Dissertation

The Acute Effect of Ingesting a Quercetin-Based Supplement on Exercise-Induced Inflammation and Immune Changes in Runners

submitted by

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Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the guidelines of "Good Scientific Practice".

Graz,

Structure of sections

1 Abstracts

1.1 Abstract in English

The Acute Effect of Ingesting a Quercetin-Based Supplement On Exercise-Induced Inflammation In Runners

Purpose: This study tested the acute anti-inflammatory influence of a quercetin-based supplement consumed by endurance athletes 15-min prior to an intense 2-h run.

Design: In this randomized, crossover study, 20 long distance runners (N=11 males, N=9 females, age 38.4±2.1 y, $VO2_{max}$ 52.6±2.0) completed two 2-h treadmill runs at 70% VO_{2max} (3 weeks apart) that incorporated a closing 15-min time trial. In double-blinded fashion, subjects ingested either four Q-chews or placebo chews (PL) 15 min prior to the treadmill runs. The four Q-chews provided 1000 mg quercetin, 120 mg epigallocatechin 3-gallate (EGCG), 400 mg isoquercetin, 400 mg eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), 1000 mg vitamin C, and 40 mg niacinamide. Subjects provided blood samples 30 min before, immediately following and 1-h post-exercise, and were analyzed for plasma quercetin concentration, total blood leukocytes (WBC), and nine inflammatory cytokines (IL-6, TNFα, GM-CSF, IFNγ, IL-1β, IL-2, IL-8, IL-10, IL-12p70) using an electrochemiluminescence based solid-phase sandwich immunoassay (Meso Scale Discovery, Gaithersburg, MD, USA).

Results: Plasma quercetin was elevated from 80.0±26.0 μg/L (pre-exercise) to 6,337±414 (post-exercise) and 4,324±310 μg/L (1-h post-exercise) after ingestion of Q-chews compared to no change in PL (interaction effect, $P<0.001$). No difference was measured between Q-chew and PL conditions in the distance run during the 15-min time trial (3.08±0.11 and 3.13±0.11 km, respectively, $P=0.370$). Exercise caused significant increases in WBCs, GM-CSF, IL-10, IL-1β, IL-2, IL-6, IL-8, and TNFα, but no differences in the pattern of change were measured between Q-chew and PL trials.

Conclusions: Acute ingestion of Q-chews 15 min before heavy exertion caused a strong increase in plasma quercetin levels but did not counter post-exercise inflammation relative to placebo. These data are in contrast to the anti-

inflammatory effect measured with this same quercetin-based supplement when consumed for two weeks prior to a 3-day period of intensified exercise. Taken together, these data imply that a prolonged Q-chew supplementation period is needed before anti-inflammatory effects can be achieved.

1.2 Abstract in German

Der akute Effekt auf bewegungsinduzierte Entzündungsparameter bei LäuferInnen nach Einnahme eines Supplements auf Quercetin- Basis

Ziel: Diese Studie untersucht den akuten anti-inflammatorischen Einfluss eines Supplements auf Quercetin- Basis bei AusdauerathletInnen bei Einnahme 15 Minuten vor einem 2-stündigen Lauf.

Studiendesign: Die Studie ist im randomisierten crossover Design. Zwanzig LangdistanzläuferInnen (N=11 männlich, N=9 weiblich, Alter 38.4±2.1 , $VO2_{max}$ 52.6±2.0) sind zwei Stunden auf dem Laufband bei 70% VO_{2max} mit einem abschließenden 15- Lauf mit selbst gewählter Geschwindigkeit gelaufen (mit 3 Wochen Zeitabstand zwischen den Testungen). In doppelblinder Version bekamen die ProbandInnen entweder Q-Kaubonbons oder Placebo- Kaubonbons (PL) 15 min vor dem Lauf auf dem Laufband. Die vier Q-Kaubonbons beinhalteten 1000 mg Quercetin, 120 mg Epigallocatechin 3-gallate (EGCG), 400 mg Isoquercetin, 400 mg Eicosapentaensäure (EPA) und Docosahexaensäure (DHA), 1000 mg Vitamin C und 40 mg Niacinamide. Es erfolgte 30 min bevor, unmittelbar danach und eine Stunde nach dem Lauf eine Blutabnahme. Plasmaquercetin, weißen Blutkörperchen, neun entzündungsrelevanten Leukine (IL-6, TNFα, GM-CSF, IFNγ, IL-1β, IL-2, IL-8, IL-10, IL-12p70) wurden mittels eines auf Elektrochemiluminescence basierenden Festphasenkondensations- Immuntests (Meso Scale Discovery, Gaithersburg, MD, USA) bestimmt.

Ergebnisse: Plasma Quercetin stieg von 80.0±26.0 µg/L (vor dem Lauf) auf 6337±414 (nach dem Lauf) und 4324±310 µg/L (1 Stunde nach dem Lauf) nach Einnahme von Q- Kaubonbons im Vergleich zu PL (Interaktionseffekt, $P<0.001$). Keine Unterschiede wurden gemessen zwischen Q-Kaubonbons und PL Bedingungen im 15-minütigen abschließenden Lauf (3.08±0.11 bzw. 3.13±0.11 km, $P=0.370$).

Die Belastung führte zum signifikantem Anstieg der weißen Blutkörperchen, GM-CSF, IL-10, IL-1β, IL-2, IL-6, IL-8 und TNFα, es konnte jedoch kein Unterschied in der Ausprägung der Veränderung zwischen Q-Kaubonbon und PL festgestellt werden.

Conclusions: Die akute Einnahme von Q-Kaubonbons 15 min vor einer intensiven physischen Belastung resultierte in einem starken Anstieg von Plasmaquercetin, reduzierte allerdings nicht den Ausprägungsgrad der belastungsinduzierten Entzündung im Vergleich zu PL. Diese Ergebnisse stehen im Gegensatz zum inflammationsreduzierenden Effekt der mit demselben Supplement nach 2-wöchiger Verabreichung und anschließender 3-tägigen intensiven körperlichen Belastungen erreicht werden konnte. Zusammenfassend lässt sich sagen, dasseine längere Einnahme des Q-Kaubonbons nötig ist um anti-inflammationsrelevante Effekte erzielen zu können.

2 Introduction

Sports and exercise impair dozens of positive effects on the human body. Despite all the pros of exercise in a long-lasting heavy training physiological change is tremendous.

Multiple components of the immune system in athletes exhibit transient dysfunction after prolonged, heavy exertion. During this "open window" of impaired immunity, pathogens may gain a foothold and increase the infection risk (Nieman, 1997; Nieman, 2000a; Nieman, Henson, Dumke, Rind, Shooter, et al. 2006).

Obviously elite athletes must train intensively to compete at the highest levels and they can benefit from immunonutritional support to bolster immunity during periods of physiological stress, like heavy training and competitions (Nieman, 2008).

In contrary non-athletes engaging in moderate physical activity programs do not require nutritional supplements, and can obtain all needed nutrients from a healthy and balanced diet (Walsh, Gleeson, Pyne, Nieman, Dhabhar, et al., 2011).

Many nutritional supplements with very different effects have been studied as countermeasures to exercise- induced immune changes and infection risk. The supplements itself vary in their time period of the intake, their composition and their physical condition.

Some question the value of using immunonutritional support for athletes because blocking the transient immune changes, oxidative stress, and inflammation following heavy exertion interferes with important signaling mechanisms for training adaptations (Ristow, Zarse, Oberbach, Kloting, Birringer, et al., 2009).

Another viewpoint is that efficacious nutritional supplements only partially block exercise-induced immune function, inflammation, and oxidative stress, analogous to the beneficial use of ice packs to reduce swelling following mild injuries (Yfanti, Akerstrom, Nielsen, Nielsen, Mounier, et al., 2010).

Taken together, this debate will hopefully spur additional research on the overall value of immunonutritional support for athletes (Walsh, et al., 2011).

The purpose of this study was to extend the findings using the Q-chew to determine if an acute dose taken 15 minutes prior to 2-h treadmill running bout would exert anti-inflammatory and immune modulating influences, as it did when consumed for two weeks prior to a 3-day period of intensified exercise (Nieman, Henson, Maxwell, Williams, McAnulty, et al., 2009).

2.1 Physiological changes while training

Already in 1902 Larrabee in exercise immunology described the large increase in blood neutrophils among four athletes who ran the Boston Marathon in 1901. Larrabee observed "the exertion had gone far beyond physiological limits" and that changes in the white blood differential counts paralleled those seen in certain diseased and inflammatory conditions (Larrabee, 1902).

Physical activity influences immune function and the risk of certain types of infection such as upper respiratory tract infections (URTI), increase of inflammation markers and an increase of oxidative stress. In contrast to moderate physical activity, prolonged and intensive exertion by endurance athletes causes numerous changes in immunity in multiple body compartments and an increased risk of URTI. As a matter of fact elite endurance athletes must train intensively and with a high frequency to compete at the highest levels and are prime candidates for immunonutrionial support to bolster the immune system function in the phase of the physiological stress (Nieman, 2008).

Many components of the immune system exhibit change after “marathon-type” exertion, reflecting the physiological stress that the body is experiencing:

- Neutrophilia and lymphopenia, including a steep drop in blood natural killer (NK) and T-cells.
- Decrease in blood and spleen NK cell cytotoxic activity and T-cell function.
- Decrease in nasal neutrophil phagocytosis and mucociliary clearance.
- Decrease in nasal and salivary IgA concentration.
- Decrease in blood granulocyte oxidative burst activity.
- Decrease in the skin delayed-type hypersensitivity response.
- Blunted MHC II expression and antigen presentation in macrophages.
- Increase in pro- and anti-inflammatory cytokines and chemokines (e.g. IL-6. IL-1rA, IL-10, IL-8, granulocyte colony-stimulating factor, monocyte chemoattractant protein 1 and macrophage inflammatory protein 1-β) (Nieman, 2000b).

Of all immune cells, NK cells, neutrophils and macrophages (of the innate immune system) exhibit the greatest changes in response to marathon competition, both in terms of numbers and function (Larrabee, 1902; Nieman, 1997).

Several mechanisms appear to be involved, including exercise-induced stress hormones, body temperature changes, increases in blood flow, lymphocyte apoptosis and dehydration. Following prolonged heavy exertion, the concentration of serum cortisol is significantly elevated for several hours and has been related to many of the cell trafficking changes in experienced athletes during recovery (Nieman, 2007).

Again, during this open window of altered immunity – which may last between 3 and 72 hours- depending on the immune measure, viruses and bacteria may gain a foothold, increasing the risk of subclinical and clinical infection (Nieman, 1997; Nieman, 2000a).

Attempts thus so far to compare resting immune function in endurance athletes and non-athletes have failed to provide evidence that athletic endeavor is linked to

clinically important changes in chronic, resting, immunity (Nieman, Buckley, Henson, Warren, Suttles, et al., 1995).

Several studies indicate that the innate immune system responds differentially to the chronic stress of intensive exercise, with NK cell activity tending to be enhanced while neutrophil function is suppressed but this is not a consistent finding (Nieman, et al., 1995; Nieman, Nehlsen-Cannarella, Fagoaga, Henson, Shannon, et al., 2000).

The adaptive immune system (resting state), in general, seems to be largely unaffected by athletic endeavor. Thus, the magnitude of change in immunity that occurs after each marathon race bout may have more clinical significance than training-induced alterations in resting immunity.

Taken together, these data indicate that there is a relationship between exercise workload and infection. Most endurance athletes should experience low to normal URTI risk during periods of regular training, with URTI risk during periods of overreaching/overtraining and competition. Other factors may amplify URTI risk for the marathon athlete, including exposure to novel pathogens, lack of sleep, severe mental stress, malnutrition and weight loss (Walsh, et al., 2011).

2.1.1 Exercise- induced inflammation

There is a strong evidence for certain cytokines to rise after endurance exercise, especially when larger muscle groups are involved (Lenn, Uhl, Mattacola, Boissonneault, Yates, et al., 2002; Urso & Clarkson, 2003).

Several studies have indicated that strenous physical exercise is accompanied by an increase in circulating levels of Interleukin 6. The physiological consequences of the increased secretion of IL-6 are uncertain (Croisier, Camus, Cenneman, Depy-Dupont, Juchmes-Ferir, et al., 2001).

It has been documented that strenuous exercise not only induces pyrogenesis but also elicits mobilization and functional augmentation of neutrophils and monocytes whereas it suppresses cellular immunity leading to increased susceptibility to infections. Although tumor necrosis factor (TNF-)- alpha and interleukin (IL-)-1 beta have traditionally been understood to be the main inducer cytokines of acute phase reactions, the majority of studies have shown that the circulating concentration of these cytokines is either unchanged following exercise, or exhibits relatively small, delayed increments. Plasma interferon (IFN)- alpha and IFN-gamma do not appear to change following exercise, whereas IL-2 decreases after endurance exercise. Endurance exercise induces systemic release of granulocyte colony- stimulating factor (G-CSF), macrophage CSF (M-CSF), IL-8 and monocyte chemotactic protein 1 (MCP-1) (Suzuki, Nakaji, Yamanda, Totsuka, Sato, et al., 2002).

The immune system reflects the physiologic stress of an endurance athlete, and as a consequence anti-inflammatory cytokines (IL-6, IL-10, IL-8, IL-1ra, granulocyte colony-stimulating factor, monocyte chemotactic protein 1, macrophage inflammatory protein 1 beta, macrophage migration inhibitory factor 1, and TNF-α) increase during heavy exertion (Nieman, Henson, Smith, Utter, Vinci, et al. 2001; Nieman, Oley, Henson, McAnulty, Davis, et al., 2006b).

Other athlete studies showed very similar data, the longer the exercise bout, the higher the cytokines. In the Western States Endurance Run IL-6, IL-8, IL-1β, IL-10 and TNF-α are very high after the 160 km competition. The greatest fold increase is experienced pre- to postrace for interleukin 6 (~130-fold), followed by interleukin 10 (~31-fold), granulocyte colony stimulating factor (G-CSF) (~26-fold), interleukin 8 (9-fold), IL-1 receptor antagonist (IL-1Ra) (7-fold), monocyte chemotactic protein-1 (MCP-1) (3-fold), macrophage inflammatory protein- 1β (MIP-1β) (2-fold), macrophage migration inhibitory factor-1 (MIF-1) (1.5 fold), and tumor necrosis factor-α (TNF-α) (1.3-fold) (Nieman, Henson, Davis, Dumke, Gross, et al., 2007; Nieman, et al., 2006b).

In contrast to the adaptive system, cells from the innate immune system are influenced by heavy exertion with a transient post-exercise increase in granulocyte phagocytosis and a decrease in granulocyte oxidative burst activity and natural killer cell lytic activity (Nieman, 1997).

By measuring the serum creatine kinase (CK) there was a correlation between the degree of muscle damage and the plasma levels for most of the cytokines. Il-6, IL-8, IL-1β and TNF-α mRNA content is increased within postexercise muscle biopsy samples, and blood leukocytes may secrete increased amounts of IL-8, IL-10 and IL-1Ra during sustained exercise (Nieman, et al., 2007).

Concerning the mechanism for cytokine gene expression there is some data, that shows that nitric oxide (NO) production is a key regulator (Steensberg, Keller, Hillig, Frosig, Wojtaszewski, et al., 2007).

There are some other potential triggers like leakage of endotoxins from the intestines, high core body temperature, elevation of catecholamines and cortisol, glycogen deficiency, other metabolic demands and oxidative stress (Nieman, Davis, Henson, Walberg-Rankin, Shute, et al., 2003a; Nieman, et al., 2005; Nieman, et al., 2006b; Nieman et al., 2003b; Steensberg et al., 2007; Suzuki, Totsuka, NaKaji, Yamada, Kudoh, et al., 1999).

Eccentric exercise in people produces delayed-onset muscle soreness (DOMS), the acute muscle damage from eccentric exercise can cause local inflammation (Mc Intyre, Reid, Lyster, Szasz, & Mc Kenzie, 1996).

There is still a debate going on in regard to benefit versus risk, a reduction in the inflammatory cytokine response to exercise through nutraceutical or pharmacologic means is a goal being pursued by several research teams (Nieman, et al., 2007).

2.1.2 Exercise and oxidative stress in athletes

During strenuous exercise, there is a dramatic increase in oxygen uptake in various organs, particularly in the skeletal muscle. The resting body is equipped with both enzymatic and non-enzymatic antioxidant reserves (Morillas-Ruiz, Villegas Garcia, López, Vidal-Guevara, & Zafrilla, 2006).

Cells continuously produce free radicals and reactive oxygen species (ROS) as part of metabolic processes. These free radicals elaborate an antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and numerous non-enzymatic antioxidants, including vitamins A, E, and C, glutathione, ubiquinone, and flavonoids. Exercise can produce an imbalance between ROS and antioxidants, which is referred to as oxidative stress. Physical activity increases the generation of free radicals in several ways. Two of 5% of oxygen used in the mitochondria forms free radicals. As oxidative phosphorylation increases in response to exercise, there will be a concomitant increase in free radicals. Catecholamines that are released during exercise can lead to free radical production. Other sources of free radical increase with exercise induced prostanoid metabolism, xanthine oxidase, NAD(P)H oxidase, and several secondary sources, such as the release of radicals by macrophage recruited to repair damaged tissue (Jackson, 2000).

Athletes use antioxidant supplementation as a mean to counteract the oxidative stress of exercise. Whether strenuous exercise does, in fact, increase the need for additional antioxidants in the diet is not clear. If the increase in free radicals is greater than the ability to neutralize them, the radicals will attack cellular components, especially lipids. The attack on lipids initiates a chain reaction called lipid peroxidation, which leads to generation of more radicals and ROS that can harm other cellular components. The body appears able to withstand a limited increase in free radicals and in fact, data suggest that an increase in ROS is necessary for muscle adaptation to occur (Jackson, 1999; Urso & Clarkson, 2003).

2.2 Immunonutrition support for athletes

Many nutritional agents have been tested in order to attenuate the immune changes after heavy exertion.

In general the strategy is similar to the immunonutrition support provided to patients recovering from trauma and surgery, and to the frail elderly (Grimble, 2005; Nieman, 2008).

2.2.1 Types and efficacy

The most commonly used dietary supplements are micronutrients. The antioxidant properties of several dietary micronutrients are of particular interest to athletes due to the support of the body ´s endogenous antioxidant defense systems that allows free radicals to be neutralized to help decrease oxidative damage (Nieman, Stear, Castell, & Burke, 2010a).

Supplements studied thus far in human athletes include zinc, N-3 polyunsaturated fatty acids (N-3 PUFAs), plant sterols, antioxidants (e.g. vitamins C and E, beta-carotene, N-acetylcysteine, and butylated hydroxyanisole), glutamine, bovine colostrum, and carbohydrate. Moreover we have the so-called "advanced supplements" such as β-glucan, curcumin and quercetin (Nieman, 2008).

Results for most nutritional supplements tested as countermeasures to exercise-induced inflammation, oxidative stress, and immune dysfunction following heavy exertion have been disappointing. Early studies focused on large dose vitamin and/or mineral supplements, and no consistent countermeasure benefit has been observed (Davison & Gleeson, 2005; Davison & Gleeson, 2006; Gleeson, Nieman, & Pedersen, 2004; Nieman, Henson, McAnulty, McAnulty, Swick, et al., 2002; Nieman, Henson, McAnulty, McAnulty, Morrow, et al., 2004).

A series of studies dating back to the mid- 1990s have shown that carbohydrate supplement ingestion before and/or during prolonged exercise attenuates increases in blood neutrophil and monocyte counts, stress hormones, and anti-inflammatory cytokines such as IL-6, IL-10, and IL-1ra but has little effect on decrements in salivary IgA output and T cell and natural killer cell function (Gleeson, 2007; Chen, Wong, Wong, Lam, Huang, et al., 2008).

So carbohydrate ingestion has an effect but more on stress hormones and inflammation but limited effects on markers of innate or adaptive immunity. Amino acids and Glutamine show no benefits when compared to placebo and are not recommended (Gleeson, 2008).

2.2.2 Timing of supplementation

An important question is about the appropriate duration of supplementation of flavonoids to archieve any positive effect. Powers et al. (2010) speak about the appropriate duration of antioxidant supplementation as one of the key issues in the guidelines to investigate the impact on exercise performance (Powers, Smuder, Kavazis, & Hudson, 2010).

There were many attempts in other human trials to counter the exercise-induced inflammation and the time period of supplemenation was as far as 60 days, the majority varied between seven and 21 days (Nieman, et al., 2010a).

Even a seven- day supplementation of quercetin is regarded as „short term“ supplementation (Davis, Murphy, Carmichael, & Davis, 2009a).

Bakker et al. (2010) showed in male, overweight subjects that even after a 5-week supplementation of an evidence based anti-inflammatory dietary mix, only mild effects of improvement were observed due to the length of the intervention (Bakker, Van Erk, Pellis, Wopereis, Rubingh, et al., 2010).

Most investigations using flavonoid-rich products or extracts in athletic settings have utilized supplementation periods of at least seven days prior to heavy exertion, and reported varying levels of success in attenuating inflammation and oxidative stress (McAnulty, McAnulty, Morrow, Khardouni, Shooter, et al., 2005; Chang, Hu, Huang, Yeh, & Liu, 2010; Goldfarb, Garten, Cho, Chee & Chambers, 2011; Nieman, et al., 2010a; Trombold, Barnes, Critchley, & Coyle, 2010).

A few studies used an acute dose of a flavonoid-rich product or extract prior to exercise (Lyall, Hurst, Cooney, Jensen, Lo, et al., 2009; Davison, Callister, Williamson, Cooper, & Gleeson, 2011; Morillas-Ruiz et al., 2006; Wiswedel, Hirsch, Kropf, Gruening, Pfister, et al., 2004).

One of the acute dose studies in athletes worked with blackcurrent extract. They had 10 moderately active subjects consuming 48 grams of anthocyanin-rich blackcurrent extract or placebo (double-blinded, crossover design) immediately before and after high-intensity rowing, and reported some success in alleviating transient post-exercise inflammation and oxidative stress. In particular, acute blackcurrant supplementation significantly reduced post-exercise cytokine production from LPS stimulated cells perhaps through partial NFKB inhibition, but no in vivo measures of inflammation were included in this study. A reduction in protein carbonyls, reactive oxygen species (ROS) generating capability, and creatine kinase (24-h post-exercise) was also reported following blackcurrant supplementation, and the authors speculated that the reduced oxidative stress and inflammation were related (Lyall, Hurst, Cooney, Jensen, Lo, et al., 2009).

In contrast, Davison et al. (2011) reported no anti-inflammatory influences of ingesting an acute 100 g dose of dark chocolate prior to 2.5 h cycling (Davison, Callister, Williamson, Cooper, & Gleeson, 2011).

As we can see, the time range of supplementation might vary a lot and there are few studies measuring the short-term effect.

Quercetin supplementation covers periods ranging from two to six weeks in untrained and trained subjects has been linked to an inconsistent influence on exercise performance (Nieman, 2010; Nieman, Williams, Shanely, Jin, McAnulty, et al., 2010b).

Short-term supplementation with EGCG has been related to a small but significant increase in maximal exercise performance (Richards, Lonac, Johnson, Schweder, &

Bell, 2010). The authors speculated that the ergogenic influence of EGCG occurred through COMT inhibition and increased sympathetic nervous system stimulation.

Taken together there are no consistent recommendations on the timing of the supplement. There is little evidence for an acute dose of polyphenols on performance and immunological changes.

2.2.3 Supplementation to counter inflammation

A reduction in blood glucose levels during intense and prolonged exertion when athletes drink plain water has been linked in multiple studies to hypothalamic-pituitary-adrenal activation, and increased release of adrenocortitrophic hormone and cortisol, increased plasma growth hormone, and increased plasma epinephrine levels (Nieman, et al., 2001; Nieman & Bishop, 2006c).

Most studies on carbohydrates indicate that carbohydrate beverage ingestion during prolonged exercise attenuates increases in blood neutrophil and monocyte counts, stress hormones, and inflammatory cytokines such as IL-6, IL-10, and IL-1ra, but it has little effect on decrements in salivary IgA output and T-cell and natural killer cell function (Nieman, et al., 2001; Bishop, Walker, Bowley, Evans, Molyneux, et al., 2005).

Carbohydrate may exert these effects through multiple mechanisms including elevation in blood glucose and tissue glucose uptake leading to diminished output of stress hormone, decrease in cytokine mRNA expression, reductions in pro-inflammatory signals, and an attenuation of IL-6 release from the working muscle tissue (Nieman, et al., 2001; Bishop, et al., 2005).

The effect of vitamin E supplementation on the inflammatory and immune response to intensive and prolonged exercise is equivocal. Vitamin E supplementation of 800 IU/d for 48 days attenuated endotoxin-induced IL-6 secretion from mononuclear

cells for 12 days after running downhill on an inclined treadmill, according to one early report (Cannon, Meydani, Fiedling, Fiatarone, Meydani, et al., 1991).

Subsequent studies showed no effect of vitamin E supplementation (800 IU/day) on the increase in plasma IL-6 following a 98-min treadmill run at 65-70% $VO_{2\ max}$. (Petersen, Ostrowski, Ibfel, Richelle, Offort, et al., 2002).

A month supplementation with Vitamin E did not counter increases in plasma cytokines in triathletes either (Nieman, et al., 2004).

Nieman (2008) showed in a review paper that a large- dose of Vitamin E actually can exacerbate inflammation during prolonged exercise at heat (Nieman, 2008a).

Beta-Glucans are polysaccharides found in the bran of oat and barley cereal grains, the cell wall of baker's yeast, certain types of fungi, and many kinds of mushrooms. A wide variety of β-glucans exists that vary in macromolecular structure, solubility, molecular weight, and biological activity (Mantovani, Bellini, Angeli, Oliveira, Silva, et al., 2008).

Receptors of β-glucans have been identified on a wide variety of cell types including macrophages, dendritic cells, natural killer cells, neutrophils, some types of T-cells, epithelial cells, vascular endothelial cells, and fibroblasts (Castro, Panilaitis, Bora, & Kaplan, 2007).

There is good evidence for animals and positive influence on the immune system, in contrast a recent study with human athletes failed to confirm these results. Trained mailed cyclists were randomized to β-glucan or placebo (doble-blind procedure) for two weeks prior and during a 3-day period in which subjects cycled for 3 h/d at a high intensity. Blood samples were taken before and after 14 days of β–glucan supplementation (chronic immunity) and immediately after the last bout of exercise and 14 h of recovery; they were assayed for a wide variety of immune function measures including natural killer and T-cells, granulocytes, and plasma cytokine levels. None of these immune measures differed by β–glucan and placebo groups (Nieman, Henson, McMahon, Wrieden, Davis, et al., 2009a).

Phillips, Childs, Dreon, Phinney, & Leeuwenburgh (2003) suggested that a

combination of natural bioactive nutrients (tocopherols, docosahexaenoate, and flavonoids as quercetin) provided before and during eccentric-exercise induced has potential anti-inflammatory properties as assessed by the attenuated levels of IL-6 and CRP. However, repair of muscle injury is dependent on inflammatory mediators, and it is therefore not clear to what extent a severe inflammatory response as seen in this study needs to be curbed for better recovery and improved function.

2.2.4 Supplementation to counter oxidative stress

As discussed already intensive and sustained exercise can create an imbalance between reactive oxygen species and antioxidant defenses, leading to oxidative stress that causes lipid peroxidation and protein oxidation (Nieman, et al., 2003b; Mastaloudis, Traver, Carstensen, & Widrick, 2006).

Although pathways between oxidative stress during heavy exertion and immune dysfunction have been described, data support is widely lacking (Nieman, et al., 2003b).

Moreover the proposed benefits of antioxidant supplementation in attenuating both oxidative stress and exercise- induced immune dysfunction remain unsubstantiated (Nieman, et al., 2003b; Mastaloudis, et al., 2006; Petersen, et al., 2002).

A two-month supplementation with Vitamin E at a dose of 800 IU/day α-tocopherol did not counter oxidative stress in triathletes competing in the Kona Triathlon World Championship. Triathletes in the vitamin E group compared to the placebo group actually experienced greater lipid peroxidation (Nieman, et al., 2004).

Curcumin is a yellow-orange pigment derived from the rhizome of Curcuma longais or turmeric, a spice often used in curry powder. Several clinical trials have been conducted using curcumin at doses up to 8 g/day, and each trial has concluded that curcumin is safe and poses minimal or no adverse effects. Curcumin acts as a free radical scavenger and anti-oxidant, inhibiting lipid peroxidation and oxidative DNA damage (Johnson & Mukhtar, 2007).

It is not clear, whether curcumin acts directly as an antioxidant in vivo. Due to its limited oral bioavailability in humans, plasma and tissue curcumin concentrations are likely to be much lower than that of other fat- soluble antioxidants. In addition to direct antioxidant activity, curcumin may function indirectly as an antioxidant by inhibiting the activity of inflammatory enzymes or by enhancing the synthesis of glutathione, an important intracellular antioxidant (Nieman, 2008a).

Taken together all the results of human studies, antioxidant supplementation to counter both oxidative stress and immune dysfunction in endurance athletes during heavy exertion cannot be recommended. The majority of investigations have failed to show that ingestion of antioxidants such as vitamins E and C have meaningful effects on exercise- induced inflammation, muscle damage, increases in plasma cytokines, and immune perturbations (Nieman, 2008a).

2.3 Quercetin

2.3.1 Quercetin Pharmacokinetics in Humans

Quercetin as one of the predominant flavonoids is widespread in our diet. Bioavailability of quercetin is poor, and the bioavailability of quercetin glycosides is dependent on the type and position of the sugar moieties (Hollman, Bijsman, van Gameren, Cnossen, de Vries, et al., 1999).

The average terminal half-life is 3.5 hours for quercetin. The total recoveries of C-Quercetin in urine, feces and exhaled air in the individuals is interestingly highly varible (Moon, Wang, DiCenzo, & Morris, 2008).

The pharmacokinetic data on doses of pure quercetin being used in clinical trials show that quercetin can be detected in plasma within 15-30 min of ingestion of a 250-500 mg quercetin chew preparation, reaching a peak concentration at

approximately 120-180 min, returning to baseline levels at 24 hours in humans (Davis, Murphy, & Carmichael, 2009b).

These results are consistent with those of others that have measured quercetin absorption and appearance in the plasma after ingestion of the pure quercetin aglycone as well as various gluconated forms contained in foods such as shallots, onions, and apples (Egert, Wolffram, Bosy-Westphal, Wagner, Frank, et al., 2008).

The natural antioxidant flavonoids constitute significant components of the diet and display a diverse array of biological effects (Kandaswami & Middleton, 1994; Korkina & Afanas´ev, 1997; Li, Fu, Dongyan, Mikovits, Ruscetti et al., 2000; Middleton, Kandaswami, Theoharides, 2000).

Polyphenolic compounds, including a large class of flavonoids, are enriched in certain vegetables, fruits, seeds, and beverages (e.g. tea and wine) and are regarded as semiessential nutrients in humans. The intake of these compounds improves the health of individuals and decreases the risk of cardiovascular disease. The beneficial effects of flavonoids have been attributed to their antioxidant and anti-inflammatory properties (Kim, Son, Chang, & Kang, 2004; Korkina & Afanas´ev, 1997; Scalbert, Johnson, & Saltmarsh, 2005).

One of these subgroups, the flavonols, includes quercetin, a widely distributed and investigated flavonoid (Nieman, 2010).

Food sources of quercetin include tea, onions, apples, peppers, blueberries, and dark green vegetables (Chun, Chung, & Song, 2007; USDA Nutrient Data Laboratory, 2007).

Quercetin accumulates in the outer and aerial tissues (skin and leaves) because its biosynthesis is stimulated by light. Human subjects can absorb significant amounts of quercetin from food or supplements, and elimination is quite slow, with a reported half-life ranging from 11 to 28 hours (Conquer, Maiani, Azzini, Raguzzini, & Holub, 1998; Manach, Williamson, Morand, Scalbert, & Remesy, 2005).

Flavonol intake is about 13 mg / day for U.S. adults, quercetin represents three-fourths of this amount. The estimated flavonoid intake ranges from 50-800 mg / day

depending on the consumption of fruits and vegetables and the intake of tea (Chun, et al., 2007).

In Spain however the average daily of flavonoids is 313 mg/d with important sources including tea, citrus fruits and juice, beers and ales, wines, melon, apples, onions, berries and bananas (Zamora-Ros, Andres-Lacueva, Lamuela-Raventos, Berenguer, Jakszyn et al., 2010).

Quercetin and its supplementation are regarded as safe. In both animal and human studies there were no adverse symptoms or harmful effects (Harwood, Danielewska-Nikiel, Borzelleca, Flamm, Williams et al., 2007; Knab, Shanely, Henson, Jin, Heinz, et al., 2011; Utesch, Feige, Dasenbrock, Broschard, Harwood, et al., 2008; Henson, Nieman, Davis, Dumke, Gross, et al., 2008).

Additional literature indicates that isoquercetin (glycosylated quercetin) is more completely absorbed than is quercetin in aglycone form, and that the simultaneous ingestion of quercetin with vitamin C, folate, and additional flavonoids improves bioavailability (Manach et al., 2005; Harwood et al., 2007; Moon & Morris, 2007).

2.3.2 Quercetin and safety

Quercetin has GRAS status (generally recognized as safe) according to criteria established by the U.S. Food and Drug Administration (FDA) (Davis, Murphy & Carmichael, 2008).

Quercetin supplementation in both animal and human studies does not cause adverse symptoms or harmful physiological effects (Harwood, et al., 2007; Knab, et al., 2011; Utesch, et al., 2008).

Long term feeding of quercetin in rats leads to an accumulation in several organs including the lungs, testes, kidneys, heart, liver, thymus, and muscle (de Boer, Dihal, van der Woude, Arts, Wolffram, et al., 2005).

This finding, however, was not replicated in pigs, with quercetin found only in organs involved in its metabolism and excretion, including small intestine, kidneys, and the liver (Bieger, Cermak, Blank, de Boer, Hollman, et al., 2008).

All these data produce some doubt about lung, heart, and muscle incorporation in humans, but biopsy or radiolabeled investigations have not been conducted (Nieman, 2010).

2.3.3 Quercetin and inflammation

Inflammation and oxidative stress are key mechanisms in the pathogenesis of certain disease states, supporting the strategy of increased flavonoid and quercetin intake either through diet enrichment or supplementation (Nieman, 2010).

There are quite some studies that focus on quercetin and exercise-induced inflammation:

After a 3 week supplementation period with pure quercetin (1000mg/d) the test subjects s raced 160 km in the Western States Endurance Run. Thirty-nine subjects (18 for quercetin, 21 for placebo) finished the race, provided blood samples the morning before the race and 15-30 min postrace. Significant pre- to postrace decreases were measured for natural killer cells (43%), granulocyte respiratory burst activity (55%) and increases for neutrophil (288%) and monocyte (211%) cell counts with no significant group differences. In conclusion the quercetin supplementation had no effect on illness rates, perturbations in leukocyte subset counts, or decreases in granulocyte respiratory burst activity and salivary IgA (Henson et al, 2007).

Quercetin also influences in vitro measures of immune function via upregulation of interferon gamma, inhibition of NF κB signaling in macrophages, and augmentation of neutrophil chemotaxis and respiratory burst activity, macrophage phagocytosis, NK cell lytic activity, and mitogen-stimulated lymphocyte proliferation (Yu, Lai, Yang, Chiang, Lu, et al., 2010).

Data on the effects of supplemental quercetin in lowering inflammation in human subjects also differs from in vitro and animal findings (Knab, et al., 2011).

Egert et al. (2008) reported no effect of two weeks quercetin supplementation (50, 100, or 150 mg/day) on inflammatory markers in 35 healthy subjects (Egert, et al., 2008).

A small decrease in IL-6 was reported following 12 weeks of 1000 mg/day quercetin supplementation, but other inflammation biomarkers including C-reactive protein, tumor necrosis factor alpha (TNF-α), monocyte chemoattractant protein (MCP), and granulocyte colony stimulating factor were unaffected (Knab, et al., 2011).

Summing up the results of the influence of quercetin on exercise-induced inflammation there is barely evidence for measures in vitro.

2.3.4 Quercetin and oxidative stress

Quercetin is a powerful in vitro antioxidant and free radical scavenger (Loke, Proudfoot, McKinley, Needs, Kroon, et al., 2008).

It hast been well-established that quercetin with its phenolic OH groups protects against free radical damage via radical scavenging activity (Santos & Mira, 2004).

However, low bioavailability and metabolic transformation reduce the likely in vivo scavenging activity (Loke et al, 2008).

McAnulty et al. (2008) could not exert protection from exercise- induced oxidative stress despite previous data demonstrating potent antioxidant actions of quercetin in vitro. Forty athletes were recruited and randomized to quercetin or placebo. Subjects consumed 1000 mg quercetin or placebo each day for 6 weeks before and during 3 d of cycling at 57% work maximum for 3 hours. The findings from this study indicate that quercetin supplementation increases circulating plasma values of quercetin, however, the increase in plasma quercetin metabolites did not affect oxidative stress, inflammation or plasma antioxidant capacity (Mc Anulty, Mc Anulty, Nieman, Quindry, Hosick, et al., 2008).

The majority of human studies indicate that supplementation with quercetin in aglycone form does not exert anti-oxidant effects even in daily doses up to 1000 mg over 12 weeks (Knab, et al., 2011; Egert, et al., 2008).

2.3.5 Effects of Quercetin on performance in humans

Cardiorespiratory endurance exercise increases active skeletal muscle mitochondrial density by 20% to 100% depending on the exercise workload (Hoppeler & Fluck, 2003).

By increasing the intracellular calcium levels this process is imparted during muscle contraction and involves the coordinated expression of mitochondrial and nuclear genes including the transcriptional coactivator peroxisome proliferator –activated receptor γ-coactivator-1 (PGC-1α) (Diaz & Moraes, 2008).

There is a good evidence to support the hypothesis that quercetin may be able to increase endurance exercise capacity. The evidence comes primarily from in vitro and in vivo studies in rodents that show that quercetin has a combination of biological properties, known to affect both physical and mental performance, and the ability to increase mitochondrial biogenesis in both the muscle and brain of mice (Davis, et al., 2009).
There are a couple of studies with supplementation of quercetin and the performance outcome in humans too. The improvement of physical performance in athletes might be because of its antioxidant properties (Cureton, Tomporowski, Singhal, Pasley, Bigelman, et al., 2009).

The results though are not consistent on performance outcome:
After a two-week supplementation with quercetin (1000 mg/ d^{-1}) vs. placebo in untrained male subjects, providing blood and muscle biopsy, was associated with a small but significant improvement in a 12-min time trial (15% treadmill with a self-

selected speed) and modest but insignificant increases in the relative copy number of mitochondrial DNA and messenger RNA levels of four genes related to mitochondrial biogenesis (Nieman, et al., 2010b).

Another study in untrained volunteers did show a modest improvement of their VO_{2max} (3.9% vs. placebo; $p < .05$) along with a substantial (13.2%) increase in ride time to fatigue ($p < .05$) after a one week supplementation with quercetin ($1000mg \cdot d^{-1}$) compared to Placebo (Davis, Carlstedt, Chen, Carmichael, & Murphy, 2010).

One study of 11 elite cyclists reported a 1.7% 30-km time trial performance enhancement above placebo following six weeks of quercetin supplementation mixed with green tea leave extract and antioxidant vitamins (MacRae & Mefferd, 2006).

In contrary an other study with trained cycler showed different results: 39 trained cycler where randomized to placebo or quercetin mixed with EGCG, took the supplement for two weeks and cycled on three consecutive days 3 hours/day. Subjects of all groups were able to maintain a mean power output of 56.9 ± 0.6% $W_{max,}$ the total time trial duration did not differ among groups, and there was no difference in mRNA expression for genes related to skeletal muscle mitochondrial biogenesis (Nieman, et al., 2009a).

In a double-blind study recreationally-active, but not endurance-trained cyclers were measured before and after a 7-16 d variable period of time ingesting 1000 mg quercetin in a sports hydration beverage or a placebo beverage. There was no significant difference ($p>0.05$) of muscle oxidative capacity, metabolic, neuromuscular and perceptual determinants of performance in prolonged exercise or cycling performance in Placebo (in a sugar-based "PowerAde") relative to quercetin. Each group hat 15 subjects, the study did not use a crossover design and the mode of exercise testing was novel to the study participants (Cureton, et al., 2009).

Another study in 40 trained cyclists randomized to 1000 mg /day quercetin or placebo for three weeks failed to show any group differences in measures of cycling efficiency or skeletal muscle mRNA expression for PGC-1α or SIRT1 (Dumke, Nieman, Utter, Rigby, Quindry, et al., 2009).

There was no effect either in a study with 39 trained cycler after a supplementation with 1000 mg quercetin a day compared to Placebo on mRNA expression for mitochondrial biogenesis or cycling time trial performance when engaging in 5-km, 110-km, and 20-km time trials at the end of three 3-h cycling bouts (Nieman, Henson, Maxwell, Williams, McAnulty, et al., 2009b).

In a meta-analysis on quercetin and its ergogenic effect data on 254 subjects did show a small but significant benefit. The mean VO2 max ranged among studies from 41-64 ml/kg/min, had median treatment duration of 14 days and a median dosage of 1083 mg/ day. Despite variability among studies, quercetin provides a small but significant benefit in physiological measures of human endurance exercise capacity (Kressler, Millard-Stafford, & Warren, 2010).

Another interesting property of quercetin that may enhance mental and physical performance is its caffeine-like psycho stimulant effect. Many studies showed a psycho stimulant like caffeine can delay fatigue (Davis, Zhao, Stock, Mehl, Buggy, et al., 2003).

In general polyphenols appear to be beneficial to athletic performance, however the exact mechanisms are unknown, as each class is likely to have a different physiological effect (Braakhuis & Hopkins, 2011; Powers, DeRuisseau, Quindry, & Hamilton, 2004).

2.3.6 Effects of Quercetin on Performance in Animals

The quercetin- related effects on performance and mitochondrial biogenesis are far better in animals:

In vitro data in mice showed that quercetin increased brain and muscle mitochondrial biogenesis and exercise tolerance. Mice were randomly assigned to placebo, 12.5 mg/kg quercetin or 25 mg/kg quercetin. After 7 days of treatment the mice were killed and soleus muscle and brain were analyzed for mRNA expression. Quercetin increased mRNA expression of PGC-1α and SIRT 1, mtDNA and cytochrome c concentration. These changes in markers of mitochondrial biogenesis

were associated with an increase in both maximal endurance capacity and voluntary wheel-running activity (Davis, Murphy, Carmichael, & Davis, 2009a).

3 Methods

3.1 Subjetcs

We did the baseline testing with 22 subjects and had two drop- outs after the first orientation. Out of these 20 subjects we worked with 9 female and 11 male athletes.

The subjects came to the Appalachian State University Human Performance Laboratory at the North Carolina Research Campus in Kannapolis for their initial testing and orientation. All the baseline testing took place within one week.

The subject recruitment process began 2 months prior to baseline testing. By the end of July 2010 several running clubs around Kannapolis were contacted via e- mail in order to ask their members to participate in the study. We also asked in several running shops for interested subjects and an ad was posted in the local newspaper.

All the communication with the subjects was either on the phone or via e- mail. We ended up with 22 runners, 11 male and 11 female subjects.

The subjects were asked to be physically fit, in overall good health, and capable of running for 2 hours on a treadmill. The subjects needed to avoid all nutritional supplements and avoid anti-inflammatory medication.

3.2 Study design

The research design is crossover, with treatments randomized, placebo controlled, and double blinded. Subjects are randomized to Q-chew or placebo, and then crossed over to the opposite condition after a 3-week washout period.

Blood samples are collected 30 minutes prior to exercise, immediately post-exercise, 1-h post-exercise (3 total blood samples during each trial). Blood samples are analyzed for inflammation markers (C-reactive protein, IL-6, IL-10, IL-8, MCP, GCSF, and F2-isoprostanes (oxidative stress). Granulocyte phagocytosis and oxidative burst activity are measured for immune function. Plasma quercetin is measured to determine plasma concentrations from the supplement.

Written informed consent was obtained from each subject, and the experimental procedures were approved by the institutional review board of Appalachian State University.

3.3 Baseline testing

After recruiting the subjects via mass media they came to an appointment in the early afternoon to the Human Performance Lab where they heard again about the study design and their pros and cons on participating in this study. They filled in all the paper work, signed the contract, received their food logs for documentation for both running bouts and had different tests on that specific day.

For the baseline the blank A1 for general data was used (see appendices).

To make sure to have “low risk subjects” concerning their health study, the ACSM/NCRC screening questionnaire was used (see A2 in appendices).

In order to get information about the training history, we used a questionnaire about their training habits and basic demographic data (A3-see appendices).

Measuring of Body Composition

To study body composition, the body mass is subdivided into two or more components. The classic two-component model divides the body mass into fat and fat-free mass. The fat mass contains all extractable lipids, and the fat-free mass includes water, protein, and mineral components (Nieman, 2011).

Body composition refers to the proportion of body weight that is from fat relative to the amount of weight that is from lean tissue (Hoffman, 2006).

For many years hydrostatic weighing was considered the gold standard of body composition analysis. Hydrostatic weighing measures the amount of water that is displaced when an individual is submerged in a tank. As a body sinks under water, it is buoyed by a counterforce equal to the weight of the water displaced. The loss of weight in water, corrected by the density of water can be used to calculate body density with certain formulas. Through various equations the percent body fat can be calculated. Since body density varies and is affected by age, growth, maturation, gender and ethnicity, equations are often specific to a population.

Recent technological advances have led to air displacement plethysmography (ADP), a system that measures air displacement rather than water displacement (Hoffman, 2006).

The BOD POD Gold Standard Body Composition Tracking System is one of them, which uses whole-body densitometry to determine body composition (fat and fat-free mass). The patented air displacement plethysmography used by the BOD POD and PEA POD is very similar in principle to hydrostatic (or "underwater") weighing. The obvious difference is that air is more convenient and comfortable than water; so

that Air Displacement Plethysmography provides a much easier and safer testing environment, better reliability, and significantly improved repeatability and accuracy. The basic operating principles of air displacement plethysmography include Densitometry, Mass Measurement, Volume Measurement, Thoracic Gas Volume, and Surface Area Artifact (Cosmed, USA. Overview and test process of Bod Pod Gold Standard, 2010).

The plethysmograph is a closed chamber that assesses body volume by measuring changes in pressure, has been found to be highly reliable (Hoffman, 2006).

Find the form we used in our study in the appendices (A4).

Measuring of VO2 max with Bruce Protocol

Selecting the appropriate exercise test becomes a critical consideration in an experimental design if the objective of the study is to determine the effects of antioxidant supplementation on exercise performance. Over the years, many studies have evaluated human performance in the laboratory, and there has been extensive debate about the correct use of exercise protocols. The use of accurate performance exercise is needed to achieve the ultimate goal of delineating links between e.g. oxidative stress and human performance (Powers, et al., 2010).

In selection an exercise test to assess changes in performance between experimental treatments, three aspects of the test must be considered: validity, reliability, and sensitivity. A valid exercise test is one that closely resembles the performance of the event being simulated (Currel & Jeukendrup, 2008).

Aerobic power, also referred to as aerobic capacity, measures a person ´s capacity for aerobic synthesis of ATP and so indicates the ability to perform, sustained, high-intensity exercise (McArdle, Katch, & Katch, 1996).

During exercise of increasing intensity (whether by running faster or up a greater incline) oxygen consumption increases. As the workload continues to increase, oxygen uptake plateaus and the athlete begins to utilize other energy sources (e.g. glycolytic) sources to produce ATP (Hoffman, 2006).

At this point, oxygen volume plateaus at what is called the $VO_{2\ max}$ (Nieman, 2008b).

Exercise physiologists consider directly measured maximum oxygen uptake ($VO_{2\ max}$) or peak VO_2 the most valid measure of functional capacity of the cardiorespiratory system. The $VO_{2\ max}$ or rate of oxygen uptake during maximal aerobic exercise, reflects the capacity of the heart, lungs, and blood to transport oxygen to the working muscles and the utilization of oxygen by the muscle during exercise (Heyward, 2002).

Aerobic capacity may be used to assess the fitness and health status of athletes.

Oxygen uptake is the difference in oxygen content between the air inspired and the air expired, expressed in ml/kg body weight/minute. In other words it is the amount of oxygen required by the body to fulfill its functions at a given time. Obviously more oxygen will be required in severe exercise and so oxygen uptake will increase. However, a point is eventually reached where the body can take up no more oxygen. At this point the value is referred to as the maximal oxygen uptake (Dick, 2007).

Directly measuring VO2 while the athlete performs a graded exercise is considered the gold standard of maximal aerobic power assessment. However, $VO_{2\ max}$ can also be determined on a cycle ergo meter or through tethered swimming. The choice of exercise depends on the sport that the athlete plays or the exercise that the individual is most accustomed to performing. If specificity is not an issue, the treadmill produces the best results (Hoffman, 2006).

Nieman (2011) summarized the $VO_{2\ max}$ as the body's capacity to transport and use oxygen during a maximal exertion involving dynamic contraction of large muscle groups, such as during running or cycling; also known as maximal aerobic power and cardiorespiratory endurance capacity (Nieman, 2011).

With increasing workload, oxygen consumption increases up to the last stage of exercise.

The $VO_{2\ max}$ is recognized as an important predictor of performance in endurance events (ACSM ´S Resource Manual for Guidelines for Exercise Testing and Prescription, 2001).

Exercise scientists and physicians use exercise tests to evaluate functional aerobic capacity ($VO_{2\ max}$) objectively. The $VO_{2\ max}$, determined from graded maximal or submaximal exercise tests is used to classify the cardiorespiratory fitness level of the client (Heyward, 2002).

Bruce Treadmill Protocol

Before beginning a maximal exercise test, the subject should warm up for a minimum of 5 minutes or until she feels ready to proceed (Hoffman, 2006).

The Bruce, Kusumi, and Hosmer (1973) exercise test is a multistaged treadmill protocol. The workload is increased by changing both the treadmill speed and percent grade. During the first stage (minutes 1-3) of the test, the normal individual walks at a speed of 2.7km/h and at 10% grade. At the start of the second stage (minutes 4-6), the grade is increased by 2% and the speed to 4km/h. In each subsequent stage of the test, the grade is increased by 2% and the speed by either 1.3 or 1.5 km/h, as dictated by the protocol, until the subject is exhausted (Heyward, 2002).

At the NCRC we used a standardized blank for VO_{2max} norms (A5- see appendices).

Rate of Perceived Exertion Scale

To obtain ratings of perceived exertions during exercise testing, we use the original (6 to 20) RPE scale. This scale allows subjects to rate their degree of exertion

subjectively during exercise. The RPE scale takes into account the linear rise in heart rate and $VO_{2\ max}$ during exercise. Ratings on the scale usually correspond with the maximum level of exercise (Heyward, 2002).

Find the standardized RPE scale in appendices (A6).

Dietary Intake

To estimate average nutrient intake, participants completed a food log of dietary intake for the three consecutive days prior to the scheduled 2-hour run. A trained nutritionist gave detailed written and oral instructions about proper dietary recording. A full description of foods and fluids was requested, including cooking or processing methods, and food items and ingredients added during preparation, and amounts. Participants estimated the amount of food or fluids consumed by referring to the weight or volume information provided on food package or by using standardized household measures. Subjects were asked to eat foods from the food list, which was divided in “foods to eat” and “foods to avoid”. Recommended foods were mostly those that are carbohydrate- rich (cereals, pasta, rice, bagels, any type of fruit or vegetables, honey) and high in fiber.

Carbohydrates are the primary energy source for exercising muscles. Anyone who exercises vigorously, especially for more than an hour per day on a regular basis, needs to consume moderate to high amounts of carbohydrates. Numerous servings of grains, starchy vegetables, and fruits provide enough carbohydrate to maintain adequate liver and muscle glycogen stores, especially for replacing glycogen losses from workouts on the previous day. Carbohydrate intake should be at least 5-7 g / kg of body weight. Athletes engaged in aerobic training and endurance activities (duration 60 minutes or more per day) may need as much as 7-9 g/kg of body weight. When exercise duration approaches several hours per day, the carbohydrate recommendation increases up to 10 g/ kg of body weight (Byrd- Bredbenner, Moe, Beshgetoor, & Berning, 2009; Jeukendrup & Gleeson, 2010; Burke, 2007).

The fat recommendations for the subjects were low, (avoidance of butter, margarine, whole milk or products made of whole milk) and no special recommendations for protein were made.

Coffee, tea, soft drinks and alcoholic beverages in moderation were fine. Subjects were asked to avoid intake of nutritional and herbal supplements as well as anti-inflammatory medications.

Our goal was to have a similar intake of macro- nutrients over the 3 days prior to both runs. In order to archive that, we used a food list (A7- see appendices).

The Dietary- record information was converted to energy and nutrients using Food Processor Food; ESHA Research, Salem, OR (see appendices for example).
This program was supplemented with information for composite dishes, commercial foods, and sports foods whenever reliable nutritional composition data could be obtained. The food record needed to be documented 3 days prior to each run (Q-Chew or control- group) and delivered to the research team on the day of the run.

In order to bring them to a comparable energy intake on the day of the run the subjects were asked to have their breakfast before 8 am (from the food list), just water during the morning and for lunch a standardized chocolate flavored boost. It is a standardized liquid meal (Boost Plus; Mead Johnson Nutritionals, Evansville, IN) at an energy level of 15 kcal/kg). Boost Plus is a nutritionally complete, high-energy oral supplement with an energy density of 1.52 kcal/ml) and 16% of energy as protein, 34% as fat, and 50% as carbohydrate. In quantities of 1000 ml, Boost Plus exceeds daily value recommendations for all major vitamins and minerals. No other food and beverage (other than water) was consumed from this meal until the end of the running bout. Subjects ingested 0.8 to 1.2 l of water during the whole run.

For these data collection days, subjects were scheduled to come in on the same day of the week as for baseline testing and all the subjects at the same time in the early afternoon.

Prior to the testing they were sent an email with a reminder of the date and time they were scheduled, including important instructions about the food log.

3.4 Test setting

The testing took place in between two weeks time, each day we had 1-4 subjects for the testing itself.

Preparation of the subjects

The subjects came in around 2.30 pm, got changed and dispensed their food logs of the last three days. While a dietician went through the food logs and checked the readability and the completeness of the food logs, the subjects provided blood and urine samples. After that we took their weight and then applied the supplement.

On the day of the testing we worked with a form to make sure that all subjects go through all steps of the study (A8- see appendices).

The supplement

The subjects either got 4 pieces of Q-chew or placebo (double blinded) exactly 15 min before they got on the treadmill. Each placebo soft chew contained brown rice syrup, evaporated cane juice, carnuba wax, natural flavors, gelatin, soy lecithin, palm oil, glycerin, xylitol, mono- and di-glyceride, corn starch, carrageenan, sucralose, and 20 kilocalories energy with citric acid to substitute for taste of vitamin C and FD&C yellow #5 and FD&C blue #1 to substitute for the quercetin color (Quercetin Pharma, Sudbury, MA). Each Q-chew soft chew contained all placebo ingredients with 250 mg quercetin and isoquercetin, 30 mg EGCG from green tea extract, and 100 mg N3-polyunsaturated fatty acids (55 mg

Eicosapentaeonic acid, and 45 mg Docosahexaeonic) from fish oil, 250 mg vitamin C, and 10 mg niacinamide.

Data from Quercetin Pharma indicate that the bioavailability of quercetin is enhanced with Vitamin C and niacin, and thus this study tested whether or not soft chews with or without the combination of quercetin, vitamin C and niacin had an influence on the outcome measures (Nieman, et al., 2007).

These food components were included to improve quercetin bioavailability and extend its bioactive effects, as determined by experiments conducted by Quercegen Pharma (Nieman, et al., 2009a).

Quercetin ´s anti-inflammatory and anti-oxidative effects may be augmented by co-ingestion of N-3 polyunsaturated fatty acids (Camuesco, Comalada, Concha, Nieto, Sierra, et al., 2006), vitamin C, vitamin E (Mostafavi-Pour, Zal, Monabati, & Vessal, 2008) and EGCG (Ivanov, Ivanova, Kalinovsky, Niedzwiecki, & Rath, 2008).

For example, the concurrent administration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and quercetin resulted in a synergistic anti-inflammatory effect with inflammatory disorders (Camuesco, et al., 2006).

Epigallocatechin Gallate

Epigallocatechin Gallate (EGCG) is a potent bioactive catechin in green tea. Quercetin increases bioavailability of EGCG (Kale, Gawande, Kotwal, Netke, Roomi, et al., 2010).

Regular ingestion of a green tea extract over several weeks has been shown to improve running and swimming capacity in endurance-trained mice (Murase, Haramizi, Shimotoyodome, Tokimitsu, & Hase, 2006).
Green tea and/ or catechins have been discussed as a means of perhaps influence exercise performance and metabolism (by modulation fatty acid oxidation). In a three-week- supplementation with a low-dose Green Tea extract beverage, however, did not influence the time to complete a time trial and energy metabolism with endurance-trained humans (Eichenberger, Mettler, Arnold, & Colombani, 2010).

EPA/DHA in this supplement

Fish oil is a natural source of long-chain omega-3 fatty acids. It contains both docosahexaenoic (DHA) and eicosapentaenoic acid (EPA). Fish oil is said to improve membrane characteristics and improve membrane function when more of these omega-3 fatty acids are incorporated into the lipid bilayer of the membrane. (Jeukendrup & Gleeson, 2010).

The fish-oil amount with 0.4 g per testing in our study is regarded as a low dose. In the literature there is mainly a longer supplementation and a higher amount to achieve an increase of fatty acids in the blood plasma.

Marsen, Pollok, Oette & Baldamus (1992) showed an increase of EPA and DHA concentration in the plasma after 28 days of ingestion a supplement of 3 g, 6 g or 12 g. Rapid increases in EPA and DHA plasma concentrations can be demonstrated at all dosages. EPA accumulated more during ingestion 12 g vs. 3 g of fish oil, whereas DHA plasma levels revealed almost identical in all. Therefore it seems unlikely that the pathogenetic mechanisms for the beneficial effects follow a clear dose- and time –dependent relationship (Marsen, Pollok, Oette, & Baldamus, 1992).

A study from 2009 had even a longer time period (6 weeks) of supplementation to raise the plasma levels. They ingested 2000 mg EPA and 400 mg DHA, cycled then for 3 hours on 3 consecutive days and where analyzed for their immune changes, their inflammation measures and their performance. There was no difference to Placebo after these 9 hours of heavy exertion on none of the parameters (Nieman, Henson, McAnulty, Jin, & Maxwell, 2009c).
For the majority of athletes, especially those at the leisure level, general guidelines should include EPA and DHA of about 1 to 2 g/d at a ratio of EPA:DHA of 2:1 (Simopoulos, 2007).

The concurrent administration of EPA, DHA, and quercetin has been reported to cause a synergistic anti-inflammatory effect in rats with intestinal inflammatory disorders (Camuesco, et al., 2006).

The subjects (n=20) arrived, changed into running clothes, gave the food record to the researcher, and were asked about their overall well-being and their training on that specific day. We then took their pre-exercise weight, and the first blood and urine samples were obtained. They either got placebo or Q-chew as designated by randomization, consumed it, and as soon as they finished the intake of the chew, we started a stopwatch for 15 minutes until they were to begin the 2-hour treadmill run. The chews were labeled with a code, which differentiated between the placebo and Q-Force chews, and only the company "Quercetin Pharma" was familiar with the identification of the group numbers.

During their first 105 minutes on the treadmill the subjects ran at 70% of their individual $VO_{2\ max}$. Every 30 minutes we recorded heart rate, pace, RPE, and ventilation and respiratory frequency as measured by the Cosmed Fitmate Pro. During the last 15 minutes the subjects completed a time trial. They were told to run as fast as they could and at a minimum of 95% of their maximal heart rate. They could see their own heart rate and control it by themselves. During the 2-hour run they could drink as much water as they wanted, but we measured closely how much they consumed and made note of the amount.

Laboratory Tests to evaluate Human Performance

When investigating endurance-race events the most common exercise-protocols are a time-to-exhaustion test and a time-trial test (i.e., target endpoint would be the distance covered). It has been argued that time trial events have greater validity than time-to-exhaustion tests because they simulate the actual race event and are highly correlated with actual performance in the athletic event (Currell & Jeukendrup, 2008; Powers, et al., 2010; Laursen, Francis, Abbiss, Newton, & Nosaka, 2007).

Recovery phase

After completing 120 minutes on the treadmill and within 2 minutes got a second blood sample drawn, followed by a urine sample, and the post-exercise weight measurement.

Each subject received a new drinking bottle with water and again they were permitted to drink ad libitum. Some athletes did some stretching; some took a shower and got changed. At exactly one hour post-exercise was the final blood draw followed by a urine sample.

After the first data collection day, subjects received blank food logs and the appropriately measured Boost supplement drink for the second visit, which was exactly three weeks later.

This is an overview about the testing:

3-day period before 2-h runs	Exercise taper; 55-60% carb diet, avoid all supplements
Day of the run: 7-8 am breakfast	15 kcal/kg; carbohydrate rich
noon	Boost, 10 kcal/kg body weight
2:30 pm	Paper work (food log, well-being, training staus), take weight,
2:45 pm	Provide blood and urine sample
3:00 pm	Ingest soft chews
3:15 pm	Start 2-h run
5:15 pm	Finish run and provide blood sample immediately after it; urine sample
5:30pm	Take weight; calculate fluid intake
6:15pm	Provide final blood and urine sample, provide Boost for the second run (only after first run)

3.5 Biochemical analysis

A critical step in the successful completion of any experiment designed to discern the effect of antioxidant supplementation on exercise performance or protection against oxidative damage is properly procuring and storing the biological material before assay. A general principal that applies to any biological sample is that the sample should be rapidly obtained and quickly frozen in liquid nitrogen. Freezing the material swiftly is important to limit its exposure to room air, which prevents the high PO2 contained in room air from oxidizing lipids and proteins in the sample. After freezing, the biological material should be stored at -80 C until assay (Powers, et al., 2010).

3.6 Complete Blood Count, Leukocyte Subset Counts, C-Reactive Protein (CRP) and Creatine Phosphokinase (CPK)

Routine complete blood counts were performed by our clinical hematology laboratory using a Coulter STKS instrument (Beckman Coulter Electronics, Brea, CA) and provided leukocyte subset counts, and hemoglobin and hematocrit for the determination of plasma volume change using the method of Dill and Costill (Dill & Costill, 1974).

CRP and CPK were measured using an LX-20 clinical analyzer (Beckman Coulter Electronics, Brea, CA).

3.7 Plasma Quercetin

Quercetin and its conjugates were measured as described (Jin, Nieman, Shanely, Knab, Austin, et al., 2010).

To each 500 µl human plasma, 10% DL-dithiothreitol solution (10 µl) was added before the addition of 50 µl of 0.58 mol/l acetic acid. The mixture was then spiked

with 10 µl of 0.356 µmol/l fisetin internal standard. To this, a mixture of 50 µl enzyme β-glucuronidase/arylsulfatase and crude extract from Helix pomatia (Roche Diagnostics Corporation, Indianapolis, IN, USA) was added. The mixture was incubated for 2 h at 37°C. Then 500 µl of 0.01 µmol/l oxalic acid was added to stop the enzymatic reactions. The microplate was vortexed for another 1 min before centrifugation at 3000 rpm for 17 min (Allegra X-22R centrifuge; Beckman Coulter, Fullerton, CA, USA). Waters (Milford, MA, USA) Oasis HLB 96-well sample extraction plates (30 mg, 30 µm) were conditioned with 1.0 ml methanol, 0.5 ml 0.01 mol/l oxalic acid, and 1.0 ml DI water sequentially. The waste was pulled through at a speed of <0.2 ml/min. The supernatant of the enzyme hydrolysis mixture was then loaded in the extraction plate, and the samples were washed with 1.0 ml of 5% methanol in 0.5 mol/l phosphoric acid and 1.0 ml of 50% methanol in 0.5 mol/l phosphoric acid solution, respectively. Finally, samples were eluted with two volumes of 0.5 ml methanol and the eluents were combined into a clean microplate with elution flow rate kept at <0.2 ml/min. A total of 10% DL-dithiothreitol solution (10 µl) was added before solvent evaporation at 30°C with N_2 blowing through. The dried samples were reconstituted into 50/50 methanol/water for HPLC analysis.

3.8 Plasma EPA and DHA

After addition of 500 µg butylated hydroxytoluene and 20 µg of heptadecanoic acid methyl ester (NU-Chek Prep, Inc., Elysian, MN) internal standards to 100 µL thawed plasma, lipids were extracted and methyl esters were formed after the mixture was added to 2 ml of methanolic 5% HCl and incubated at 80°C for 2 hr in an OLS200 Shaking Waterbath (Grant Instruments Ltd., Shepreth, Cambridgeshire, England) (Nieman, Cayea, Austin, Henson, Mc Anulty, et al., 2009d).

The samples were cooled to room temperature on completion of the incubation, and the methyl esters were extracted twice with 2 ml of n-hexane. The top layer of the supernatant was combined and dried with NEVAP116 nitrogen evaporator (Organomation Associates, Inc., Berline, MA). The dried extract was then reconstituted in 200 µl of n-hexane supplemented with 0.05%

butylated hydroxytoluene, of which 1 µl was injected into an HP 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a 5975B Inert

XL MSD mass spectrometer detector. A DB-23 GC column (60 m × 250 μm × 0.25 μm) from J&W Scientific (Agilent Technologies) was used to separate the methyl esters of the extracted fatty acids. Fatty-acid concentrations were calculated in relation to the heptadecanoic acid methyl ester internal standard peak. The concentration of each phospholipid fatty acid was expressed as a concentration (μg/ml plasma).

3.9 Plasma Cytokines

Total plasma concentrations of nine inflammatory cytokines (IL-6, TNFα, GM-CSF, IFNγ, IL-1b, IL-2, IL-8, IL-10, IL-12p70) were determined using an electrochemiluminescence based solid-phase sandwich immunoassay (Meso Scale Discovery, Gaithersburg, MD, USA). The MSD assays provide a rapid and convenient method for measuring the levels of protein targets within a single small-volume sample. MSD provides a plate that has been pre-coated with capture antibody on spatially distinct spots- antibodies for IL-2, IL-8, IL-12p70, IL-1β, GM-CSF, IFN-γ, IL-6, IL-10 and TNF-α. The sample need to be added and moreover a solution containing the labeled detection antibodies- anti- IL-2, anti-IL-8, anti-IL-12p70, anti-IL-1β, anti-GM-CSF, anti-IFN-γ, anti-IL-6, anti-IL-10 and anti-TNF-α labeled with an elctochemiluminescent compound, MSD SULFO-TAG ^{TM} label- over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface, recruitment of the labeled detection antibodies by bound analytes completes the sandwich.

All samples and provided standards were analyzed in duplicate. The minimum detectable concentration of IL-6 was <2.2 $pg \cdot ml^{-1}$, TNFα <1.0 $pg \cdot ml^{-1}$, GM-CSF <0.42 $pg \cdot ml^{-1}$, IFNγ <0.96 $pg \cdot ml^{-1}$, IL-1b <0.29 $pg \cdot ml^{-1}$, IL-2 <2.7 $pg \cdot ml^{-1}$, IL-8 <41 $pg \cdot ml^{-1}$, IL-10 <0.47 $pg \cdot ml^{-1}$, and IL-12p70 <1.1 $pg \cdot ml^{-1}$. Pre- and post-exercise samples for the cytokines were analyzed on the same assay plate to decrease inter-kit assay variability (Handbook, Mesoscale; Gaithersburg).

3.10 Granulocyte and Monocyte Phagocytosis and Oxidative Burst Activity

Simultaneous measurement of granulocyte and monocyte phagocytosis and oxidative burst activity was performed using a modified flow cytometric assay (34). For each sample, 100 µl heparinised whole blood were dispensed into two 15 x 75mm (5 ml) tubes. To each tube, 10 µl hydroethidine working solution (10 µg hydroethidine/ml in PBS–glucose; Invitrogen Corporation, Carlsbad, CA, USA) were added. The tubes were vortexed briefly, incubated in a 37°C water-bath for 15 min, and cooled in a 4°C ice-water-bath for 12 min. After the hydroethidine-loaded blood samples were cooled, 20 µl of working bacteria–FITC solution (Staphylococcus aureus labelled with FITC, diluted in PBS to 1·33 x 10^8 particles/ ml; Invitrogen Corporation, Carlsbad, CA, USA)
were added to both tubes and vortexed briefly. Tube 2 (test) was transferred to a 37°C water-bath, and tube 1 (control) was left in the ice. The tubes were incubated for 20 min, placed in an ice-water-bath, and 100 µl ice-cold Quench Solution (0·025% Trypan blue in 0·1 M-citrate buffer, pH 4·0) were added to each tube. The tubes were vortexed for 10 s and incubated for 1 min to quench the FITC fluorescence of any noninternalised bacteria, after which the cells were washed twice with ice-cold PBS and re-suspended in 50 µl cold fetal bovine serum. Samples were processed on a Q-Prep™ Workstation (Beckman Coulter, Inc., Fullerton, CA, USA), which lysed the
erythrocytes and stabilized and fixed the leucocytes. Tubes were stored at room temperature in the dark until flow cytometric analysis, which was performed within 24 h of blood collection for all samples. Analysis of samples was performed using a Beckman Coulter FC-500 flow cytometer with CXP software (Fullerton, CA, USA). FITC emits a green fluorescence; therefore, cells that phagocytize the FITC-labelled bacteria are detected as fluorescence channel 1-positive. Similarly, cells stimulated by the bacteria will undergo a respiratory burst, oxidizing the non-fluorescent hydroethidine to ethidium bromide, which emits a red fluorescence that can be detected on fluorescence channel 3. After gating on the granulocytes using forward scatter and side scatter, the mean fluorescence intensity (x-mean) for each channel was determined and shifts in

x-mean were calculated by subtracting the control (4°C) x-mean from the test (37°C) x-mean for fluorescence channel 1 and fluorescence channel 3. Typically, 5000 granulocytes were counted for each reaction tube. The mean intra-assay CV was ±7% for phagocytosis and ±10·5% for GOBA.

4 Results

Twenty subjects (11 males and 9 females) finished all aspects of the study. Subject characteristics are listed for each gender group in Table 1, and indicate that they were highly experienced in long distance training and capable of running 2-h on treadmills in a laboratory setting. Age and gender specific norms for VO_{2max} indicate a high level of aerobic fitness and acceptable body fat levels for runners (Nieman, 2011).

Three-day food records before each of the two exercise trials revealed no significant differences in energy or macronutrient intake (data not shown). Energy intake was 2123±90.6 kcal/day (8.88±0.38 MJ/day) and 2154±93.9 kcal/day (9.02±0.39 MJ/day), with carbohydrate representing 59.0±1.5% and 59.4±1.7%, protein 16.1±0.7% and 16.0±0.7%, and fat 25.3±1.3% and 24.1±1.5% of total energy for Q-chew and placebo trials, respectively.

Performance data for the 1 h 45 min exercise preload period and the 15-min running time trial did not differ between the Q-chew and placebo trials for both male and female runners as summarized in Table 2. During the preload period, subjects averaged 70.8±2.3% and 68.9±2.4% VO_{2max} and 82.9±1.2% and 81.6±0.8% HR_{max} during the Q-chew and placebo trials, respectively (P=0.231, P=0.176). Plasma volume shifts were less than 2% following exercise and did not differ between trials (P=0.924).

Genders were combined for all other statistical analyses. Plasma quercetin increased strongly following exercise in the Q-chew trial relative to placebo, as summarized in Figure 1 (interaction effect, $P<0.001$). The patterns of change in plasma EPA and DHA did not differ between Q-chew and placebo conditions, indicating that the Q-chew supplement did not increase blood levels of these fatty acids when ingested just prior to exercise (data not shown).

Data for total blood leukocytes and plasma cytokines are listed in Table 3. Significant increases post-exercise were measured for WBCs, CRP, IL-2, IL-8, IL-10, IL-1β, GM-CSF, and TNFα, but no differences in the pattern of change were found between Q-chew and placebo trials. The pattern of change for plasma IL-6 was different between trials (interaction effect, P=0.040), but no significant time point differences were noted post- and 1-h-post-exercise (Figure 2). The pattern of increase for serum CPK did not differ significantly between groups (interaction effect, P=0.917).

Exercise caused significant increases in granulocyte (GR-PHAG) and monocyte phagocytosis (MO-PHAG), and decreases in granulocyte and monocyte oxidative burst activity (GR-OBA, MO-OBA), but no differences in the pattern of change were measured between Q-chew and PL trials (Table 4 and Figure 3).

Table 1 Subject Characteristics (Mean±SE) (Konrad, et al., 2011)

Variable	**Female**	**Male**
	N=9	**N=11**
Age (years)	35.8±2.7	40.5±3.0
Height (m)	1.63±1.7	1.77±1.3
Body mass (kg)	57.3±1.4	72.0±3.1
Body composition (% fat)	24.7±1.6	14.5±1.7
Peak aerobic power ($ml \cdot kg^{-1} \cdot min^{-1}$)	47.9±2.4	56.4±2.6
Maximal heart rate ($beats \cdot min^{-1}$)	184±2.9	172±3.9
Maximal ventilation ($L \cdot min^{-1}$)	102±3.7	152±4.6
Maximal respiratory frequency ($breaths \cdot min^{-1}$)	49.6±1.8	49.6±1.5
Marathon race time (h)	4.3±0.2	3.5±0.3
Marathons raced	2.6±1.2	9.1±4.7

Training distance (km/wk)	51.9±4.3	43.0±3.8
Training experience (yr)	11.7±2.7	9.9±2.3

Table 2 Exercise performance data (mean±SE) (Konrad, et al., 2011).

A. 1.75 h exercise preload data

	Males (N=11)		**Females(N=9)**	
	Q-chew	**Placebo**	**Q-chew**	**Placebo**
Variable				
VO_2 ($ml \cdot kg \cdot min^{-1}$)	27.7±1.0	25.9±1.0	19.5±0.6	19.7±0.7
Heart rate ($beats \cdot min^{-1}$)	141±3.0	140±3.3	154±2.9	151±2.7
Ventilation ($liters \cdot min^{-1}$)	65.3±3.9	67.6±3.9	52.1±1.9	52.7±2.2
Respiratory rate ($breaths \cdot min^{-1}$)	34.8±1.3	35.0±1.6	41.0±2.8	42.7±3.0
Rating perceived exertion	11.9±0.3	11.6±0.4	12.2±0.3	11.8±0.2
Pace (km/h)	11.3±0.4	11.3±0.4	11.0±0.4	11.0±0.3

B. 15-minute time trial

VO_2 ($ml \cdot kg \cdot min^{-1}$)	37.0±1.8	36.4±1.8	25.3±0.8	25.2±1.6
Heart rate ($beats \cdot min^{-1}$)	166±2.7	166±3.9	176±1.1	174±2.6
Ventilation ($liters \cdot min^{-1}$)	119±7.2	110±8.0	79.7±3.4	77.6±3.2

Rating perceived exertion	16.0±0.4	15.6±0.5	16.0±0.4	16.1±0.6
Respiratory rate (breaths·min^{-1})	55.2±0.7	53.2±1.1	55.5±3.9	55.6±2.9
Pace (km/h)	13.9±0.5	13.8±0.4	11.0±0.4	11.0±0.3
Distance covered (km)	3.4±0.1	3.5±0.1	2.7±0.09	2.7±0.09

Table 3: Total blood leukocytes (WBC) and plasma cytokines in runners during Q-chew and placebo trials (Konrad, et al., 2011).

Variable	**Pre-exercise**	**Post-exercise**	**1-h Post-exercise**	**Time; interaction effects; p-value**
WBC, $10^9 \cdot L^{-1}$				
Q-chew	6.21±0.28	12.2±0.69	11.7±0.68	<0.001; 0.939
Placebo	6.04±0.30	11.8±0.64	11.5±0.79	
CRP (mg·L^{-1})				
Q-chew	0.61±0.13	0.67±0.15	0.60±0.13	0.002; 0.934
Placebo	0.54±0.12	0.60±0.14	0.54±0.13	
IL-1ß, pg·ml^{-1}				
Q-chew	0.23±0.05	0.44±0.15	0.42±0.09	0.042; 0.852
Placebo	0.33±0.08	0.43±0.08	0.47±0.11	
IL-2, pg·ml^{-1}				
Q-chew	0.80±0.09	1.17±0.10	1.16±0.12	<0.001; 0.850
Placebo	0.85±0.12	1.16±0.14	1.12±0.14	

IL-8, $pg \cdot ml^{-1}$				
Q-chew	2.80±0.17	8.74±0.52	9.77±0.82	<0.001; 0.066
Placebo	2.68±0.20	7.63±0.64	8.94±0.83	
IL-10, $pg \cdot ml^{-1}$				
Q-chew	1.67±0.11	9.73±2.59	14.9±3.12	<0.001; 0.195
Placebo	1.73±0.14	8.42±2.68	11.2±2.53	
IL-12-p70, $pg \cdot ml^{-1}$				
Q-chew	1.69±0.24	2.01±0.26	1.94±0.21	0.089; 0.605
Placebo	1.72±0.15	1.82±0.27	1.87±0.27	
GM-CSF, $pg \cdot ml^{-1}$				
Q-chew	0.92±0.11	1.14±0.11	1.36±0.14	<0.001; 0.426
Placebo	0.96±0.14	1.24±0.13	1.25±0.14	
IFNγ, $pg \cdot ml^{-1}$				
Q-Chew	1.12±0.17	1.20±0.16	1.30±0.18	0.108; 0.242
Placebo	1.28±0.17	1.48±0.20	1.30±0.21	
TNF-α, $pg \cdot ml^{-1}$				
Q-Chew	4.83±0.22	6.00±0.34	6.51±0.40	<0.001; 0.556
Placebo	4.71±0.24	5.53±0.29	6.17±0.30	

Table 4: Granulocyte (GR) and monocyte (MO) phagocytosis (PHAG), and monocyte oxidative burst activity (OBA) in runners during Q-chew and placebo trials. Units are in mean fluorescence intensity (MFI) (Konrad, et al., 2011).

Variable	**Pre-exercise**	**Post-exercise**	**1-h Post-exercise**	**Time; interaction effects; p-value**

GR-PHAG				
Q-chew	135±16.4	163±22.2	139±17.1	0.001; 0.684
Placebo	115±10.9	148±12.9	133±15.5	
MO-PHAG				
Q-chew	64.5±8.0	76.6±13.0	82.1±9.9	<0.001; 0.859
Placebo	59.2±7.5	72.3±8.9	80.7±9.7	
MO-OBA				
Q-chew	10.7±0.6	10.3±0.5	8.98±0.7	<0.001; 0.641
Placebo	10.9±0.6	11.0±0.6	8.68±0.6	

Figure 1

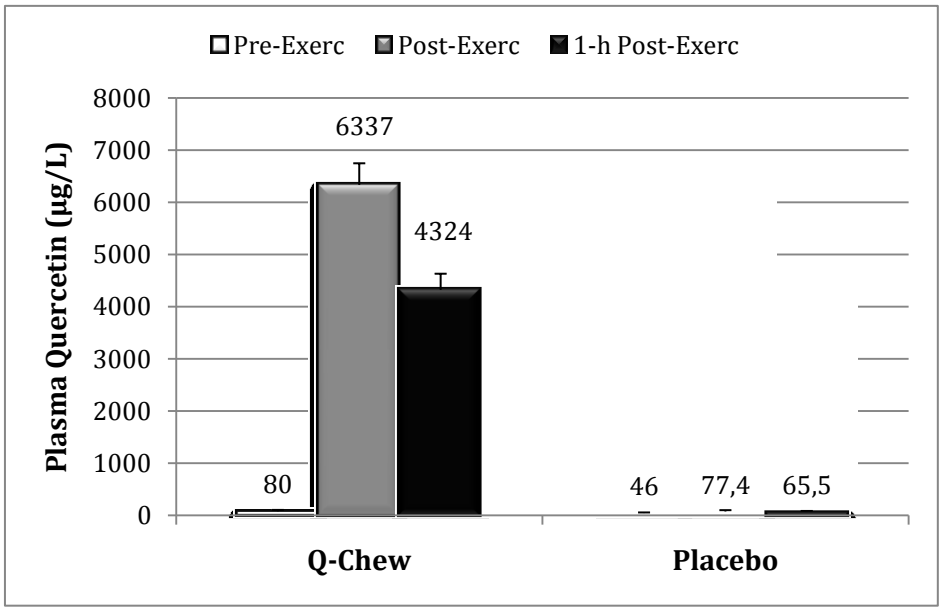

Figure 1 Plasma quercetin levels before, after, and 1-h after running 2 hours in the Q-chew and placebo trials (Konrad, et al., 2011).

Figure 2

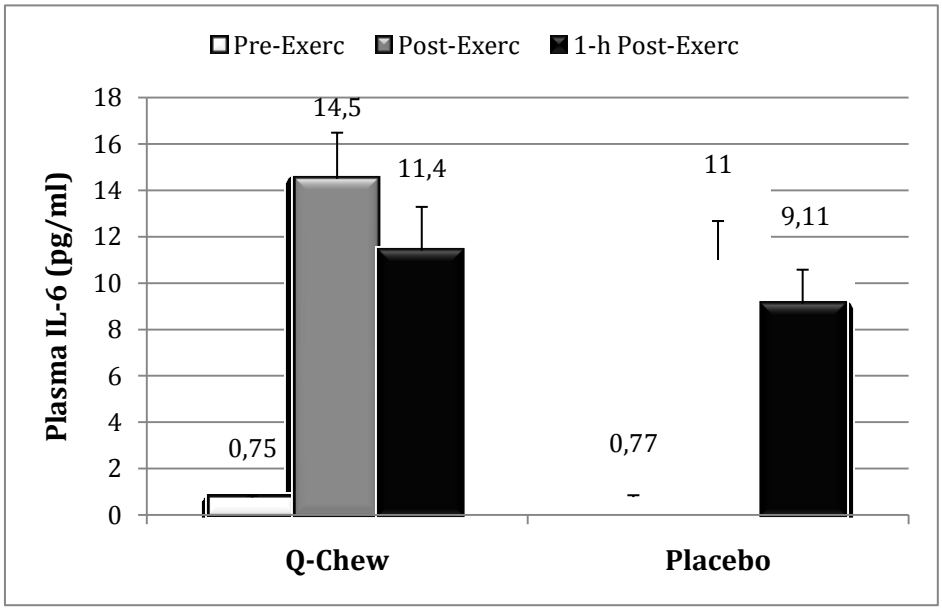

Figure 2 Plasma IL-6 levels before, after, and 1-h after running 2 hours in the Q-chew and placebo trials (Konrad, et al., 2011).

Figure 3

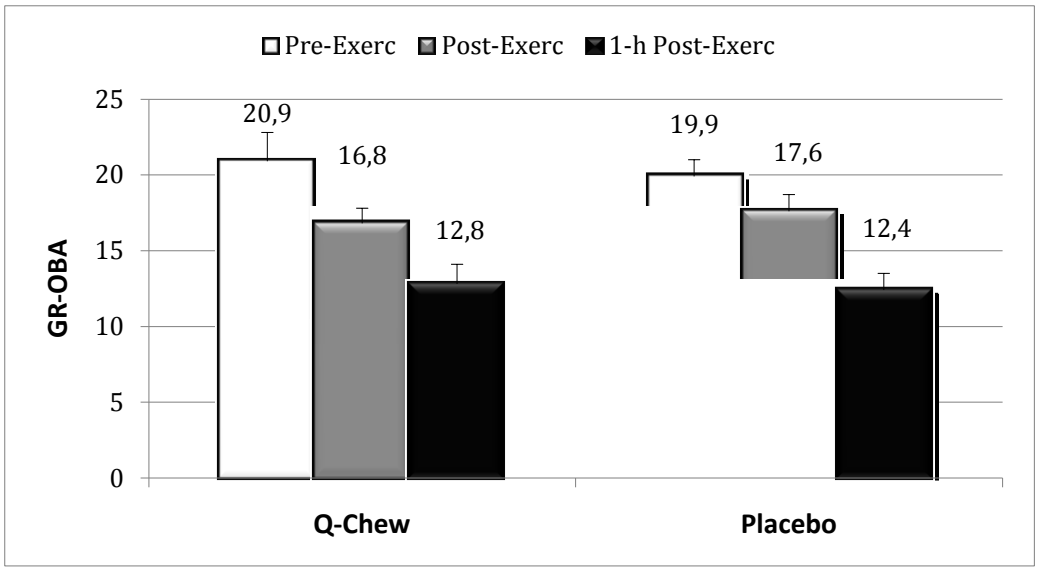

Figure 3 Granulocyte oxidative burst activity before, after, and 1-h after running 2 hours in the Q-chew and placebo trials (Konrad, et al., 2011).

Data are expressed as mean±SE and were analyzed using a 2 (condition) x 3 (time) repeated-measures ANOVA, within-subjects design. When interaction effects were significant ($P \leq 0.05$), changes from pre-exercise to post-exercise and 1-h post-exercise within conditions were compared between trials using paired t-tests, with significance set after Bonferroni adjustment at $P \leq 0.025$.

5 Discussion

In a prior study with the Q-chew, supplementation for two weeks before a 3-day period of intensified exercise (3 h/day) was effective in attenuating post-exercise inflammation (Nieman et al. 2009a). Our goal in this study was to determine if an acute dose of the Q-chew supplement consumed 15-minutes before a 2-h treadmill run would also diminish exercise-induced inflammation, but results were not supportive despite a strong increase in plasma quercetin levels. Exercise caused significant increases in WBCs, CRP, GM-CSF, IL-10, IL-1β, IL-2, IL-6, IL-8, TNFα, GR-PHAG, and MO-PHAG, and decreases in GR-OBA and MO-OBA, but no differences in the pattern of change were measured between Q-chew and PL trials. Taken together, these data imply that a prolonged supplementation period is needed for the Q-chew to function as an anti-inflammatory post-exercise, or that the

Q-chew is more effective during a 3-day intensified period of training compared to one bout of heavy exertion.

We hypothesized that an acute dose of Q-chew would exert post-exercise anti-inflammatory influences, and that a loading period would not be necessary for athletes interested in avoiding the use of NSAIDS. Our data indicate that the acute dose of Q-chew ingested pre-exercise quickly elevated blood quercetin concentrations as reported previously by Moon et al. (2008), but was insufficient to exert anti-inflammatory influences, perhaps due to extensive conjugation (Tribolo, Lodi, Connor, Suri, Wilson, et al., 2008) or an insufficient time to stimulate adaptive anti-inflammatory mechanisms. The concurrent administration of EPA, DHA, and quercetin has been reported to cause a synergistic anti-inflammatory effect in rats with intestinal inflammatory disorders (Camuesco, Comalada, Concha, Nieto, Sierra, et al., 2006). Acute supplementation with the Q-chew supplement did not result in a measurable increase in plasma EPA and DHA during the 2-h run, implying that a longer loading period is needed to allow the potential anti-inflammatory influences of quercetin and fish oil to occur. We did not measure plasma EGCG concentrations, but others have reported that plasma levels rise quickly following intake, especially when combined with quercetin (Kale et al., 2010).

Most investigations using flavonoid-rich products or extracts in athletic settings have utilized supplementation periods of at least seven days prior to heavy exertion, and reported varying levels of success in attenuating inflammation and oxidative stress (Chang, Hu, Huang, Yeh, & Liu, 2010; Goldfarb, Garten, Cho, Chee, & Chambers, 2011; Nieman et al., 2010a; Trombold, Barnes, Critchley, & Coyle, 2010). A few studies used an acute dose of a flavonoid-rich product or extract prior to exercise (Davison, et al., 2011; Lyall et al. 2009; Morillas-Ruiz, Villegas-Garcia, López, Vidal-Guevara, & Zafrilla, 2006; Wiswedel, Hirsch, Kropf, Gruening, Pfister, et al., 2004). Lyall et al. (2009) had 10 moderately active subjects consume 48 grams of anthocyanin-rich blackcurrent extract or placebo (double-blinded, crossover design) immediately before and after high-intensity rowing, and reported some success in alleviating transient post-exercise inflammation and oxidative stress. In particular, acute blackcurrant supplementation significantly reduced post-exercise cytokine production from LPS-stimulated cells perhaps through partial NFKB inhibition, but no *in vivo* measures of inflammation were included in this study. A reduction in protein carbonyls, reactive oxygen species (ROS) generating

capability and creatine kinase (24-h post-exercise) was also reported following blackcurrant supplementation, and Lyall et al. (2009) speculated that the reduced oxidative stress and inflammation were related. In contrast, Davison et al. (2011) reported no anti-inflammatory influences of ingesting an acute 100 g dose of dark chocolate prior to 2.5 h cycling. Taken together with our data, the strategy of using a single serving of a flavonoid-rich product just prior to intensive exercise to reduce exercise-induced inflammation has limited support.

The increase in plasma cytokines and granulocyte/monocyte phagocytosis, and decrease in oxidative burst activity found in our runners after running for 2 hours parallels what we and others have reported (Nieman, 1997). The major difference in this trial was that CRP remained at levels below 1 mg/L in contrast to much higher and sustained levels following 3 days of heavy exertion or ultramarathon race events (Nieman, Henson, Davis, Dumke, Gross, et al., 2007a; Nieman et al., 2009a). In our prior study with the Q-chew, CRP increased to ~7-8 mg/L in the placebo group following the third day of 3-h intensive cycling, and remained at this level 14 h post-exercise. Two weeks of supplementation with the Q-chew reduced post-exercise CRP by approximately 50%. Although post-exercise increases in IL-6 and other cytokines were similar between the acute dosing and 2-week dosing studies, the lack of significant increase in CRP from just one 2-h bout of running implies lower chronic inflammation and therefore less room for the Q-chew to exert anti-inflammatory effects.

Quercetin supplementation over periods ranging from two to six weeks in untrained and trained subjects has been linked to an inconsistent influence on exercise performance (for review, see Nieman, 2010; Nieman, et al. 2010b). This is the first study to report that an acute dose of Q-chew had no influence on performance during a 15-minute time trial following a 1.75-h exercise pre-load. Short-term supplementation with EGCG has been related to a small but significant increase in maximal exercise performance (Richards, Lonac, Johnson, Schweder, & Bell, 2010). The authors speculated that the ergogenic influence of EGCG occurred through catechol-O-methyltransferase (COMT) inhibition and increased sympathetic nervous system stimulation. Quercetin has also been related in vitro to COMT inhibition (Zhu & Lhier, 1996), but our data do not support that an acute quercetin dose has ergogenic effects.

In summary, ingestion of a quercetin-based supplement 15 minutes prior to a 2-h treadmill run did not attenuate exercise-induced inflammation and immune changes,

or improve performance during a 15-minute time trial. A previous study showed that the same supplement reduced post-exercise inflammation when ingested for two weeks prior to a 3-day period of intensified exercise (9 h intensive cycling) (Nieman et al., 2009a). Flavonoids may require a week or longer to be incorporated into tissues such as muscle and adipose tissue, and then influence cytokine production during exercise. Taken together with other studies utilizing flavonoid-rich products, single servings consumed just prior to intensive training or competition for purposes of attenuating inflammation and oxidative stress are less efficacious than when ingested daily for one week or longer.

6 References

ACSM ´S Resource Manual for Guidelines for Exercise Testing and Prescription. Philadelphia: Lippincot Williams & Williams; 2001.

Bakker, G.C.M, Van Erk, M.J., Pellis, L., Wopereis, S., Rubingh, C.M., Cnubben, N.H.P., Kooistra, T., Van Ommen, B., Hendriks, H.F.J. (2010). An anti-inflammatory dietary mix modulates inflammation and oxidative and metabolic stress in overweight men: a nutrigenomics approach. *American Journal of Clinical Nutrition. 91*. 1044-1059.

Bieger, J., Cermak, R., Blank, R., de Boer, V.C., Hollman, P.C., Kamphues, J., Wolffram, S. (2008). Tissue distribution of quercetin in pigs after a long-term dietary supplementation. *Journal of Nutrition. 138.* 1417-1420.

Bishop, N.C., Walker, G.J., Bowley, L.A., Evans, K.F., Molyneux, K., Wallace, F.A., Smith, A.C. (2005). Lymphocyte responses to influenza and tetanus toxoid in vitro following intensive exercise and carbohydrate ingestion on consecutive days. *Journal of Applied Physiology. 99.* 1327-1335.

Braakhuis, A.J., Hopkins, W.G. (2011). Impact of Vitamin C and other dietary Antioxidants on Sport Performance

Burke, L. Practical Sports Nutrition. Champaign: Human Kinetics; 2007.

Byrd- Bredbenner, C., Moe, G., Beshgetoor, D., Berning, J. Wardlaw´s Perspectives in Nutrition. 8th ed. Columbus: Mc Graw-Hill; 2009.

Camuesco, D., Comalada, M., Concha, A., Nieto, A., Sierra, S., Xaus, J., Zarzuelo, A., Galvez, J. (2006). Intestinal anti-inflammatory activity of combined quercitrin and dietary olive oil supplemented with fish oil, rich in EPA and DHA (n-3) polyunsaturated fatty acids, in rats with DSS-induced colitis. *Clinical Nutrition. 25.* 466-476.

Cannon, J.G., Meydani, S.N., Fiedling, R.A., Fiatarone, M.A., Meydani, M., Farhangmehr, M., Orencole, M., Blumberg, J.B., Evans, W.J. (1991). Acute phase

responding to exercise. II. Associations between vitamin E, cytokines, and muscle proteolysis. *American Journal of Physiology. 260*. 1235-1240.

Castro, G.R., Panilaitis, B., Bora, E., Kaplan, D.L. (2007). Controlled release biopolymers for enhancing the immune response. *Molecular Pharmacology. 4.* 33-46.

Chang, W.H., Hu, S.P., Huang, Y.F., Yeh, T.S., & Liu, J.F. (2010). Effect of purple sweet potato leaves consumption on exercise-induced oxidative stress and IL-6 and HSP72 levels. *Journal of Applied Physiology. 109.* 1710-1715.

Chen, Y.J., Wong, S.H., Wong, C.K., Lam, C.W., Huang, Y.J., Siu, P.M. (2008). The effect of a pre-exercise carbohydrate meal on immune responses to an endurance performance run. *British Journal of Nutrition. 100*. 1260-1268.

Chun, O.K., Chung, S.J., Song, W.O. (2007). Estimated dietary flavonoid intake and major food sources of U.S. adults. *Journal of Nutrition. 137*. 1244-1252.

Conquer, J.A., Maiani, G., Azzini, E., Raguzzini, A., Holub, B.J. (1998). Supplementation with quercetin markedly increases plasma quercetin concentration

without effect on selected risk factors for heart disease in healthy subjects. *Journal of Nutrition. 128.* 593-597.

Cosmed, USA. Overview and test process of Bod Pod Gold Standard. [Internet; cited 2010 October 2nd] Available from: http://www.bodpod.com/products/overviewBodpod

Croisier, J.L., Camus, G., Venneman, I., Deby-Dupont, G., Juchmès-Ferir, A., Lamy, M., Deby, C., Duchateau, J. (2001). Markers of inflammation and myofibrillar proteins following eccentric exercise in humans. *European Journal of Applied Physiology. 84(3).* 180-186.

Cureton, K.J., Tomporowski, P.,D., Singhal, A., Pasley, J.D., Bigelman, K.A., Lambourne, K., Trilk, J.L., McCully, K.K., Arnaud, M.J., Zhao, Q. (2009). Dietary Quercetin Supplementation is not ergogenic in untrained men. *Journal of Applied Physiology 107(4).* 1095-1104.

Currel, K. & Jeukendrup, A.E. (2008). Validity, reliability, and sensitivity of measures of sporting performance. *Sports Medicine. 38.* 296-316.

Davis, J.M., Zhao, Z., Stock, H.S., Mehl, K.A., Buggy, J., Hand, G.A. (2003). Central nervous system effects of caffeine and adenosine on fatigue. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology. 284.* 399-404.

Davis, J.M., Murphy & E.A., Carmichael (2008). Effects of the Dietary Flavonoid Quercetin Upon Performance and Health. *Current Sports Medicine Reports. 8(4).* 206-213.

Davis, J.M., Murphy, E.A., Carmichael, M.D., Davis, B. (2009a). Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance. *American Journal of Regulatory, Integrative and Comparative Physiology. 296.* 1071-1077.

Davis, J.M, Murphy, E.A., Carmichael, M.D. (2009b). Effects of the dietary flavonoid quercetin upon performance and health. *Current Sports Medicine Reports. 8(4).* 206-213.

Davis, J.M., Carlstedt, C.J., Chen, S., Carmichael, M.D., Murphy, E.A. (2010). The dietary flavonoid quercetin increases VO2max and endurance capacity. *International Journal for Sport Nutrition and Exercise Metabolism. 20(1)*. 56-62.

Davison, G., Callister, R., Williamson, G., Cooper, K.A., & Gleeson, M. (2011). The effect of acute pre-exercise dark chocolate consumption on plasma antioxidant status, oxidative stress, and immunoendocrine responses to prolonged exercise. *European Journal of Nutrition (in press).*

Davison, G. & Gleeson, M. (2005). Influence of acute vitamin C and/or carbohydrate ingestion on hormonal, cytokine, and immune responses to prolonged exercise. *International Journal of Sport Nutrition and Exercise Metabolism. 15.* 465-479.

Davison, G. & Gleeson, M. (2006). The effect of 2 weeks vitamin C supplementation on immunoendocrine responses to 2.5 h cycling exercise in man. *European Journal of Applied Physiology. 97*. 454-461.

De Boer, V.C., Dihal, A.A., van der Woude, H., Arts, I.C., Wolffram, S., Alink, G.M, Rietjens, I.M., Keijer, J., Hollman, P.C. (2005). Tissue distribution of quercetin in rats and pigs. *Journal of Nutrition. 135.* 1718-1725.

Diaz, F. & Moraes, C.T. (2008). Mitochondrial biogenesis and turnover. *Cell Calcium. 44.* 24-35.

Dill, D.B., Costill, D.L. (1974). Calculation of percentage changes involumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology. 37*. 247–248.

Dick, F.W. Sports Training Principles. 5th ed. London: A&C Black. 2007.

Dumke, C.L., Nieman, D.C., Utter, A.C., Rigby, M.D., Quindry, J.C., Triplett, N.T., McAnulty, S.R., McAnulty, L.S. (2009). Quercetin´s effect on cycling efficiency and substrate utilization. *Applied Physiology, nutrition and metabolism. 34.* 993-1000.

Egert, S., Wolffram, S., Bosy-Westphal, A., Wagner, A.E., Frank, J., Rimbach, G., Mueller, M.J. (2008). Daily quercetin supplementation dose-dependently increases plasma quercetin concentrations in healthy humans. *Journal of Nutrition. 138(9).* 1615-1621.

Eichenberger, P., Mettler, S., Arnold, M., Colombani, P.C. (2010). No effects of three-week consumption of a green tea extract on time trial performance in endurance-trained men. *International journal for vitamin and nutrition research. 80(1)*. 56-64.

Gleeson, M. (2007). Immune function in sport and exercise. *Journal of Applied Physiology. 103*. 693-699.

Gleeson, M. (2008). Dosing and efficacy of glutamine supplementation in human exercise and sport training. *Journal of Nutrition. 138*. 2045-2049.

Gleeson, M., Nieman, D.C. & Pedersen, B.K. (2004). Exercise, nutrition and immune function. *Journal of Sports Sciences. 22.* 115-125.

Goldfarb, A.H., Garten, R.S., Cho, C., Chee, P.D., & Chambers, L.A. (2011). Effects of a fruit/berry/vegetable supplement on muscle function and oxidative stress. *Medicine and Science in Sports and Exercise. 43*. 501-508.

Grimble, R.F. (2005). Immunonutrition. *Current Opinion in Gastroenterology. 21*. 216-222.

Cosmed, USA. Overview and test process of Bod Pod Gold Standard. [Internet; cited 2010 October 2nd] Available from: http://www.bodpod.com/products/overviewBodpod

Handbook- Brochures and Handouts , Mesoscale;

[Internet; cited 2011 February 14th] Available from: http://www.mesoscale.com/CatalogSystemWeb/WebRoot/literature/brochures.aspx

Harwood, M., Danielewska-Nikiel, B., Borzelleca, J.F., Flamm, G.W., Williams, G.M., Lines, T.C. (2007). A critical review of the data related to the safety of quercetin and lack of evidence of in vivo toxicity, including lack of genotoxic/carcinogenic properties. *Food and chemical toxicology. 45(11).* 2179-2205.

Henson, D.A., Nieman, D.C., Davis, J.M., Dumke, C.L., Gross, S.J., Murphy, E.A., Carmichael, M.D., Jenkinns, D.P., Quindry, J.C., McAnulty, S.R., McAnulty, L.S., Utter, A.C., Mayer, E.P. (2008). Post-160-km race illness rates and decreases in granulocyte respiratory burst and salivary IgA output are not countered by quercetin ingestion. *International Journal of Sports Medicine. 29(10).* 856-863.

Heyward, V. Advanced fitness assessment and exercise prescription. 4thed. Champaign: Human Kinetics; 2002.

Hoffman, J. Norms for Fitness, Performance, and Health. Champaign: Human Kinetics; 2006.

Hoppeler, H. & Fluck, M. (2003). Plasticity of skeletal muscle mitochondria: structure and function. *Medicine and Science in Sports and Exercise. 35*. 95-104.

Hollman, P.C., Bijsman, M.N., van Gameren, Y., Cnossen, E.P., de Vries, J.H., Katan, M.B. (1999). The sugar moiety is a major determinant of the absorption of dietary flavonoid glycosides in man. *Free Radical Research. 31.* 569-573.

Ivanov, V., Ivanova, S., Kalinovsky, T., Niedzwiecki, A., Rath, M. (2008). Plant-derived micronutrients suppress monocyte adhesion to cultured human aortic endothelia cell layer by modulating its extracellular matrix composition. *Journal of Cardiovascular Pharmacology. 52*. 55-65.

Jackson, M.J. (1999). Free radicals in skin and muscle: damaging agents or signals for adaptation? *Proceedings of the Nutrition society. 58(3).* 673-676.

Jackson, M.J. Exercise and oxygen radical production by muscle. In: Hanninen, O., Packer, L., Sen, C.K., editors. Handbook of Oxidants and Antioxidants in Exercise. Amsterdam: Elsevier; 2000.

Jeukendrup, A. & Gleeson, M. Sport Nutrition. 2nd ed. Champaign: Human Kinetics; 2010.

Jin, F., Nieman, D.C., Shanely, R.A., Knab, A.M, Austin, M.D. Sha, W. (2010). The Variable Plasma Quercetin Response to 12-Weeks Supplementation in Humans. *European journal of clinical nutrition. 64(7).* 692-697.

Johnson, J.J. & Mukhtar, H. (2007). Curcumin for chemoprevention of colon cancer. *Cancer Letters. 255*. 170-181.

Kale, A., Gawande, S. Kotwal, S., Netke, S., Roomi, W., Ivanov, V., Niedzwiecki, A., Rath, M. (2010). Studies on the effects of oral administration of nutrient mixture, quercetin and red onions on the bioavailability of epigallocatechin gallate from green tea extract. *Phytotherapy Research. 24(1).* 48-55.

Kandaswami, C., Middleton, J. (1994). Free radical scavenging and antioxidant activity of plant flavonoids. *Advances in Experimental Medicine and Biology. 366*. 351-376.

Kim, H.P., Son, K.H., Chang, H.W., Kang, S.S. (2004). Anti-inflammatory plant flavonoids and cellular action mechanisms. *Journal of Pharmacological Sciences. 96*. 229-245.

Knab, A.M., Shanely, R.A., Henson, D.A., Jin, F., Heinz, S.A., Austin, M.D., Nieman, D.C. (2011). Influence of quercetin supplementation on disease risk factors in communitiy-dwelling adults. *American Journal of Clinical Nutrition. 111(4).* 542-549.

Korkina, L. G., Afanas´ev. I.B. (1997). Antioxidant and chelating properties of flavonoids. *Advances in Pharmacology. 38.* 151-163.

Kressler, J., Millard-Stafford, M., Warren, G.L. (2010). Quercetin improves aerobic capacity and endurance performance: a meta-analysis. *Medicine & Science in Sports & Exercise. 42(10).* 33.

Larrabee, R.C. (1902). Leucocytosis after violent exercise. *The Journal of medical research. 7.* 76-82.

Laursen, P.B., Francis, G.T., Abbiss, C.R., Newton, M.J., Nosaka, K. (2007). Reliability of time-to-exhaustion versus time-trial running tests in runners. *Medicine & Science in Sports & Exercise. 39.* 1374-1379.

Lenn, J., Uhl, T., Mattacola, C., Boissonneault, G., Yates, J., Ibrahim, W., Bruckner, G. (2002). The effects of fish oil and isoflavones on delayed onset muscle soreness. *Medicine & Science in Sports & Exercise. 34(10).* 1605-1613.

Li, B.Q., Fu, T., Dongyan, Y., Mikovits, J.A., Ruscetti, F.W., Wang, J.M. (2000). Flavonoid baicalin inhibits HIV-1 infection at the level of viral entry. *Biochemical and Biophysical Research Communications. 276.* 534-538.

Loke, W.M., Proudfoot, J.M., McKinley, A.J., Needs, P.W., Kroon, P.A., Hodgson, J.M., Croft, K.D. (2008). Quercetin and its in vivo metabolites inhibit neutrophil-mediated low-density lipoprotein oxidation. *Journal of Agricultural and Food Chemistry. 56*. 3609-3615.

Lyall, K.A., Hurst, S.M., Cooney, J., Jensen, D., Lo, K., Hurst, R.D., & Stevenson, L.M. (2009). Short-term blackcurrant anthocyanins extract consumption modulates exercise-induced oxidative stress and lipopolysaccharide- stimulated inflammatory responses. *American Journal of Physiology- Regulatory, Integrative, Comparative Physiology. 297*. 70-81.

Mac Intyre, D.L., Reid, W.D., Lyster, D.M., Szasz, I.J., Mc Kenzie, D.C. (1996). Presence of WBC, decreased strength, and delayed soreness in muscle after eccentric exercise. *Journal of Applied Physiology. 80(3).* 1006-1013.

MacRae, H.S. & Mefferd, K.M. (2006). Dietary antioxidant supplementation combined with quercetin improves cycling time trial performance. *International Journal of Sport Nutrition, Exercise and Metabolism. 16*. 405-419.

Manach, C., Williamson, G., Morand, C., Scalbert, A. Remesy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. 1. Review of 97 bioavailability studies. *American Journal of Clinical Nutrition. 81.* 230-242.

Mantovani, M.S., Bellini, M.F., Angeli, J.P., Oliveira, R.J., Silva, A.F., Ribeiro, L.R. (2008). Beta-glucans in promoting health: prevention against mutation and cancer. *Mutation Research. 658(3).* 154-161.

Marsen, T.A., Pollok, M., Oette, K., Baldamus, C.A. (1992). Pharmacokinetics of omega-3-fatty acids during ingestion of fish oil preparations. *Prostaglandins, Leukotrienes and Essential Fatty Acids. 46(3).* 191-196.

Mastaloudis, A., Traver, M.G., Carstensen, K., Widrick, J.J. (2006). Antioxidants did not prevent muscle damage in response to an ultramarathon run. *Medicine & Science in Sports & Exercise. 38.* 72-80.

McAnulty, S.R., McAnulty, L.S., Morrow, J.D., Khardouni, D., Shooter, L., Monk, J., Gross, S., & Brown, V. (2005). Effect of daily fruit ingestion on angiotensin converting enzyme activity, blood pressure, and oxidative stress in chronic smokers. *Free Radical Research. 39*. 1241-1248.

Mc Anulty, S.R., Mc Anulty, L.S., Nieman, D.C., Quindry, J.C., Hosick, P.A., Hudson, M.H., Still, L., Henson, D.A., Milne, G.L., Morrow, J.D., Dumke, C.L., Utter, A.C., Triplett, N.T., Dibarnardi, A. (2008). Chronic quercetin ingestion and exercise-induced oxidative damage and inflammation. *Applied Physiology, Nutrition, and Metabolism. 33.* 254-262.

McArdle, W.D., Katch, F.I., Katch, V.L. Exercise physiology. Energy, nutrition, and human performance. 4th ed. Baltimore: Williams & Wilkins; 1996.

Middleton, E., Jr., Kandaswami, C., Theoharides, T.C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological Reviews. 52*. 673-751.

Moon, Y.J., & Morris, M.E. (2007). Pharmacokinetics and bioavailibility of the bioflavonoid biochanin A: effects of quercetin and EGCG on biochanin A disposition in rats. *Molecular Pharmaceutics. 4*. 865-872.

Moon, Y.J., Wang, L., DiCenzo, R., Morris, M.E. (2008). Quercetin Pharmakokinetics in humans. *Biopharmaceutics & Drug disposition. 29.* 205-217.

Morillas-Ruiz, J.M., Villegas Garcia, J.A., López, F.J., Vidal-Guevara, M.L., Zafrilla, P. (2006). Effects of polyphenolic antioxidants on exercise-induced oxidative stress. *Clinical Nutrition. 25.* 444-453.

Mostafavi-Pour, Z., Zal, F., Monabati, A., Vessal, M. (2008). Protective effects of a combination of quercetin and vitamin E against cyclosporine A-induced oxidative stress and hepatotoxicity in rats. *Hepatology Research. 38.* 385-392.

Murase, T., Haramizi, S., Shimotoyodome, A., Tokimitsu, I., Hase, T. (2006). Green tea extract improves running endurance in mice by stimulating lipid utilization during exercise. *American Journal of Physiology - Regulatory, Integrative Physiology. 290(6).* 1550-1556.

Nieman, D.C. (1997). Immune response to heavy exertion. *Journal of Applied Physiology. 82*.1385-1394.

Nieman, D.C. (2000a). Is infection risk linked to exercise workload? *Medicine & Science in Sports & Exercise. 32(7).* 406-411.

Nieman, D.C. (2000b). Special feature for the Olympics: effects of exercise on the immune system. Exercise effects on systemic immunity. *Immunology & Cell Biology. 78.* 496-501.

Nieman, D.C. (2007). Marathon Training and immune function. *Sports Medicine. 37(4-5).* 412-415.

Nieman, D.C. (2008a). Immunonutrition support for athletes. *Nutritional Review. 66.* 310-320.

Nieman, D.C. Fitness and your health. 4th ed. Dubuque: Kendall/Hunt Publishing; 2008b.

Nieman, D.C. (2010). Quercetin´s bioactive effects in human athletes. *Current Topics in Nutraceutical Research. 8*. 33-44.

Nieman, D. C. Exercise Testing and Prescription. New York: Mc Graw Hill; 2011

Nieman, D.C., Buckley, K.S., Henson, D.A., Warren, B.J., Suttles, J., Ahle J.C., Simandle, S., Fagoaga, O.R., Nehlsen-Cannarella, S.L. (1995). Immune function in marathon runners versus sedentary controls. *Medicine & Science in Sports & Exercise. 27.* 986-992.

Nieman, D.C., Nehlsen-Cannarella, S.L., Fagoaga, O.R., Henson, D.A., Shannon, M., Hjertman, J.M., Schmitt, R.L., Bolton, M.R., Austin, M.D., Schilling, B.K., Thorpe. R. (2000). Immune function in female elite rowers and non-athletes. *British Journal of Sports Medicine. 34*.181-187.

Nieman, D.C., Henson, D.A., Smith, L.L., Utter, A.C., Vinci, D.M., Davis, J.M., Kaminsky, D.E., Shute, M. (2001). Cytokine changes after a marathon race. *Journal of Applied Physiology. 91*. 109-114.

Nieman, D.C., Henson, D.A., McAnulty, S.R., McAnulty, L., Swick, N.S., Utter, A.C., Vinci, D.M., Opiela, S.J., Morrow, J.D. (2002). Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. *Journal of Applied Physiology. 92*. 1970-1977.

Nieman, D.C, Davis, J.M, Henson, D.A, Walberg-Rankin, J., Shute, M., Dumke, C.L., Utter, A.C., Vinci, D.M., Carson, J.A., Brown, A., Lee, W.J., McAnulty, S.R., McAnulty, L.S. (2003a). Carbohydrate ingestion influences skeletal muscle cytokine mRNA and plasma cytokine levels after a 3-h run. *Journal of Applied Physiology 94(5).* 1917-1925.

Nieman, D.C., Dumke, C.L., Henson, D.A., McAnulty S.R., McAnulty, L.S., Lind, R.H., Morrow, J.D. (2003b). Immune and oxidative changes during and following the Western States Endurance Run. *International Journal of Sports Medicine. 24.* 541-547.

Nieman, D.C., Henson, D.A., McAnulty, S.R., McAnulty, L.S., Morrow, J.D., Ahmed, A., Heward, C.B. (2004). Vitamin E and immunity after the Kona Triathlon World Championship. *Medicine & Science in Sports & Exercise. 36.* 1328-1335.

Nieman, D.C., Dumke, C.L., Henson, D.A., McAnulty, S.R., Gross, S.J., Lind, R.H. (2005). Muscle damage is linked to cytokine changes following a 160-km race. *Brain, Behavior, and Immunity. 19.* 398-403.

Nieman, D.C., Henson, D.A., Dumke, C.L., Rind, R.H., Shooter, L.R., Gross, S.J. (2006a). Relationship between salivary IgA secretion and upper respiratory tract infection following a 160 –km race. *Journal of Sports Medicine and Physical Fitness. 46.* 158-162.

Nieman, D.C., Oley, K., Henson, D.A., Dumke, C.L., McAnulty, S.R., Davis, J.M., Murphy, E.A., Lind, R.H. (2006b). Ibuprofen use, endotoxemia, inflammation and

plasma cytokines during ultramarathon competition. *Brain, Behavior, and Immunity. 20.* 578-584.

Nieman, D.C. & Bishop, N.C. (2006c). Nutritional strategies to counter stress to the immune system in athletes, with special reference to football. *Journal of Sport Sciences. 24*. 763-772.

Nieman, D.C., Henson, D.A., Davis, J.M., Dumke, C.L., Gross, S.J., Jenkins, D.P., Murphy, E.A., Carmichael, M.D., Quindry, J.C., Mc Anulty, S.R., Mc Anulty L.S., Utter, A.C., Mayer, E.P. (2007). Quercetin ingestion does not alter cytokine changes in athletes competing in the Western States Endurance Run. *Journal of Interferon & Cytokine Research. 27*. 1003-1011.

Nieman, D.C., Henson, D.A., McMahon, M., Wrieden, J.L., Davis, J.M., Murphy, E.A., Gross, S.J., McAnulty, L.S., Dumke, C.L. (2008). Beta-glucan, immune function, and upper respiratory tract infections in athletes. *Medicine & Science in Sports & Exercise. 40(8).* 1463-1471.

Nieman, D.C., Henson, D.A., Maxwell K., Williams, A.S., McAnulty, S.R., Jin, F., Shanely, A., Lines, T.C. (2009a). Effects of quercetin and EGCG on Mitochondrial Biogenesis and Immunity. *Medicine & Science in Sports & Exercise. 41.* 1467-1475.

Nieman, D.C., Henson, D.A., Maxwell, K., Williams, A., McAnulty, S.R., Jin, F., Shanely, R.A. Lines, T. (2009b). Influence of supplemental quercetin and epigallocatechin 3-gallate on exercise performance, mitochondrial biogenesis, immunity, inflammation and oxidative stress. *Medicine and Science in Sports and Exercise. 41*. 1467-1475.

Nieman, D.C., Henson, D.A., McAnulty, S.R., Jin, F. & Maxwell, K. (2009c). N-3 Polyunsaturated Fatty acids do not alter immune and inflammation measures in endurance Athletes. *International Journal of Sport Nutrition and Exercise Metabolism. 19(5).* 536-546.

Nieman, D.C., Cayea, E.J., Austin, M.D., Henson, D.A., McAnulty, S.R., Jin, F. (2009d). Chia Seed Does Not Promote Weight Loss or Alter Disease Risk Factors in Overweight Adults. *Nutrition Research. 9*. 414-418.

Nieman, D.C., Stear, S.J., Castell, L.M., Burke, L.M. (2010a) A-Z of nutritional supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and perfromance: part 15. *British Journal of Sports Medicin. 44(16).* 1202-1205.

Nieman, D.C., Williams, A.S., Shanely, R.A., Jin, F., McAnulty, S.R., Triplett, N.T., Austin, M.D., & Henson, D.A. (2010b). Quercetin ´s influence on exercise

performance and muscle mitochondrial biogenesis. *Medicine and Science in Sports and Exercise. 42.* 338-345.

Petersen, E.W., Ostrowski, K., Ibfel, T., Richelle, M., Offort, E., Halkjaer-Kristensen, J., Pedersen, B.K. (2002). Effect of vitamin supplementation on cytokine response and on muscle damage after strenuous exercise. *American Journal of Physiology- cell physiology. 280.* 1570-1575.

Phillips,T., Childs, A.C., Dreon, D.M., Phinney, S., and Leeuwenburgh, C. (2003). A dietary supplement attenuates IL-6 and CRP after eccentric exercise in untrained males. *Medicine & Science in Sports & Exercise. 35(12).* 2032-2037.

Powers, S.K., DeRuisseau, K.C., Quindry, J., Hamilton, K.L. (2004). Dietary antioxidants and exercise. *Journal of Sports Sciences. 22(1).* 81-94.)

Powers, S.K., Smuder, A.J., Kavazis, A.N., Hudson, M.B. (2010). Experimental guidelines for studies designed to investigate the impact of antioxidant supplementation on exercise performance. International *Journal for Sport Nutrition and Exercise Metabolism. 20(1).* 2-14.

Richards, J.C., Lonac, M.C., Johnson, T.K., Schweder M.M., & Bell, C. (2010). Epigallocatechin-3-gallate increases maximal oxygen uptake in adult humans. *Medicine and Science in Sports and Exercise. 42*. 739-744.

Ristow, M., Zarse, K., Oberbach, A., Kloting, N., Birringer, M., Kiehntopf, M., Stumvoll, M., Kahn, C.R., Bluher, M. (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. *Proceedings of the National Academy of Sciences USA. 106.* 8665-8670.

Santos, M.R., Mira, L. (2004). Protection by flavonoids against the peroxynitrite-mediated oxidation of dihydrorhodamine. *Free Radical Research 38(9)*. 1011-1018.

Scalbert, A., Johnson, I.T., Saltmarsh, M. (2005). Polyphenols: antioxidants and beyond. *American Journal of Clinical Nutrition. 81.* 215-217.

Simopoulos, A.P. (2007). Omega-3 fatty acids and athletics. Current Sports Medicine Reports. 6. 230-236.

Steensberg, A., Keller, C., Hillig, R., Frosig, C., Wojtaszewski, J.F.P., Pedersen, B.K., Pilegaard, H., Sander, M. (2007). Nitric oxide production is a proximal signalling event controlling exercise-induced mRNA expression in human skeletal

muscle. *The Journal of the Federation of American Societies for Experimental Biology. 21*. 2683-2694.

Suzuki, K., Totsuka, M., NaKaji, S., Yamada, M., Kudoh, S., Liu, Q., Sugawara, K., Xamaya, K., Sato, K. (1999). Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage. *Journal of Applied Physiology. 87*. 1360-1367.

Suzuki, K., Nakaji, S., Yamanda, M., Totsuka, M., Sato, K., Sugawara, K. (2002). Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. *Exercise Immunology Review. 8*. 6-48.

Tribolo, S., Lodi, F., Connor, C., Suri, S., Wilson, V.G., Taylor, M.A., Needs, P.W., Kroon, P.A., & Hughes, D.A. (2008). Comparative effects of quercetin and its predominant human metabolites on adhesion molecule expression in activated human vascular endothelial cells. *Atherosclerosis, 197,* 50-56.

Trombold, J.R., Barnes, J.N., Critchley, L., & Coyle, E.F. (2010). Ellagitannin consumption improves strength recovery 2-3 d after eccentric exercise. *Medicine & Science in Sports & Exercise. 42*. 493-498.

Walsh, N.P., Gleeson, M., Pyne, D.B., Nieman, D.C., Dhabhar, F.S., Shephard, R.J., Oliver, S.J., Bermon, S., Kajeniene, A. (2011). Position statement. Part two. Maintaining immune health. *Exercise Immunology Review*. 17. 64-103.

Wiswedel, I., Hirsch, D., Kropf, S., Gruening, M., Pfister, E., Schewe, T., & Sies, H. (2004). Flavanol-rich cocoa drink lowers plasma F(2)-isoprostane concentrations in humans. *Free Radical Biology and Medicine. 37*. 411-421.

USDA Nutrient Data Laboratory. USDA Database for the flavonoid content of selected foods. Beltsville, Md.: U.S. Department of Agriculture. [Internet; cited 2010 August 12th] Available from: http://www.ars.usda.gov/nutrientdata;

Urso, M.L. & Clarkson, P.M. (2003). Oxidative stress, exercise, and antioxidant supplementation. *Toxicology. 189 (1-2).* 41-54.)

Utesch, D., Feige, K., Dasenbrock, J., Broschard, T.H., Harwood, M., Danielewska-Nikiel, B., Lines, T.C. (2008). Evaluation of the potential in vivo genotoxicity of quercetinn. *Mutatation Research. 654*. 38-44.

Yfanti, C., Akerstrom, T., Nielsen, S., Nielsen, A.R., Mounier, R., Mortensen, O.H., Lykkesfeldt, J., Rose, A.J., Fischer, C.P., Pedersen, B.K. (2010). Antioxidant

supplementation does not alter endurance training adaptation. *Medicine & Science in Sports & Exercise. 42.* 1388-1395.

Yu, C.S., Lai, K.C., Yang, J.S., Chiang, J.H., Lu, C.C., Wu, C.L., Lin, J.P., Liao, C.L., Tang, N.Y., Wood, W.G., Chung, J.G. (2010). Quercetin inhibited murine leukemia WEHI-3 cells in vivo and promoted immune response. *Phytotherapy Research. 24(2).* 163-168.

Zamora- Ros, R. Andres-Lacueva C., Lamuela-Raventos, R.M., Berenguer, T., Jakszyn, P., Barricarte, A., Ardanaz, E., Amiano, P., Dorronsoro, M., Larrañaga, N., Martínez, C., Sánchez, M.J., Navarro, C., Chirlaque, M.D., Tormo, M.J., Quirós, J.R., González, C.A. (2010) Estimation of dietary sources and flavonoid intake in Spanish adult population (EPIC- Spain). *Journal of American Dietetic Association. 110.* 390-398.

Zhu, B.T. & Liehr, J.G. (1996). Inhibition of catechol *O*-methyltransferase-catalyzed *O*-methylation of 2- and 4-hydroxyestradiol by quercetin. *Journal of Biological Chemistry, 271,* 1357-1363.

7 Appendices

7.1 Questionnaires and forms

A1: General data form

Appalachian State University
NCRC-Human Performance Lab
Q-Force Immune Running Study Data

Date_____ **TEST SESSION** □ **Baseline**

Name _____

ID Number_____

Height (inches)_____ **Weight (pounds)**_____

Body Fat%_____ **BMI**_____

Gender _____ **DOB**_____ **Age**_____

Approximate Mileage per week _____

□ **Bod Pod**
□ **VO2 Max**

Food list explained? _____

Verify two test date appointments? _____

Boost given for next visit? _____

Informed Consent signed? _____

Screening Questionnaire Completed? _____

A2: ACSM Screening Questionnaires

ASU/NCRC SCREENING QUESTIONNAIRE*

YOUR NAME _____

Assess your health status by marking all true statements

MEDICAL HISTORY

_____ a heart attack
_____ heart surgery
_____ cardiac catheterization
_____ coronary angioplasty (PTCA)
_____ pacemaker/implantable cardiac
_____ defibrillator/rhythm disturbance
_____ heart valve disease
_____ heart failure
_____ heart transplantation
_____ congenital heart disease

SYMPTOMS

_____ You experience chest discomfort with exertion.
_____ You experience unreasonable breathlessness.
_____ You experience dizziness, fainting, or blackouts.
_____ You take heart medications.

OTHER HEALTH ISSUES

_____ You have diabetes.
_____ You have asthma or other lung disease.
_____ You have burning or cramping sensation in your lower legs when walking.
_____ You have musculoskeletal problems that limit your physical activity.
_____ You have concerns about the safety of exercise.
_____ You take prescription medication(s).
_____ You are pregnant.

If you marked any of these statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a medically qualified staff.

CARDIOVASCULAR RISK FACTORS

_____ You are a man older than 45 years.
_____ You are a woman older than 55 yrs, had a hysterectomy, or are postmenopausal.
_____ You smoke, or quit smoking within the previous 6 months.
_____ Your blood pressure is >140/90 mm Hg, or take medication.
_____ Your blood cholesterol level is >200 mg/dL (borderline or high), or take meds.
_____ You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister).
_____ You are physically inactive (i.e., you get <30 minutes of physical activity on at least 3 days per week).
_____ You are >20 pounds overweight.

If you did not mark any of these, you should be able to exercise safely without consulting an MD in a self-guided program or almost any facility that meets your exercise program needs. If you marked two or more of the statements in this section you should consult your physician or other appropriate health care provider before engaging in exercise. You might benefit from using a facility with a professionally qualified exercise staff to guide your exercise program.

**2010 AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire*

2010 ACSM Risk Stratification

Low Risk – individuals classified as Low Risk are those who do not have signs/symptoms of or have diagnosed cardiovascular, pulmonary, and/or metabolic disease, and have no more than one (i.e., <1) CVD risk factor. The risk of an acute cardiovascular event in this population is low and a physical activity/exercise program may be pursued safely without the necessity for medical examination and clearance.

Moderate Risk – individuals classified as Moderate Risk do not have signs/symptoms of or diagnosed cardiovascular, pulmonary, and/or metabolic disease, but have two or more (i.e., >2) risk CVD factors. The risk of an acute cardiovascular event in this population is increased although in most cases individuals at moderate risk may safely engage in low to moderate intensity physical activities without the necessity for medical examination and clearance. However it is advisable to have a medical examination and an exercise test before participation in vigorous intensity exercise (i.e., >60% VO2max).

High risk – individuals classified as High Risk are those who have one or more signs/symptoms of, or have diagnosed cardiovascular, pulmonary, and/or metabolic disease. The risk of an acute cardiovascular event in this population is increased to the degree that a thorough medical examination should take place and clearance given before initiating physical activity or exercise at any intensity.

A3: Form for training history and demographic data

Training History and Basic Demographics

Date _____

Time of pre-run blood sample _____

Name _____ **Gender:** Male Female

Height _____ **Weight** _____ **Age** _____

Telephone: _____

E-mail address (please print carefully): _____

Current Exercise Program

1. How many years have you been running seriously (for races)? _____

2. How many miles per week have you run on average in the previous year? _____

3. How many marathons have you raced? _____

4. What is your best marathon race time? _____

5. List and describe any significant injuries or illness you have had in the previous year, and list any medications you are using:

_____

_____

_____

6. When is the last time you had a cold, sore throat, or the flu (including today):

_____

_____

7. Please list any vitamin/mineral/herb supplements that you use on a regular basis (at least 3 times a week or more):

_____

_____

_____

A4: Body Composition Measurement Norms

Body Composition Measurement: Norms

Appalachian State University/NCRC Human Performance Lab
Room 1201, 600 Laureate Way, NCRC, Kannapolis, NC 28081; email: ASU-NCRC@appstate.edu

Name_____ **Date** _____

Age ___ **Sex** _____ **Weight** _____ **Height**_____

Body Mass Index: _____ ***kg/m²***

<20 Underweight; 20-24.9 Normal; 25-29.9 Overweight; ≥30 Obese

DEXA, BodPod, or BIA Measurement: %Fat _____

Skinfold Measurement: %Fat _____

Body Fat Ranges for Ages 18 Years and Older

	Males	Females
Unhealthy range -- too low	≤5%	≤8%
Acceptable range -- lower end	6-15%	9-23%
Acceptable range -- higher end	16-24%	24-31%
Unhealthy range -- too high	≥25%	≥32%

Average Body Fat Ranges for Elite Athletes	**Males**	**Females**
Long distance runners	4-9%	6-15%
Gymnasts	4-10%	10-17%
Body builders	6-10%	10-17%
Swimmers	5-11%	14-24%
Basketball athletes	7-11%	18-27%
Tennis players	14-17%	19-22%

Source: Nieman DC. Exercise Testing and Prescription. McGraw Hill, 2010

A5: Form for Bruce Protocol and VO_{2max}

Aerobic Fitness Measurement: $V0_{2Max}$, Bruce Protocol

Appalachian State University/NCRC Human Performance Lab
Room 1201, 600 Laureate Way, NCRC, Kannapolis, NC 28081; email: ASU-NCRC@appstate.edu

Name_____ Age_____ Sex____ (M) ____ (F) Date_____

Weight_____; Height _____; Predicted MHR_____; Resting BP ____/____

STAGE	MPH	GRADE	METS	MIN	H R	BP	RPE	VO2	RER	VE	RR
I	1.7	10%	4-5			/					
II	2.5	12%	6-7			/					
III	3.4	14%	8-10			/					
IV	4.2	16%	10-12			/					
V	5.0	18%	14-16			/					
VI	5.5	20%	18-20			/					
VII	6.0	22%	21-23			/					
Rec				2		/					
Rec				4		/					

Exercise Counselor's Evaluation of Fitness Data: Exercise Prescription

Total Time Till Exhaustion_____min/sec. Max. H. R._____bpm

$V0_2$Max_____ = Rating_____

Training HR = MHR_____ -RHR _____ x 60%/70%/80% + RHR____=_____-_____

Aerobic Fitness Measurement: $V0_{2Max}$ Norms

Appalachian State University/NCRC Human Performance Lab
Room 1201, 600 Laureate Way, NCRC, Kannapolis, NC 28081; email: ASU-NCRC@appstate.edu

Name_____

$V0_2$MAX NORMS

(in milliliters of oxygen per kilogram of body weight per minute)

Age	Low	Fair	Average	Good	High	Athletic	Olympic
Women							
20-29	<28	29-34	35-43	44-48	49-53	54-59	60+
30-39	<27	28-33	34-41	42-47	48-52	53-58	59+
40-49	<25	28-31	32-40	41-45	46-50	51-56	57+
50-65	<21	22-28	29-36	37-41	42-45	46-49	50+
Men							
20-29	<38	39-43	44-51	52-56	57-62	63-69	70+
30-39	<34	35-39	40-47	48-51	52-57	58-64	65+
40-49	<30	31-35	36-43	44-47	48-53	54-60	61+
50-59	<25	26-31	32-39	40-43	44-48	49-55	56+
60-69	<21	22-26	27-35	36-39	40-44	45-49	50+

Source: Adapted from Astrand *ACTA Physiol Scand 49* (Suppl): 169, 1960. Reprinted with permission from Blackwell Scientific Publications Ltd.

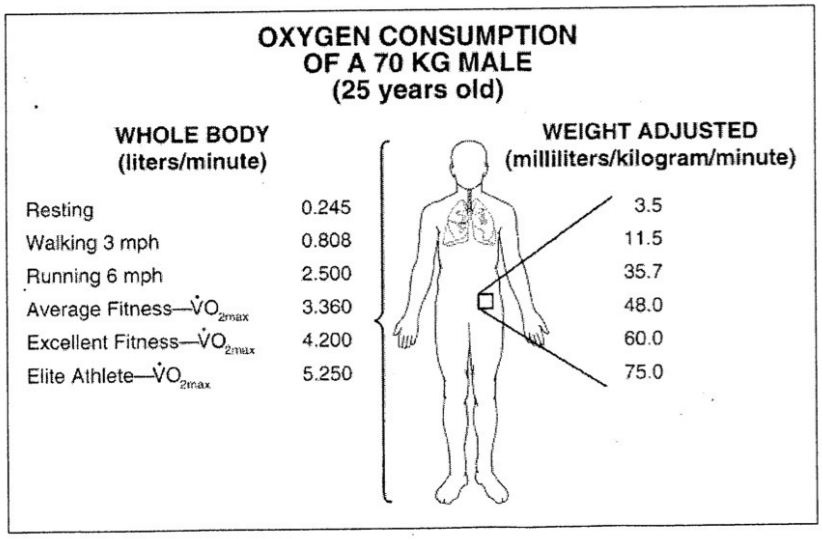

FIGURE 4.1
The oxygen consumption ($\dot{V}O_{2max}$) can be expressed in units of liters per minute for the entire body or in units of milliliters per kilogram of body weight per minute, to represent the oxygen consumption for each kilogram of body weight.

Aerobic Fitness Measurement: $V0_{2Max}$ Norms

Appalachian State University/NCRC Human Performance Lab
Room 1201, 600 Laureate Way, NCRC, Kannapolis, NC 28081; email: ASU-NCRC@appstate.edu

Name_____

$V0_2$MAX NORMS

(in milliliters of oxygen per kilogram of body weight per minute)

Age	Low	Fair	Average	Good	High	Athletic	Olympic
Women							
20-29	<28	29-34	35-43	44-48	49-53	54-59	60+
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40-49	<25	28-31	32-40	41-45	46-50	51-56	57+
50-65	<21	22-28	29-36	37-41	42-45	46-49	50+
Men							
20-29	<38	39-43	44-51	52-56	57-62	63-69	70+
30-39	<34	35-39	40-47	48-51	52-57	58-64	65+
40-49	<30	31-35	36-43	44-47	48-53	54-60	61+
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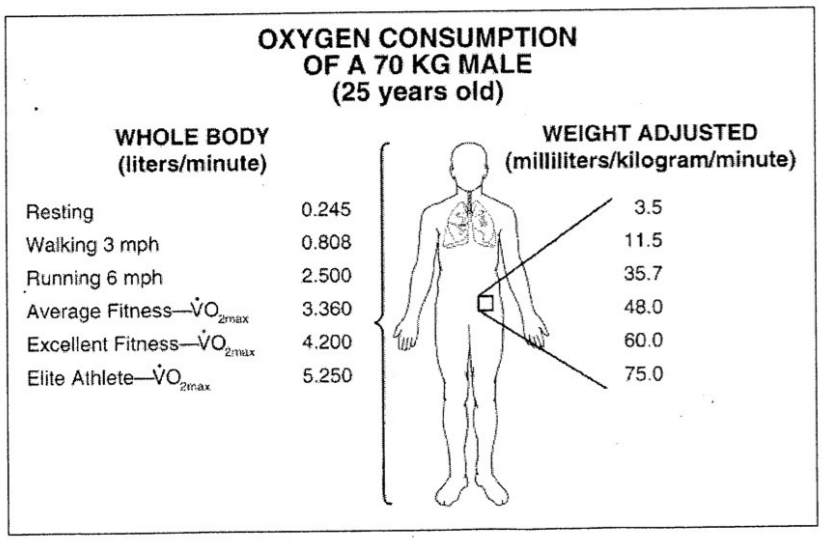

FIGURE 4.1
The oxygen consumption ($\dot{V}O_{2max}$) can be expressed in units of liters per minute for the entire body or in units of milliliters per kilogram of body weight per minute, to represent the oxygen consumption for each kilogram of body weight.

Aerobic Fitness Measurement: $V0_{2Max}$ Norms

Appalachian State University/NCRC Human Performance Lab
Room 1201, 600 Laureate Way, NCRC, Kannapolis, NC 28081; email: ASU-NCRC@appstate.edu

Name_____

$V0_2$MAX NORMS

(in milliliters of oxygen per kilogram of body weight per minute)

Age	Low	Fair	Average	Good	High	Athletic	Olympic
Women							
20-29	<28	29-34	35-43	44-48	49-53	54-59	60+
30-39	<27	28-33	34-41	42-47	48-52	53-58	59+
40-49	<25	28-31	32-40	41-45	46-50	51-56	57+
50-65	<21	22-28	29-36	37-41	42-45	46-49	50+
Men							
20-29	<38	39-43	44-51	52-56	57-62	63-69	70+
30-39	<34	35-39	40-47	48-51	52-57	58-64	65+
40-49	<30	31-35	36-43	44-47	48-53	54-60	61+
50-59	<25	26-31	32-39	40-43	44-48	49-55	56+
60-69	<21	22-26	27-35	36-39	40-44	45-49	50+

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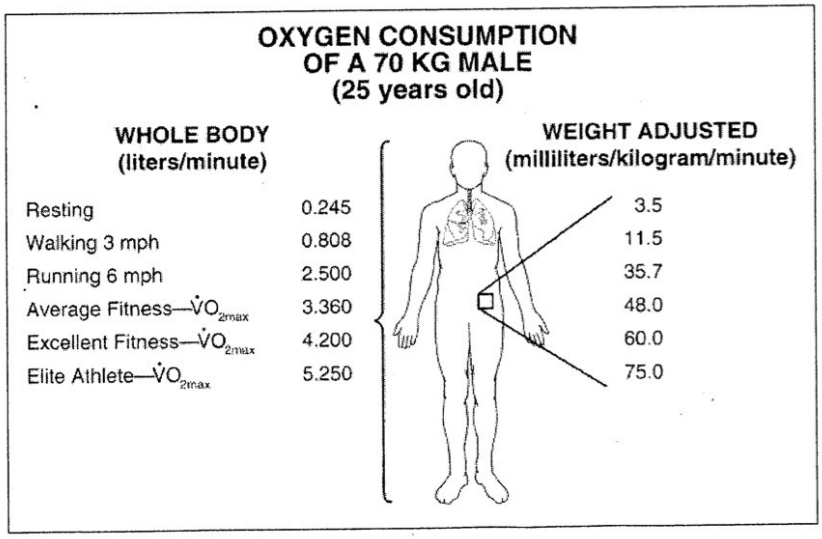


FIGURE 4.1
The oxygen consumption ($\dot{V}O_{2max}$) can be expressed in units of liters per minute for the entire body or in units of milliliters per kilogram of body weight per minute, to represent the oxygen consumption for each kilogram of body weight.

A6: Ratings of Perceived Exertion (RPE) and Category – Ration Scale

Table 4.2 Ratings of Perceived Exertion Scale and Category-Ratio Scale

RPE scale		CR10 scale		
6	No exertion at all	0	Nothing at all	"No P"
7		0.3		
	Extremely light	0.5	Extremely weak	Just noticeable
8		1	Very weak	
9	Very light	1.5		
10		2	Weak	Light
11	Light	2.5		
12		3	Moderate	
13	Somewhat hard	4		
14		5	Strong	Heavy
15	Hard (heavy)	6		
16		7	Very strong	
17	Very hard	8		
18		9		
19	Extremely hard	10	Extremely strong	"Max P"
20	Maximal exertion	11		
		↑		
	Borg RPE scale © Gunnar Borg, 1970, 1985, 1994, 1998	●	Absolute maximum	Highest possible
			Borg CR10 scale © Gunnar Borg, 1981, 1982, 1998	

For correct instruction and administration see Borg's book: Borg, G. (1998), *Borg's Perceived Exertion and Pain Scales,* Champaign, IL: Human Kinetics.

A 7: The food list for the subject

The Acute Anti-Inflammatory Effects of Q-Force Immune in Athletes Running Two Hours

Food List During The 2-Day Period Prior To and the Day of the 2-h Treadmill Runs; ASU- NCRC Human Performance Lab

All subjects require similar diets (moderately low in fat and high in carbohydrates) prior to the 2-h treadmill runs. Please use foods that conform to the list given below for the 2-day period and breakfast prior to the test dates. Also remember to taper exercise during the two days before the test dates. LUNCH on the test date will be Boost Plus (nothing else). Avoid all supplements and medications (unless cleared through Dr. Nieman, 828-773-0056).

Breakfast Foods:	
Foods to eat:	Foods to avoid:
• Cold cereals (Whole grains, wheat, grape nuts, etc.) • Hot cereals (oatmeal, grits, etc.) • Low fat milk, cottage cheese, cheese (made with skim milk) • Egg Substitutes (EggBeaters, etc.) • Fresh and dried fruits or vegetables and juices • Seeds and nuts • Whole grain breads, bagels • Honey, peanut butter • Fat-free margarine/butter spreads or sprays (I Can't Believe It's Not Butter, etc.) • Coffee or tea	• Fatty meats (bacon, sausage, etc.) • Oils, margarine, butter • Whole milk, regular cheese • Eggs • Nutritional and herbal supplements • Anti-inflammatory medications
Lunch and Supper Foods	
Foods to eat:	Foods to avoid:
• Low fat meats broiled, baked, or grilled (chicken, turkey, fish, etc.) • Whole grains breads • Pastas and rice • Low fat sauces (Tomato or herb sauces) • Low fat cheese, sour cream, and milk • Low fat Mayonnaise and salad dressings • Cooked and Raw vegetables of all kinds (seasonings only, not gravies or butter) • Fruits • Soft drinks • Coffee or tea • Alcoholic beverages (moderate)	• High fat meats (beef, pork, sausage, luncheon and fried or blackened meats) • Oils, margarine, butter, gravies, high fat salad dressings, etc. • Meat or cream sauces • Eggs, regular cheese, whole milk • Nutritional and herbal supplements • Anti-inflammatory medications

A8: The general form to check the different steps of the study

Appalachian State University
NCRC-Human Performance Lab
Q-Force Immune Running Study Data

Date_____ **TEST SESSION** ☐ **#1** ☐ **#2**

Name _____

ID Number_____

Height (inches)_____ **Weight (pounds)**_____

☐ **Urine (pre)**	☐ **Blood (pre)**
☐ **Urine (post)**	☐ **Blood (post)**
☐ **Urine (1 hr post)**	☐ **Blood (1 hr post)**

Q-Force Immune chews (4) given and ingested? _____

Food list adhered to 2 days prior to today's run? _____

Boost ingested prior to today's run? _____

Boost given for next visit? _____

7.2 Subject ´s report

Dept HLES, PO Box 32071

North Carolina Research Campus

111 River Street, Boone, NC 28608

600 Laureate Way, Kannapolis, NC 28081

http://www.hles.appstate.edu

http://www.ncrc.appstate.edu

11-5-2010

Dear *subject´s name*,

Thank you once again for participating in the research project, "**The Acute Anti-Inflammatory Effects of Q-Chew in Athletes Running Two Hours".**

The study was a success, and the purpose of this letter is to provide a summary of basic findings with detailed information regarding your personal data.

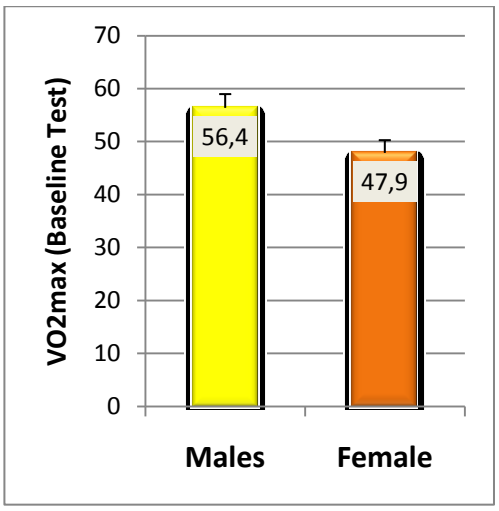
Attached to the end of this letter is an abstract that will be presented at the American College of Sports Medicine conference during June, 2011. In general, we found that ingesting four Q- chews (containing quercetin, green tea extract, fish oil, vitamin C, and niacin) 15-min before running 2-h on the treadmill did NOT counter the inflammation that occurred after the exercise bout. We had hoped that this supplement that in a previous study was strongly anti-inflammatory after 2-weeks of daily ingestion (4 chews per day) would be efficacious after just one dose, but this hypothesis was not supported. This is very useful information, and our next step will be to test whether one week of supplementation is effective. Plant molecules such as quercetin appear to need a period of time before they saturate the tissues and help reduce inflammation.

Here are some summary graphs of interesting data collected during the study. Your results are listed in the data boxes and you can compare to the group averages in the graphs.

1. Baseline $VO2_{max}$

During your first session in the lab, we measured your VO_{2max} (maximal oxygen consumption when fatigued at the end of the graded treadmill test). Compare your values noted below with the group averages.

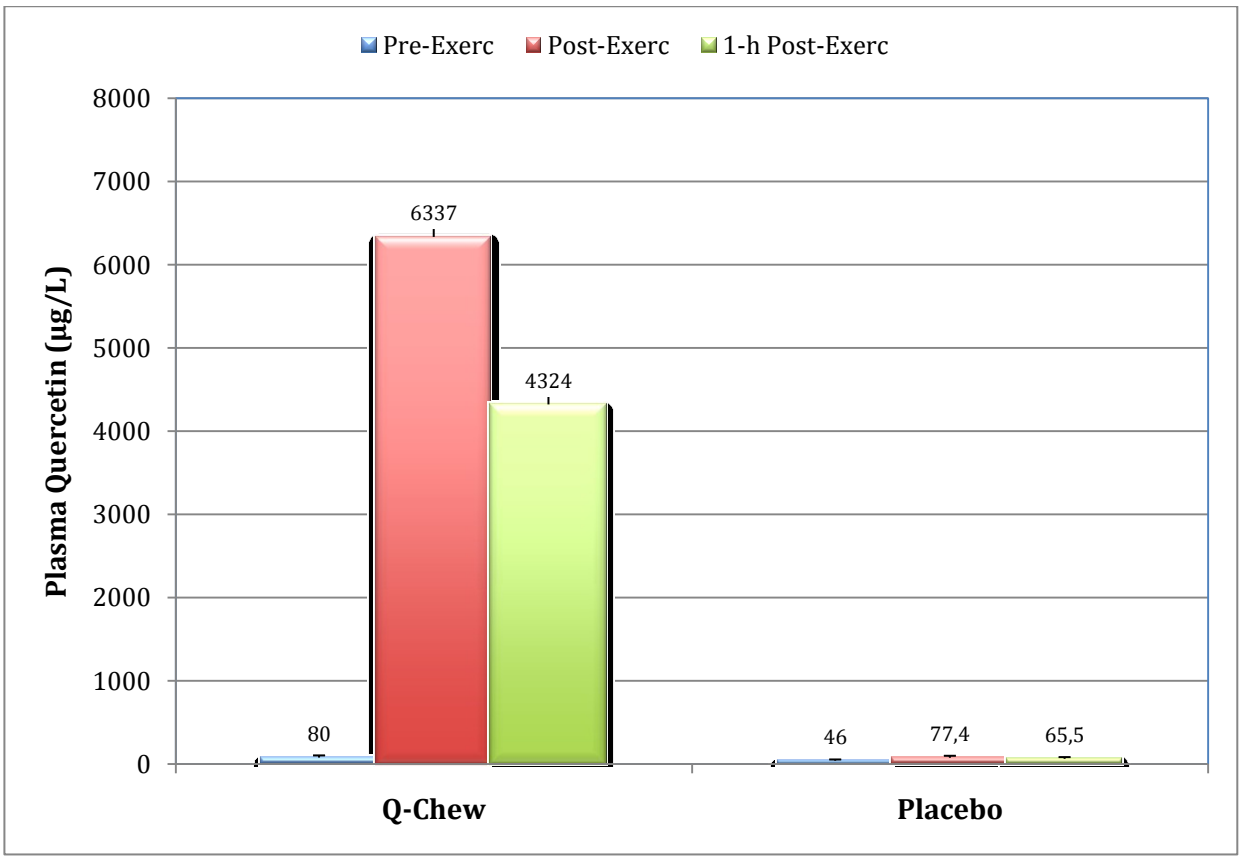
Your VO_{2max} was 54 ml/kg/min (compare with gender-specific mean in graph).



2. Plasma quercetin data.

(The Q-chew caused a huge increase in plasma quercetin levels. We had hoped that this would produce an immediate anti-inflammatory effect, but other studies (including our own) indicate that 2 or more weeks of Q-chew ingestion may be needed to saturate the tissues leading to an anti-inflammatory effect every time you train). Here are your personal plasma quercetin values (there was no male or female difference). People absorb quercetin differently, and this appears to be due to receptors on the intestinal cells.

	Pre-Exercise	**Post-Exercise**	**1-h Post-Exercise**
Q-chew	2.5 ug/L	6805.8 ug/L	4175.3 ug/L
Placebo	14.6 ug/L	32.9 ug/L	10.0 ug/L

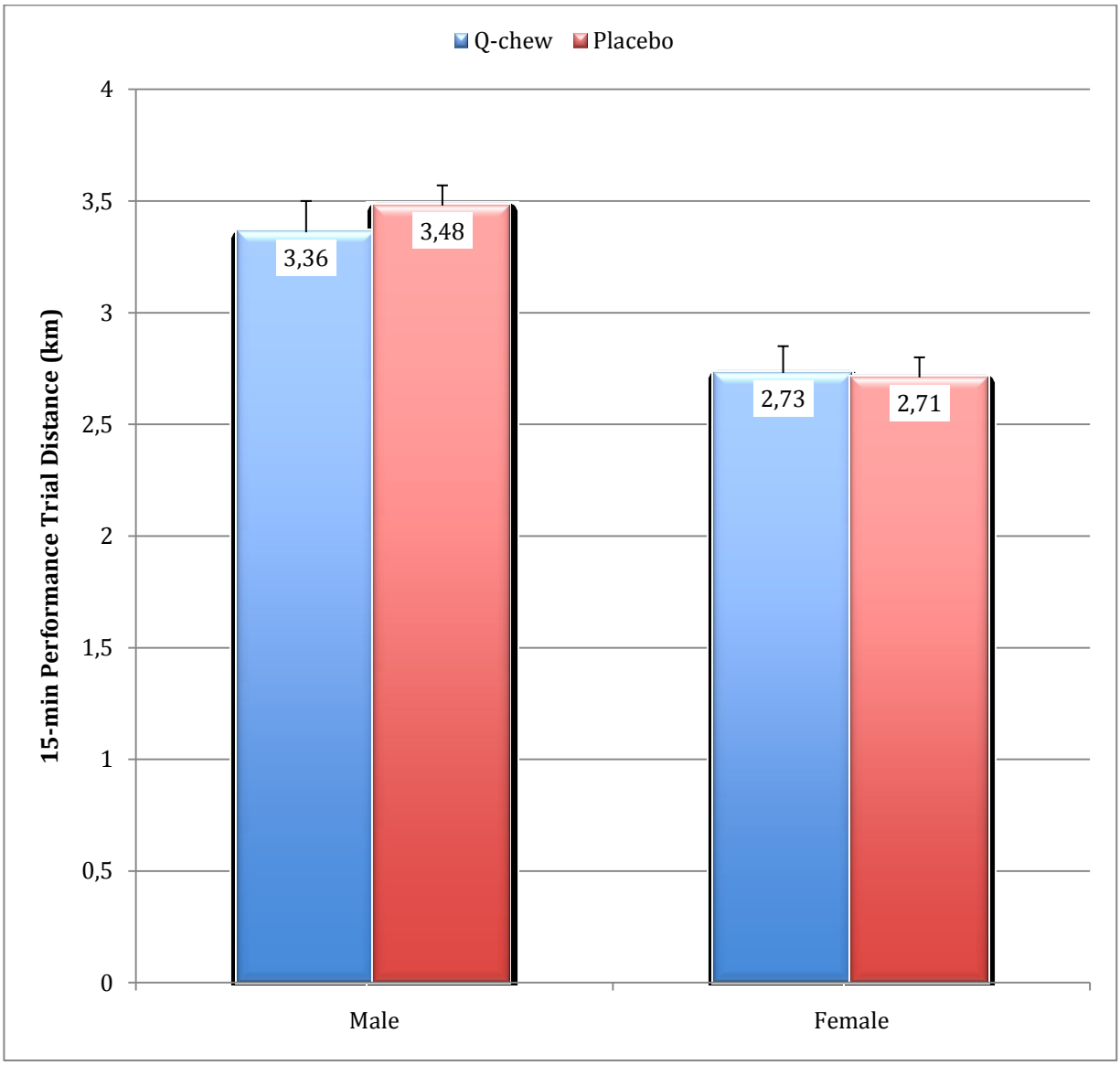


3. Performance During the 15-min Time Trials (after running 1h 45 min)

(The distance covered during the 15-min time trials (end of the 2-h runs) did not differ between Q-chew and placebo trials, indicating that consuming the Q-chew just before running does not improve performance).

Your distance covered:

	Female
Q-chew	3.12 km
Placebo	3.07 km

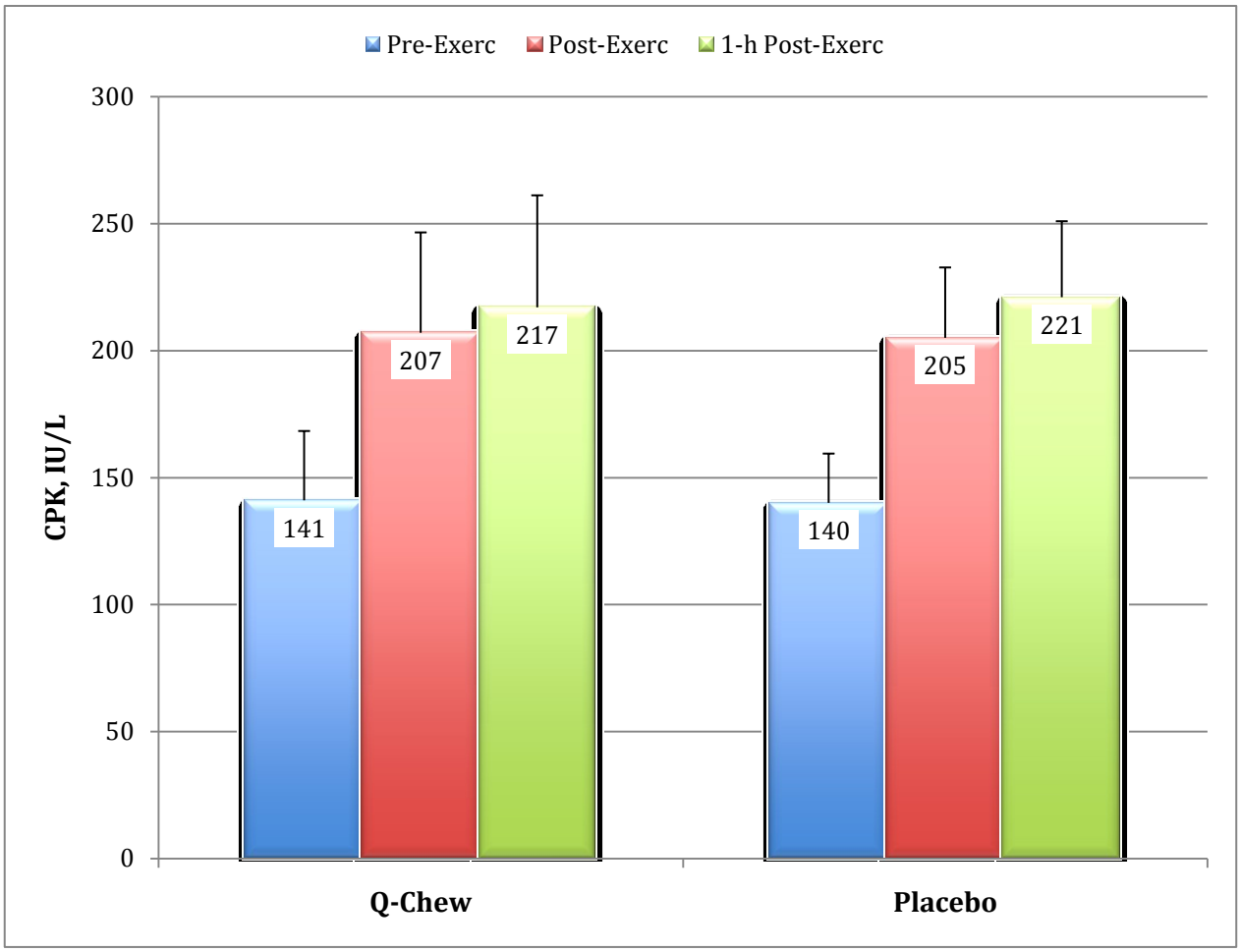


4. Blood Creatine Phosphokinase (CPK) Levels

CPK is an enzyme in the muscle that leaks out into the blood when the muscle experiences injury and damage. High levels indicate significant muscle cell damage, pain, and inflammation. Thus low levels are good. We saw no differences between Q-chew and placebo trials.

Your CPK values (IU/L):

	Pre-Exerc	Post- Exerc	1-h-Exerc
Q-chew	116	144	136
Placebo	193	339	404



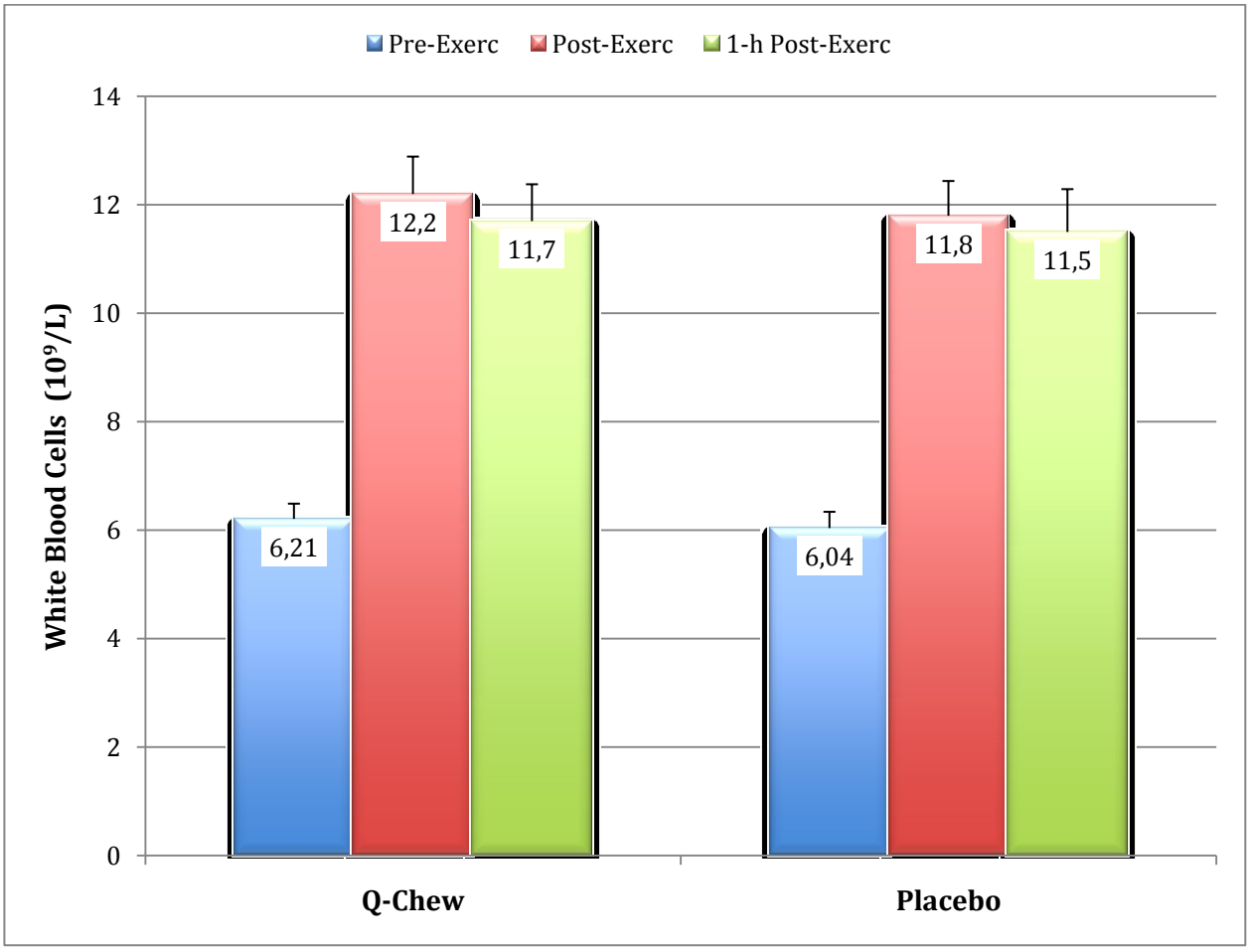
5. Total White Blood Cell Count

The white blood cell (WBC) count rises during exercise as neutrophils, lymphocytes, monocytes, and other cells surge into the blood compartment. A high

WBC count is an indication of unusually high inflammation. Thus lower WBC counts during exercise are better than high counts. There were no differences between Q-chew and placebo trials.

Your WBC counts (compare to the graph) (10^9/L):

	Pre-Exerc	**1-h Exerc**	**Post-Exerc**
Q-chew	5.4	8.3	6.6
Placebo	5.5	9.4	8.3

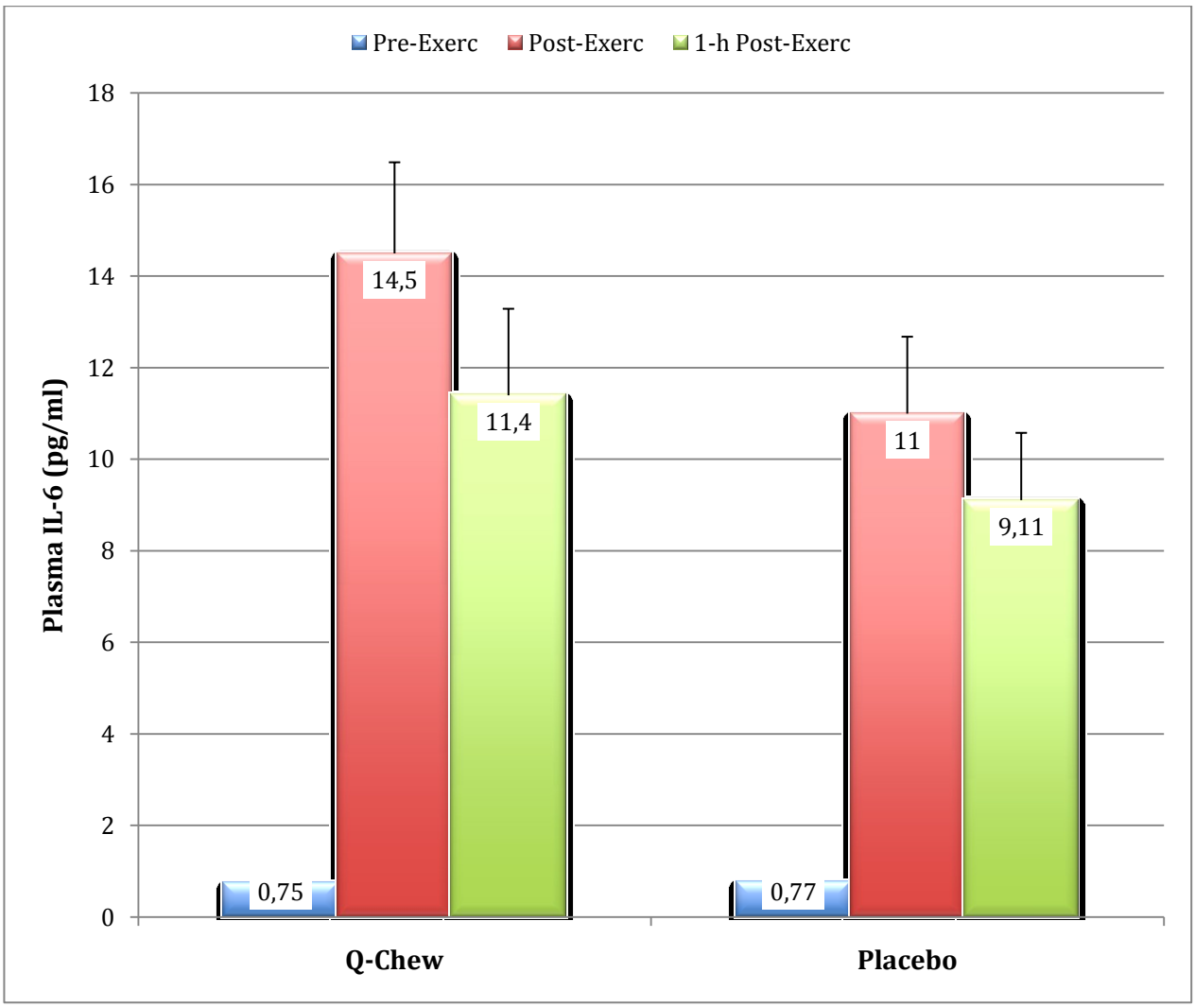


6. Blood Interleukin-6 (IL-6) (pg/ml):

IL-6 is an inflammatory cytokine. High levels during exercise are indication of high physiologic stress. Thus lower values are better, and represent reduced inflammation.

Your IL-6 levels (compare to the graph) (pg/ml):

	Pre-Exerc	**1-h Exerc**	**Post-Exerc**
Q-chew	1.18	6.65	5.29
Placebo	1.16	8.32	7.29



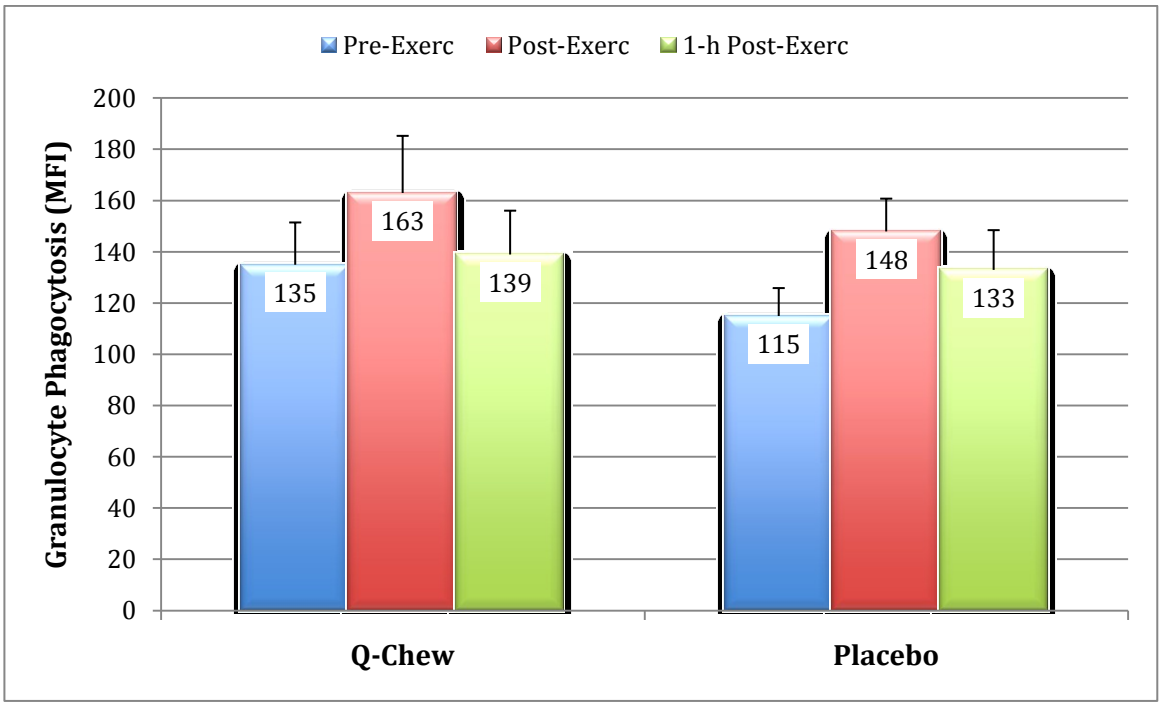
7. Granulocyte Phagocytosis

Granulocytes (primarily neutrophils) engulf bacteria, a process called phagocytosis. High levels during exercise are indication of muscle cell breakdown, inflammation, and high physiologic stress. Thus lower values are better, and represent reduced inflammation.

Your granulocyte phagocytosis levels (compare to the graph) (MFI):

	Pre-Exerc	**1-h Exerc**	**Post-Exerc**

Q- chew	135	203	153
Placebo	95.7	139	75.6



Thank you once again for serving as a subject in this research project. We look forwarding to working with you again soon in another project.

Sincerely,

David C. Nieman

David C. Nieman, DrPH
Director, Human Performance Lab
North Carolina Research Campus, Kannapolis, NC 28081
Phone: 828-773-0056; E-mail: niemandc@appstate.edu

www.ncrc.appstate.edu

Manuela Konrad, Doctoral candidate

Medical University Graz, Austria

Manuela.konrad@fh-Joanneum.at

7.2.1 Posters and Papers

Poster:

Konrad M, Nieman DC, Henson DA, Kennerly KM, Jin F, Shanely RA, Wallner-Liebmann, SJ.: The Acute Effect of Ingesting a Quercetin-Based Supplement on Exercise-Induced Inflammation in Runners. Annual Meeting of American College of Sports Medicine, Denver, Colorado, USA. Supplement zu Med&Sci in Sports& Exercise, Vol 43, Number 5.

Papers:

Manuela Konrad, David C. Nieman, Dru A. Henson, Krista M. Kennerly, Fuxia Jin, Sandra J. Wallner-Liebmann (2011).**The Acute Effect of Ingesting a Quercetin-Based Supplement on Exercise-Induced Inflammation and Immune Changes in Runners.** International. Journal for Sport Nutrition and Exercise Metabolism. *In press.*

David C. Nieman, Manuela Konrad, Dru A. Henson, Krista Kennerly, R. Andrew Shanely, Sandra J. Wallner-Liebmann (2011).**Variance in the acute inflammatory**

response to prolonged cycling is linked to exercise intensity. Journal of Interferon & Cytokine Research. *In press.*

7.2.2 Declaration of my duties

My duties on the first paper were:

development of study design

recruitment of the subjects

email and personal conversation with subjects

baseline testing (including paper work, co-measuring of VO_{2max}, measuring body composition)

communication with subjects

counseling on nutritional intake

testing itself with paperwork

tube labeling for eliquots

coordinate interns for wet lab work

eliquot samples and freeze them in liquid nitrogen

lab work assistance (Quercetin & inflammation markers)

provide data sheets

data entry into excel

calculating food logs

writing the subject ´s reports

literature research for the manuscript

provide tables and graphs

writing the manuscript (under supervision)

My duties on the 2nd paper were:

co-development of study design

recruitment of the subjects

email and personal conversation with subjects

baseline testing (including paper work, co-measuring of VO_{2max}, measuring body composition)

communication with subjects

counseling on nutritional intake

testing itself with paperwork

tube labeling for eliquots

coordinate interns for wet lab work

eliquot samples and freeze them in liquid nitrogen

lab work assistance (inflammation markers)

literature research for the manuscript

co-authoring the manuscript