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THE EFFECTS OF QUERCETIN ON CYCLING TIME TRIAL PERFORMANCE

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THE EFFECTS OF QUERCETIN ON CYCLING TIME TRIAL PERFORMANCE

by

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ABSTRACT

THE EFFECTS OF QUERCETIN ON CYCLING TIME TRIAL PERFORMANCE

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Quercetin is a flavonoid found in commonly consumed fruits and vegetables that has exhibited powerful antioxidant and anti-inflammatory properties in rodents and *in vitro*. In humans, the ergogenic effects of antioxidant supplementation on exercise performance and adaptations are still equivocal and need to be further investigated. A powerful antioxidant such as quercetin may inhibit the high levels of oxidative stress associated with the high volume and intensity of exercise training seen with trained individuals.

PURPOSE: To determine the effect of 28 days of daily quercetin supplementation on cycling time trial performance and the associated exercise performance variables.

METHODS: Thirteen trained cyclists (VO_{2peak} 58.8 ± 3.9 ml/kg/min) were recruited for this study from the University of Texas at Austin and the local Austin, Texas community and participated in this placebo controlled, randomized, crossover designed study. After initial

assessment of baseline data (VO_{2peak}, lactate threshold, and two familiarization time trials), participants began daily supplementation of either an antioxidant supplement containing vitamins and quercetin (Q-VIT: 1000mg quercetin, 820mg Vitamin C, 40mg Vitamin B3) or the same vitamin supplement without quercetin (VIT: 820mg Vitamin C, 40mg Vitamin B3). A simulated time trial using an electromagnetically braked cycle ergometer in which subjects had to complete a set amount of work (kJ) as fast as possible was performed on the last day of supplementation. Measured performance variables included: time to completion, average power output, average oxygen consumption (VO_2), Respiratory Exchange Ratio (RER), gross mechanical efficiency (GE), heart rate (HR), and rating of perceived exertion (RPE).

RESULTS: Quercetin had no effect on HR, RER, power output, or RPE. There was also no difference in time to complete the time trial between treatments. However, an approximately ~2% higher, but not significantly different, VO_2 during Q-VIT supplementation significantly lowered the GE compared to VIT (Q-VIT: 20.49 ± 0.26 % and 19.94 ± 0.33 %; VIT: 20.9 ± 0.24 % and 20.37 ± 0.33 %; $p < .01$) at 15 and 30 min respectively.

CONCLUSION: Chronic supplementation for 28 days with a quercetin based antioxidant supplement lowered cycling gross efficiency in well trained cyclists, but it did not affect performance time. The results of the current study suggest that chronic supplementation with quercetin does not influence aerobic exercise performance in well trained athletes.

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INTRODUCTION

In an attempt to improve human performance, exercise physiologists and athletes have experimented with a plethora of ergogenic aids. Recently, there has been surge of research into naturally occurring substances that may not only provide clinical health benefits, but also induce positive changes in exercise performance and training adaptations. Common plant-derived substances provide a safe, easily accessible, and less expensive alternative to preventing disease, maintaining health, and possibly increasing mental and physical performance. Epidemiological data suggests evidence that diets rich in fruits and vegetables can lower the incidence of some Cardiovascular Disease risk factors due to the high concentration of polyphenolic compounds in the diet[1]. Flavonoids are a well-defined group of polyphenolic compounds that show potential for use in the treatment, prevention, and attenuation of disease through multiple actions, including possible anti-oxidant and anti-inflammatory activities, and have been the recent focus of clinical and performance investigations. Specifically, there has been a large interest in the potential health and exercise performance benefits of quercetin, a common flavonoid found in grapes, apples, cherries, onions, citrus fruits, broccoli, and tea [2]. The flavonoid quercetin has been associated with numerous potential health benefits in rodents and *in vitro* including anti-viral[3], hepatoprotective, antifibrogenic[4], antiatherogenic[5], anti-inflammatory[6], anti-apoptotic, and potent antioxidant capabilities[7]. Furthermore, quercetin feeding in mice has indicated that quercetin may induce increased aerobic endurance capacity and wheel running activity through proliferation of mitochondrial mRNA, proliferator-activated receptor-ϒ coactivator (PGC-1α), and sirtuin 1 (SIRT 1)[8]. In vitro observations have also led researchers to believe that there may be stimulatory effects of quercetin on the central nervous system similar to caffeine, that may have the potential to improve human exercise performance [9].

Recent research has focused on the seemingly more promising potential quercetin may have in reducing the oxidative stress induced by high intensity exercise.

The combination of potent antioxidant and anti-inflammatory mechanisms have not only created interest into the potential health benefits of flavonoids in prevention and treatment of disease, but have also initiated investigations regarding Quercetin's potential as an ergogenic aid to improve athletic performance in humans. Current research is difficult to interpret in relation to human exercise performance as there have been a variety of results and discussions presented thus far [10, 11]. However, current data suggests that quercetin is a modest ergogenic aid in untrained [12, 13] and possibly trained individuals [14]. We hypothesize that the powerful antioxidant properties of quercetin will improve exercise performance in trained cyclists during a time trial after daily supplementation for 28 days.

REVIEW OF LITERATURE

The following literature review will discuss the flavonoid quercetin and its associated properties in cell culture, animal, and human experimental trials. Specifically, the review will focus on the effects of exercise on oxidative stress and the proposed mechanism by which quercetin can improve the physiological response to this stress; therefore, potentially improving exercise performance. Studies that have evaluated the effect of quercetin based supplements on exercise performance will be analyzed.

Exercise and Oxidative Stress

Exercise and the inherent associated muscular contractions induces oxidative stress through an increase in the production of free radicals [15, 16]. Muscle tissue, xanthine oxidase, NAD(P)H oxidase, oxygen within the mitochondria, the release of radicals by macrophages and/or neutrophils recruited to repair damaged tissue, contribute to the generation of free radicals [17, 18]. The term free radical refers to reactive oxygen species (ROS) and reactive nitrogen species (RNS) that include oxygen or nitrogen as the reactive center. Known ROS and RNS include, but are not limited to, superoxide, hydrogen peroxide, hydroxyl radicals, singlet oxygen, nitric oxide, peroxynitrite, and hyperchlorite [19]. These molecules have an unpaired valence electron that makes them highly reactive and volatile to surrounding cells and tissues. The subsequent increase in free radicals above the resting levels created as a by-product of cellular respiration leads to a disruption of redox signaling and molecular damage [20, 21]. This disruption leads to a series of disturbances that increase muscular fatigue, muscular damage,

DNA damage, and peroxidation of lipid membranes, thus effecting the functioning and recovery of damaged tissues and cells [18, 22-24].

During exercise, especially high intensity aerobic exercise, there are a large amount of free radicals produced by the process of aerobic respiration within the mitochondria. The oxidative stress from these free radicals reduces the efficiency of the electron transport chain (ETC) and therefore reduce the efficiency of NADH:NAD^+ cycling [25, 26]. In turn, the efficiency of ATP production is reduced. The free radicals also have detrimental effects on the tricarboxylic acid (TCA) cycle by inhibiting the actions of enzymes such as α-ketoglutarate dehydrogenase (KGDH) and succinate dehydrogenase (SDH) [27]. Supplementation with an antioxidant such as quercetin may have the potential to mitigate the disruption of ATP production and assist in the maintenance of energy efficiency during exercise.

However, there are investigations and discussions contrasting the evidence that the exercise induced increases in oxidative stress are completely detrimental. There appears to be an optimal level of oxidative stress necessary for some cellular and bodily functions to function appropriately. Reid et al. [23] has shown in vitro that muscular force and fatigability were improved with the appropriate levels of reactive oxygen species (ROS). However, during an intense exercise bout, the large increase in oxidative stress may then become detrimental to the contractility of the muscle fibers.

Furthermore, an increase in oxidative stress seems necessary to induce favorable adaptations of the body's endogenous antioxidant defense system. Ji et al.[28] examined the effects of ROS on pathways involved in the activation of nuclear factor kappa-B (NF-KB) and MAP kinases within the muscle cell and suggested that the pathways initiated by oxidative stress

are essential for the body to produce advantageous adaptations pertaining to endogenous antioxidants and cytoprotective proteins. There are beneficial endogenous adaptations regarding the protection of cellular redox homeostasis that occur following an acute bout of exercise, especially through the activation of NF-KB [29, 30]. Blockage of RNS production attenuates up-regulation of superoxide dismutase, an important endogenous antioxidant, along with eNOS and iNOS [31]. Preventing oxidative stress during an exercise bout prevents not only the production of an improved endogenous protection of oxidative stress but can limit acute exercise adaptations. Regular exercise training produces advantageous adaptations essential for improved oxidative stress protection [32, 33]. The accumulation of the acute exercise response appears to induce favorable protective, chronic improvements in protection against high levels of exercise induced oxidative stress. Most evidence suggests that there needs to be the appropriate balance between antioxidant capacity and oxidative stress. The question remains whether chronic or acute supplementation of exogenous antioxidants is detrimental or beneficial to human exercise performance and human exercise adaptations.

Exercise and Inflammation

The immune system responds to strenuous exercise and instigates the release of pro- and anti-inflammatory cytokines following an exercise bout [34]. Plasma cytokines shown to increase during or after an exercise bout include IL-6, IL-10, IL-8, IL-1ra, granulocyte colony stimulating factor (G-CSF), C Reactive Protein (CRP), monocyte chemoatractic protein (MCP-1), and tumor necrosis factor alpha (TNF-α) [35]. Elevations in these cytokines after exercise are considered normal; however chronic elevations lead to detrimental health outcomes and increased sickness, especially with pro-inflammatory cytokines such as IL-6 and CRP.

A relationship exists between oxidative stress and the inflammatory process. There are intricate redundant cellular pathways mediated by the increase in oxidative stress seen with exercise and the production of oxidative stress from macrophages and neutrophils [17]. After an intense exercise bout, a large increase in oxidative stress may increase cellular damage, thereby increasing inflammation at the site of injury. The resulting macrophage and neutrophil invasion can then increase oxidative stress and cytokine signaling in a cycle of response to the exercise stress. Supplementation with an antioxidant may prevent the overabundance of oxidative stress; therefore, systemic inflammation may be lower after the intense exercise bout.

Quercetin and an Antioxidant and Anti-inflammatory Agent

As mentioned, quercetin has potent antioxidant and anti-inflammatory effects *in vitro* due to its chemical structure[2, 36, 37]. Multiple hydroxyl groups on the molecule allow for it to be a potent electron acceptor. The hypothesis of many physiologists is that the antioxidant and anti-inflammatory capabilities of quercetin may provide a buffer against excess oxidative stress and immune response to acute bouts of exercise or high volumes of strenuous exercise training. By attenuating the stress response, a faster recovery of performance may be incurred and allow for greater training adaptations or acute exercise performance. The efficiency of ATP production may also be augmented by the prevention of excess oxidative stress and allow for an improved exercise performance during the exercise bout.

Bioavailability of Quercetin

Typical intake of flavonoids in the human diet can vary greatly depending on the location, demographics, supplementation, and activity level of the population studied: The range of typical daily intake can vary from an estimated 5 – 230mg/day [38, 39]. Large scale

epidemiological studies support the availability of quercetin in human plasma and serum when volunteers were supplemented with doses ranging from 50-1000mg/day. Quercetin serum and plasma levels rose in a direct dose dependent manner [40, 41]. Jin et al. [41] reported that the net increase in blood was 332 and 516 micrograms/L, respectively, for the 500 and 1,000 mg/d dose. However, the levels of quercetin within the plasma varied greatly among the population with no relationship to demographic or lifestyle factors [41].

The chemical transformation and alterations of quercetin that occur in the intestinal mucosa of humans may contribute greatly to its absorption into human plasma. Absorption seems to be greatly facilitated in combination with its conjugation with a sugar group [42]. The glucosidated form has a greater capacity for absorption then the commonly found aglycone form found in fruits and vegetables [43]. Strong arguments have also been made for an increased bioavailability of quercetin through vitamin "cocktails": a mix of vitamins and minerals that may be beneficial in the absorption of quercetin as the bioavailibilty of quercetin aglycone alone may not exhibit the same effects in vivo due to changes in absorption and chemical structure [37]. Without appropriate sugars, vitamins, or fatty acids, quercetin may undergo conjugation in the intestinal mucosa and liver that may weaken its anti-oxidant and anti-inflammatory effects [37, 44, 45]. There is still more work needed to investigate whether there can be direct associations made between the *in vitro* studies that greatly support a multitude of actions of quercetin and the associated changes that may occur on the physical and chemical structure of quercetin upon absorption in the intestinal mucosa. It may need to be administered with an appropriate mixture of ingredients to be optimally absorbed and metabolized in humans.

Quercetin and Exercise Performance in Rodents

A large interest in the potential effects of quercetin on human exercise performance has arisen from not only the in vitro studies that have shown numerous potential health benefits, but also the potent effects seen in mice. Davis et al. [8] examined the effects of 7 days of quercetin feedings on markers of mitochondrial biogenesis in skeletal muscle and brain and the associated effects on exercise endurance performance. No exercise training programs were implemented on the mice so that the potential benefits of only quercetin ingestion on fitness and endurance capacity of the mice were examined. After 7 days of the quercetin treatment, the mice on quercetin treatment exhibited increased mitochondrial DNA, PGC-1α mRNA, expression of PGC-1α, SIRT 1, and cytochrome c concentration within the brain and skeletal muscle. These biochemical markers are well established adaptations exemplifying an increase in the metabolic pathways responsible for the creation of new mitochondria; one of the main adaptations that occur in skeletal muscle in response to exercise training to increase exercise capacity [46, 47]. The changes in the markers of mitochondrial biogenesis were associated with an increase in endurance performance and increased voluntary wheel running activity versus the placebo controlled group mice [8]. The benefit that quercetin elicited suggest that the in vitro benefits may transfer into a biological system and provide substantial improvements in endurance exercise performance independent of exercise training.

Quercetin and Exercise Performance in Humans

Chronic Supplementation Improving Performance

The aforementioned evidence from Davis et al. [8] and a host of in vitro studies suggest that quercetin might have the potential to be a substantial ergogenic aid for human exercise

performance; specifically, it may have protective effects against the high levels of oxidative stress experienced during strenuous aerobic exercise: There may even be potential for positive changes in exercise capacity and performance independent of exercise training. However, human experiments utilizing quercetin in exercise performance trials have only raised more questions rather than elucidate its effectiveness in exercise performance. The evidence that is presented from in vitro studies and rodents does not translate into definitive improvements in exercise capacity in humans. Current evidence on quercetin's influence on human exercise is inconclusive thus far.

A study in humans by MacRae et al.[14] added quercetin to an antioxidant cocktail and cyclists ingested 600mg/day for 6 weeks and compared their performance to a placebo supplementation. They found that although performance was improved compared to a baseline practice trial, the addition of quercetin, did not improve performance compared to the antioxidant without quercetin.

More recently, Davis et al. [12] performed a placebo controlled, double blind, crossover study and had twelve untrained subjects ingest 500mg/day for 7 days in vitamin cocktail and compared changes in VO2max and endurance capacity. The untrained subjects showed significant improvements in VO2max and time to fatigue while supplementing with quercetin versus placebo[12]. However, there were no analyses of plasma or muscle tissue to assess possible mechanisms behind these changes or those seen in the trained cyclists from MacRae et al. [14].

A more comprehensive cross-sectional design by Nieman et al. examined changes in human exercise performance with quercetin supplementation in untrained subjects [13]. Twenty

six untrained individuals ingested 1000mg/day Quercetin for 2 weeks and placebo for 2 weeks. At the end of each supplementation period the subjects performed a time trial in which they attempted to run the farthest distance possible on a treadmill. Subjects showed a small, but significant increase in the amount of distance covered on the treadmill during the quercetin trial. Muscle tissue analysis of markers of improved aerobic capacity showed small non-significant elevations in mRNA for PGC-1α, SIRT1, cytochrome c, and citrate synthase [13]. Although the findings were small and the mRNA data were insignificant, it leads to possible questions as to whether a longer supplementation period may have produced significant data. As discussed earlier, MacRae et al.[14] reported improved performance in trained cyclists utilizing a 6 week supplementation period, yet, only one week of supplementation was enough to elicit significant changes in VO2max in untrained subjects in the crossover design by Davis et al [12].

The improvements in performance in both untrained and trained individuals with a variety of supplementation periods gives some evidence, although unclear, that quercetin may be an effective ergogenic aid for increasing human aerobic exercise performance.

Chronic Supplementation Without Improvement in Performance

In contrast to Davis et al.[12], Ganio et al. [48] reported that 5 days of quercetin supplementation did not raise VO_2max in 11 untrained men and women. Although the supplementation period was 2 days less then Davis et al, a very similar design resulted in no effects on VO_2max. Of interest is the method in which the quercetin was orally delivered. Davis et al. [12] mixed quercetin in a vitamin enriched Tang solution while Ganio et al. [48] used food bars that contained quercetin. Perhaps the bioavailability of the flavonoid differed among the

studied groups caused by differences in metabolic transformation of the quercetin molecule [37, 45].

Another investigation of short term feeding of quercetin by Cureton et al. [49] showed no effects of the supplementation on performance in 30 untrained but recreationally active participants. Time of supplementation varied from 9-16 days with 1000mg/day of quercetin mixed in a sports hydration beverage. Subjects cycled for 1 hour at 50% VO_2 max, then performed a 10 minute cycling bout in which as much work was performed as possible. This design is similar to the type of protocol utilized by Nieman et al [13] by assessing the maximal amount of work that can be completed after a submaximal exercise session. Opposite of Nieman et al [13], Cureton et al. [49] was unable to find a difference in the work performed between the quercetin and placebo trials. After cycling testing, subjects performed isometric strength tests of the knee extensors and revealed no differences when comparing the quercetin supplementation to the placebo [49].

Additional negative findings regarding the flavonoid's effects on human exercise performance is a study that used 40 trained cyclists in a double-blind, cross sectional, placebo controlled investigation that attempted to elucidate quercetin's effects on exercise performance. In 2 of the 3 primary evaluations, the primary outcome was not exercise performance, per se, but measurements were taken to investigate the effect of 3 weeks of quercetin on 3 days of cycling at 57% of VO_2max for 3 hours each day [50, 51]. Quercetin exhibited no influence on exercise-induced changes in plasma cytokines and muscle and leukocyte cytokine mRNA after 3 weeks of supplementation [50]. After 6 weeks of supplementation, there were no differences in any markers of oxidative stress or total antioxidant status before, during, and after the exercise bout [51]. The study administered quercetin in doses of 1000mg/day in a vitamin enriched Tang

mixture. Dumke et al. [52] examined performance parameters using the same study design by analyzing heart rate(HR), respiratory exchange ratio, oxygen consumption (VO2), cadence, power output, substrate utilization, and cycling gross efficiency. No differences were observed between quercetin and placebo trials regarding the performance data [52]. Although these results may provide insight into quercetin's lack of efficacy on exercise induced inflammatory and oxidative response, the exercise bout employed may also not be an adequate or appropriate for making claims on the possible effects the flavonoid may have on exercise endurance performance. Other performance tests such as time trials may be a more appropriate method for assessing quercetin's performance effects on trained cyclists [53].

Nieman et al [44] employed the same exercise protocol as previously described [50] to investigate quercetin's influence on exercise induced acute inflammatory response and markers of mitochondrial biogenesis. Thirty-nine trained cyclists supplemented for 2 weeks on placebo, quercetin, or quercetin with epigallocatechin 3-gallate (EGCG). No markers of mitochondrial biogenesis were elevated, however, there were significantly lower levels of CRP, IL-6, and IL-10 after exercise in the quercetin with EGCG group immediately following exercise [44]. The combination of the quercetin with EGCG may have improved the absorption of quercetin and worked in a synergistic manner to improve the anti-inflammatory effects. No effects on the markers of mitochondrial proliferation may be due to the use of trained individuals that possibly are closer to their highest potential of mitochondria; untrained individuals may be more sensitive to the actions of quercetin on SIRT1 and PGC-1α.

The previously described studies have mostly had a primary focus on endurance exercise performance. Abbey et al [54] considered the effect quercetin may have on the maintenance of repeated sprint performance; an important factor in high caliber performance during many sports

games such as soccer. Fifteen recreationally active young adult men participated in the crossover, placebo controlled experiment and supplemented with 1000mg/day of quercetin for one week. No differences were seen in the maintenance of sprint performance or levels of xanthine oxidase and IL-6 in the quercetin group compared to placebo [54]. Abbey et al had hypothesized that the possible actions on the adenosine receptors may play a role in maintaining sprinting performance. Bigelman et al. [55] had also examined the effects of 6 weeks of quercetin supplementation on various modalities of exercise performance in ROTC cadets progressing through the rigors of military training and observed no differences in exercise performance or improvement when compared to the placebo.

Acute Supplementation

Although the primary focus of most exercise experiments in human models have been related to chronic supplementation of quercetin, there have been some investigations into the effect of acute supplementation on exercise. Cheuvront et al [56] examined a large dose of 2000mg quercetin on cycling performance during heat stress compared to caffeine and placebo. There was no effect of quercetin on cycling performance. However, there was no effect of the caffeine on performance despite a large amount of literature supporting the helpful ergogenic effect of caffeine on endurance performance [57, 58]. The dosage of caffeine administered (9 mg/kg) has been proven to have positive ergogenic effects on exercise performance [58]. Nonetheless, heat stress has also been shown to nullify the potential benefits of caffeine on endurance performance [59]. It is difficult to interpret if the quercetin had no stimulatory effect or if the heat stress masked the potential benefit that quercetin may have had on exercise performance.

The potential antioxidant, anti-inflammatory, and immune-modulating influence of a single dose of a quercetin based supplement on an acute bout of exercise was examined by Konrad et al. in a crossover designed study on 20 endurance trained runners [60]. A mixture of 1000mg quercetin, 120mg EGCG, and other vitamins provided no benefit to the post-exercise immune changes or inflammation. While the primary outcome of this study was not performance, per se, it contributes evidence that quercetin may not alter the immune or oxidative stress incurred with exercise, or there are mechanisms unique to chronic supplementation that makes quercetin elicit progressive effects on adaptation to exercise training.

There have also been investigations into the effects of chronic supplementation with quercetin on immune perturbations and inflammation following the western states endurance run. This race is a 160 km race that places a large oxidative stress on the participating racers. No effects of the supplementation were found for performance, immune perturbations, or acute inflammation following the race; although, the incidence of illness was reduced in the quercetin group [61-63].

METHODS

Subjects

Endurance-trained male (n = 11) and female (n = 2) cyclists (age 30.1 ± 7.1 y, body mass 67.8 ± 10.81 kg, height 173.41 ± 9.15 cm, VO_{2max} 58.83 ± 3.93 ml/kg/min) were recruited for this study from the University of Texas at Austin and local Austin, Texas community. A trained cyclist was defined as an individual that regularly competes in cycling racing and competitions and had a minimum VO_2max of 53 ml/kg/min for men and 45 ml/kg/min for women on the cycle ergometer. Subjects had to be training a minimum of 7 hours per week on the bike and between 18 and 40 years old. During the entire duration of the study, the subjects were required to maintain their current volume and intensity of cycling training. Any potential participants that were planning on tapering or vastly increasing volume or intensity of training during the 12 weeks following the screening appointment were not included in the study. Subjects completed a questionnaire prior to participation to assure they met the inclusion criteria, which included the following: must be healthy, non-smoking, and have no history of chronic disease. Consumption of anti-inflammatory (Advil, Motrin, etc.) or anti-oxidant supplements (supplemental vitamins/minerals, etc.) could not be consumed during the entire testing period. Subjects must have no history of hypertension, history of or current Angiotensin Converting Enzyme (ACE) inhibitor use, or use of lipid-lowering, or anti-inflammatory steroid medication; no active weight loss > 5 kg in the prior 3 months, and no history of kidney dysfunction. Subjects must not use Selective Serotonin Reuptake Inhibitors (SSRI) during the testing period. The study was conducted under a protocol approved by the University of Texas at Austin Institutional Review Board, and each subject provided written informed consent.

Design

This study was a double blind, randomized, placebo controlled, crossover experiment. There were 2 testing periods lasting 28 days with a 1 week washout between them. Preliminary testing for determination of each subject's maximal oxygen consumption (VO_{2max}) and lactate threshold (LT) along with 2 familiarization time trials (FAM1, FAM2) were performed in a 2 week period prior to the start of the testing period. During each testing period, the subject would be randomly assigned to ingestion of a daily quercetin based vitamin supplement (Q-VIT) or a vitamin supplement without quercetin (VIT). On the last day of each testing period (Day 28) the subject returned to the lab to perform a simulated time trial on a cycle ergometer. Combined with the preliminary testing, there were a total of 6 visits to the Human Performance Laboratory for each subject. The preliminary phase of testing consisted of the first 4 visits: VO_{2max} testing, LT testing, FAM1, and FAM2. The testing period consisted of 2 visits to the lab in which the subjects would perform the simulated time trial on Day 28 of supplementation (TT1 and TT2). Data collection during the time trials included gas measurements (VO_2 and VCO_2), heart rate, power output, Borg scale Rating of Perceived Exertion (RPE), and time to completion. Respiratory Exchange Ratio (RER) and cycling Gross Efficiency (GE) were also evaluated.

Weeks 1 - 2	Weeks 3 - 6	TT	Week 7	Weeks 8 - 11	TT
VO2max	Supplementation 1 (Q or P)		Washout	Supplementation 2 (Q or P)	
LT	TT on last day			TT on last day	
2 Fam TT					

Preliminary testing

Maximal Oxygen Consumption (VO_{2max})

There was a minimum of 48 hours between each of the preliminary visits. The first visit consisted of the determination of VO_{2max} on an electromagnetically braked cycle ergometer (Lode; Gronigen, Netherlands). It was determined by having each subject perform a submaximal cycling protocol with 4, 5 min stages at 80, 120, 160 and 200 watts or 100, 140, 160 and 220 watts. Following a 10-15 min rest period, each subject performed a VO_{2max} test. Briefly, the first four minute stage was set at a workload to achieve ~75-80% of predicted VO_{2max} based on the submaximal heart rate and oxygen consumption *vs.* workload regression equations and age predicted max heart rate (208-0.7*age). Following the first four-minute stage, the workload was increased every 2 minutes until 10 minutes and then increased at 1 min increments thereafter until volitional fatigue. Peak oxygen consumption was determined from the final 30 seconds prior to exhaustion during the maximal protocol. The subjects breathed through a 2 way valve while the volume of inspired air was determined by a pnuemotachometer (Hans Rudolph Inc., Model 4813; Kansas City, MO). Expired air entered a mixing chamber and was analyzed for oxygen (AEI Technologies, Oxygen Analyzer S-3A/I, Pittsburg, PA) and carbon dioxide (AEI Technologies, Carbon Dioxide Analyzer CO-3A, Pittsburg, PA). The analyzers were connected to a computer and computer software was utilized for the output of gas measurements.

Lactate Threshold (LT)

The second visit to the lab consisted of the determination of the subjects lactate threshold (LT) on the cycle ergometer. Each subject completed 5, 5 minute stages of cycling with a progressively increasing workload with each stage. Workload at each stage consisted of

wattages meant to elicit 50, 60, 70, 80, and 90 percent of the subjects VO_{2max}. During the last minute of each stage, a small droplet of blood was obtained by pricking the end of the subject's finger and analyzed for blood lactate utilizing a handheld blood lactate test meter (Arkray Inc., Lactate Pro, Kyoto, Japan). LT was defined by graphing the VO_2 and lactate relationship and determining the workload that elicited a rise in lactate 1mM above the baseline lactate values as previously described by Coyle et al [64].

Determination of Time Trial Workrate ($TT_{workrate}$)

After determination of the percent VO_2max at lactate threshold (% VO_2max at LT) and subsequent workload that lactate threshold occurred, the workload utilized for the first half of the time trial was determined. It was accomplished by calculating the workload that would elicit a VO_2 10% above the lactate threshold VO_2 ($TT_{workrate}$). This was done using the VO_2 versus workload regression equation determined during the maximal test. This workrate was utilized to pace the participants for the first half of the time trial and has been successfully utilized with cyclists within our lab in previous investigations.

Exercise Trial

Upon arrival to the laboratory, subjects were seated and rested for 5 minutes before initiation of any measures or activity. Before and after a 20 minute warmup ride on the cycle ergometer at 70% VO_2max, subjects were seated and blood was drawn via venipuncture of an antecubital vein. After the second blood draw, the subjects began the exercise time trial on the cycle ergometer. The goal of the time trial was to complete a set amount of work as quickly as possible. The required Kilojoules that the participant needs to complete during the time trial was calculated from the equation below:

- $TT_{workrate}$ = watts = Joules/sec
- Joules/sec X 2400sec = Joules
- Joules/1000 = Time Trial kJ (TT kJ)

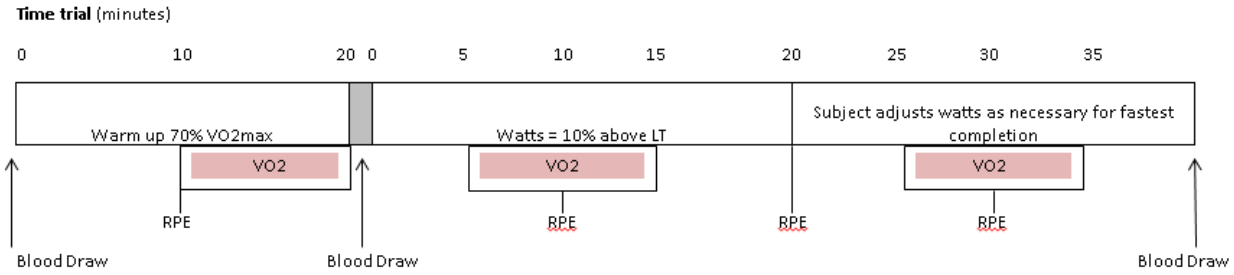
The first 20 minutes of the time trial were set at a fixed workrate equal to the aforementioned $TT_{wokrate}$. Computer software (Lode Ergometer Manager V 9.1, Lode; Gronigen, Netherlands) was used to program the cycle ergometer to have a fixed power (fixed power mode) independent of cadence during the first 20minutes. After the first 20 minutes, the cycle ergometer would change to a cadence dependent mode (linear mode). In this mode, the subjects could increase or decrease power output (watts) by increasing or decreasing cadence from a previously established preferred cadence or by requesting the investigators to raise the $TT_{workrate}$. Workrate was raised or lowered in pre-determined increments equal to 5% of their $TT_{workrate}$. The subjects were told when they had completed 60, 70, 80, 90, and 95 percent of the work they must complete and the approximate time left until completion at their current power output. Subjects were verbally encouraged at these intervals to complete the time trial as fast as possible. Time to completion was recorded as the total time to complete TT kJ. The subject would immediately move off of the cycle ergometer to a seated position for the final blood draw. Aside from the pacing information, the subjects were blinded from all performance information until participation in the study was complete.

Gas measurements were collected from minute 10 to 15 and from minute 25 to 30. Subjects were instructed on the use of hands to signal to request changes in the workrate during the gas collection from minute 25 to 30. Heart rate (HR) was continuously recorded each second during the time trial using a wireless receiver and was recorded using custom programmed

software. Heart rate was averaged for 10 seconds and recorded at minute 10, 15, 20, 25, 30, and 35. Borg scale (6 – 20) was ascertained at minute 15, 30, and 35.

The caloric expenditure per minute (kcal/min) was calculated [65] and then used to calculate the cycling gross efficiency (GE) [66]:

- Kcal/min = $3.716(VO_2) + 1.332(VCO_2)$
- GE (%) = (watts/69.7) / (kcal/min)



Blood Collection

Blood was taken via venipuncture from an antecubital vein before the warmup, after the warmup, and after completion of the time trial. Whole blood was separated into Vacuum sealed tubes (Vacutainer, Franklin Lakes, NJ) containing K_2EDTA. The tubes were centrifuged immediately at 1000 g for 15 minutes at 4^o C for separation of plasma and red blood cells. The plasma was allocated into micro-centrifuge tubes and stored immediately at -80^o C.

Activity and Diet Control

Subjects were asked to refrain from any major changes in exercise training for the duration of the study. Subjects recorded daily training during the first supplementation periods

that had to be closely matched during the second treatment period. All subjects were required to refrain from exercise the day before their time trial.

Diet was recorded during the 3 days leading up to the time trial during the first treatment period. This diet had to be replicated 3 days prior to the time trial during the second treatment period. A minimum of 2 hours was required between the time trial and the subject's last meal.

Supplementation

Subjects supplemented twice daily with a chewable flavored tablets. Two tablets were ingested in the morning and 2 were ingested in the evening. The Q-VIT tablets each contained 250 mg quercetin, 205 mg Vitmain C, and 10 mg Vitamin B3 for a total of 1000 mg/day quercetin, 820 mg/day Vitamin C, and 40 mg/day Vitamin B3. The placebo chews (VIT) contained identical vitamin ingredients minus the quercetin. The placebo chews had a similar external appearance and flavor. On the final day of supplementation, subjects were instructed to ingest all 4 of the daily chews 2 hours before the time trial.

Statistical Analysis

Performance time and average power output were analyzed with a paired, two-tailed Student's t-test. All other performance variables were analyzed with a two-way repeated measures ANOVA to analyze interaction of treatment and time. The criterion for significance was set at $p < 0.05$. All statistics were calculated with IBM SPSS Statistics software, version 19. Results are reported as means ± SEM.

RESULTS

Time to Complete Simulated Time Trial

Time to complete the pre-determined amount of kilojoules (kJ) on the cycle ergometer was reported in minutes (min). The time to complete the work on quercetin (Q-VIT) and placebo (VIT) was 39.36 ± 0.39 min and 39.61 ± 0.42 min, respectively. There were no significant differences between the treatments ($p = 0.47$). (Table 1)

Power Output min 20 – end

Average power output from minute 20 until the end of the time trial was reported as the average watts produced. The average power output from minute 20 until the completion of the time trial on Q-VIT and VIT was 247 ± 11 watts and 251 ± 12 watts, respectively. There were no significant differences between the treatments ($p = 0.41$). (Table 2)

Oxygen Consumption (VO2)

VO2 was measured from minute 10 until 15 and from minute 25 until 30 during the time trials and was expressed as liters of oxygen consumed per minute (L/min). The average oxygen consumption for the Q-VIT for minutes 10-15 and 25 – 30 were 3.37 ± 0.15 L/min and 3.44 ± 0.16, respectively. The VO2 values for the same data points during the VIT trials were 3.30 ± 0.14 L/min and 3.38 ± 0.17 L/min. There were no significant differences between the treatments ($p = .13$). (Figure 1)

Gross Efficiency (GE)

Gross efficiency was measured from minute 10 until 15 and from minute 25 until 30 during the time trial and was expressed as the percent of caloric expenditure that was being used for production of power. The average GE for Q-VIT for minutes 10-15 and 25 – 30 were 20.49 ± 0.26 % and 19.94 ± 0.33 %, respectively. The GE values for the same data points during the VIT trials were 20.9 ± 0.24 % and 20.37 ± 0.33 %. The GE at minutes 10 – 15 and minutes 25 – 30 was significantly higher in VIT compared to Q-VIT ($p < .01$). (Figure 2)

Respiratory Exchange Ratio (RER)

RER (VCO_2/VO_2) was assessed and averaged from minute 10-15 and from minute 25-30 during the time trial. The average RER from minute 10-15 and minutes 25-30 for Q-VIT was 0.95 ± 0.1 and 0.93 ± 0.1, respectively. The RER for the same time points during the VIT time trial were 0.95 ± 0.1 and 0.94 ± 0.1. There were no differences between the treatments ($p = 0.19$). (Figure 3)

Heart Rate (HR)

Heart rate was recorded at minute 10, 15, 20, 25, 30, and 35. There were no significant differences in heart rate between the treatments ($p = .51$). There was an overall effect of time on heart rate ($p < 0.01$). Heart rate at 10 minutes was significantly lower than all other time points ($p < 0.01$). At 15 minutes, heart rate was significantly higher than at 10 minutes ($p < 0.01$); significantly lower than heart rate at 20 minutes ($p = 0.02$); and significantly lower than heart rate at minutes 25, 30, and 35 ($p <.01$). At minute 20, the heart rate was significantly lower than minute 35 ($p < 0.01$). At minute 25, heart rate was significantly lower than heart rate at minute

35 ($p = 0.04$). At minute 30, heart rate was significantly lower than heart rate at minute 35 ($p < 0.01$). (Figure 4)

Rating of Perceived Exertion (RPE)

RPE was recorded at minute 15, 25 and 30 during the time trial. The average RPE for VIT at minute 15, 25, and 30 were 15.6 ± 0.3, 17.5 ± 0.3, and 18.4 ± 0.3, respectively. The values for Q-VIT at the same time points were 15.1 ± 0.3, 17.4 ± 0.3, and 18.5 ± 0.3, respectively. There were no differences between treatments ($p = .39$). (Figure 5)

Plasma Quercetin

Supplementation with Q-VIT was successful in significantly raising plasma quercetin concentration when compared to the VIT treatment. Q-VIT quercetin plasma concentration was 2197 ± 409 ng/mL and VIT quercetin plasma concentration was 27 ± 9.69 ng/ml ($p < 0.01$).

Table 1- Time (min) to complete set amount of work

Subject	VIT	Q-VIT
1	37.07	38
3	39.48	42.17
4	41.35	39.47
5	40.27	39.53
6	39.1	39.15
7	38.97	38.78
8	40.55	42.8
9	37.86	37.75
11	40.2	39.9
12	36.98	37.93
13	39.95	40.03
16	40.95	40.4
19	39	39.07
MEAN	39.36	39.61
± SEM	0.39	0.42

Table 2- Average power (W) during time trial

Subject	VIT	Q-VIT
1	305	290
3	300	264
4	201	221
5	282	292
6	309	308
7	172	174
8	199	180
9	225	226
11	228	232
12	289	274
13	256	255
16	234	240
19	258	257
MEAN	251	247
± SEM	12	11

Figure 1

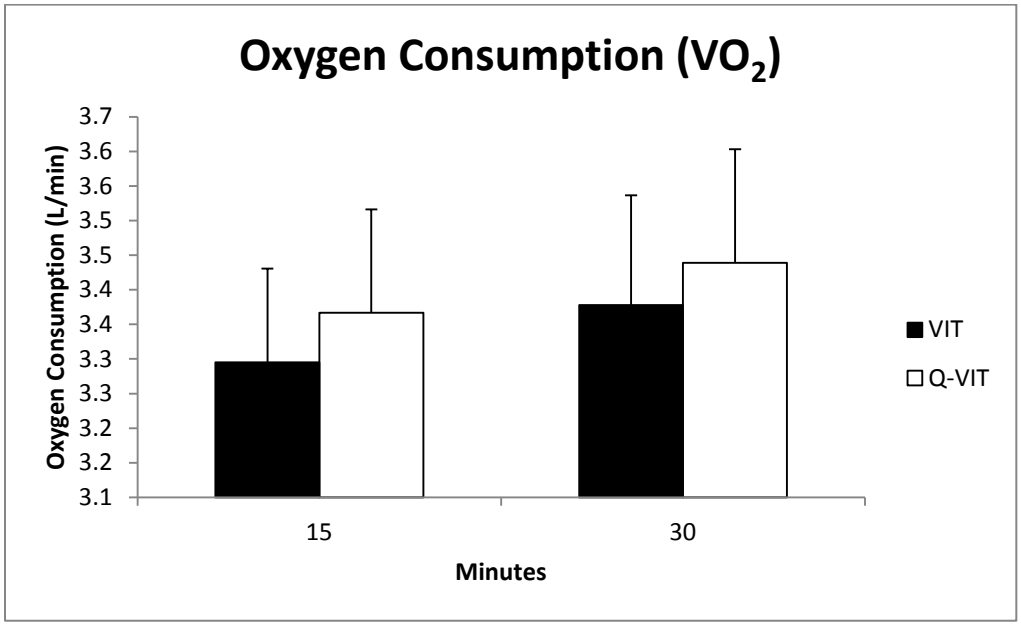


Figure 2

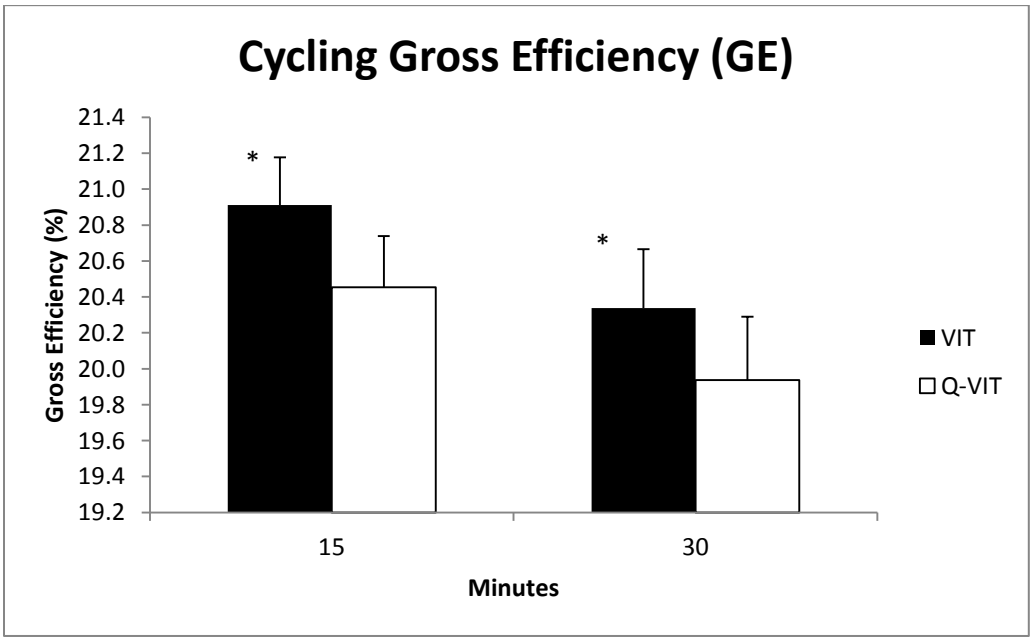


Figure 3

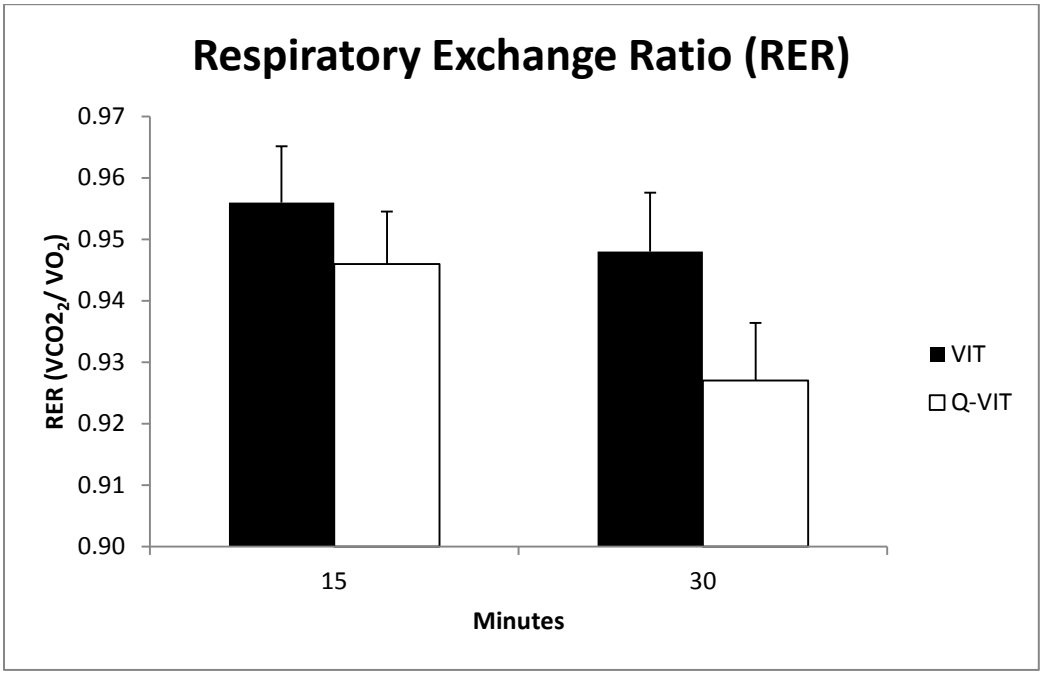


Figure 4

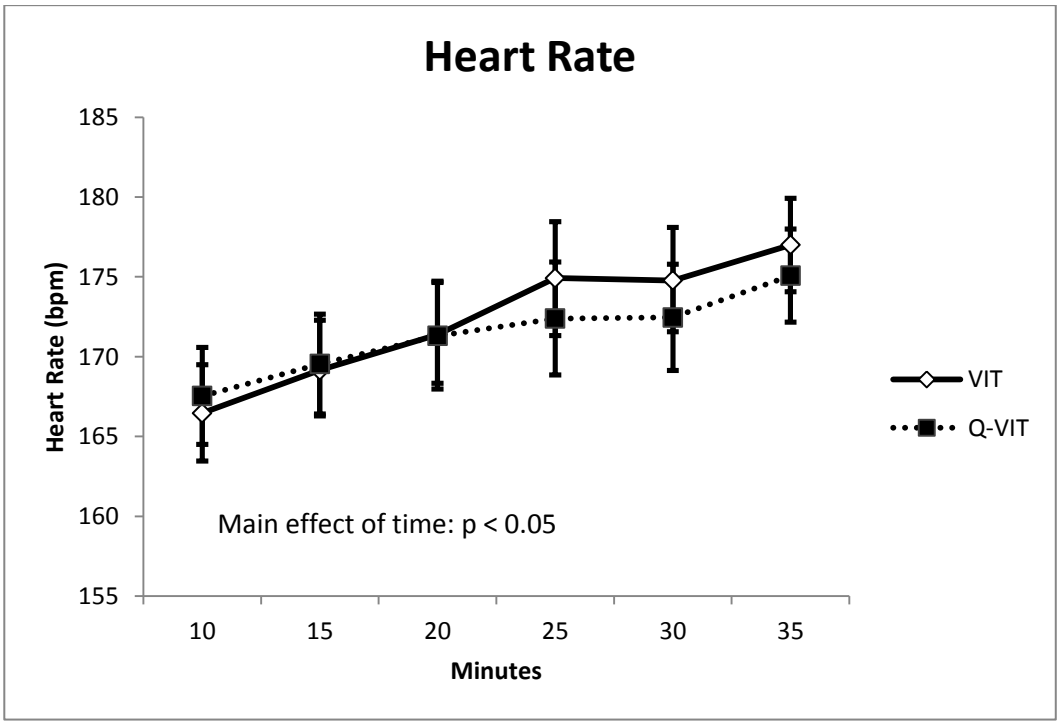
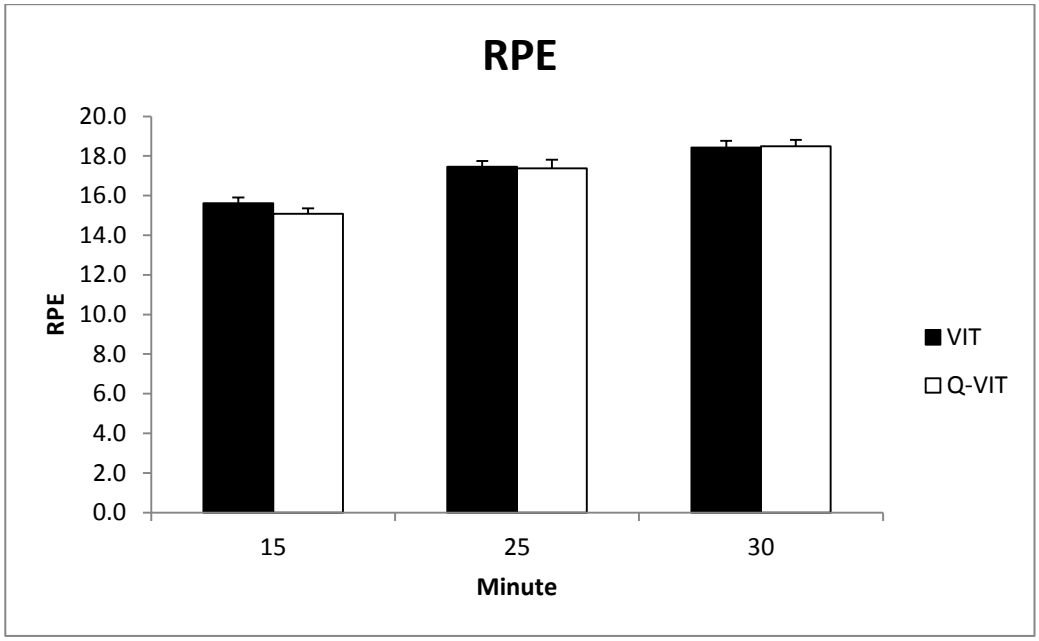


Figure 5


RPE
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
RPE
15
25
30
Minute
VIT
Q-VIT

DISCUSSION

The primary finding of this study was the negative effect of quercetin supplementation on Gross Efficiency (GE) when cycling in trained cyclists without effecting performance measured as the time to complete a set amount of work; therefore, there were no changes in the overall performance in the trained cyclists in regards to completion of the time trial. GE was lowered from 20.9 to 20.49% during minutes 10 – 15 and from 20.37 to 19.94% during minutes 25 – 30 when cyclists were supplemented with the Q-VIT compared to VIT. The change in gross efficiency was driven mostly by the non-significant increase in oxygen consumption seen at these time points; oxygen consumption was ~2.1% and ~1.8% higher, respectively. No other measured parameters of performance were significantly affected by the treatment. Our results also show that the Q-VIT supplement increased plasma concentrations of quercetin by 81 fold in the subjects compared to VIT ($p < .01$).

To our knowledge, this is the first study to show a decrement in gross mechanical efficiency in conjunction with supplementation of a quercetin based antioxidant supplement. There have been equivocal findings thus far regarding the ergogenic effect of either acute or chronic supplementation of quercetin on exercise performance. Improvements in exercise performance with quercetin supplementation have been seen in untrained subjects utilizing only 1 week [12] or 2 weeks [13] of supplementation. The results of these studies are difficult to associate to the current study as the response of individuals that are not actively exercise training may not be comparable. MacRae et al. found an increased performance on a cycling time trial with a quercetin based supplement in trained cyclists when compared to a baseline time trial [14]. However, there were no differences between the performance times of the quercetin based

supplement versus the same supplement without quercetin (the placebo). Also, there were confounding factors involving the composition of the supplements as they contained caffeine. Caffeine has been shown to be a powerful ergogenic aid in regards to aerobic exercise performance [57, 58]. However, the supplement with quercetin did exhibit better performance compared to the baseline time trial without supplementation when compared to the placebo.

There have been previous claims from Dumke et al. that chronic quercetin supplementation has no effect on cycling GE [52], but the methods utilized for examining the potential ergogenic effects of quercetin on cycling performance were not optimal for assessing changes in exercise performance. In a cross-sectional design, trained cyclists supplemented with a quercetin based antioxidant supplement or placebo for 3 weeks and were asked to perform 3 days of 3 hours of cycling at 57% $VO2_{peak}$ before and after supplementation. This type of exercise bout may not be the optimal design for the examination of cycling performance. Time trials similar to the one utilized in the current study have been validated as reliable and reproducible and optimal for the assessment of performance on a cycling ergometer [53]. A simulated time trial in which as much work as possible is completed during a set amount of time or a set amount of work must be completed as quickly as possible may be a more valid exercise performance test: The latter type of design was chosen or the current study.

Cureton et al. utilized a time trial in which untrained subjects performed as much work as possible in 10 minutes on a cycle ergometer after cycling at 50% VO2peak to investigate the effects of chronic quercetin supplementation [49]. There were no differences on any measured performance variables including VO2, RER, work completed, and other markers of substrate utilization. The overall conclusion of this study was that chronic supplementation with a quercetin based antioxidant supplement was not ergogenic in untrained, recreationally active

individuals. However, an inherent flaw of this study was the varying duration of the supplementation period between subjects. The duration of quercetin supplementation ranged from 9 to 16 days. Although one can debate the veracity of claims that this flaw negates their findings, it does question the consistency of the results obtained due to differing exposure to the quercetin supplementation. Their results are in contrast to the findings of the previously discussed findings from Nieman et al [13] and Davis et al [12] in which quercetin significantly improved the exercise performance of untrained subjects.

While the chemical properties of the flavonoid quercetin exhibit astounding and repeatable effects in vitro by scavenging free radicals, reducing inflammation [6], and lowering oxidative stress [7, 67], attempts at examining the in vivo antioxidant potential of quercetin without focusing on the exercise performance per se, have not elucidated quercetin's powerful antioxidant and anti-inflammatory properties. Most have found no effect of quercetin supplementation on markers of oxidative stress in trained [50, 51, 63] or untrained individuals [54, 68]. Only in combination with epigallocatechin 3-gallate (EGCG) has quercetin been shown to significantly decrease markers of oxidative stress and inflammation in trained cyclists [44]. The authors concluded that quercetin must be taken with the appropriate mixtures of vitamins and sugars for optimal absorption in the gastrointestinal tract. Vitamin mixtures are suggested for optimal absorption of quercetin in humans [37, 45], such as the form of the supplement utilized in this study. Without a measure of oxidative stress, it cannot be concluded if the presently reported decrease in efficiency can be linked to changes in oxidative status.

In our study, we have teased out the effect of quercetin itself by using a vitamin mixture as the placebo group (VIT) and have shown that the flavonoid quercetin can reduce efficiency of exercise. As mentioned, no other studies have shown these detrimental effects in gross

efficiency in trained cyclists or in untrained individuals thus far. Reasons for our novel finding may be due to our strict adherence of screening and selecting only well qualified trained cyclist, consistency of the quercetin supplement, type of exercise test utilized, and / or the duration of supplementation.

Due to the evidence in untrained individuals that quercetin may elicit improvements in exercise performance, we hypothesized that supplementation with the powerful antioxidant quercetin would improve exercise performance in endurance trained subjects. However, the reduced gross efficiency of the quercetin treatment in the trained cyclists in this study indicate that these results may not be comparable to the untrained population. No claims can be made based on our data that the quercetin supplementation was detrimental to performance per se, but the efficiency of the energy production during the time trials were reduced by quercetin supplementation.

Although oxidative stress may cause damage to cellular membranes and increases in muscular fatigue, DNA damage, and peroxidation of lipid membranes, it may also be necessary in moderate amounts to induce adaptive responses [33]. Trained individuals have been shown to have and increased endogenous antioxidant capacity when compared to sedentary controls [32, 69, 70]. That is, both the acute and chronic exposure to exercise training stimulates cellular pathways that can elevate concentrations and activities of superoxide dismutase, catalase, and glutathione peroxidase in skeletal muscle [19, 71]. It seems plausible that increased cellular concentrations of these antioxidants will reduce the risk of cellular injury, improve performance, and delay muscle fatigue. Exposure to higher levels of oxidative stress has also been shown to effect DNA repair enzymes and the subsequent expression of cytoprotective proteins such as heat shock proteins[18]. Interestingly, it has been established in vitro that quercetin can inhibit

the regulation of heat shock proteins [72-74]. These results were proven in a recent human study in which chronic supplementation with quercetin inhibited heat adaptation and worsened thermotolerance at the cellular and systemic level [75].

It has been suggested that the pathways initiated by oxidative stress are essential for the body to produce advantageous adaptations in endogenous antioxidants and cytoprotective proteins through the activation of nuclear factor kappa-B (NF-KB) and MAP kinases within the muscle cell [28]. There are beneficial endogenous adaptations regarding the protection of cellular redox homeostasis that occur following an acute bout of exercise, especially through the activation of NF-KB [29, 30]. Blockage of RNS production attenuates up-regulation of superoxide dismutase, an important endogenous antioxidant, along with eNOS and iNOS [31]. Preventing oxidative stress during an exercise bout may prevent the production of an improved endogenous protection against oxidative stress.

Although the subjects that participated in this study were actively training and competing in cycling racing, they were not undertaking any training programs meant to elicit improvements in their aerobic capacity. Rather, the trained cyclists were participating in this study during the mid to late portion of the cycling competition season and were required to maintain their fitness status constant during their commitment to the study. Cyclists were typically undergoing training to maintain their aerobic capacity rather than vastly increasing volume and intensity of training during a period of daily racing competitions. Perhaps the aforementioned regulation of cellular adaptation through ROS and RNS mediated pathways was blunted and the subjects' normal exercise training failed to elicit the same stimulus during the Q-VIT versus the VIT. The longevity of decrement in the exercise stimulus may have caused a maladaptive response to their normal training and triggered the decrease in gross efficiency.

In conjunction with the reduction in stimulation of the ROS and RNS mediated adaptation pathways, the functioning of the mitochondria and electron transport chain (ETC) may have been effected. The exercise induced oxidative stress may have reduced the efficiency of the electron transport chain (ETC) and therefore reduced the efficiency of NADH:NAD^+ cycling [25, 26]. In turn, the efficiency of ATP production would have been reduced. The free radicals can also have detrimental effects on the tricarboxylic acid (TCA) cycle by inhibiting the actions of enzymes such as α-ketoglutarate dehydrogenase (KGDH) and succinate dehydrogenase (SDH)[27]. It may seem intuitive that the quercetin supplementation should improve the efficiency of the ETC by mitigating the detrimental effects of the free radicals on the functioning of the ETC; however, chronic supplementation may have caused the down regulation of endogenous antioxidant enzymes and actually increased the levels of oxidative stress upon the ETC. In a feedback mechanism, the lowered levels of oxidative stress through quercetin's scavenging of free radicals may have lowered the activation of the ROS and RNS mediated pathways of cellular adaptation [28, 33, 71]. Pharmacological studies have also found inverse relationships between the concentration of glutathione and the amount of superoxide and hydrogen peroxide production [76, 77] which may be a reflection of the events occurring in the trained cyclists during the down regulation of their endogenous antioxidant factors.

The primary finding of this study was unique in that the reduction in gross efficiency did not affect the time to complete the time trial. It has been well established that cycling gross efficiency is a major determinant of cycling performance in well trained and elite cyclists and is highly correlated to the amount and functioning of type I muscle fibers [66, 78, 79]. It has also been shown that small increments or decrements in cycling efficiency may manifest changes in endurance performance [80]. Work from Mogensen et al. [81] has further investigated the

mechanisms behind the increased efficiency and their results implied that it may be the result of the efficiency of transferring the chemical energy from ATP hydrolysis into physical work rather than mitochondrial efficiency [82]. Nevertheless, it is difficult to interpret how this may be affected by the evidence that mitochondrial complex I function and hampered cycling of NAD:NADH may affect these processes. The possible down regulation of the endogenous antioxidant defenses may contribute to lessened mitochondrial efficiency and / or conversion of the energy from ATP hydrolysis into physical work. However, it can only be speculated from our results since no mechanisms involved in this process were directly measured.

The absolute changes in GE were ~0.42% at both measured timepoints during the time trial. This is not a huge decremement, however it was a statistically significant finding ($p < 0.01$) with 17 out of the 20 measured timepoints from all of the subjects exhibited a lower efficiency on quercetin supplementation. The 3 timepoints that did not follow this trend were dispersed amongst 3 separate subjects. This decrement in GE did not elicit any differences between the treatments in the performance time of the time trial in the trained subjects. The trained cyclists exhibited higher oxygen consumption while supplementing with quercetin during equivalent power output between treatments, although this finding did not reach statistical significance. In essence, the cyclists could maintain equivalent power output, heart rates, and time to complete the time trials although the efficiency was slightly lower when taking quercetin.

One possible explanation is the change in the efficiency was too small to impact their performance time during this type of time trial. Subjects were performing at an average of ~85% $VO2_{peak}$ or higher during most of the last half of the time trial. This very high intensity and average duration of ~39.5 minutes may have been too short and of an exercise intensity that is too high to elucidate the small change that was seen in the GE. A longer duration exercise trial

of a lesser exercise intensity, such as a time to fatigue test, may have exposed differences in the performance times.

There were limitations that must be realized pertaining to the application of the results of this study. The placebo (VIT) contained Vitamin C and Vitamin B3 in order to expound the effects of quercetin itself on the cyclists' performance, however the effect of the vitamin supplementation itself on the exercise performance of the cyclist cannot be determined with this design. It cannot be concluded if the effect of Q-VIT may have been more detrimental to performance and / or efficiency compared to their baseline performance variables. Aside from the previously discussed mechanisms in this discussion, readers are directed to more comprehensive reviews on vitamin supplementation and exercise performance for a full discussion on this still debated topic [83, 84].

Also, highly elevated quercetin was present in the plasma at the time of the exercise performance testing in Q-VIT compared to VIT, therefore it can be difficult to interpret if that may have affected the GE. While there is evidence to support the lack of an ergogenic effect of an acute dosage of quercetin on exercise performance [54, 56], it is prudent to claim that it may not have affected the performance of the cyclists.

In conclusion, daily supplementation with the flavonoid quercetin for 28 days lowered cycling gross efficiency without affecting the time to complete a time trial in trained cyclists. This may be an important finding as the use of antioxidant supplementation in highly trained endurance athletes has become a popular method of nutritional augmentation due to the high levels of oxidative stress experienced during exercise training. Although the decrease in gross efficiency failed to affect the performance time of the time trial, the exercise bout utilized in this

study was short and of a relatively high intensity. Further research is needed to validate and examine the mechanisms behind these changes in efficiency.

APPENDIX: DATA TABLES

Performance Data

	V-IT						**Q-VIT**					
	minutes 10-15				minute 25-30				minute 25-30			
Subject	VO2 (L/min)	VCO2 (L/min)	RER	GE(%)	VO2 (L/min)	VCO2 (L/min)	RER	GE(%)	VO2 (L/min)	VCO2 (L/min)	RER	GE(%)
1	3.44	3.24	0.94	21.82	3.95	3.95	1	21.44	3.5	3.41	0.97	21.26	3.97	3.95	0.99	20.79
3	3.85	3.68	0.96	21.81	4.11	3.94	0.96	20.49	3.94	3.8	0.96	21.26	3.67	3.32	0.90	20.89
4	2.8	2.76	0.99	21.91	2.58	2.42	0.94	21.84	2.85	2.7	0.95	21.74	2.87	2.7	0.94	21.73
5	3.83	3.67	0.96	21.38	3.72	3.42	0.92	21.08	3.97	3.78	0.95	20.66	4	3.8	0.95	20.52
7	2.33	2.02	0.87	20.61	2.38	2.1	0.88	20.46	2.36	2.12	0.90	20.17	2.47	2.2	0.89	19.91
8	2.95	2.93	0.99	19.79	2.98	2.79	0.94	18.33	3.04	2.93	0.96	19.35	2.73	2.44	0.89	18.74
11	3.21	2.92	0.91	20.72	3.24	2.88	0.89	20.71	3.16	2.93	0.93	21.09	3.28	2.95	0.90	20.30
13	3.54	3.38	0.95	20.72	3.5	3.29	0.94	20.71	3.65	3.51	0.96	20.06	3.76	3.62	0.96	19.54
16	3.59	3.42	0.95	19.64	3.64	3.48	0.96	18.56	3.76	3.44	0.91	18.94	3.75	3.34	0.89	18.03
19	3.41	3.31	0.97	20.58	3.68	3.6	0.98	20.04	3.44	3.35	0.97	20.38	3.89	3.77	0.97	18.93
Mean	**3.30**	**3.13**	**0.95**	**20.90**	**3.38**	**3.19**	**0.94**	**20.37**	**3.37**	**3.20**	**0.95**	**20.49**	**3.44**	**3.21**	**0.93**	**19.94**
± SEM	**0.14**	**0.14**	**0.01**	**0.24**	**0.17**	**0.18**	**0.01**	**0.33**	**0.15**	**0.15**	**0.01**	**0.26**	**0.16**	**0.18**	**0.01**	**0.33**

TT watts
20-end

Subject	871	532
1	305	290
3	300	264
4	201	221
5	282	292
6	309	308
7	172	174
8	199	180
9	225	226
11	228	232
12	289	274
13	256	255
16	234	240
19	258	257
Mean	**251**	**247**
± SEM	**12**	**11**

Time

Subject	871	532
1	37.07	38
3	39.48	42.17
4	41.35	39.47
5	40.27	39.53
6	39.1	39.15
7	38.97	38.78
8	40.55	42.8
9	37.86	37.75
11	40.2	39.9
12	36.98	37.93
13	39.95	40.03
16	40.95	40.4
19	39	39.07
Mean	**39.36**	**39.61**
± SEM	**0.39**	**0.42**

Rating of Percieved Exertion

	VIT				Q-VIT			
Subject	15min	30 min	35 min	overall	15min	30 min	35 min	overall
1	14	18	20	20	15	20	20	20
3	15	17	19	18	16	19	19	18
4	17	17	18	18	16	18	19	18
5	17	17	18	19.5	16	18	19	17
6	15	16	19	17	14	15	18	17
7	16	19	19.5	18.5	15	18	19	20
8	15	17	17	18	15	16	16	17
9	16	19	19	20	16	19	19.5	19.5
11	17	17	17	17	16	17	18	18
12	16	18	20	18	17	19	20	19.5
13	15	17	18	17	15	16	18	16
16	15	16	16	18	15	16	17	15
19	14	17	19	20	13	19	19	20
Mean	**15.5**	**17.3**	**18.4**	**18.4**	**15.3**	**17.7**	**18.6**	**18.1**
± SEM	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**	**0.4**	**0.3**	**0.5**

HR

	VIT						Q-VIT					
Subject	10 min	15 min	20 min	25 min	30 min	35 min	10 min	15 min	20 min	25 min	30 min	35 min
1	177	175	179	185	186	190	176	178	179	185	186	188
3	174	176	181	177	174	177	164	166	170	180	176	180
4	174	181	181	177	179	177	174	178	178	180	180	180
5	154	158	156	154	157	163	148	150	148	144	151	155
6	158	163	163	170	170	174	154	158	160	165	165	170
7	163	159	165	163	166	167	175	172	175	171	169	171
8	171	175	184	186	181	182	171	175	182	174	169	172
9	191	191	194	203	202	201	185	188	190	196	196	198
11	155	159	162	163	167	172	158	160	161	163	168	170
12	160	160	164	173	169	176	162	163	163	171	164	171
13	175	178	177	180	177	177	179	183	182	182	184	182
16	155	162	161	161	163	162	159	160	163	162	160	167
19	167	172	172	179	179	180	163	163	165	171	176	175
Mean	**167**	**170**	**172**	**175**	**175**	**177**	**167**	**169**	**170**	**173**	**173**	**175**
± SEM	**3**	**3**	**3**	**4**	**3**	**3**	**3**	**3**	**3**	**4**	**3**	**3**

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