EFFECTS OF DIETARY QUERCETIN SUPPLEMENTATION ON PHYSICAL PERFORMANCE, MOOD, SLEEP, AND ILLNESS DURING MILITARY PHYSICAL TRAINING

by

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(Under the Direction of Kirk J. Cureton)

ABSTRACT

Quercetin is a naturally-occurring polyphenolic flavonol, commonly consumed in the human diet and associated with numerous health benefits. Quercetin supplementation has been shown to increase muscle oxidative capacity, endurance, and voluntary physical activity in mice, but its effect on physical performance and other important variables in moderately-trained humans is unknown. Using a randomized, double-blind, repeated-measures, placebo-controlled design, this research was conducted to determine the effects of chronic quercetin supplementation on physical performance, mood, sleep, and illness in 58 healthy, moderately-trained young men and women undergoing regular military physical training. In the first study, the effects of 6 weeks of quercetin supplementation on peak oxygen consumption ($\dot{V}O_{2peak}$) during maximal-effort uphill treadmill running, four physical performance tests [Army Physical Fitness Test (APFT), Baumgartner Modified Pull-Up Test (BMPU), Wingate Anaerobic Test (WAnT), and 36.6-m sprint], and a simple reaction time test [Walter Reed palm-held psychomotor vigilance test] were evaluated before and after supplementation with 1 g/d of quercetin with vitamins and

other substances in a soft chew or a placebo chew. Pretreatment-to-posttreatment changes in $\dot{V}O_{2peak}$ and physical performance were not significantly different ($p > 0.05$). Pre-, mid-, and posttreatment changes in simple reaction time were not significantly different between groups ($p > 0.05$). In the second study, the effects of 6 weeks of chronic quercetin supplementation on the transient moods of energy and fatigue, sleep, and illness were examined prior to, in the middle, at the end, and 2 weeks following supplementation with 1 g/d of quercetin with vitamins and other substances in a soft chew or a placebo chew. Changes in energy and fatigue, sleep quality, and self-reported illness rate and severity were not significantly different ($p > 0.05$) between groups. Outcome measures were not influenced by the sex of the participants. In conclusion, these results indicate that chronic dietary quercetin supplementation in moderately-trained young men and women conducting regular military physical training does not improve $\dot{V}O_{2peak}$, physical performance, or simple reaction time. Results also show that the transient moods of energy and fatigue, sleep quality, and illness rate and severity are unaffected by chronic quercetin supplementation in this population.

INDEX WORDS: Quercetin, Peak oxygen uptake, Physical performance, Mood, Fatigue, Sleep, Illness, Military physical training, Vigilance

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DEDICATION

To my beautiful wife, Karen. Thank you for your love, support, and encouragement throughout graduate school. I will always cherish this unique Army assignment here in Athens, Georgia.

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I would like to thank the members of my committee, Dr. Kirk J. Cureton, Dr. Kevin K. McCully, and Dr. Patrick J. O'Connor, for their assistance in making this project stronger. More importantly, I would like to thank each of them for inspiring me to become a better researcher and changing the way I think about finding solutions to research problems. I will carry this knowledge, influence, and passion for research with me as I work with the next generation of Army leaders at the United States Military Academy at West Point, New York.

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I am also extremely grateful to the future United States Army and Air Force officers and cadre members from the University of Georgia's ROTC programs who participated in this project. Without their support, this endeavor would not have been possible.

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CHAPTER 1

INTRODUCTION

Quercetin (3,3',4',5,7-pentahydroxyflavone) is a naturally-occurring, plant-derived flavonol, belonging to the larger class of polyphenolic flavonoids. As a flavonoid, quercetin is characterized by two benzene rings (A and B in Figure 1.1) joined by an oxygen-containing pyrene ring (C in Figure 1.1). Quercetin occurs in plants in either the aglycone form (Figure 1.1), or glycosylated with a sugar compound at the 3-position of the unsaturated C-ring, as in the quercetin glycoside quercitrin (Figure 1.2) (26). In plants, quercetin is most commonly found in the glycosylated form (24), with the different sugar compounds determining the rate of intestinal absorption in humans (22).

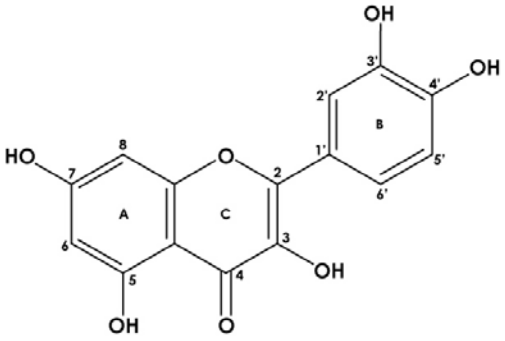

Figure 1.1

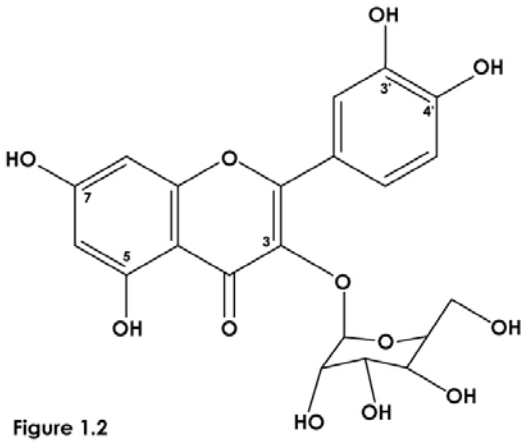

Figure 1.2

Quercetin accumulates in the skin and leaves of plants, as its biosynthesis is stimulated by exposure to sunlight (42). As a plant secondary metabolite, quercetin is not

required for organism growth and development, but is produced in response to plant stress in order to increase survivability (4, 66). Quercetin, like other flavonoids, functions to counteract stress in plants in a variety of ways including providing protection from ultraviolet radiation, oxidative cell damage, and invasion by pathogens (4, 12, 24).

Quercetin has a long history of consumption by humans (26) and is the most common flavonol in the diet (23). It is found in many regularly-consumed food and beverage sources including onions, red apples, blueberries, hot peppers, red grapes, fruit juices, red wine, and black tea (26, 50, 66). Although not commonly consumed, capers and lovage are two of the most richest plant sources of quercetin (66). National dietary records from the United States, Australia, Japan, Italy, the Netherlands, Finland, and Croatia indicate that the mean daily intake of quercetin in a typical diet varies from less than 5 mg to 40 mg, but daily levels as high as 200-500 mg may be obtained by those consuming large quantities of fruits and vegetables (26).

Quercetin consumption is associated with numerous health benefits including anti-oxidant, anti-cancer, anti-pathogen, anti-inflammation, anti-stroke, anti-hypertension, and cardio-protective properties (14, 16, 18, 26, 43, 51). Recent human research has focused on the possible ergogenic effects of quercetin, after improvements in muscle oxidative capacity, endurance, and voluntary physical activity were reported in mice following quercetin supplementation (15). However, a majority of the human research has involved extremely well-trained male cyclists and runners. Treatment durations have also been short, typically 3 weeks or less, and very little quercetin research has involved females.

Purpose

To date, no published research has investigated the effects of chronic quercetin supplementation on measures of physical performance, moods of energy and fatigue, sleep quality, and illness rate and severity in young men and women involved in systematic military physical training. These physical and psychological variables were chosen to determine possible effects of quercetin on the mitigation of potential battlefield stressors. If quercetin supplementation enhances measures of physical performance, mood, sleep, and illness, it may have important implications for optimization of soldier performance, as success in combat is strongly associated with these variables. Additionally, studying the effects of quercetin on this moderately-trained population fills an existing gap in the literature and may potentially allow for generalization of quercetin's effects beyond well-trained male cyclists and runners. Therefore, this study has two primary objectives: 1) To examine the ergogenic effects of quercetin supplementation on peak oxygen uptake ($\dot{V}O_{2peak}$) and measures of muscular endurance, power, speed, and simple reaction time and 2) To examine the effects of quercetin supplementation on the moods of energy and fatigue, sleep quality, and illness.

Specific Aims

1. To determine if 6 weeks of dietary quercetin supplementation influences $\dot{V}O_{2peak,}$ four physical performance tests [Army Physical Fitness Test, Baumgartner Modified Pull-Up Test, Wingate Anaerobic Test, and 36.6-m sprint], and a simple reaction time test [Walter Reed palm-held psychomotor vigilance test] in moderately-trained young men and women undergoing military physical training.

2. To determine if 6 weeks of dietary quercetin supplementation affects the transient moods of energy and fatigue, sleep quality, and self-reported illness rate and severity in moderately-trained young men and women undergoing military physical training.

Hypotheses

Study 1. The hypotheses for Study 1 were:

1. $\dot{V}O_{2peak}$ increases following quercetin supplementation.

2. Physical performance on tests assessing muscular endurance, power, and speed improves after quercetin supplementation.

3. Simple reaction time decreases following quercetin supplementation.

Study 2. The hypotheses for Study 2 were:

1. Quercetin supplementation improves measures of energy and decreases measures of fatigue.

2. Sleep quality decreases following quercetin supplementation.

3. Self-reported illness rate and severity decrease following quercetin supplementation.

Limitations

There are several limitations of this study. One limitation is that the level of physical activity was not completely controlled. At a minimum, participants were required to conduct three organized military physical training sessions per week. However, some participants attended five organized training sessions per week. Additionally, physical activity outside of the organized training sessions was not

regulated. Future studies may attempt to better control participant physical activity levels to determine if quercetin supplementation affects voluntary physical activity.

Another limitation of this study is that markers of immune function were not obtained. Illness data recorded in this study is self-reported symptomatology by participants, and not corroborated with blood or saliva markers of immune function. Had these measures been obtained, the illness data collected in this experiment would have been potentially strengthened.

A third limitation was that the sample size was restricted by the number of Army and Air Force Reserve Officers' Training Corps (ROTC) cadets available on the University of Georgia campus, and by the number of participants that could be tested in one academic semester.

CHAPTER 2

REVIEW OF THE RELATED LITERATURE

Health Effects of Quercetin

Quercetin is reported to have anti-oxidant, anti-inflammatory, and anti-hypertensive properties (21, 23, 26). Although the precise mechanisms associated with these beneficial effects remain unclear (23), they appear to be related to the chemical structure of quercetin and its glycosides, specifically the location of the hydroxyl groups and B-ring (26). Studies in humans and mice suggest that as an anti-oxidant, quercetin reduces or inhibits oxidation of low-density lipoprotein (LDL) cholesterol, reducing the risk for atherosclerosis (10, 27). As an anti-inflammatory agent, quercetin has been shown to reduce edema in animals (25) and decrease proliferation of macrophage cells involved in the inflammation process associated with irritable bowel disease in humans (11). Quercetin supplementation has also been reported to have anti-hypertensive effects in hypertensive rats (18), and has reduced systolic, diastolic, and mean arterial pressures in stage 1 hypertensive patients after 28 days of treatment (21).

Additionally, quercetin has anti-carcinogenic, anti-stroke, and cardio-protective properties (26, 36, 63). Several epidemiological studies offer support for the health benefits associated with flavonol consumption. In a national prospective case-control study conducted in Scotland, there was a 32% reduction in cases of colorectal cancer among those individuals with the highest quartile of quercetin intake (63). A multi-ethnic prospective cohort study of 183,518 persons followed for 8 years reported that total

flavonol intake was associated with a 23% risk reduction for pancreatic cancer (52). In the Zutphen study of 552 elderly males, dietary flavonoid (mainly quercetin) consumption was inversely associated with incidence of stroke, after adjusting for confounders including anti-oxidant vitamin intake (36). Another prospective cohort study of 10,054 Finnish men and women found that persons with higher quercetin intake had lower mortality from ischemic heart disease and lower incidence of asthma. Additionally, the males in this study who consumed the highest levels of quercetin had the lowest incidence of lung cancer (37). Finally, a meta-analysis of seven prospective cohort studies involving 105,000 persons and 2,087 fatal coronary heart disease (CHD) events, suggested that individuals in the top third of dietary flavonol consumption had a 20% risk reduction for CHD mortality compared to individuals in the bottom third (31).

As a result of these health benefits, quercetin combined with other substances has been patented in the United States and made commercially available as an anti-oxidant dietary supplement or pharmaceutical formulation (40). Pure quercetin is harvested from the fava d'anta (*Dimorphandra* spp.) and uncaria (*Uncaria elliptica*) plants (W. Waddell, personal communication, March 9, 2009) through the extraction of quercetin glycosides, followed by hydrolysis to release the aglycone portion, and subsequent purification (26). Following purification, quercetin has been marketed using several oral delivery methods, either in a dry or liquid form. Potential quercetin delivery vehicles include tablets, capsules, soft chews, gels, or as additives in food and beverage products including tea, soft drinks, juice, milk, coffee, jelly, ice cream, cookies, cereals, chocolates, and snack bars (40).

In United States Patent Application 20080032987 entitled "Quercetin-containing compositions", the manufacturer claims that quercetin, in conjunction with vitamins and other substances designed to increase its bioavailability, enhances physical and mental performance. The United States Patent Application describes physical performance improvements in measures of strength, speed, endurance, power, flexibility, balance, focus, coordination, fatigue recovery, and reaction time. Similarly, the application reports mental performance improvements following quercetin supplementation in sharpness, attention span, mental alertness, cognitive function, mood elevation, and recovery or reduction of mental fatigue. The manufacturer asserts that consumption of the quercetin cocktail results in greatly enhanced physical and mental performance without deleterious side effects.

Early research from 1977 suggested that quercetin was carcinogenic *in vitro*, as it showed mutagenicity in *Salmonella typhimurium* during the Ames Test (5). However, as a food and beverage supplement, quercetin is self-affirmed by the manufacturer as generally recognized as safe (GRAS) (26) and numerous *in vivo* studies suggest quercetin is not carcinogenic (32). In human clinical trials involving quercetin or plant extracts containing quercetin glycosides, oral ingestion of doses ranging from 3-1000 mg/d for up to 12 weeks resulted in no reports of ill effects. These trials also confirmed that there were no variations in subjects' hematology, clinical chemistry, and urinalysis parameters following dietary quercetin supplementation (26). Similar conclusions regarding the safety of quercetin for human consumption were obtained by an independent laboratory analysis, with results presented in September, 2008 at a United States Army Research

Institute for Environmental Medicine (USARIEM) conference in Natick, MA (W. Waddell, personal communication, March 9, 2009).

Although numerous beneficial health effects associated with quercetin consumption have been reported (6), there is limited research on the effects of quercetin supplementation on physical performance, mood, sleep, and illness.

Physical Performance

Research involving the ergogenic effects of quercetin on physical performance is not conclusive, and has yielded conflicting results. Additionally, the observed effects of quercetin supplementation on physical performance may be different in animals than in humans. If quercetin does have an ergogenic effect, it may be related to several possible mechanisms including the initiation of skeletal muscle mitochondrial biogenesis, its effects as an anti-oxidant, and/or its properties as an *in vitro* adenosine A_1 receptor.

Recently, Davis et al. (15) reported that 7 days of quercetin feedings (12.5 and 25 mg/kg/d) in mice increased messenger ribonucleic acid (mRNA) of coactivators of mitochondrial biogenesis [sirtuin 1 (SIRT1) and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α)], mitochondrial DNA (mtDNA) content in brain and skeletal muscle, mitochondrial protein (cytochrome-c) concentration, and maximal endurance capacity. The increase in skeletal muscle and brain oxidative capacity observed in this study was similar to the increase observed in mice treated with resveratrol, another naturally-occurring polyphenol found in red grape skins and red wine. Lagouge et al. (38) reported that mice treated with resveratrol significantly increased their aerobic capacity, measured by increased treadmill running time and muscle oxygen consumption. Transgenic sedentary mice with high levels of skeletal muscle PGC-1α had

greater mitochondrial content, peak rate of oxygen consumption, and capacity for exercise compared to wild-type mice (7). These studies of mice treated with quercetin and resveratrol indicate that polyphenols have effects on skeletal muscle, aerobic capacity, and endurance performance. It is possible that these effects, similar to those observed following exercise training (30, 44), carry over to humans after dietary supplementation with certain polyphenols.

In humans, the ergogenic effects of quercetin supplementation are less clear. If quercetin initiates mitochondrial biogenesis, mitochondrial enzyme activity would be expected to increase. However, 3 weeks of quercetin supplementation in humans did not increase mitochondrial cytochrome-c concentration in well-trained cyclists (20). If mitochondrial biogenesis is associated with quercetin supplementation in humans, it is also plausible that physical performance might be improved. However, in field trials, finish times for a 160-km ultra-marathon were unaffected by 3 weeks of quercetin supplementation (1 g/d) in trained male and female runners (28, 49). It may be that quercetin increases muscle oxidative capacity in untrained to less-trained, but not in well-trained, individuals because of the high training-induced mitochondrial level already present in aerobically-trained individuals.

In two studies of untrained individuals, there was no effect of short-duration quercetin supplementation. In one of these studies involving sedentary college-aged males, treatment with quercetin (1 g/d) did not improve muscle oxidative capacity, or metabolic and perceptual determinants during prolonged cycle performance, or 10-min cycling time trial performance (K. Cureton, personal communication, December 23, 2008). Similarly, Ganio et al. (M. Ganio, personal communication, February 18, 2009)

reported that 5 days of quercetin supplementation (1 g/d) by sedentary men and women did not influence $\dot{V}O_{2max}$ and related physiological variables. However, Chen et al. (8) observed that 7 days of quercetin ingestion (1 g/d) by healthy, fit, but not highly-trained college students was associated with small, but significant increases in $\dot{V}O_{2max}$, along with a 13.2% increase in cycling ride time to fatigue. Further study of the effects of quercetin supplementation by fit, but not highly-trained, individuals is warranted.

Few studies have examined the anti-oxidant effects of quercetin on physical performance. While some studies show that dietary anti-oxidant supplementation improves swimming time to exhaustion in animals (2, 53, 54), there is little evidence that anti-oxidant supplementation improves physical performance in humans (57). MacRae and Mefferd (41) found that 6 weeks of dietary supplementation with an anti-oxidant health drink containing quercetin (FRS®, The FRS Company, Foster City, CA) improved 30-km cycling time trial performance by 3.1% over a control test in 11 elite male cyclists, an effect attributed to quercetin's anti-oxidant properties. However, the performance improvement was not significantly greater than the improvement by the control. Therefore, it is not clear that quercetin improved performance relative to the control.

It has been hypothesized that dietary anti-oxidant supplementation may improve exercise performance by minimizing damage to skeletal muscle membranes, and structural and contractile proteins. If this is true, quercetin may limit the acute, negative, fatiguing effects of increased reactive oxygen species generated during high intensity endurance exercise (41). As such, anti-oxidant supplementation may reduce muscle damage and soreness following exercise and facilitate recovery. The literature on this topic is equivocal (35, 57), with some studies suggesting soreness (64, 65) may be

improved, and others finding no effect of quercetin (29). Further study is needed to determine whether quercetin supplementation minimizes soreness and facilitates recovery, thereby enhancing physical performance, in those participating in habitual physical training.

Quercetin has also been shown *in vitro* to be an adenosine A_1 receptor antagonist (1), and thus, may have analgesic effects similar to caffeine. Therefore, it could affect physical performance during exercise by reducing pain and the perception of effort, as occurs with caffeine (13, 17, 45). Quercetin may also have direct effects on muscle by augmenting force production capability (33). Although two studies with well-trained cyclists and runners (9, 67) do not suggest ratings of perceived exertion during prolonged endurance exercise are reduced by quercetin supplementation, more research is required to examine quercetin's effect on perception of effort in moderately-trained young men and women.

A critical component related to certain types of physical performance is reaction time (58), or how quickly individuals are able to respond to changes in their environment. It is not well established how this aspect of cognition is affected by quercetin, but animal studies suggest that quercetin supplementation may have some positive effect on cognitive function (46, 56, 61). Naidu et al. (46) observed beneficial effects on memory in a maze learning task following quercetin supplementation, while Patil et al. (56) reported better avoidance behavior and memory in a maze learning task in old, but not young mice, after quercetin consumption. In these studies, quercetin supplementation reversed or offset age-related declines in memory. Animal studies also support the benefits of other sources of flavonoids (e.g., blueberries, grape juice, strawberries,

spinach) on motor learning after exposure to oxidative stress and inflammation interventions (3, 19, 60). It is hypothesized that flavonoids impact cognitive function in older animals by altering kinase activation and neuronal communication (34).

In humans, epidemiological evidence suggests a relationship between flavonoid consumption and protection against the onset of age-related cognitive decline (62) and stroke (14, 36). However, few studies have examined the specific effects of quercetin on cognitive function. One published abstract (59) reported that quercetin protected against mental fatigue and loss of sustained attention following 3 days of heavy exercise. In unpublished work by Cureton et al., quercetin supplementation was associated with more rapid response time (marginally significant; $p = 0.072$) and fewer choice response errors (significant; $p = 0.021$) in the Switch-Task Test, a measure of cognition (K. Cureton, personal communication, December 23, 2008). Results from another unpublished study by Durak and Bell (E. Durak, personal communication, February 21, 2009) suggested that 3 weeks of dietary supplementation with an anti-oxidant health drink containing quercetin (FRS®) in an occupational setting improved several work-related measures, including concentration. It is unknown how quercetin supplementation affects simple reaction time in moderately-trained young men and women.

Moods of Energy and Fatigue

Quercetin's effects on the transient moods of energy and fatigue are unknown. Davis et al. (15) reported that short-duration quercetin feedings (12.5 and 25 mg/kg/d) in mice increased both muscle and brain markers of mitochondrial biogenesis, mtDNA content, and cytochrome-c concentration in a dose-dependent fashion. There was also an increase in voluntary wheel running performance (distance run, time on wheel, and peak

speed), partially attributed to quercetin's effect on the central nervous system. The authors hypothesized that the increased brain mitochondrial activity might enhance cerebral metabolism, affecting motivation and the moods of energy and fatigue, thereby resulting in more voluntary physical activity.

In humans, studies of the effects of quercetin on energy and fatigue are limited and offer conflicting results. After 3 days of heavy exercise, 3 weeks of quercetin supplementation afforded protection in trained cyclists against mental fatigue and loss of vigilance (59). Durak and Bell reported that 3 weeks of supplementation with FRS® in an occupational setting improved measures of work performance, work frustration, fatigue status, and concentration (E. Durak, personal communication, February 21, 2009). In another study, Durak and Taguchi observed that 3 months of FRS® supplementation improved perceived levels of energy and fatigue in those with advanced cancers (E. Durak, personal communication, February 21, 2009).

These positive results differ from a published thesis (55) examining varying doses of acute caffeine and quercetin ingestion on mood and vigilance, which reported that quercetin supplementation was not associated with improvements in either mood or vigilance. Similarly, unpublished research from USARIEM suggested that consumption of a quercetin-containing food bar had no positive or beneficial effect on either vigor or fatigue levels (G. Adam, personal communication, March 3, 2009). It may be that the effect of quercetin on the moods of energy and fatigue is not observed after acute supplementation, but requires chronic feeding.

The mechanisms behind any effect of quercetin on the moods of energy and fatigue are unknown. Similar to reaction time, quercetin's effect on transient feelings in

the brain may be associated with alteration of kinase activation and neuronal communication (34). It may also be related to its properties as an adenosine A_1 receptor antagonist (1). In a study conducted during U.S. Navy SEAL training, the A_1 receptor antagonist caffeine was associated with improvements in self-reported moods of vigor and fatigue during exposure to intense physical and mental stressors (39). Nonetheless, the effects of chronic quercetin ingestion on the moods of energy and fatigue remain unexplored in moderately-trained persons subjected to military physical training.

Sleep

There are no published research studies examining the effect of quercetin on measures associated with sleep. It is plausible that as an adenosine A_1 receptor antagonist, quercetin may have a negative effect on sleep, as the A_1 receptor is closely associated with regulation of sleep and alertness (39). Lieberman et al. (39) reported that caffeine, an adenosine receptor antagonist, reduced self-reported sleepiness during intense Navy SEAL training. However, in an unpublished pilot study conducted by Durak and Taguchi (E. Durak, personal communication, February 21, 2009), supplementation with a quercetin-containing cocktail (FRS®) in those with advanced cancers slightly improved sleep. More research is required to determine quercetin's effect on sleep quality and duration in this population.

Illness

Studies *in vitro* have shown that quercetin reduces both infectivity and replication of many respiratory viruses, adenoviruses, coronaviruses, rhinoviruses, the parainfluenza virus type 3, and severe acute respiratory syndrome (16). Quercetin's anti-viral properties appear to be related to its ability to bind to viral proteins and disrupt the early stage of

viral DNA synthesis (24, 51). Another possible explanation for quercetin's effects may be related to its ability to inhibit signaling of the protein NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), a transcription factor that regulates genes involved in immune and inflammatory process (47).

In animals, Davis et al. (16) reported that short-duration quercetin supplementation decreased susceptibility for respiratory infection following stressful exercise in mice. After exposure to 3 consecutive days of exercise to fatigue, the mice displayed an increase in susceptibility to the influenza virus. However, infection susceptibility, characterized by mice morbidity, mortality, and symptom severity, was offset by 7 days of quercetin feedings. In mice that rested and were not stressed, there was also a trend toward possible benefits of quercetin supplementation on infection susceptibility, but this trend was not statistically significant (16).

Recent human research examining illness and immune function determined that quercetin reduced the incidence of upper respiratory tract infection (URTI) following 3 days of exhaustive exercise by well-trained cyclists, but it did not significantly alter several markers of immunity or inflammation (51). In the 2 weeks following heavy exercise among those cyclists supplementing with quercetin, the URTI rate was 5%. URTI rate in the control group was significantly higher at 45% (51). Similar, but less dramatic, illness trends were observed for athletes supplementing with quercetin and competing in the 160-km Western States Endurance Run (48). Since markers of immunity or inflammation were not significantly altered, the authors suggested that quercetin may have reduced the URTI rate via anti-pathogenic pathways. The effects of

quercetin supplementation on illness rate and/or severity in young men and women exposed to regular physical training are unknown and merit further study.

As summarized, there is limited and conflicting research on the effects of quercetin supplementation on physical performance, mood, sleep, and illness in humans. Treatment duration, state of training, and quercetin ingestion schedule (acute vs. chronic) make interpretation and generalization of the results challenging. To date, there is no published research on the effects of 6 weeks of dietary quercetin supplementation on physical performance, mood, sleep, and illness parameters in moderately-trained young men and women exposed to regular military physical training. This gap in the literature is addressed in the following chapters.

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CHAPTER 3

EFFECTS OF SIX WEEKS OF QUERCETIN SUPPLEMENTATION ON PHYSICAL PERFORMANCE IN ROTC CADETS[1]

[1] Bigelman, Kevin, A., Eugene H. Fan, Donald P. Chapman, Eric C. Freese, Jennifer L. Trilk, and Kirk J. Cureton. To be submitted to *Military Medicine.*

Abstract

Objective: To investigate the effects of 6 weeks of quercetin supplementation on physical performance in men and women enduring military physical training. **Methods:** Using a randomized, double-blind, repeated-measures, placebo-controlled design, 58 healthy, moderately-trained young men and women undergoing regular military physical training were randomly assigned to Quercetin (Q) and Placebo (P) groups. Peak oxygen uptake ($\dot{V}O_{2peak}$) during maximal-effort uphill treadmill running, four physical performance measures [Army Physical Fitness Test (APFT), Baumgartner Modified Pull-Up Test (BMPU), Wingate Anaerobic Test (WAnT), and 36.6-m sprint], and a simple reaction time test [Walter Reed palm-held psychomotor vigilance test] were evaluated before and after 42-54 days of supplementation with 1 g/d of quercetin with vitamins and other substances in a soft chew or a placebo chew. **Results:** Pretreatment-to-posttreatment changes in $\dot{V}O_{2peak}$ and performance on the APFT, BMPU, WAnT, and 36.6-m sprint were not significantly different ($p > 0.05$) in Q and P. Pre-, mid-, and posttreatment changes on the Walter Reed palm-held psychomotor vigilance test were not significantly different ($p > 0.05$) between groups. **Conclusion:** Six weeks of dietary quercetin supplementation in moderately-trained young men and women involved in regular military physical training does not improve $\dot{V}O_{2peak}$, performance on the APFT, BMPU, WAnT, 36.6-m sprint, or simple reaction time.

Keywords: Quercetin, Flavonoids, Oxygen consumption, Reaction time, Military physical training, ROTC

Introduction

Quercetin (3,3',4',5,7-pentahydroxyflavone) is a naturally-occurring polyphenolic flavonol, belonging to the larger class of flavonoids, regularly consumed in the human diet (1, 23). Quercetin is found in numerous fruit, vegetable, and beverage sources including apples, berries, red grapes, red onions, spinach, fruit juices, red wine, and black tea (23, 42, 62). Quercetin accumulates in the skin and leaves of fruits and vegetables, as its biosynthesis is stimulated by exposure to sunlight (35). According to national dietary records, mean daily quercetin intake in the typical diet varies from less than 5 mg to 40 mg, but levels as high as 200-500 mg/d may be obtained by those consuming large quantities of fruits and vegetables (23).

Quercetin has anti-oxidant, anti-inflammatory, anti-carcinogenic, and cardio-protective properties (22, 23, 36, 57). It also reduces susceptibility to upper respiratory tract infections and influenza following intense exercise (18, 43). Based on its health-promoting properties, quercetin is marketed as a nutritional supplement and food and beverage additive.

A limited number of recent studies have also investigated the ergogenic effects of quercetin on physical performance in animals. In sedentary mice, Davis et al. (17) reported that 7 days of quercetin feedings (12.5 and 25 mg/kg/d) increased mRNA expression of coactivators of mitochondrial biogenesis (sirtuin 1 (SIRT1) and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α)), mitochondrial protein (cytochrome-c) concentration, DNA in the brain and skeletal muscle, and treadmill run time to exhaustion by 36-37%. The increase in muscle oxidative capacity and endurance with quercetin supplementation was similar to that found with resveratrol, another

polyphenol found in red grape skins. Lagouge et al. (33) reported that mice treated with resveratrol also increased skeletal muscle oxidative capacity and treadmill endurance performance. Resveratrol initiates mitochondrial biogenesis in skeletal muscle by activation of the intracellular signaling proteins SIRT1 and PGC-1α (7, 33). Compared to wild-type mice, transgenic sedentary mice with high levels of skeletal muscle PGC-1α have greater mitochondrial content, peak rate of oxygen consumption, and capacity for exercise (11). These studies on mice treated with quercetin and resveratrol offer the possibility that dietary supplementation with certain polyphenols by humans may induce mitochondrial biogenesis and enhance exercise performance in a manner similar to endurance training (26, 38).

In humans, however, the ergogenic effects of quercetin supplementation on physical performance are less clear. If quercetin increases skeletal muscle oxidative capacity, it may increase maximal oxygen uptake ($\dot{V}O_{2max}$) by increasing the maximal arteriovenous oxygen content difference. However, prior studies find no significant changes in $\dot{V}O_{2max}$ following quercetin supplementation (K. Cureton, personal communication, 23 December, 2008, M. Ganio, personal communication, February 18, 2009, (34)). If quercetin induces mitochondrial biogenesis, mitochondrial enzyme activity would be expected to increase. In well-trained cyclists, however, Dumke et al. (21) reported that 3 weeks of quercetin supplementation (1 g/d) had no effect on mitochondrial cytochrome-c concentration. It is also plausible that physical performance in field trials might be improved if quercetin has an ergogenic effect in humans, though finish times for a 160-km ultra-marathon were unaffected by 3 weeks of quercetin supplementation (1 g/d) in trained male and female runners (24, 41). It may be that

quercetin supplementation increases skeletal muscle oxidative capacity through mitochondrial biogenesis and enhances endurance performance in untrained to moderately-trained individuals, but not in the well-trained, as a physiological ceiling for mitochondrial content may already exist in these individuals (10, 27).

There are very few studies on the effect of quercetin in less highly-trained individuals. In healthy, untrained college males, Cureton et al. (K. Cureton, personal communication, December 23, 2008) observed that short-duration chronic quercetin supplementation (1 g/d) did not improve muscle oxidative capacity. Metabolic and perceptual determinants of performance during prolonged cycling, as well as 10-min cycling time trial performance, were also unaffected by quercetin supplementation (K. Cureton, personal communication, December 23, 2008). Similarly, Ganio et al. (M. Ganio, personal communication, February 18, 2009) reported that 5 days of quercetin supplementation (1 g/d) by sedentary men and women did not influence $\dot{V}O_{2max}$ and its related physiological variables. However, Chen et al. (12) observed that 7 days of quercetin ingestion (1 g/d) by healthy, fit, but not highly-trained college students was associated with a small, but significant increase in $\dot{V}O_{2max}$, along with a 13.2% increase in cycling ride time to fatigue. Further study of the chronic effects of quercetin supplementation on $\dot{V}O_{2max}$ by fit, but not highly-trained, individuals is warranted.

If quercetin has an ergogenic effect in humans, it could be related to its anti-oxidant properties. While some animal studies show that dietary anti-oxidant supplementation improves swimming time to exhaustion (19, 44, 45), there is little evidence that anti-oxidant supplementation improves human physical performance (48). Only MacRae and Mefferd (34) found that 6 weeks of dietary supplementation with an

anti-oxidant health drink containing quercetin (FRS®, The FRS Company, Foster City, CA) improved 30-km cycling time trial performance by 3.1% above a baseline test in elite male cyclists, an effect they attributed to its anti-oxidant properties. However, the performance was not significantly greater than the improvement by the control, so it is unclear that it was quercetin that improved performance.

It is possible that dietary anti-oxidant supplementation may improve exercise performance by minimizing damage to skeletal muscle membranes, and structural and contractile proteins. If this is true, quercetin may limit the acute, negative, fatiguing effects of increased reactive oxygen species generated during high-intensity endurance exercise (34). As such, anti-oxidant supplementation may reduce muscle damage and soreness following exercise and facilitate more rapid recovery. The literature on this topic is small and equivocal (30, 48), with some studies suggesting soreness (58, 59) may be improved, and others finding no effect of quercetin (25). One study demonstrated that quercetin supplementation did not attenuate the loss of force that occurs as a result of prolonged endurance exercise (K. Cureton, personal communication, December 23, 2008). Quercetin may also have direct effects on skeletal muscle by augmenting force production capability, as observed in one animal study (29). To date, the effects of quercetin supplementation on power, speed, and muscular endurance are uninvestigated. It is plausible that quercetin may reduce oxidative damage to muscle or augment force production capability, thereby improving these measures of physical performance.

If quercetin does have an ergogenic effect, it could be related to its property as an *in vitro* adenosine A_1 receptor antagonist (1), resulting in analgesic effects similar to caffeine. As such, it may affect physical performance during exercise by reducing the

perception of effort and pain, as occurs with caffeine (16, 20, 39). However, Cheuvront et al. (13) recently reported that an acute quercetin dose (2 g) had no effect on perceptual measures of pain and effort during endurance exercise in the heat. Although two studies with trained cyclists and runners (14, 63) also suggested that ratings of perceived exertion during prolonged endurance exercise were not reduced by quercetin, examination of the effects of chronic quercetin ingestion on the perception of effort in moderately-trained individuals is warranted.

Quercetin's effect on cognition is also unknown. Animal studies suggest that quercetin supplementation may have some positive effect on memory (40, 47, 55). One published abstract of a study in humans (52) reported that quercetin protects against post-exercise mental fatigue and loss of vigilance after intense exercise. To date, no research has examined the effects of quercetin supplementation on simple reaction time in moderately-trained humans.

The objective of this study was to examine the ergogenic effects of 6 weeks of quercetin supplementation on peak oxygen uptake ($\dot{V}O_{2peak}$) and four physical performance tests [Army Physical Fitness Test (APFT), Baumgartner Modified Pull-Up Test (BMPU), Wingate Anaerobic Test (WAnT), and 36.6-m sprint], in moderately-trained individuals subjected to regular military physical training. The effect of simple reaction time, a component of certain types of physical performance (49), was also evaluated. It was hypothesized that quercetin supplementation would improve $\dot{V}O_{2peak}$, all measures of physical performance, and simple reaction time compared to 6 weeks of placebo ingestion. If the ergogenic effects of quercetin can be confirmed in this

population, it may have important implications for the military, as success on the battlefield is strongly linked to these outcome measures.

Methods

Participants. Fifty-eight healthy, moderately-trained males and females, aged 18-40 y, recruited from Army and Air Force Reserve Officers' Training Corps (ROTC) programs completed this study. Moderately-trained individuals were selected to participate because the effects of quercetin on $\dot{V}O_{2peak}$, the primary outcome measure, in this population are unknown. Using a mixed-model repeated measures analysis of variance (ANOVA), this sample size was sufficient to detect a moderate Group x Treatment Time interaction effect for $\dot{V}O_{2peak}$ during graded treadmill running of approximately 0.33 standard deviation with an alpha of 0.05 and a power of 0.8 (46), assuming a correlation between repeated trials of 0.95 (56).

After signing an informed consent statement approved by the Institutional Review Board, participants completed physical activity history, medical history, and diet questionnaires. Physical activity history was assessed using a 7-day physical activity recall (7-D PAR) questionnaire (8) and an athletic history questionnaire. Participants reported no contraindications to maximal exercise testing and all met the low risk health stratification standard for exercise testing recommended by the American College of Sports Medicine (2). A food frequency questionnaire, based on the Youth-Adolescent Questionnaire (53, 54), was administered to assess pretreatment habitual dietary quercetin intake. Food models were provided to ensure accurate portion-size reporting.

Participants were asked to abstain from non-prescription drugs, vitamins, herbs, and other dietary supplements for the duration of the study. Dietary quercetin intake was

not controlled, as low levels of quercetin are found in fruits, vegetables, and beverages (62) commonly consumed by this demographic. No financial compensation was given for participation.

The study commenced at the beginning of the academic year. In accordance with ROTC programs of instruction, participants were engaged in systematic military physical training a minimum of three times per week for the duration of the treatment period. Individual training sessions lasted 1 h and consisted of muscular strength and endurance exercises, as well as cardio-respiratory and flexibility training.

Research design. A randomized, double-blind, repeated-measures, placebo-controlled design was used for this experiment. A repeated-measures, cross-over design, with increased statistical power, was not used because of the lengthy treatment duration and the time required to conduct all pretest-posttest outcome measures. Additionally, the time needed for washout of the effects of chronic quercetin ingestion is unknown.

To ensure equal aerobic capacity within each group, men and women were separately ranked according to $\dot{V}O_{2peak}$ and subsequently divided into matched pairs. One individual from each pair was randomly assigned to the experimental group consuming quercetin (Q) and the other to the placebo group (P). Randomization was performed using Research Randomizer (www.randomizer.org). Q and P each contained 22 men and 7 women. The effects of the treatment intervention were evaluated by assessing $\dot{V}O_{2peak}$ and four physical performance tests, before and immediately prior to the end of treatment. Physical performance tests assessed aerobic capacity, muscular endurance, power, and speed of participants. Additionally, the effect of the treatment on simple reaction time, or how quickly participants responded to changes in their environment,

was evaluated before, during, and immediately prior to the end of treatment. Evaluation of simple reaction time occurred at three time periods to increase the likelihood of detecting an effect.

Treatment. Participants in Q ingested four individually-wrapped chews daily (two with breakfast, two with dinner) during the treatment period. The purpose of the multiple feedings was to maintain plasma quercetin levels throughout the day (37). Each chew contained 250 mg quercetin, 100 mg isoquercetin, 100 mg omega-3 fatty acids (eicosapentaenoic acid (EPA) & docosahexaenoic acid (DHA)), 30 mg epigallocatechin gallate (EGCG), a vitamin mixture, sucrose, and other ingredients in a carnuba wax and soy lecithin base (Quercegen Pharma, Newton, MA). Participants in P also ingested four chews daily at the same time periods. Chews for P were similar in appearance and identically-wrapped, but contained no quercetin, isoquercetin, EPA & DHA, EGCG, or vitamin mixture. Participants turned in chew wrappers weekly for verification of ingestion. Investigators and participants were blinded to the treatment identity until data collection and analysis were completed.

Participants remained on the treatment for 42-54 days, beginning immediately after all pretest measures were completed and continuing until the conclusion of all posttest measures. Pretests and posttests required between 8-12 days per person for administration, with a minimum of 48 h between each physical test. As a result, some participants remained on the treatment several days longer than others. Mean treatment duration was 52 days, while overall duration of the study was approximately 10 weeks.

Outcome measures. In order to evaluate the potential effects of dietary quercetin supplementation on physical performance relative to a control, $\dot{V}O_{2peak}$ during uphill

treadmill running, performance on the APFT, muscular endurance, power, speed, and reaction time were measured. $\dot{V}O_{2peak}$ and APFT 3,219-m (2-mile) run time were used to assess changes in whole-body aerobic capacity. Changes in muscular endurance, power, and speed measures due to quercetin supplementation were used to assess any potential reductions in oxidative damage to muscle, as force loss is one of the best markers of muscle damage (64). Changes in reaction time were measured to determine quercetin's effect on one aspect of cognitive function. The 7-D PAR was completed weekly to measure differences in physical activity levels between groups.

Preliminary measures. Participants attended a preliminary test session at which physical characteristics and body composition were assessed, and procedures used in assessment of outcome measures were practiced. Body height was measured using a wall stadiometer, nude body mass was determined using an electronic scale (model FW-150KA1, A&D Co., Ltd., Tokyo), and body composition was estimated using dual-energy x-ray absorptiometry (iDXA, GE Healthcare-Lunar, Madison, WI). Participants then practiced three of the unfamiliar physical performance tests ($\dot{V}O_{2peak}$, WAnT, and BMPU), use of the perceived exertion and pain scales during exercise, and the Walter Reed palm-held psychomotor vigilance test.

Physical performance and reaction time test preparation. Participants were asked to abstain from the potentially confounding effects of caffeine, alcohol, and nicotine 24 h before testing. Strenuous exercise was also limited 48 h prior to testing. Mid- and posttest sessions were conducted at the same time of day as pretest sessions.

Peak oxygen uptake ($\dot{V}O_{2peak}$). $\dot{V}O_{2peak}$ was used to assess whole-body aerobic capacity. Upon arrival at the laboratory, participants completed a 24-h history form to

assess compliance with pretest instructions. Diet records were kept for 2 days prior and for the day of the $\dot{V}O_{2peak}$ test, and this diet was replicated for the posttest session. Euhydration was ensured by instructing participants to drink liberally the day before and to drink one 240-mL glass of water 1 h prior to testing. After a 5-min low intensity running warm-up on a treadmill (Trackmaster, JAS Fitness System, Newton, KS), a graded treadmill running test following the modified Åstrand protocol (3) was administered to evaluate $\dot{V}O_{2peak}$. Treadmill speed was 3.129 m/s (male) or 2.682 m/s (female) and initial treadmill grade was 0%. After each 2-min interval, grade was increased by 2.5% while speed remained constant. Participants ran until volitional exhaustion.

Oxygen uptake and related gas exchange measures were obtained by open-circuit spirometry using a Parvo Medics TrueOne 2400 Metabolic Measurement System (Parvo Medics, Inc., Salt Lake City, UT) and averaged at 1-min intervals. Standard gases of known composition were used to calibrate the oxygen and carbon dioxide analyzers, and a 3-L syringe was used to calibrate the pneumotachometer prior to each test session. All tests were conducted at the same temperature (21-24^{o}C) and relative humidity (<58%). Heart rate (HR), rating of perceived exertion (RPE), and thigh muscle pain were measured every 2 min, and at test completion. HR was measured with a Polar® Vantage XL heart rate monitor (Polar Electro, Inc., model 145900, Woodbury, NY). RPE was measured by the Borg 15-point category scale and thigh muscle pain was assessed with a validated 0-10 pain intensity category scale described by Cook et al. (15). Three minutes after exercise, a capillary blood sample from the index finger was obtained for determination of peak blood lactate concentration [La] (Lactate Pro Test Meter, model

LT-1710, Arkray, Inc., Kyoto, Japan). All participants met at least 3 of the 4 criteria for maximal effort: [La] ≥ 7-9 mmol/L, respiratory exchange ratio (RER) ≥ 1.10, RPE ≥ 18 on the Borg scale, or HR within 5% (10 bt/min) of age-predicted maximum.

Army Physical Fitness Test (APFT). The APFT is an important assessment tool used by United States Army commanders to determine physical fitness readiness of soldiers. Performance on the APFT has been strongly associated with an individual's fitness level and ability to do fitness-related tasks (61). Participants completed a standard APFT, at an outdoor 400-m oval track, consisting of the maximum number of push-ups and sit-ups possible in 2 min and a 3,219-m (2-mile) run for time. The APFT was conducted in accordance with U. S. Army Field Manual 21-20 and scored out of a maximum of 300 points based on age and gender-specific scales using Department of the Army Form 705 (61). Push-up repetitions were used to estimate muscular endurance of the chest, shoulders, and triceps. Sit-up repetitions were used to estimate muscular endurance of the abdominal muscles and hip flexors. Run time was used as an additional means to assess changes in whole-body aerobic capacity (32).

Baumgartner Modified Pull-Up Test (BMPU). The BMPU was used to assess changes in arm and shoulder girdle muscular endurance. Male and female participants performed the maximum number of modified pull-up repetitions, as described by Baumgartner (5, 6), until they reached volitional failure, unable to raise their chin over a horizontal bar positioned on a 30-degree inclined board. The BMPU was selected over traditional pull-ups as 92-95% of college-aged females cannot perform a single pull-up (50).

Wingate Anaerobic Test (WAnT). The WAnT was used to assess changes in estimated mean anaerobic power output. The test was conducted on a mechanically-braked cycle ergometer (Monark Ergomedic 874E, Stockholm, Sweden) with resistance set at 0.098 kp/kg (male) or 0.085 kp/kg (female), as recommended by Bar-Or (4). Upon arrival at the laboratory, participants completed a 24-h history form to assess compliance with pretest instructions. After a 5-min warm-up of unloaded cycling, participants performed 5 s of loadless pedaling to achieve maximum cadence. Participants then completed 30 s of maximal pedal revolutions against the applied resistance. Monark Anaerobic Test software v. 2.22 (Monark Exercise AB, Vansbro, Sweden) was used to obtain peak and mean power output, and fatigue percentage (9).

36.6-m sprint. The 36.6-m (40-yd) sprint was used to assess changes in participants' speed. After a 10-min warm-up consisting of low-intensity running, stretching, and several acceleration runs, participants performed two timed, maximum-effort 36.6-m sprints on an indoor gymnasium wooden floor, with a 5-min recovery period between each trial. Participants assumed a crouched start with both feet behind the start line. Distance was determined using a measuring wheel (Measure Master, Rolatape MM-12M, Spokane, WA) and a wireless sprint timing system (Brower Timing System, model IRD-TI75, Draper, UT) was activated at the initiation of movement across the start line, recording participants' times to the nearest $1/100^{th}$ s at the finish.

Walter Reed palm-held psychomotor vigilance test. This portable 4-min vigilance test (Walter Reed Army Institute of Research, Silver Springs, MD), run on a personal digital assistant (PDA, Palm PVT 2.0.0), was used to assess simple reaction time (60). The test was conducted in a quiet room, free of distractions, with the participant seated in

a desk. Participants were asked to respond to a stimuli as quickly as possible by touching a stylus to the screen of the PDA. Reaction times measured by this test are sensitive to sleep deprivation, circadian rhythms, time-on-task, fatigue, and order effects (60). Data collected for each test session included sequential trial number, foreperiod, response time, response type (e.g., valid, anticipatory, wrong button), and elapsed time from the start of the session.

Blood samples. Ten mL venous blood samples were obtained by phlebotomists from a superficial antecubital vein. Blood collection occurred after a 12-h fast with participants seated and the arm in a standarized position. Samples were obtained pre-treatment, mid-treatment, and immediately prior to the conclusion of treatment to measure plasma quercetin concentration. Whole blood samples were collected in lithium heparin tubes and immediately centrifuged for 10 min at 2,000 rpm. Two mL plasma samples were aliquoted into two Corning®-cryogenic vials and frozen at -70^{o}C until quercetin analysis.

Plasma quercetin. Total plasma quercetin (quercetin and its primary conjugates) was measured following solid-phase extraction by reverse-phase high performance liquid chromatography (HPLC) in a procedure similar to that described by Ishii et al. (28). Quercetin conjugates were hydrolyzed by incubating 500 µL plasma with 10 µL 10% DTT solution, 50 µL 0.5 M acetic acid, 10 µL 0.356 mM fisetin internal standard, and 50 µL enzyme β-glucuronidase/arylsulfatase for 2 h at 37^{o}C. After incubation, 500 µL 0.01 M oxalic acid was added to stop the reaction, and each sample was centrifuged for 5 min at 10,000 rpm. One mL of supernatants were then applied to solid-phase extraction (SPE) cartridges (Oasis HLB 1cc SPE cartridge, Waters, Milford, MA) that were

preconditioned with 1 mL 5% methanol, 0.5 mL 0.01 M oxalic acid, and 1 mL distilled water drawn through at a rate of 0.2 mL/min using a vacuum manifold (Waters, Milford, MA). SPE cartridges were then washed twice with 1 mL 5% methanol in 0.5 M phosphoric acid solution and 50% methanol in 0.5 M phosphoric acid solution, and purged with air for several minutes to ensure full removal of wash solutions. Ten µL 10% DTT solution were added to the combined eluent, and the samples were gently vortexed for 1 min and placed into a vacuum concentrator until the methanol was completely evaporated. The residue was reconstituted with 150 µL 50/50 methanol/water mixture. After centrifuging for 2 min at 3,000 rpm, 150 µL were transferred to a conical autosampler. Fifty µL injections of this solution were used for HPLC analysis.

Chromatographic analysis was performed using a Waters Breeze HPLC system (Waters, Milford, MA) consisting of a Waters 1525 Binary HPLC pump, 2487 ultraviolet detector, and Symmetry C_{18} (particle size: 5-µm steel) 4.6 x 150-mm column. Quantification of the quercetin peak was based on the standard addition method using both plasma and methanol. Both standards and samples were treated identically.

Statistical analysis. All data were analyzed using the statistical software SPSS v. 15.0 (SPSS, Inc., Chicago, IL). Differences between Q and P in physical characteristics, body composition, and physical activity history at the pretest were determined using *t*-tests for independent samples. Differences between Q and P in pretreatment-posttreatment changes on the outcome measures were analyzed using a two-way (Group x Treatment Time) or three-way (Group x Treatment Time x Test Time) mixed-model ANOVA with repeated measures for the factors involving time. Analysis of covariance (ANCOVA) was used to evaluate the effects of plasma quercetin levels and body weight,

which may have caused different effects among individuals. All tests were considered significant at alpha level < 0.05.

Results

Participant characteristics and physical activity. The physical characteristics of participants (height, mass, fat-free mass, % body fat, bone mineral density), $\dot{V}O_{2peak}$, and average weekly energy expenditure in Q and P were not different prior to the treatment (Table 3.1). There were, however, significant age and years of self-reported organized athletic history differences between groups. These differences occurred because Q contained two older participants (aged 37 y and 40 y) with many more years (23 y and 28 y) of participation in organized athletics. When these individuals' ages and years of athletic participation were treated as outliers, there were no age ($p = 0.138$) or athletic history ($p = 0.490$) differences between groups. These data indicate that randomization created groups nearly-equivalent in physical characteristics, aerobic fitness, and physical activity prior to treatment. During treatment, no significant physical activity differences existed between groups according to 7-D PAR. After the treatment, neither body mass nor physical activity, measured by 7-D PAR, was significantly different ($p > 0.05$) compared to pretest values, in either group.

Dietary and plasma quercetin. The food frequency questionnaire, used to assess pretreatment habitual dietary quercetin intake, classified participants as either low, medium, or high consumers of fruits, vegetables, and beverages containing significant quantities of quercetin. Q contained 11 low, 10 medium, and 8 high quercetin consumers, while P contained 14 low, 8 medium, and 7 high quercetin consumers. When proportional numeric values were assigned to foods and beverages, based on their

estimated quercetin quantity, and the various portion sizes, mean (± SD) quercetin intake values were 778.9 ± 458.4 units for Q and 732.4 ± 666.9 units for P. These data suggest that randomization created groups with nearly-equal pretreatment habitual dietary quercetin intake.

Plasma quercetin was measured from whole blood samples taken before, at the midpoint, and immediately prior to the end of treatment. A series of planned comparisons using post-hoc independent *t*-tests were performed revealing no differences in plasma quercetin concentration between groups pretest, but significant differences at mid- and posttest time points ($p < 0.000$) (Figure 3.1). Total plasma quercetin in Q increased significantly above baseline by 20.6-fold at midtreatment and 15.7-fold at posttreatment, and decreased in P. The decrease in plasma quercetin levels in Q from mid- to posttreatment measures may be due to differences in chew ingestion times and when blood samples were taken on the two occasions, since the plasma half-life of quercetin (6-12 h) is relatively short (23). These results still demonstrate that ingestion of the experimental chew had the intended effect of significantly elevating total plasma quercetin. Additionally, ANCOVA revealed no effect of body weight on plasma quercetin levels ($p > 0.05$).

$\dot{V}O_{2peak}$. Prior to treatment, mean percentile values for $\dot{V}O_{2peak}$ for young men and women in this study were approximately 70%, indicating that participants were moderately trained with "above average" aerobic fitness (2). All participants met the established criteria (3 of 4 conditions met for maximal effort: [La] ≥ 7-9 mmol/L, RER ≥ 1.10, RPE ≥ 18 on Borg scale, or HR within 5% (10 bt/min) of age-predicted maximum) for achievement of $\dot{V}O_{2peak}$ on each test. Data on the effect of the treatment on $\dot{V}O_{2peak}$

and related measures are presented in Table 3.2 and Figure 3.2. The interaction between Group x Treatment Time for $\dot{V}O_{2peak}$ was non-significant ($p > 0.05$) when expressed in absolute terms (L/min; $p = 0.238$) and relative to body weight (mL/kg min; $p = 0.132$). When taking sex into account, results were also non-significant (L/min: male $p = 0.533$, female $p = 0.084$; mL/kg min: male $p = 0.414$, female $p = 0.078$).

The mean (± SD) of individual changes from pretreatment to posttreatment in $\dot{V}O_{2peak}$ was 1.4 ± 2.4 mL/kg min (2.8 ± 10.1%) or 89 ± 180 mL/min (2.4 ± 4.2%) in Q and 0.4 ± 2.5 mL/kg min (0.8 ± 3.8%) or 33 ± 180 mL/min (0.9 ± 1.5%) in P. The mean change for time on the graded exercise test, a measure of work capacity, was 0.3 ± 0.9 min (3.5 ± 2.9%) in Q and 0.2 ± 0.9 min (2.6 ± 10.5%) in P. However, the Group x Treatment Time interactions were not statistically significant ($p > 0.05$) for these measures, indicating pretreatment-to-posttreatment changes were not different between Q and P.

There were no significant Group x Treatment Time interactions for ventilation ($\dot{V}_E$), RER, HR, thigh pain, and [La] at $\dot{V}O_{2peak}$, indicating the changes from pretreatment-to-posttreatment were not different between the two groups. The means for $\dot{V}_E$, RER, HR, peak thigh pain, and [La] at $\dot{V}O_{2peak}$ were typical of those expected, indicating that participants in both groups gave similar maximal efforts during both tests. Analysis of RPE at $\dot{V}O_{2peak}$ revealed a significant Group x Treatment Time interaction ($p = 0.047$), but this finding is of little relevance as RPE was only used as one indicator of maximal effort during the treadmill test.

Physical performance measures. APFT, BMPU, WAnT, and 36.6-m sprint physical performance measures are presented in Table 3.3. There were no statistically

significant differences ($p > 0.05$) in pretreatment-to-posttreatment changes for any aerobic capacity, muscular endurance, power, or speed measures. The effect of gender also had no influence on these measures ($p > 0.05$).

Simple reaction time. The mean (± SD) of individual changes from pretreatment to posttreatment in simple reaction time was -15.06 ± 5.5 ms or -5.39% in Q and -3.36 ± 1.4 ms or -1.21% in P (Figure 3.3). While Q showed a trend in reduction of simple reaction time at each time point, these differences were not statistically significant ($p > 0.05$). Additionally, there were no significant differences between groups in the number of errors committed at each time point ($p > 0.05$).

Discussion

The purpose of this investigation was to determine the effects of 6 weeks of dietary supplementation with a chew containing quercetin on physical performance in moderately-trained individuals undergoing military physical training. Our primary finding was that dietary quercetin supplementation by moderately-trained men and women did not have an ergogenic effect, as assessed using $\dot{V}O_{2peak}$, four physical performance measures, and a simple reaction time test. All pre- to posttreatment changes between and within groups were non-significant. Our data do not support the hypothesis that 6 weeks of quercetin supplementation improves measures of physical performance compared to a placebo.

Although not statistically significant, mean performance on three of the evaluated outcome measures improved compared to pretreatment results. However, the improvements in $\dot{V}O_{2peak}$, APFT, and BMPU performance were consistent with the improvement expected following 10 weeks of military physical training focusing on

muscular endurance and cardio-respiratory training, and likely unrelated to any ergogenic effect of quercetin supplementation. The decline in WAnT and 36.6-m sprint performance from pretreatment to posttreatment may be attributed to the lack of training focus placed on power and speed development during physical training sessions.

There are several possible reasons that the ergogenic effects of quercetin supplementation observed in mice did not appear in moderately-trained humans. It is possible that the quercetin dosage used in this experiment was not optimal. Davis et al. (17) reported that sedentary mice fed quercetin at a dose of 12.5 and 25 mg/kg/d increased skeletal muscle and brain mitochondrial enzyme (cytochrome-c) activities by 20-30%, and treadmill run time to exhaustion by 36-37%. By simple conversion based on body weight, this translates to 937.5 mg/d for a 75-kg human, or slightly less than the 1000 mg/d dosage used in this study, regardless of body weight.

However, in a recent review by Reagan-Shaw et al. (51), the authors recommend that animal dosage not be extrapolated to a human equivalent dose solely based on body weight conversion. A more appropriate approach for conversion between animals uses the body surface area normalization method. When this approach is utilized, the optimal dose translation between mice and humans is 1.01 mg/kg, or 76.0 mg/d for a 75-kg human. It is possible that the dose used in this experiment was too high and may have interfered with the activation of genes that initiate oxidative phosphorylation and mitochondrial biogenesis.

It is also plausible that the treatment duration used in this experiment was too long. The only research to show an ergogenic effect of quercetin supplementation in fit college men and women utilized a treatment duration of 7 days (12). It is possible that the

improvement observed in peak or maximal oxygen uptake occurs within the first week of supplementation. It is conceivable that the lengthy treatment duration used in this experiment made it impossible to capture any significant changes in oxygen uptake, or performance on the other physical measures that may have occurred earlier and subsequently returned to pretreatment levels.

In this experiment, there were small, but insignificant, differences between mean $\dot{V}O_{2peak}$ values between Q and P. The mean (± SD) of individual changes in $\dot{V}O_{2peak}$ from pretreatment to posttreatment was 1.4 ± 2.4 mL/kg min (2.8 ± 10.1%) in Q and 0.4 ± 2.5 mL/kg min (0.8 ± 3.8%) in P. It is possible that the ergogenic effect of quercetin supplementation on $\dot{V}O_{2peak}$ is smaller than the biological and technological variability associated with measurement of this value. With biological and technological variations in measures of maximal aerobic power estimated at ± 5.6% (31), the magnitude of the effects of quercetin treatment observed in this experiment can be considered below the detectable limit.

Quercetin's effect on simple reaction merits further exploration. Although Q and P showed no statistically-significant differences between the vigilance parameters assessed in this study, there was a trend for decreased reaction time in Q. Future research should focus on the optimal dosage to achieve a reaction time effect. Longer treatment durations should also be studied to determine quercetin's effect on reaction time.

Using a randomized, double-blind, repeated-measures placebo-controlled group design, we demonstrated that 6 weeks of quercetin supplementation (1g/d) did not influence $\dot{V}O_{2peak}$, APFT performance, muscular endurance, power, speed, or simple reaction time. Future studies should focus on the appropriate dose for quercetin

supplementation, as well as varying durations of supplementation. The effects of quercetin taken in conjunction with exercise training of different modalities, as well as its effects on other populations in varying states of training, in a variety of environmental conditions is also warranted. Additionally, the study of quercetin's effect on simple reaction time requires further investigation, with focus on dose and treatment duration.

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Disclosures

The experimental and placebo chews used in this experiment were provided by Quercegen Pharma, Newton, MA.

Table 3.1: Participant characteristics (mean ± SD).

Variable	Quercetin (n=29) 22 males, 7 females	Placebo (n=29) 22 males, 7 females	*t*-test *p*-value
Age (y)	22.0 ± 5.1	20.3 ± 1.6	0.009*
Height (cm)			
Male (M):	178.8 ± 5.8	177.3 ± 6.8	0.543
Female (F):	166.4 ± 6.0	165.6 ± 3.6	0.150
Body mass (kg)			
M:	82.9 ± 11.2	76.3 ± 9.5	0.336
F:	67.2 ± 7.7	62.9 ± 8.2	0.761
Fat-free mass (kg)			
M:	64.8 ± 6.0	62.2 ± 6.9	0.465
F:	48.3 ± 6.2	46.3 ± 4.8	0.811
Body fat (%)			
M:	23.2 ± 6.5	19.4 ± 5.9	0.819
F:	30.6 ± 7.9	27.9 ± 3.6	0.164
Bone mineral density (g/cm^2)			
M:	1.25 ± 0.11	1.25 ± 0.10	0.940
F:	1.23 ± 0.12	1.20 ± 0.10	0.207
$\dot{V}O_{2peak}$ (mL/kg min)			
M:	50.2 ± 6.4	50.6 ± 5.8	0.587
F:	42.2 ± 3.8	44.4 ± 4.3	0.684
7-D PAR (kcal/kg wk)			
M:	311.2 ± 62.4	295.2 ± 68.5	0.811
F:	302.5 ± 126.0	332.3 ± 103.3	0.997
Self-reported organized physical activity history (y)	7.2 ± 5.7	4.9 ± 2.1	0.034*

*Significant independent *t*-test *p*-value at $p < 0.05$.

Table 3.2: $\dot{V}O_{2peak}$ and related measures (mean ± SD).

Variable	Pretreatment	Posttreatment	Group x Time Interaction *p*-value
$\dot{V}O_{2peak}$ (L/min)			
Q	3.80 ± 0.72	3.89 ± 0.69	0.238
P	3.59 ± 0.66	3.62 ± 0.67	
$\dot{V}_{E\ peak}$ (L/min)			
Q	131.6 ± 23.9	135.9 ± 22.0	0.650
P	121.8 ± 22.5	127.6 ± 21.4	
RER_{peak}			
Q	1.15 ± 0.06	1.16 ± 0.05	0.791
P	1.16 ± 0.05	1.16 ± 0.05	
HR_{peak} (bt/min)			
Q	192 ± 8	194 ± 8	0.448
P	196 ± 9	196 ± 9	
RPE_{peak} (6-20 Borg scale)			
Q	18.5 ± 1.4	18.2 ± 1.3	0.047*
P	17.9 ± 1.3	18.3 ± 1.2	
[La] (mmol/L)			
Q	12.4 ± 2.6	12.9 ± 2.7	0.447
P	12.4 ± 2.6	12.4 ± 2.3	
Test time (min)			
Q	7.9 ± 1.7	8.2 ± 1.7	0.745
P	7.8 ± 1.6	8.0 ± 1.4	

Q=quercetin group, P=placebo group. **F*-ratio of Group x Time interaction significant at $p<0.05$.

Table 3.3: Physical performance measures (mean ± SD).

Variable	Pretreatment	Posttreatment	Group x Time Interaction *p*-value
APFT total score (points)			
Q	255.1 ± 42.0	265.0 ± 40.0	0.740
P	239.2 ± 30.4	246.8 ± 33.9	
APFT 2-mile run (min)			
Q	15.03 ± 1.93	14.92 ± 1.74	0.442
P	15.47 ± 1.52	15.17 ± 1.69	
BMPU repetitions			
Q	27.0 ± 12.4	31.4 ± 14.2	0.439
P	27.0 ± 10.6	30.3 ± 12.6	
WAnT mean power (W)			
Q	587.03 ± 139.26	551.72 ± 110.99	0.444
P	532.69 ± 102.15	513.21 ± 104.98	
WAnT % fatigue rate			
Q	45.68 ± 9.32	50.49 ± 8.41	0.468
P	45.81 ± 9.84	52.76 ± 7.76	
36.6-m sprint (s)			
Q	5.69 ± 0.45	5.78 ± 0.50	0.479
P	5.64 ± 0.43	5.76 ± 0.52	

Q=quercetin group, P=placebo group, APFT=Army Physical Fitness Test, BMPU=Baumgartner Modified Pull-Up Test, WAnT=Wingate Anaerobic Test.

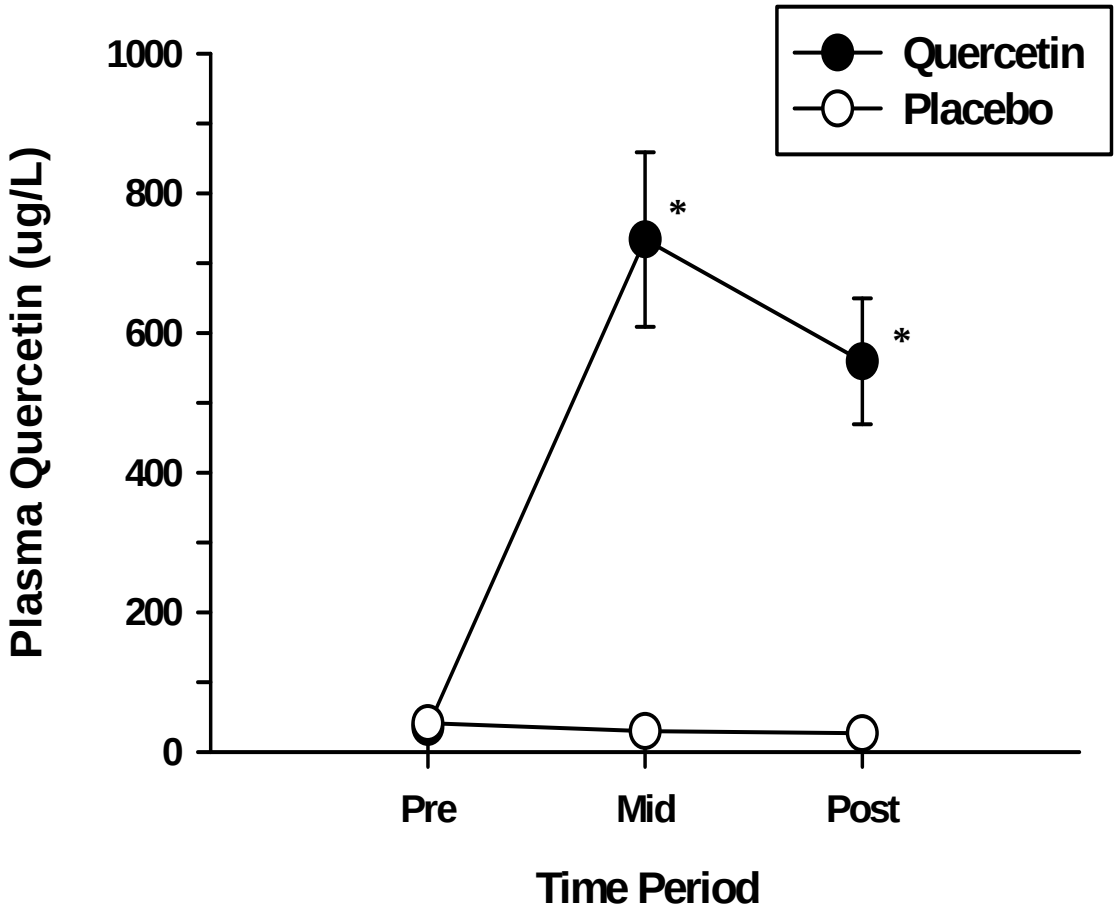

Figure 3.1: Mean (± SEM) plasma quercetin at pre-, mid-, and posttreatment points for Quercetin (Q) and Placebo (P) groups.

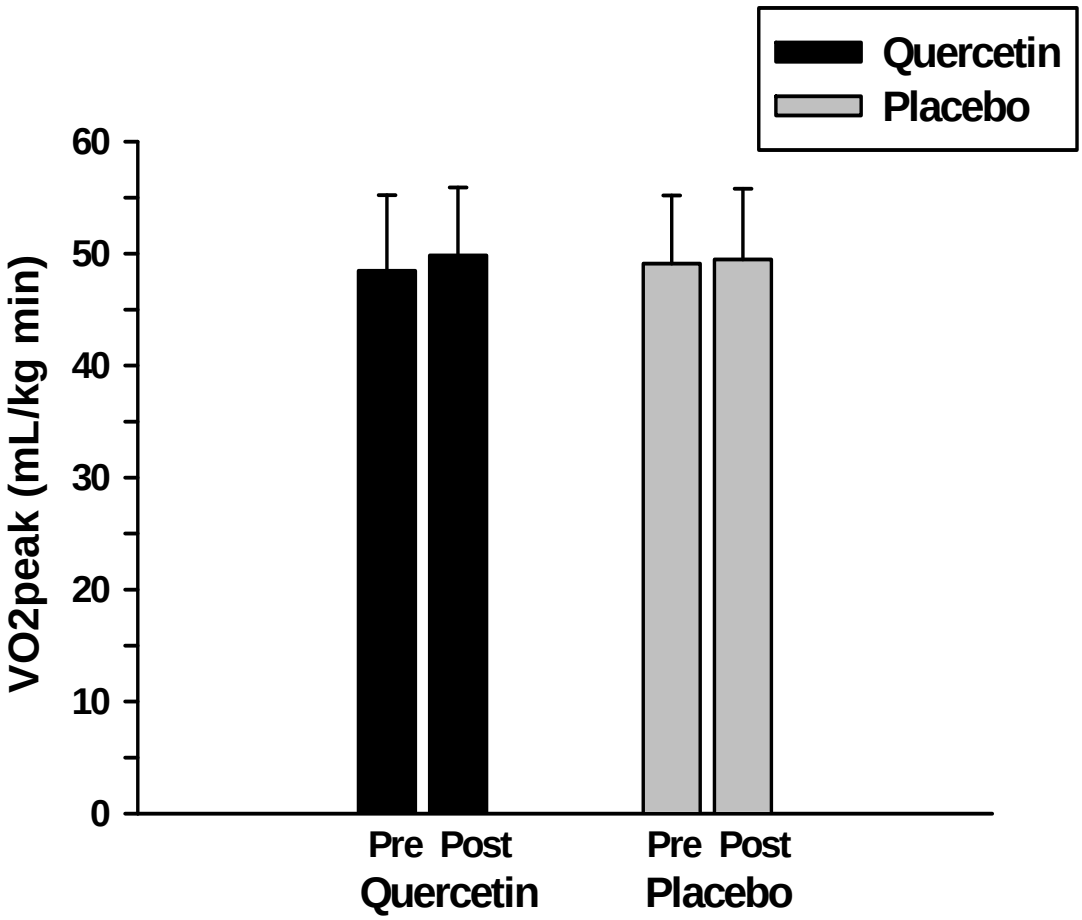

Figure 3.2: Mean (± SD) pretreatment-posttreatment $\dot{V}O_{2peak}$ (mL/kg min) in the Quercetin (Q) and Placebo (P) groups.

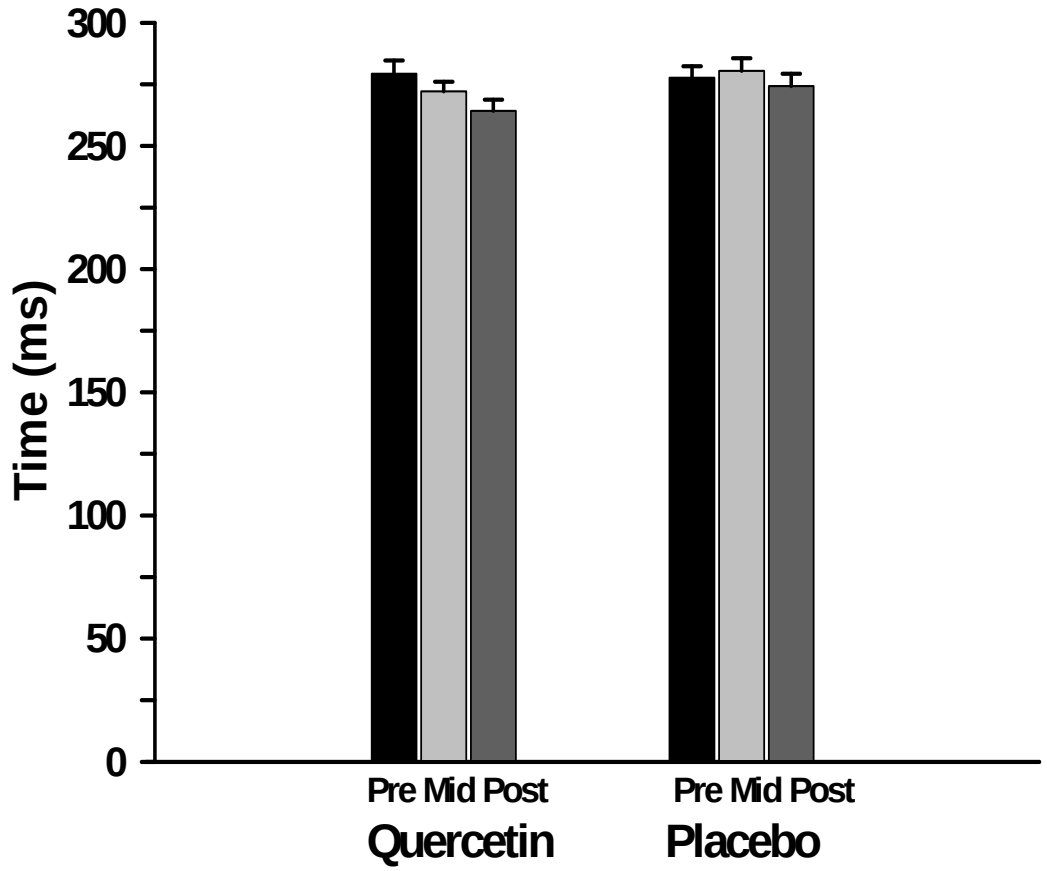

Figure 3.3: Mean (± SEM) reaction times (ms) at pre-, mid-, and posttreatment points for Quercetin (Q) and Placebo (P) groups.

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CHAPTER 4

EFFECTS OF SIX WEEKS OF QUERCETIN SUPPLEMENTATION ON MOOD, SLEEP, AND ILLNESS IN ROTC CADETS[1]

[1] Bigelman, Kevin, A., Donald P. Chapman, Eric C. Freese, Jennifer L. Trilk, and Kirk J. Cureton. To be submitted to *Military Medicine.*

Abstract

Purpose: To investigate the effects of 6 weeks of dietary quercetin supplementation on the moods of energy and fatigue, sleep quality, and self-reported illness in men and women enduring military physical training. **Methods:** Using a randomized, double-blind, repeated-measures, placebo-controlled design, 58 healthy, moderately-trained young men and women undergoing regular military physical training were randomly assigned to Quercetin (Q) and Placebo (P) groups. Moods of energy and fatigue, as well as sleep quality and self-reported incidences of illness were evaluated prior to (T1), in the middle (T2), at the end (T3), and 2 weeks following (T4) 42-54 days of supplementation with 1 g/d of quercetin with vitamins and other substances in a soft chew or a placebo chew. **Results:** Changes in energy and fatigue, assessed by the Profile of Mood States-Brief form (POMS-B) and the Mental and Physical State Energy and Fatigue Scales, and changes in sleep quality, measured by the Pittsburgh Sleep Quality Index (PSQI) were not significantly different ($p > 0.05$) in Q and P. Illness rate and severity, recorded using the Wisconsin Upper Respiratory Symptom Survey (WURSS), were also not significantly different ($p > 0.05$) between groups. **Conclusion:** Six weeks of chronic dietary quercetin supplementation in moderately-trained young men and women conducting regular military physical training does not improve moods of energy and fatigue. Sleep quality, as well as illness rate and severity, was also unaffected by quercetin supplementation in this population.

Keywords: Quercetin, Energy, Fatigue, Sleep, Illness, Flavonoids, Military physical training, ROTC

Introduction

Quercetin (3,3',4',5,7-pentahydroxyflavone) is a naturally-occurring flavonol, a member of the larger class of polyphenolic flavonoids, regularly consumed in the human diet (1). Quercetin is found in numerous fruit, vegetable, and beverage sources including apples, red onions, spinach, fruit juices, red wine, and black tea (15, 24, 40). National dietary assessments indicate that mean daily quercetin intake in the typical diet varies from less than 5 mg to 40 mg, but levels as high as 200-500 mg may be obtained by heavy fruit and vegetable consumers (15).

Quercetin has anti-oxidant, anti-inflammatory, anti-carcinogenic, and cardio-protective properties (13, 15, 20, 39). It also reduces susceptibility to upper respiratory tract infections (URTI) and influenza following strenuous exercise (9, 25). These biological effects are related to the chemical structure of quercetin, specifically the location of its 4 hydroxyl groups (-OH) and catechol B-ring (15). Based on its health-promoting properties, quercetin is marketed as a nutritional supplement and food and beverage additive.

A limited number of recent studies have examined quercetin's influence on cognitive function. In animals, chronic quercetin supplementation appears to have some positive effect on cognition (22, 32, 38). Davis et al. (8) recently reported that 7 days of quercetin feedings (12.5 and 25 mg/kg/d) in mice increased both skeletal muscle and brain markers of mitochondrial biogenesis (intracellular signaling proteins sirtuin 1 (SIRT1) and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), mitochondrial DNA (mtDNA), and cytochrome-c concentration) in a dose-dependent fashion. There was also an increase in voluntary physical activity, partially attributed to

quercetin's effect on the central nervous system. It was hypothesized that increased brain mitochondrial activity may enhance cerebral metabolism, which "has important consequences on motivation, mood (e.g. vigor, fatigue, anxiety, depression) and central motor drive from the cortex" (8).

In humans, the effects of quercetin on mental function are equivocal. One published abstract (34) reported that 3 weeks of quercetin supplementation protected against mental fatigue and reductions in sustained vigilance following 3 days of heavy exercise. Durak and Bell suggested that 3 weeks of dietary supplementation with an anti-oxidant health drink containing quercetin (FRS®, The FRS Company, Foster City, CA) in an occupational setting improved measures of work performance, work frustration, fatigue status, and concentration (E. Durak, personal communication, February 21, 2009). Similarly, Durak and Taguchi determined that 3 months of supplementation with the same quercetin cocktail improved perceived levels of energy and fatigue, as well as sleep in those with advanced cancers (E. Durak, personal communication, February 21, 2009). On the contrary, a published thesis (30) examining varying doses of acute caffeine and quercetin ingestion reported that quercetin supplementation was not associated with any improvements in either mood or vigilance. Unpublished research from the United States Army Research Institute of Environmental Medicine (USARIEM) also reported that acute ingestion of food bars containing quercetin had no positive or beneficial effect on either vigor or fatigue levels (G. Adam, personal communication, March 3, 2009).

If quercetin does have an effect on the brain, the exact mechanisms are unknown. It is hypothesized that flavonoids may impact mood, cognition, or behavior by altering brain kinase activation and neuronal communication (16). Quercetin's influence on the

brain also may be associated with its properties as an adenosine A_1 receptor antagonist (1). The effects of chronic quercetin ingestion on the transient moods of energy and fatigue, as well as sleep remain unexplored in moderately-trained persons performing regular physical training.

Quercetin's effect on health is also not well studied. *In vitro*, quercetin has been shown to reduce infectivity and replication of a number of respiratory viruses (9). In humans, recent research examining illness and immune function determined that quercetin reduced the incidence of URTI following 3 days of intense exercise (3 h/d at ~57% maximum W) by well-trained cyclists, but it did not significantly alter markers of immunity or inflammation (25). In the 2 weeks following the heavy exercise among those cyclists supplementing with quercetin, URTI rate was 5%, compared to 45% in the control group. Similar, but less dramatic, illness trends were observed for athletes supplementing with quercetin and competing in the 160-km Western States Endurance Run (23). Since markers of immunity or inflammation were not significantly altered, the authors suggested that quercetin may have reduced the URTI rate via "anti-pathogenic pathways" (24), as observed *in vitro*. Studies *in vitro* indicate that quercetin blocks the early stage of viral replication via several mechanisms (25). Further research is needed to examine the effects of quercetin supplementation on illness rates in those undergoing less intense regular physical training.

The United States Army has demonstrated interest (12) in determination of quercetin's possible ergogenic effects. To date, however, no published research has examined the efficacy of chronic quercetin ingestion on other important variables linked to military performance. The purpose of this investigation was to examine the effects of 6

weeks of dietary quercetin supplementation on the transient moods of energy and fatigue, as well as sleep quality and self-reported illness rates. It was hypothesized that quercetin supplementation would improve measures of energy and decrease measures of fatigue compared to 6 weeks of placebo ingestion. It also was hypothesized that sleep quality would be disturbed by quercetin ingestion, due to its action as an *in vitro* adenosine A_1 receptor antagonist. Illness rates and severity were hypothesized to decrease following quercetin ingestion, based on its effects observed *in vitro* and in humans following intense exercise. If these hypotheses can be confirmed, it may have important implications for the military, as success on the battlefield is strongly influenced by these outcome variables.

Methods

Participants. Fifty-eight healthy, moderately-trained males and females, aged 18-40 y, recruited from Army and Air Force Reserve Officers' Training Corps (ROTC) programs completed this study. Fifty-seven participants were full-time university students, while one was a ROTC instructor. This sample size had enough statistical power to detect a moderate Group x Treatment Time interaction effect of approximately 0.33 standard deviation with an alpha of 0.05 and a power of 0.8, assuming a repeated trials correlation of 0.95 for the outcome measure (31).

After signing an informed consent statement approved by the Institutional Review Board, participants completed questionnaires concerning physical activity history, medical history, and diet. Physical activity history was assessed using a 7-day physical activity recall (7-D PAR) questionnaire (5) and an instrument designed to assess habitual physical activity (UPACS) (28). Medical screening indicated participants had no

contraindications to maximal exercise testing and met the low risk health stratification standard for exercise testing recommended by the American College of Sports Medicine (2). A food frequency questionnaire, based on the Youth-Adolescent Questionnaire (35, 36), was used to estimate pretreatment habitual dietary quercetin intake. Plastic food models were utilized to ensure accurate portion-size reporting.

Participants were asked to abstain from non-prescription drugs, vitamins, herbs, and other nutritional supplements for the duration of the study. Dietary quercetin intake was not controlled, as low levels of quercetin are found in fruits, vegetables, and beverages (40) commonly consumed by this demographic. No financial compensation was given for participation.

In accordance with the ROTC programs of instruction, participants were subjected to organized military physical training a minimum of three times per week for the duration of the treatment period. Individual training sessions lasted 1 h and consisted of muscular strength and endurance exercises, as well as cardio-respiratory and flexibility training.

Research design. A randomized, double-blind, repeated-measures, placebo-controlled design was used for this experiment. To ensure equal aerobic capacity within each group, men and women were separately first ranked according to peak oxygen uptake ($\dot{V}O_{2peak}$) and subsequently divided into matched pairs. One individual from each pair was then randomly assigned to the experimental group consuming quercetin (Q) and the other to the placebo group (P). Randomization was performed using Research Randomizer (www.randomizer.org). Q and P each contained 22 men and 7 women.

The effects of the treatment intervention were evaluated by two instruments assessing moods of energy and fatigue, a questionnaire designed to assess components of sleep, and a survey recording the impact and severity of illness. Critical time periods for obtaining outcome measures included pretreatment (T1), the midpoint of treatment (T2), immediately prior to the end of treatment (T3), and 2 weeks after cessation of treatment (T4). Energy and fatigue instruments, as well as the sleep questionnaire, were completed at each of these time points. The illness survey was completed online daily for the duration of treatment.

Treatment. Participants in Q ingested four individually-wrapped chews daily (two with breakfast, two with dinner) during the treatment period. The purpose of the multiple feedings was to maintain plasma quercetin levels throughout the day (21). Each chew contained 250 mg quercetin, 100 mg isoquercetin, 100 mg omega-3 fatty acids (eicosapentaenoic acid (EPA) & docosahexaenoic acid (DHA)), 30 mg epigallocatechin gallate (EGCG), a vitamin mixture, sucrose, and other ingredients in a carnuba wax and soy lecithin base (Quercegen Pharma, Newton, MA). Participants in P also ingested four chews daily at the same time periods. Chews for P were similar in appearance and identically-wrapped, but contained no quercetin, isoquercetin, EPA, DHA, EGCG, or vitamin mixture. Participants turned in chew wrappers weekly for accountability and as a means of encouraging ingestion. Investigators and participants were blinded to the treatment identity until data collection and analysis were completed. Participants also completed a weekly questionnaire to determine whether they believed they were ingesting the quercetin or placebo chew. Blinding percentages for both groups were calculated with the sum of the number of correct responses each week in the numerator

and 174 (29 participants in each group multiplied by 6 treatment weeks) in the denominator.

Treatment duration lasted 42-54 days, beginning immediately after all pretest measures were completed and continuing until the conclusion of posttest measures. Pretests and posttests required between 8-12 days for administration. As a result, some participants remained on the treatment several days longer than others while completing posttest measures. Mean treatment duration was 52 days and overall duration of the study was approximately 10 weeks.

Outcome measures. In order to evaluate the effects of dietary quercetin supplementation on the moods of energy and fatigue, sleep, and illness relative to a control, a series of psychometric instruments were administered at regular intervals. Energy, fatigue, and sleep instruments were administered in a quiet, classroom environment, while the illness instrument was completed by participants using their personal computers.

Moods of energy and fatigue. The fluctuating, self-reported feelings of energy and fatigue were analyzed as unipolar factors in this experiment. The authors of this study used the definition of energy as "feelings of having the capacity to complete mental or physical activities", while fatigue was defined as "feelings of having a reduced capacity to complete mental or physical activities" (27).

The Profile of Mood States-Brief Form (POMS-B) questionnaire (19) and the Mental and Physical State Energy and Fatigue Scales (28) were used to assess the transient moods of energy and fatigue at T1, T2, T3, and T4. Each instrument was also completed weekly by participants during treatment. The POMS-B and the Mental and

Physical State Energy and Fatigue scales have demonstrated acceptable reliability and validity (19, 28).

The POMS-B is a 30-question inventory of mood states (19), assessing moods of tension, depression, anger, vigor, fatigue, confusion, and providing a total mood disturbance (TMD) score. Research suggests that POMS-B vigor and fatigue scores measure the intensity of energy and fatigue mood states (27). Participants were asked to rate a series of adjectives using a five-point scale, based on how they felt "over the past week." Total possible scores in each mood dimension ranged from 0 to 20, with higher scores reflecting stronger moods. Only POMS-B vigor and fatigue data are reported in this study.

The Mental and Physical State Energy and Fatigue Scales were used to assess state physical and mental energy and fatigue. Participants answered 12 items, based on the intensity of current feelings, to measure four energy and fatigue states: physical energy state (PES); physical fatigue state (PFS); mental energy state (MES); and mental fatigue state (MFS). Participants used a 10-cm visual analog scale to record the intensity of each of these feelings along a continuum and these were scored on a 0-100 scale. There were three items per sub-scale so possible total scores ranged from 0 to 300 in each category, with higher scores indicating more intense feelings of the energy or fatigue state. This instrument was used as an additional means of assessing energy and fatigue, as it may be more sensitive to measuring change over time since it utilizes a visual analog scale format (28).

Sleep. The Pittsburgh Sleep Quality Index (PSQI) was used to assess changes in sleep quality between groups over the preceding specified time period (7). The PSQI

administered at T1 and T3 assessed sleep quality during the preceding 30 days. The PSQI administered at T2 recorded sleep quality during the first 3 weeks of treatment, while the PSQI at T4 assessed sleep quality for the 2 weeks after treatment cessation. The PSQI, consisting of 4-point scales for 19 items, generated seven "component" scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleep medication, and daytime dysfunction. Each component was scored from 0 to 3, and the sum of these components yielded a global PSQI score ranging from 0 to 21. Higher scores indicated lower quality of sleep, and scores > 5 identified a poor sleeper with severe difficulty in at least two component areas, or moderate difficulty in more than three component areas. The PSQI has established test-retest reliability and validity (7).

Wisconsin Upper Respiratory Symptom Survey (WURSS). The WURSS, an empirically derived, illness-specific, reliable and valid quality-of-life instrument, was completed daily to assess the occurrence and impact of the common cold (4). With the author's permission, an online version of the 21-question WURSS was created using a commercially-available website (www.surveymonkey.com) designed to collect survey data for comparison between groups. Participants received a daily e-mail with a link to the survey website throughout the treatment period. If participants did not complete the survey within 24 h, a follow-up e-mail, text message, or phone call was used to encourage timely survey completion.

Physical activity. The self-administered 7-D PAR (5) was completed at T1, T2, T3, and T4, as well as weekly during treatment, to measure levels of physical activity (kcal/kg wk) between groups. Participants indicated the time they spent sleeping and engaged in moderate, hard, and very hard physical activities.

Preliminary measures. Participants attended a preliminary session during which physical characteristics and body composition were assessed pretreatment. Body height was measured using a wall stadiometer, nude body mass was determined using an electronic scale (model FW-150KA1, A&D Co., Ltd., Tokyo), and body composition was estimated using dual-energy x-ray absorptiometry (iDXA, GE Healthcare-Lunar, Madison, WI). Pretreatment energy, fatigue, and sleep surveys were also completed during this session.

On a separate day, participants reported to the laboratory to assess pretreatment whole-body aerobic capacity, measured with a test of peak oxygen uptake ($\dot{V}O_{2peak}$). Upon arrival at the laboratory, participants completed a 24-h history form to assess compliance with pretest instructions. Euhydration was ensured by instructing participants to drink liberally the day before and to drink one 240-mL glass of water 1 h prior to testing. After a 5-min low intensity running warm-up on a treadmill (Trackmaster, JAS Fitness System, Newton, KS), a graded treadmill running test following the modified Åstrand protocol (3) was administered to evaluate $\dot{V}O_{2peak}$. Treadmill speed was 3.129 m/s (male) or 2.682 m/s (female) and initial treadmill grade was 0%. After each 2-min interval, grade was increased by 2.5% while speed remained constant. Participants ran until volitional exhaustion.

Oxygen uptake and related gas exchange measures were obtained by open-circuit spirometry using a Parvo Medics TrueOne 2400 Metabolic Measurement System (Parvo Medics, Inc., Salt Lake City, UT) and averaged at 1-min intervals. Standard gases of known composition were used to calibrate the oxygen and carbon dioxide analyzers, and a 3-L syringe was used to calibrate the pneumotachometer prior to each test session. All

tests were conducted at approximately the same temperature (23-24^{o}C) and relative humidity (<58%). Heart rate (HR) and rating of perceived exertion (RPE) were measured every 2 min, and at test completion. HR was measured with a Polar® Vantage XL heart rate monitor (Polar Electro, Inc., model 145900, Woodbury, NY). RPE was measured using the well-validated Borg 15-point category scale (6). Three minutes after exercise, a capillary blood sample from the index finger was obtained for determination of peak blood lactate concentration [La] (Lactate Pro Test Meter, model LT-1710, Arkray, Inc., Kyoto, Japan). All participants met at least 3 of the 4 criteria for maximal effort: [La] ≥ 7-9 mmol/L, respiratory exchange ratio (RER) ≥ 1.10, RPE ≥ 18 on the Borg scale, or HR within 5% (10 bt/min) of age-predicted maximum.

Statistical analysis. All data were analyzed using the statistical software SPSS v. 15.0 (SPSS, Inc., Chicago, IL). Differences between Q and P in physical characteristics, body composition, and physical activity history were determined using *t*-tests for independent samples. Differences between Q and P in the outcome measures were analyzed using a two-way (Group x Treatment Time) repeated measures analysis of variance (ANOVA). All tests were considered significant at alpha level < 0.05.

Results

Participants completed all required psychometric instruments at T1, T2, and T3. At T4, all participants except one, a member of P, completed all required surveys. To complete statistical analysis without missing data, this participant's survey results from T3 were carried over and used at T4.

Participant characteristics and physical activity. The physical characteristics of participants (height, mass, fat-free mass, % body fat, bone mineral density), $\dot{V}O_{2peak}$, and

average weekly energy expenditure in Q and P were not different at T1 prior to treatment (Table 4.1). There were, however, significant age differences between groups. These differences occurred because Q contained two older participants (aged 37 y and 40 y). When these individuals' ages were treated as outliers, there were no age differences (p = 0.138) between groups. These data indicate that randomization created groups nearly-equivalent in physical characteristics, aerobic fitness, and physical activity at T1.

During treatment, no significant physical activity differences existed between groups, assessed by 7-D PAR results. At T3, neither body mass nor physical activity was significantly different ($p > 0.05$) compared with pretest values in either group.

Energy and fatigue. POMS-B scores over the duration of the study are presented in Figure 4.1. Mean vigor and fatigue scores at T1 were approximately equal, indicating no difference between groups prior to treatment. Compared to published norms that exist for this instrument, both Q and P began the study reporting higher than average scores for vigor and lower scores for fatigue (19).

During the 6 weeks of treatment, self-reported vigor decreased in both groups. At T3, vigor scores were reduced 16.1% in Q and 3.5% in P, compared with T1 scores. Self-reported fatigue was also reduced over the same time period, decreasing 50.0% in Q and 39.2% in P. However, neither vigor nor fatigue scores changed significantly differently between groups ($p > 0.05$; with $p = 0.327$ for Vigor, and $p = 0.966$ for Fatigue) over time. Two weeks following treatment at T4, both groups reported small increases in vigor (4.3% in Q, 1.8% in P) and fatigue (23.1% in Q, 9.7% in P) compared to values at T3.

The results from the Mental and Physical State Energy and Fatigue Scales are reported in Figure 4.2 and Figure 4.3. Compared to average normative data by sex, male

participants began the study with less physical and mental energy, and higher physical and mental fatigue. On average, female participants began the study with more physical and less mental energy, and less physical and slightly higher mental fatigue compared to normative female values.

At T1, Q and P were approximately equal in PES and MES. By the end of treatment at T3, PES and MES in Q increased by 7.7% and 12.3%, respectively. During the same time period, P reported an increase of 19.7% for physical energy and 20.4% for mental energy. However, these differences between groups were not statistically significant ($p > 0.05$). From T3 to T4, the changes for PES and MES in the two groups were not different.

State physical and mental fatigue showed similar trends in the two groups. Q began treatment with more physical and mental fatigue than P. Over the course of treatment, mental and physical fatigue declined in both groups. In Q, there was a 27.3% percent reduction in PFS and a 31.2% reduction in MFS compared to T1 values. In P, PFS was reduced 15.1% and MFS was reduced 28.5% compared to pretreatment values. The differences between groups in the changes, however, were not statistically significant ($p > 0.05$). After treatment at T4, there were non-significant differences between groups for PFS and MFS.

Sleep. Prior to treatment, average PSQI sleep duration (mean ± SD) was 6.4 ± 1.0 h in Q and 6.3 ± 0.9 h in P. These values are similar to results obtained using the 7-D PAR sleep sub-component, with sleep durations (mean ± SD) for Q of 6.2 ± 0.8 h during weekdays and 7.5 ± 1.5 h during weekends and sleep durations for P of 6.4 ± 0.9 h during weekdays and 7.1 ± 1.6 h during weekends.

Global PSQI scores (mean ± SD) at T1 were nearly identical in both groups (Figure 4.4). At T2, each group experienced an increase in global PSQI score, with P having a slightly greater increase than Q. At T3, there was no change in the global PSQI score for Q compared to its value at T1. However, P decreased by 20.4% from T1, indicating a reduction in sleep disturbance. At T4, global PSQI decreased slightly in Q and increased slightly in P. However, none of these changes were statistically significant ($p > 0.05$). When the components of the PSQI were analyzed by two-way ANOVA (Group x Treatment Time), no significant differences were identified in any of the seven areas ($p > 0.05$).

At T3 just prior to the end of treatment, average PSQI sleep duration (mean ± SD) in Q was 6.2 ± 1.1 h and 6.6 ± 0.8 h in P. These values are similar to results obtained using the 7-D PAR sleep sub-component, with sleep durations (mean ± SD) for Q of 6.4 ± 1.0 h during weekdays and 7.2 ± 1.4 h during weekends and sleep durations for P of 6.5 ± 0.9 h during weekdays and 7.3 ± 1.2 h during weekends. Changes in sleep duration between groups during the treatment were not statistically significant ($p > 0.05$).

Illness. Average daily online survey compliance rate for the duration of the study was 94.2%, with an average completion rate of 93.2% in Q and 95.3% in P. Differences between groups in the total number of days of illness by severity are presented in Figure 4.5. ANOVA revealed no statistically significant differences between Q and P ($p > 0.05$).

Blinding. Over the duration of the study, participants in Q correctly indicated whether they had consumed quercetin 72.9% of the time. Participants in P correctly indicated whether they had consumed the placebo 59.2% of the time.

Discussion

The purpose of this investigation was to determine the effects of 6 weeks of dietary supplementation with a chew containing quercetin on energy, fatigue, sleep, and illness in moderately-trained individuals undergoing military physical training. Our primary findings were that dietary quercetin supplementation did not affect the transient moods of energy and fatigue, sleep quality, or illness rate or severity in this population. Our findings do not support the hypothesis that quercetin has effects on mood, sleep, physical activity, or illness in those undergoing military physical training. These null findings are important, nonetheless, because they suggest that quercetin's effect on cerebral metabolism observed in mice may not carry over to humans.

Energy and fatigue. Epidemiological and experimental evidence suggest that there is a positive association between regular, moderate physical activity and feelings of increased energy and decreased fatigue (29). It was hypothesized that quercetin supplementation would improve self-reported energy and decrease fatigue levels, above the levels expected in a population subjected to regular, moderate physical training. However, there were no differences between Q and P in changes in either energy or fatigue levels.

When analyzed individually, POMS-B vigor and the PES and MES scales showed differing trends. POMS-B vigor scores decreased for both groups during the treatment period, while both PES and MES increased during the same time period. The most likely explanation for these different results may be that the POMS-B assessed feelings of vigor over the past week, while the PES and MES scales captured current (right now) feelings of physical and mental energy. Since moods of energy often last for hours, but may vary

from minutes to weeks (27), the time period assessed by the instruments may have resulted in the observed differences.

When fatigue data were analyzed, POMS-B fatigue and the PFS and MFS scale results both showed similar decreasing trends over the course of treatment. These results were unexpected for individuals undergoing regular, moderate physical training with no change in weekly physical activity. When changes in Q and P due to the treatment were analyzed using ANOVA, no differences were observed, suggesting that quercetin had no effect on levels of fatigue. This is contrary to one published abstract (34) that reported that quercetin protects against mental fatigue following heavy exercise. It may be that the effect of quercetin is dependent on the intensity of exercise, only showing an effect with more intense exercise.

Additionally, it is possible that the magnitude of the treatment effect of quercetin on energy and fatigue was too small to detect using the psychometric instruments used in this experiment. Effect sizes ($\Delta mean_Q$-$\Delta mean_P/SD_P$) were low (-0.34 SD units for POMS-B vigor; -0.20 SD units for POMS-B fatigue). However, our results are consistent with two other studies that utilized the Profile of Mood States (G. Adam, personal communication, March 3, 2009, (30)), each reporting that energy and fatigue levels were not significantly changed following acute quercetin supplementation.

It is also plausible that this particular population made detection of the effects of quercetin supplementation on energy and fatigue more difficult, if not impossible. This can be seen in the large variability in the responses on both the POMS-B and the Mental and Physical State Energy and Fatigue Scales, indicating that this population reported a

wide range of intensities of energy and fatigue. As such, a small effect of quercetin may have been statistically insignificant due to the large variability in measurement.

Physical activity. Mean weekly physical activity levels (kcal/kg wk) in this study were higher than results from other studies using the 7-D PAR to examine physical activity in college students (11). This can be attributed to the mandatory physical training required for this particular population.

There were no significant differences between groups in 7-D PAR results collected weekly during the experiment. This suggests that quercetin supplementation did not influence levels of voluntary physical activity in humans, as was observed in 7 days of quercetin feedings in mice by Davis et al. (8). Our null findings compared to those observed by Davis et al. might be related to differences in quercetin treatment duration used in this experiment. However, treatment duration is likely not the issue as there were no significant differences between groups when data from the first and second weeks of treatment were analyzed.

Another possibility for our null findings is that the dosage used in this experiment was not optimal, because quercetin's bioavailability, metabolism, and tissue distribution in humans may be different from that in mice (10). According to recent research by Reagan-Shaw et al. (33), the optimal dose for translation from animals to humans is based on body surface area, rather than weight, as was done in this experiment. When this approach is used based on the effects observed by Davis et al.(8), the mouse to human dosage translation is 1.01 mg/kg, or 76.0 mg/d for a 75-kg human. The dose used in this experiment may have been too high, perhaps interfering with the activation of

genes that initiate mitochondrial biogenesis in the brain and enhancement of cerebral metabolism, in turn resulting in no change in the moods of energy and fatigue.

Sleep. Mean global PSQI scores in this population indicated poor sleep among both groups at T1 and T2. At T3, mean global PSQI scores indicated poor sleep among Q, and nearly poor sleep in P. These results are expected, as a majority of the participants were college students, living in a university setting, with full academic course loads. Additionally, all participants attended mandatory early morning (0600 h) physical training sessions 3-5 times per week. The combination of the college lifestyle, with associated academic demands, and early morning physical training sessions likely resulted in poor self-reported sleep quality scores for both groups at T1-T2.

It was also hypothesized that as an *in vitro* adenosine A_1 receptor antagonist (1), quercetin may have an effect on alertness similar to that observed with caffeine (18), and thereby have a negative, disturbing effect on sleep quality. However, our results do not support this hypothesis for the dose of quercetin used in this study. ANOVA revealed no significant differences ($p > 0.05$) between Q and P in changes over time. The mean global PSQI score for Q remained nearly consistent at each time point throughout the experiment, indicating quercetin had no effect on sleep quality. When male and female participants were analyzed separately by group, no significant differences in changes over time were noted ($p > 0.05$). To our knowledge, this is the first experiment to report that quercetin does not influence the sleep quality of moderately-trained individuals.

When the PSQI was further examined by its seven components, there were no significant differences in changes between groups ($p > 0.05$). The slight rise in mean global PSQI score at T2 in P was attributed to decreases in habitual sleep efficiency and

sleep duration, and increases in sleep disturbances and daytime dysfunction, compared to Q. The reduction in mean global PSQI score at T3 in P compared to Q was attributed to better sleep quality, latency, duration, and efficiency, as well as a decrease in daytime dysfunction and no use of sleep medications.

Two weeks posttreatment at T4, Q and P reported improved sleep quality compared to T1, with mean T4 global PSQI scores in each group under 5. However, it is unclear why sleep quality improved in Q and decreased in P following cessation of treatment.

Illness. Quercetin supplementation had no effect on illness rates and severity in this population, as ANOVA revealed no significant differences ($p > 0.05$) between groups. Exercise immunologists contend that chronic heavy exercise reduces immune function, increasing susceptibility for viral infection, while habitual moderate physical activity confers a strengthening of immune function and resistance to viral infection (37). In a randomized trial of 36 mildly obese women, moderate exercise for 15 weeks was associated with 50% less symptom days of URTI in those that exercised compared to sedentary controls (26). It is possible that null findings were obtained in this experiment because Q and P had enhanced immune function conveyed from regular physical activity, thus preventing detection of any possible quercetin effect on URTI.

Additionally, the time of year that the experiment was conducted may have also influenced results. Large seasonal variations in immune function and immune system variables have been in reported in humans (17). This experiment took place during the fall in a mild climate. URTIs were not prevalent during this time period and may have precluded detection of any quercetin effect on illness rates and severity between groups.

It is also possible that no effect was observed because participants who suffered from illness during the experiment were too sick to complete the online survey. However, survey compliance by group was high and not likely influenced by an individual being too sick to comply. Additionally, if a participant did not complete the survey within 24 h, a follow-up phone call, text message, or e-mail encouraged survey completion within the subsequent 24 h period.

There were several limitations of the illness analysis in this study. First, since participants were not required to consume a specified diet throughout the treatment period, the effect of proper nutrition on maintenance of health and prevention of illness was not considered. Future quercetin research might closely control dietary intake to determine if an illness effect can be detected. Second, the effect of stress on immune function was not considered. Hall et al. (14) suggested that electroencephalography (EEG)-assessed sleep may be a significant correlate of the stress-immune relationship in an older population. While there were no significant differences in illness rates between groups in this experiment when PSQI sleep duration was used as a covariate, future quercetin research should examine the impact of stress on the immune response.

Other study limitations. An important limitation of this study, potentially influencing all outcome measures, was the blinding process. Based on results from the blinding questionnaire, participants in Q correctly indicated whether they had consumed quercetin 72.9% of the time, while P correctly indicated they had consumed the placebo 59.2% of the time. Slight texture differences between the chews, and close contact among all participants early in the experiment, may have caused ineffective blinding. However, ineffective blinding strengthens the null findings at this dosage of quercetin, as no

improvements in any of the outcome measures were observed, despite some participants' knowledge of being in the experimental group.

In summary, this investigation demonstrated that 6 weeks of dietary supplementation with 1 g/d of quercetin does not affect the transient moods of energy and fatigue, sleep quality, or illness rate or severity in moderately-trained men and women. Voluntary physical activity was also not influenced by quercetin consumption, in contrast to the findings observed in the animal study by Davis et al. (8). Future quercetin research should examine varying dosages and exercise training intensities to determine their effects on outcome measures. The effects of diet and stress should also be considered when examining quercetin's effect on illness.

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Disclosures

The experimental and placebo chews used in this experiment were provided by Quercegen Pharma, Newton, MA.

Table 4.1: Participant characteristics (mean ± SD) at pretreatment (T1) and *p*-values.

Variable	Quercetin (n=29)	Placebo (n=29)	*t*-test *p*-value
Age (y)	22.0 ± 5.1	20.3 ± 1.6	0.009*
Height (cm)			
Male (M):	178.8 ± 5.8	177.3 ± 6.8	0.543
Female (F):	166.4 ± 6.0	165.6 ± 3.6	0.150
Body mass (kg)			
M:	82.9 ± 11.2	76.3 ± 9.5	0.336
F:	67.2 ± 7.7	62.9 ± 8.2	0.761
Fat-free mass (kg)			
M:	64.8 ± 6.0	62.2 ± 6.9	0.465
F:	48.3 ± 6.2	46.3 ± 4.8	0.811
Body fat (%)			
M:	23.2 ± 6.5	19.4 ± 5.9	0.819
F:	30.6 ± 7.9	27.9 ± 3.6	0.164
Bone mineral density (g/cm^2)			
M:	1.25 ± 0.11	1.25 ± 0.10	0.940
F:	1.23 ± 0.12	1.20 ± 0.10	0.207
$\dot{V}O_{2peak}$ (mL/kg min)			
M:	50.2 ± 6.4	50.6 ± 5.8	0.587
F:	42.2 ± 3.8	44.4 ± 4.3	0.684
7-D PAR (kcal/kg wk)			
M:	311.2 ± 62.4	295.2 ± 68.5	0.811
F:	302.5 ± 126.0	332.3 ± 103.3	0.997
Usual physical activity composite score (UPACS)			
M:	216.2 ± 20.9	213.7 ± 20.6	0.913
F:	215.1 ± 22.5	220.4 ± 36.5	0.368

*Significant independent *t*-test *p*-value at $p < 0.05$.

Table 4.2: PSQI component scores (mean ± SD) and associated *p*-values.

Variable	T1	T2	T3	T4	*p*-value
Sleep quality					
Q	1.0 ± 0.6	0.9 ± 0.5	0.9 ± 0.6	1.0 ± 0.6	0.500
P	0.9 ± 0.5	0.9 ± 0.5	0.7 ± 0.6	1.0 ± 0.7	
Sleep latency					
Q	1.0 ± 0.8	0.8 ± 0.8	0.9 ± 0.8	1.0 ± 0.9	0.426
P	1.1 ± 0.8	0.9 ± 0.8	0.8 ± 0.8	0.8 ± 0.8	
Sleep duration					
Q	1.1 ± 1.1	1.4 ± 0.9	1.2 ± 1.0	1.2 ± 0.9	0.407
P	1.1 ± 0.9	1.3 ± 0.9	0.9 ± 0.7	0.9 ± 0.8	
Sleep efficiency					
Q	0.1 ± 0.3	0.2 ± 0.4	0.2 ± 0.5	0.0 ± 0.2	0.126
P	0.3 ± 0.8	0.5 ± 0.9	0.1 ± 0.3	0.3 ± 0.5	
Sleep disturbance					
Q	1.1 ± 0.4	1.0 ± 0.5	1.0 ± 0.5	0.8 ± 0.5	0.232
P	0.9 ± 0.3	1.0 ± 0.2	0.9 ± 0.5	0.9 ± 0.4	
Sleep medications					
Q	0.1 ± 0.4	0.3 ± 0.7	0.2 ± 0.4	0.1 ± 0.3	0.100
P	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.0	0.1 ± 0.3	
Daytime dysfunction	1.0 ± 0.7	0.8 ± 0.7	1.0 ± 0.7	0.8 ± 0.7	0.232
Q	1.0 ± 0.7	1.1 ± 0.7	0.9 ± 0.6	0.8 ± 0.5	
P					

Q=quercetin group, P=placebo group, T1=pretreatment, T2=midtreatment, T3=immediately prior to end of treatment, T4=2 weeks posttreatment.

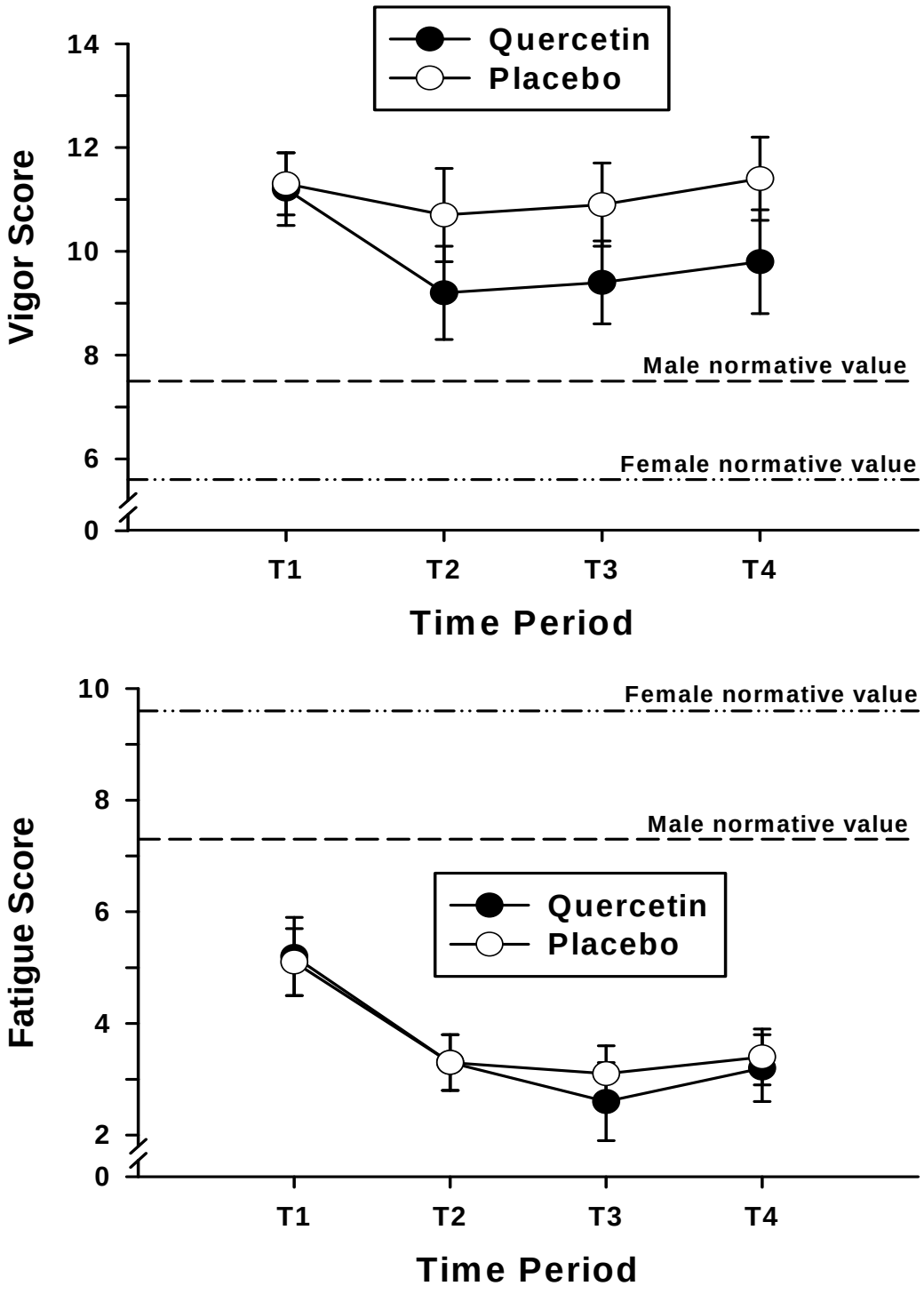

Figure 4.1: Mean (± SEM) POMS-B Vigor and Fatigue scores in the Quercetin (Q) and Placebo (P) groups at T1 (pretreatment), T2 (midtreatment), T3 (immediately prior to end of treatment), and T4 (2 weeks following treatment).

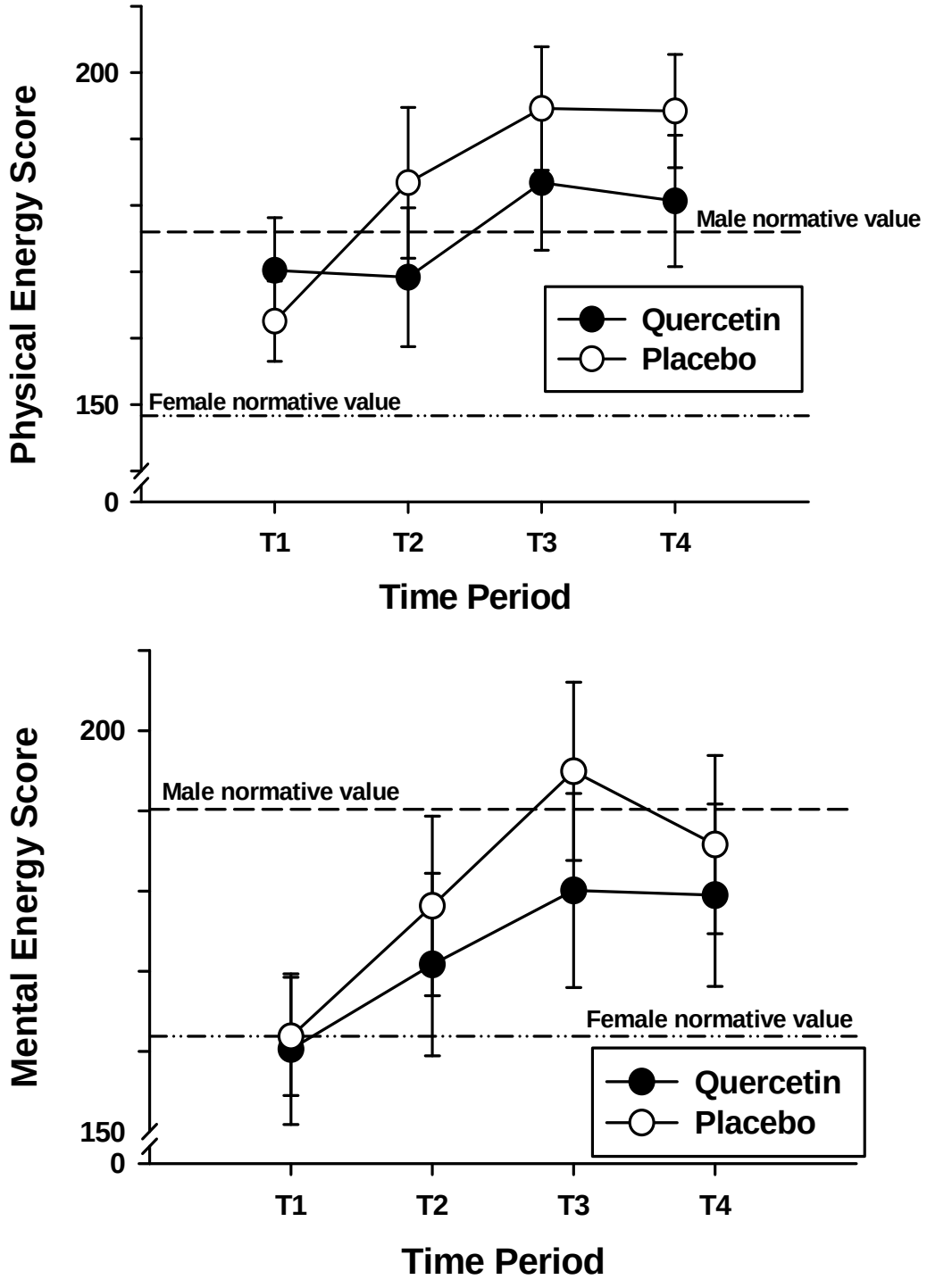

Figure 4.2: Mean (± SEM) State Physical and Mental Energy scores in the Quercetin (Q) and Placebo (P) groups at T1 (pretreatment), T2 (midtreatment), T3 (immediately prior to end of treatment), and T4 (2 weeks following treatment).

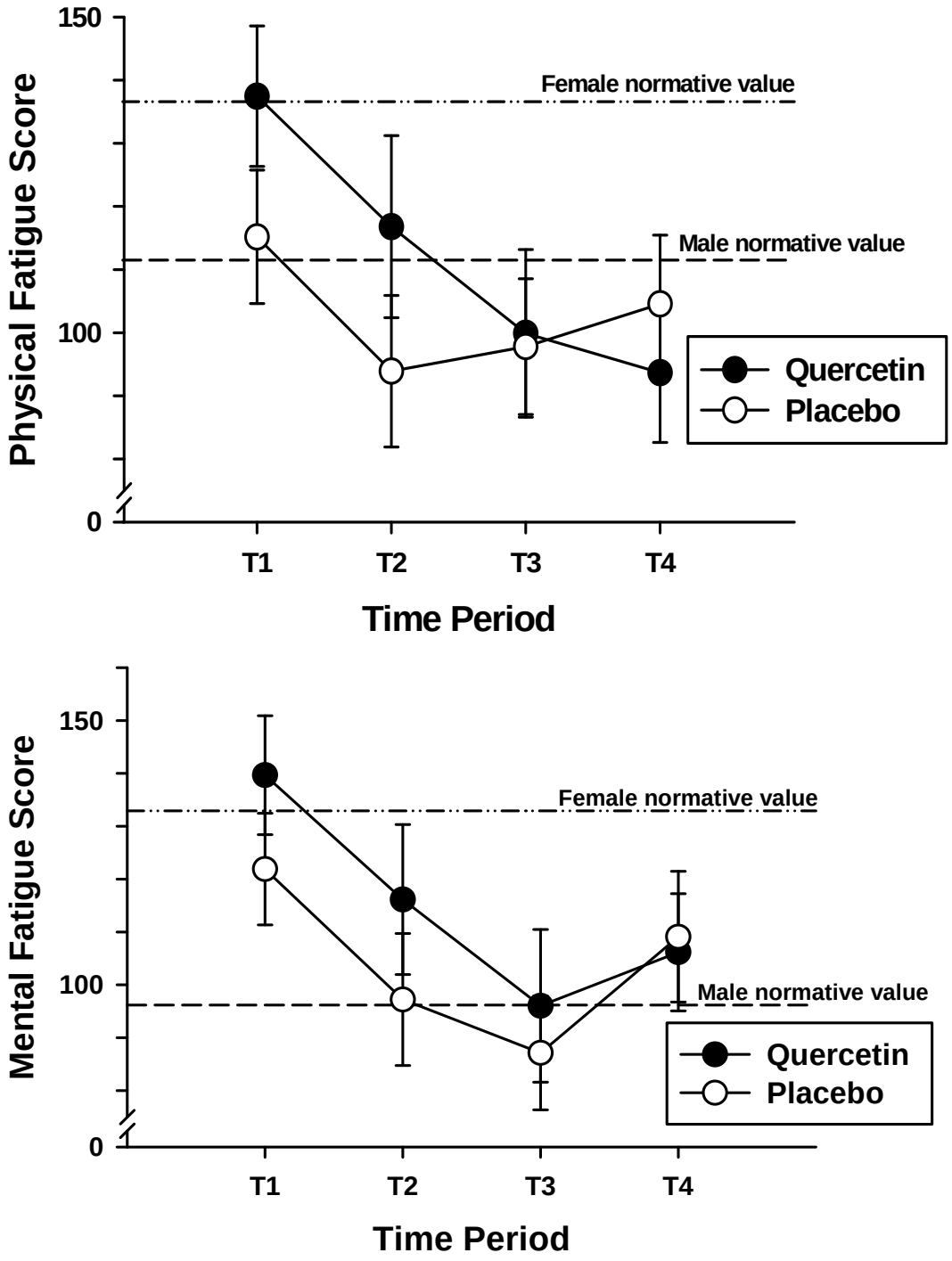

Figure 4.3: Mean (± SEM) State Physical and Mental Fatigue scores in the Quercetin (Q) and Placebo (P) groups at T1 (pretreatment), T2 (midtreatment), T3 (immediately prior to end of treatment), and T4 (2 weeks following treatment).

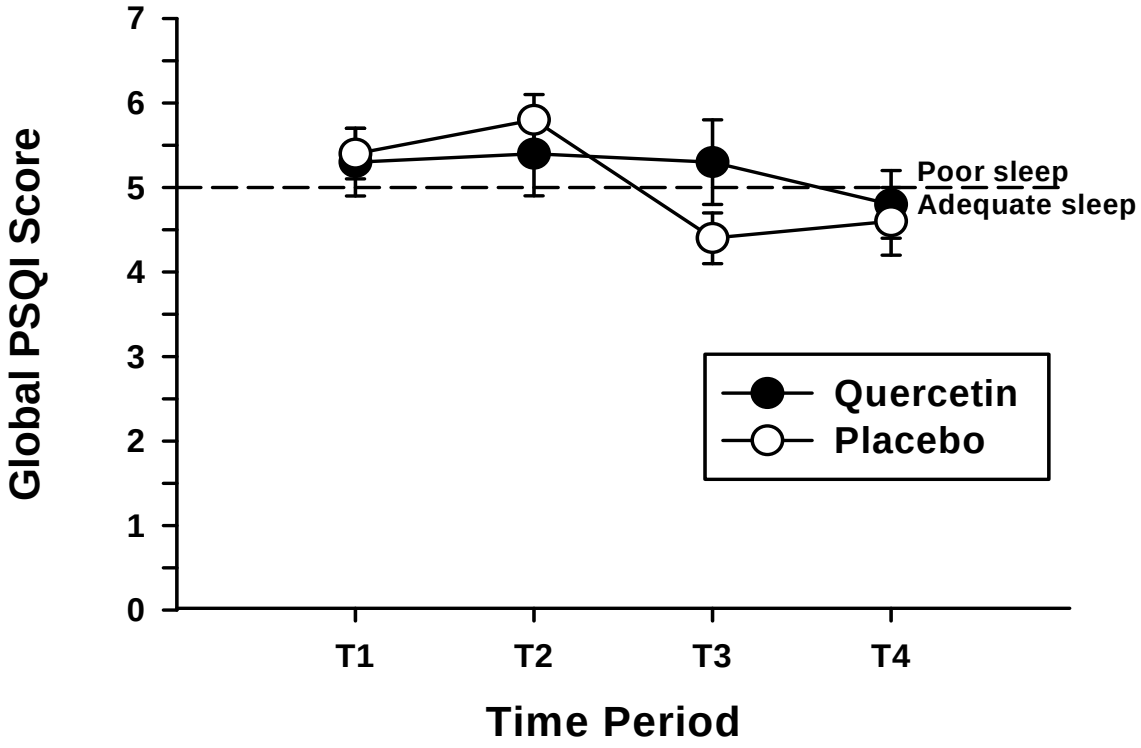

Figure 4.4: Mean (± SEM) global PSQI scores in the Quercetin (Q) and Placebo (P) groups at T1 (pretreatment), T2 (midtreatment), T3 (immediately prior to end of treatment), and T4 (2 weeks following treatment).

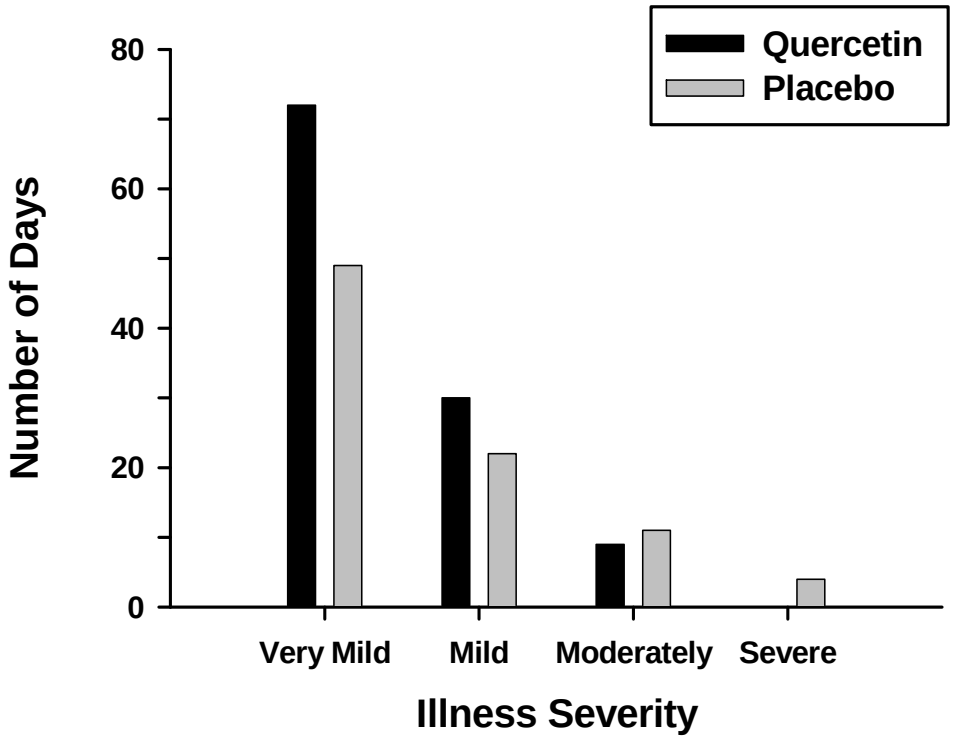

Figure 4.5: Total days of illness by severity (very mild to severe) in the Quercetin (Q) and Placebo (P) groups.

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CHAPTER 5

SUMMARY AND CONCLUSIONS

Although ergogenic aids offer the potential for improving physical and mental performance, the effectiveness of many such dietary aids is questionable. Quercetin is a dietary supplement that, if proven to have ergogenic properties, could be especially beneficial for use by competitive athletes, members of the military, or those unable to exercise due to disease status. While there are numerous health benefits related to quercetin consumption obtained from natural plant sources, the benefits of commercially-prepared, large-dose dietary quercetin supplementation are unknown. Additionally, while ergogenic effects associated with quercetin supplementation have been observed in mice, these results have yet to be examined or replicated in moderately-trained humans exposed to regular exercise training. There is also limited research concerning the effect of quercetin on mood states, sleep, or illness.

The primary purpose of this research was to examine the ergogenic effects of chronic quercetin supplementation on peak oxygen uptake ($\dot{V}O_{2peak}$), measures of physical performance assessing muscular endurance, power, speed, and simple reaction time in moderately-trained young men and women. Additionally, the effects of quercetin supplementation on the moods of energy and fatigue, sleep quality, and illness were examined. A randomized, double-blind, repeated-measures, placebo-controlled design was used to determine the effects of 6 weeks of quercetin supplementation on 58 healthy, moderately-trained young men and women undergoing regular military physical training.

In the first study, the effects of quercetin supplementation on $\dot{V}O_{2peak}$ during maximal-effort uphill treadmill running, four physical performance tests [Army Physical Fitness Test (APFT), Baumgartner Modified Pull-Up Test (BMPU), Wingate Anaerobic Test (WAnT), and 36.6-m sprint], and simple reaction time [Walter Reed palm-held psychomotor vigilance test] were evaluated before and after treatment with 1 g/d of quercetin with vitamins and other substances in a soft chew or a placebo chew. Pretreatment-to-posttreatment changes in $\dot{V}O_{2peak}$ and physical performance tests were not significantly different ($p > 0.05$). Pre-, mid-, and posttreatment changes in simple reaction time were not significantly different between groups ($p > 0.05$).

In the second study, the effects of quercetin on the transient moods of energy and fatigue, sleep quality, and illness were examined prior to, in the middle, at the end, and 2 weeks following supplementation with 1 g/d of quercetin with vitamins and other substances in a soft chew or a placebo chew in the same population. Changes in the moods of energy and fatigue, sleep quality, and self-reported illness rate and severity were not significantly different ($p > 0.05$) between groups.

The results of this research support the following conclusions:

1). There was a significant increase in plasma quercetin levels at midtreatment and following 6 weeks of quercetin chew ingestion.

2). Quercetin supplementation for 6 weeks did not improve $\dot{V}O_{2peak}$ or treadmill run time in moderately-trained young men and women undergoing regular military physical training.

3). Physical performance measures of muscular endurance, power, and speed were not influenced by quercetin supplementation.

4). Simple reaction times did not differ significantly between groups, although there was a trend toward faster reaction times in those ingesting quercetin.

5). Energy and fatigue, sleep quality, and illness rate and severity were not influenced by 6 weeks of quercetin supplementation.

Future studies should examine the appropriate dose for quercetin supplementation, as well as different treatment durations. The effects of quercetin taken in conjunction with exercise training of different modalities, as well as its effects on other populations of varying states of training, in a variety of environmental conditions, is also warranted. Finally, examination of quercetin's effect on simple reaction time requires further exploration.