\alpha$  and consequently increases PGC-1 $\alpha$  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 $\alpha$  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.

## Discussão

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 $\alpha$  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 $\alpha$  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 $\alpha$  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 $\alpha$  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 $\alpha$  expression is linked to the demand for mitochondrial ATP production and intracellular calcium levels (12, 25), both of which are known to increase under physiologically demanding conditions such as exercise and energy deprivation (12). PGC-1 $\alpha$  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 $\alpha$  to promote mitochondrial biogenesis (24); SIRT1 interacts with and deacetylates PGC-1 $\alpha$  at multiple lysine sites, increasing PGC-1 $\alpha$  activity. This information has led to great interest in developing drugs to target the SIRT1-PGC-1 $\alpha$  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 $\alpha$  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 $\alpha$  or SIRT1; however, changes in mRNA of these factors generally reflect changes in protein and activity (13).

This increase in SIRT1 and PGC-1 $\alpha$  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  $\beta$ -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).  $\text{VO}_{2\text{max}}$  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 exercise 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<sub>1</sub> 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<sub>1</sub> 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 A<sub>1</sub> 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.