

Quercetin Reduces Illness but Not Immune Perturbations after Intensive Exercise

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ABSTRACT

NIEMAN, D. C., D. A. HENSON, S. J. GROSS, D. P. JENKINS, J. M. DAVIS, E. A. MURPHY, M. D. CARMICHAEL, C. L. DUMKE, A. C. UTTER, S. R. MCANULTY, L. S. MCANULTY, and E. P. MAYER. Quercetin Reduces Illness but Not Immune Perturbations after Intensive Exercise. *Med. Sci. Sports Exerc.*, Vol. 39, No. 9, pp. 1561–1569, 2007. **Purpose:** To investigate the effects of quercetin supplementation on incidence of upper respiratory tract infections (URTI) and exercise-induced changes in immune function. **Methods:** Trained male cyclists ($N = 40$) were randomized to quercetin ($N = 20$) or placebo ($N = 20$) groups and, under double-blind procedures, received 3 wk quercetin (1000 mg·d⁻¹) or placebo before, during, and for 2 wk after a 3-d period in which subjects cycled for 3 h·d⁻¹ at approximately 57% W_{max} . Blood and saliva samples were collected before and after each of the three exercise sessions and assayed for natural killer cell activity (NKCA), PHA-stimulated lymphocyte proliferation (PHA-LP), polymorphonuclear oxidative-burst activity (POBA), and salivary IgA output (sIgA). **Results:** Pre- to postexercise changes in NKCA, PHA-LP, POBA, and sIgA did not differ significantly between quercetin and placebo groups. URTI incidence during the 2-wk postexercise period differed significantly between groups (quercetin = 1/20 vs placebo = 9/20, Kaplan–Meier analysis statistic = 8.31, $P = 0.004$). **Conclusion:** Quercetin versus placebo ingestion did not alter exercise-induced changes in several measures of immune function, but it significantly reduced URTI incidence in cyclists during the 2-wk period after intensified exercise. **Key Words:** CYCLING, NATURAL KILLER CELLS, T LYMPHOCYTES, NEUTROPHILS, SALIVARY IgA

The physiologic stress induced by prolonged and intensive exertion is reflected in transient yet significant immune system perturbations in multiple body compartments (12,23–26). These exercise-induced immune changes occur at the same time the human body is experiencing oxidative stress, inflammation, muscle microtrauma, and suppressed function against foreign pathogens (3,24).

The influence of several nutritional countermeasures to exercise-induced immune alterations have been investigated including carbohydrate beverages, omega-3 fatty acids, zinc, glutamine, bovine colostrum, and various types of antioxidants such as vitamins C and E (22,25,26,28,31). Of these, carbohydrate ingestion has proven most effective against outcome measures related to inflammation, with

little effect on exercise-induced downturns in T and natural killer (NK) cell function and other aspects of immunity such as salivary IgA output (22).

Polyphenolic compounds are abundant throughout the plant kingdom and are found in a wide variety of human foods. The flavonoids, which are the best defined group of polyphenols in the human diet, comprise a large and complex group, all of which contain a three-ring structure with two aromatic centers and a central oxygenated heterocycle (17,18). The physiologic effects of dietary flavonols such as quercetin are of great current interest for their antioxidative, antiinflammatory, antipathogenic, cardioprotective, and anticarcinogenic activities (4,9,14,19,21).

Flavonol consumption by U.S. adults is approximately 20–35 mg·d⁻¹, with quercetin contributing three fourths of this amount (18,27). Significant food sources include apples, onions, berries, leafy green vegetables, hot peppers, red grapes, and black tea (17,18). Quercetin accumulates in the outer and aerial tissues (skin and leaves) because biosynthesis is stimulated by light (17). Human subjects can absorb significant amounts of quercetin glycosides (the predominant form in plant foods) (10,18,32). Quercetin exists primarily in conjugated forms in human plasma, with only trace amounts as aglycone (17,18). Elimination of quercetin metabolites is quite slow, with reported half-lives of 11–28 h and a primary elimination route as CO₂

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(16,18,32). Long-term feeding of quercetin in rats leads to an accumulation of quercetin in several different organs, particularly the lungs, testes, kidney, heart, liver, thymus, and muscle (8).

Quercetin possesses antioxidant, free radical-scavenging, and antiinflammatory properties that may influence immune system competence and resistance to pathogens, although most data come from *in vitro* and animal experiments (1,5,20). Such experiments suggest that quercetin increases neutrophil chemotaxis and respiratory-burst activity, macrophage phagocytosis, NK cell lytic activity, and mitogen-stimulated lymphocyte proliferation (1,11,20,21). Quercetin regulates the expression of some genes including transcription factor nuclear factor-kappa B (NF6B), and may thus attenuate induction of cytokine transcription for interleukin (IL)-1 β , tumor necrosis factor (TNF)- α , monocyte chemoattractant protein (MCP), and macrophage inflammatory protein (MIP) (3,6,21).

Quercetin in culture with target cells and pathogens exerts antipathogenic activities against a wide variety of viruses and bacteria (7). For example, several culture studies showed that quercetin reduced infectivity of target cells and replication at an early stage for herpes simplex viruses (HSV)-1 and -2, adenoviruses (ADV) -3, -8, and -11, and coronavirus (5,9). Other culture studies show that various quercetin conjugates reduced human immunodeficiency (HIV), rhinovirus, and severe acute respiratory syndrome (SARS) virus replication through various pathways, including reduced lipase activity, binding of viral capsid proteins, and inhibition of DNA gyrase and proteases (4,7,9). Quercetin's antiviral effects may also be mediated through induction of interferon (20). When cultured with human peripheral blood mononuclear cells (PBMC), quercetin induces the gene expression and production of helper T lymphocyte-1 (Th-1)-derived interferon gamma (IFN γ), and it downregulates Th-2 derived IL-4 (20).

No published human studies exist regarding the influence of quercetin ingestion on exercise-induced immune dysfunction. One study showed that 6 wk of quercetin supplementation improved 30-km time-trial performance by 3.1% in 11 male cyclists, and the authors speculate that the performance advantage with quercetin use may have been related to an attenuation of oxidative stress and muscle IL-6 release (15).

Given quercetin's antioxidant, free radical-scavenging, and antiinflammatory properties, and its potential to influence immune function and inhibit infectivity and replication of a broad spectrum of viruses, we hypothesized that quercetin compared with placebo ingestion would attenuate immune dysfunction and counter the risk of upper-respiratory tract infection (URTI) in endurance athletes engaging in repeated and sustained heavy exertion.

METHODS

Subjects. Forty trained male cyclists were recruited as experimental subjects through local and collegiate cycling

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

Research design. Two to three weeks before the first test session, subjects reported to the ASU human performance lab for orientation and measurement of body composition and cardiorespiratory fitness. Body composition was assessed by hydrostatic weighing using an electronic load cell system (Exertech, Dresbach, MN) (29). $\dot{V}O_{2max}$ was determined using a graded maximal protocol (25-W increase every 2 min, starting at 150 W), with the subjects using their own bicycles on CompuTrainer Pro Model 8001 trainers (RaceMate, Seattle, WA). Oxygen uptake and ventilation were measured using the MedGraphics CPX metabolic system (MedGraphics Corporation, St. Paul, MN). Heart rate was measured using a chest heart rate monitor (Polar Electro Inc., Woodbury, NY). Basic demographic and training data were obtained through a questionnaire.

Subjects agreed to avoid the use of large-dose vitamin/mineral supplements (above 100% of recommended dietary allowances), nutritional supplements, ergogenic aids, herbs, and medications known to affect immune function for 3 wk before, during, and 2 wk after the 3-d period of intensified exercise. Subjects were asked to train normally during both the 3-wk period preceding and the 2-wk period after intensified exercise. During orientation, a dietitian instructed the subjects to follow a diet moderate in carbohydrate (using a food list) during the 3 wk before and during the 3-d test sessions. Subjects recorded food intake in a 3-d food record before the first exercise test session. The food records were analyzed using a computerized dietary assessment program (Food Processor, ESHA Research, Salem, OR).

The cyclists were randomized to quercetin ($N = 20$) or placebo ($N = 20$) groups. Under double-blind procedures, subjects received quercetin (1000 mg \cdot d $^{-1}$) or placebo supplements for 3 wk before, during, and 2 wk after the 3-d period of intensified exercise. Tang powder and 500 mg of pure quercetin powder (QU995, Quercegen Pharma, Newton, MA) were mixed with powdered food coloring and placed in plastic vials. Subjects mixed the contents of the vials with 8 ounces of water in green plastic bottles, and they consumed the beverage before their first and last meals of each day to achieve an intake of 1000 mg \cdot d $^{-1}$ of quercetin. Subjects returned empty vials to the study dietitian to verify compliance with the supplementation regimen.

Subjects came to the lab for three consecutive days after the 3-wk quercetin or placebo supplementation period. Subjects cycled for 3 h at approximately 57% W_{max} , or 57% of the maximal watts attained during graded maximal protocol. Subjects reported to the lab at 2:00 p.m. having not ingested energy in any form after 12:30 p.m. Blood samples were collected about 30 min before exercise and about 15 min after exercise. Subjects ingested 0.5–1.0 L of

water per hour during the 3-h cycling bout, with no other beverages or food ingested during the test sessions.

During the test sessions, experimental subjects cycled using their own bicycles on CompuTrainer Pro Model 8001 trainers (RaceMate, Seattle, WA) with the exercise load set at approximately 57% W_{max} . Metabolic measurements were made every 30 min of cycling using the MedGraphics CPX metabolic system to verify workload.

Blood cell counts, hormones. Blood samples were drawn from an antecubital vein with subjects in the supine position. Routine complete blood counts (CBC) were performed by our clinical hematology laboratory, and provided leukocyte subset counts, hemoglobin, and hematocrit. Other blood samples were centrifuged in sodium heparin or EDTA tubes, with plasma aliquoted and then stored at -80°C . Plasma concentrations of cortisol were determined using competitive solid-phase 125I radioimmunoassay (RIA) technique (Diagnostic Products Corporation, Los Angeles, CA) with cortisol-specific Ab-coated tubes (Coat-A-Count tubes). Intraassay (CV intra) and interassay (CV inter) coefficients of variation were 4.5 and 5%, respectively. Assay sensitivity was 5.5 nM ($0.2 \mu\text{g}\cdot\text{dL}^{-1}$). For plasma epinephrine and norepinephrine, blood samples were drawn into chilled tubes containing EGTA and glutathione, centrifuged, and the plasma was stored at -80°C until analysis. Plasma concentrations of epinephrine and norepinephrine were determined by competitive enzyme immunoassay (EIA) (Labor Diagnostika Nord, Nordhorn, Germany) using the microtiter plate format. CV intra and CV inter for epinephrine and norepinephrine were 11 and 14%, and 16 and 15%, respectively. Sensitivity of the epinephrine and norepinephrine assays for plasma samples are 11 and 44 $\text{pg}\cdot\text{mL}^{-1}$, respectively.

Plasma quercetin. Total plasma quercetin (quercetin and its primary conjugates) was measured after solid-phase extraction via reverse-phase HPLC, with UV detection as described by Quercegen Pharma (Newton, MA, personal communication, April 2006). This procedure is similar to that previously published by Ishii et al. (13). Quercetin conjugates were hydrolyzed by incubating 250- to 500- μL plasma aliquots with 10 μL of 10% DL-dithiothreitol (DTT) solution, 50 μL of 0.58 M acetic acid, 50 μL of a mixture of β -glucuronidase and arylsulfatase, and crude extract from *Helix pomatia* (Roche Diagnostics GmbH, Mannheim, Germany) for 40 min at 37°C . After incubation, 500 μL of 0.01 M oxalic acid was added, and then each sample was vortexed and centrifuged for 5 min at 10000 rpm. One milliliter of supernatants was then applied to solid-phase extraction cartridges (Oasis HLB, 1 mL, 30 mg, SPE cartridge; Waters Corporation, Milford, MA) that were preconditioned with 1 mL of methanol (MeOH), 0.5 mL of 0.01 M oxalic acid, and 1 mL of dH_2O , and drawn through at a rate of $0.5 \text{ mL}\cdot\text{min}^{-1}$ using a vacuum manifold (Waters Corp., Milford, MA). Cartridges were then washed with 0.5 mL of $\text{MeOH} \times 2$. Eluant was collected into 1.5-mL

microcentrifuge tubes. Ten microliters of 10% DTT solution was added to the combined eluant, and the samples were then vortexed for 1 min and placed into a vacuum concentrator (Savant Speed Vac SC 110 Savant Instruments Inc., Farmingdale, NY) until MeOH was completely evaporated. The residue was reconstituted with 150 μL of $\text{MeOH}/\text{dH}_2\text{O}$ (1/1). Fifty-microliter injections were used for HPLC analysis.

Chromatographic analysis was performed using a Waters Breeze system (Waters Corporation, Milford, MA) consisting of a Waters 1525 binary HPLC pump, 2487 UV detector, and symmetry C18, 5- μm , $4.6 \times 150\text{-mm}$ column. The analysis data were acquired and processed using Breeze software (v. 3.02). A mixture of acetonitrile with 0.1% formic acid (A) and dH_2O with 0.1% formic acid (B) was used as the mobile phase. The gradient elution was programmed as follows: 0–1.5 min, 10/90% A/B; 1.5–9.5 min, 40/60% A/B; 9.5–11.5 min, 90/10% A/B; 11.5–15 min, 10/90% A/B. The column temperature was maintained at 30°C . The flow rate was $1.0 \text{ mL}\cdot\text{min}^{-1}$. Quantitation of the quercetin peak was based on the standard addition method, using both plasma and MeOH, with similar results. Both standards and samples were treated in an identical manner.

Lymphocyte subsets. The proportions of T cells (CD3+) and NK cells (CD3–CD56+) were determined in whole-blood preparations, and absolute numbers were calculated using the CBC data to allow group comparison on counts of circulating cells. For lymphocyte preservation, 1 mL of whole blood collected in EDTA was added to 1 mL of Cyto-Chex reagent (Streck Laboratories, Omaha, NE). Cyto-Chex reagent is a solution that is formulated to preserve the white blood cells in blood samples without reducing the activity of the antigenic sites. Samples treated with Cyto-Chex can be maintained for 7 d before processing and analysis by flow cytometric methods. Lymphocyte phenotyping was accomplished by one- or two-color fluorescent labeling of cell-surface antigens with mouse antihuman monoclonal antibodies (CD3/CD56, CD19) and isotype control, conjugated to fluorescein isothiocyanate (FITC) or phycoerythrin derivative (RD-1) (Beckman Coulter, Fullerton, CA). Briefly, 100 μL of EDTA-anticoagulated whole blood was incubated with 10 μL of monoclonal antibodies for 10 min at room temperature in $12 \times 75\text{-mm}$ polypropylene tubes. After incubation, the sample was processed on a Q prep Instrument (Beckman Coulter, Fullerton, CA) using ImmunoPrep Reagent (Beckman Coulter, Fullerton, CA). This allows for erythrocyte lysis, as well as stabilizing and fixing of the lymphocytes in one step without the trauma of mixing, vortexing, and centrifuging of samples. Samples were analyzed on a Beckman Coulter Epics XL-MCL flow cytometer using forward and side scatter; a total of 5000 events were counted in each reaction tube.

NK cell activity. NK cell activity (NKCA) was assessed using a modification of a flow cytometry assay (2).

Peripheral blood mononuclear cells (effector cells, 3.75×10^6 cells per milliliter) were isolated from heparinized blood by density gradient centrifugation with Ficoll-Hypaque. K562 target cells (1×10^6 cells per milliliter) were labeled for 20 min with DiO reagent (0.01 mL of 3 mM DiOC18 (3), 3,3'-diocetadecyloxycarbocyanine perchlorate (DiO) (Sigma Chemical Company, St. Louis, MO) in DMSO per milliliter of cell suspension) washed and resuspended to a concentration of 5×10^5 cells per milliliter. Effector cells and target cells were added to individual tubes (0.9-mL final volume) to yield E:T ratios of 60:1, 30:1, 15:1, 7.5:1, 3.8:1, and 1.9:1. All tubes received 0.1 mL of a $500\text{-}\mu\text{g}\cdot\text{mL}^{-1}$ solution of propidium iodide (PI) (Sigma Chemical Company, St. Louis, MO). The tubes were vortexed, and the cells were pelleted by centrifugation for 15 s, then incubated for 2 h at 37°C in a 5% CO_2 incubator. At the end of the incubation, the tubes were vortexed and placed on ice, and the cells were analyzed on a flow cytometer within 30 min. DiO-labeled target-cell membranes emit a green fluorescence (FL1), and PI-labeled compromised cells emit a red fluorescence (FL3). The percentage of target (green) cells that were also compromised (red) was determined. Control tubes received target cells and PI alone, and effector cells and PI alone. The results were acceptable if the percentage of compromised cells in these controls was $< 5\%$. Plots of the percentage of compromised target cells at the various E:T ratios were constructed, and linear regression analysis was performed. The results are expressed as percent lysis of DiO-stained K562 cells at a 20:1 E:T ratio.

PHA-stimulated lymphocyte proliferation. The mitogenic response of lymphocytes was determined in whole-blood culture using phytohemagglutinin (PHA) at optimal and suboptimal doses, which were previously determined by titration experiments. Heparinized venous blood was diluted 1:10 with complete media consisting of RPMI-1640 supplemented with 5% heat-inactivated fetal bovine serum, penicillin, streptomycin, sodium pyruvate, L-glutamine, β -2-mercaptoethanol, and Mito+ serum extender (Becton Dickinson Immunocytometry Systems, San Jose, CA). PHA was prepared in RPMI-1640 media at a concentration of $1\text{ mg}\cdot\text{mL}^{-1}$ and then further diluted with complete media to the optimal and suboptimal working concentrations (25 and $12.5\text{ }\mu\text{g}\cdot\text{mL}^{-1}$, respectively). A $100\text{-}\mu\text{L}$ aliquot of the diluted blood was dispensed into each of triplicate wells of a 96-well flat-bottom microtiter plate. To each well, $100\text{ }\mu\text{L}$ of the appropriate mitogen dose was added. Control wells received complete media instead of mitogen. After a 72-h incubation at 37°C and 5% CO_2 , the cells were pulsed with $1\text{ }\mu\text{Ci}$ of thymidine (methyl)-3H (New England Nuclear, Boston, MA) prepared with RPMI-1640. After pulsing, cells were incubated for an additional 4 h before harvesting. The radionucleotide incorporation was assessed by a Wallac 1209 RackBeta Liquid Scintillation Counter (Gaithersburg, MD), with the results expressed as experimental minus control counts per minute (cpm).

Whole-blood polymorphonuclear oxidative-burst activity assay. For each sample, $100\text{ }\mu\text{L}$ of whole blood was dispensed into two $12 \times 75\text{-mm}$ tubes labeled 1 and 2. Twenty five microliters of working dihydrorhodamine 123 (DHF-123) solution (Sigma Chemical Company, St. Louis, MO) was added to each of tubes 1 (endogenous respiratory-burst activity) and 2 (stimulated respiratory-burst activity). The DHF-123 was prepared from a stock solution of $5\text{ mg}\cdot\text{mL}^{-1}$ as follows: $10\text{ }\mu\text{L}$ of the stock solution was added to $990\text{ }\mu\text{L}$ of a 1:1 ratio of phosphate buffered saline and glucose. The final concentration of DHF-123 was $1\text{ }\mu\text{g}\cdot\text{mL}^{-1}$. The tubes were incubated at 37°C for 15 min. After the incubation period, $10\text{ }\mu\text{L}$ of the working phorbol 12-myristate 13-acetate (PMA) solution (Sigma Chemical Company, St. Louis, MO) was added to tube 2. The PMA was prepared from a stock solution of $1\text{ mg}\cdot\text{mL}^{-1}$, and $10\text{ }\mu\text{L}$ of the stock solution was added to $990\text{ }\mu\text{L}$ of a 1:1 ratio of phosphate-buffered saline and glucose. The final concentration of PMA was $100\text{ ng}\cdot\text{mL}^{-1}$. Tubes were incubated again for 15 min at 37°C . After the incubation period, the tubes were placed on ice. Samples were processed on a Q prep instrument (Beckman Coulter, Fullerton, CA) using ImmunoPrep Reagent (Beckman Coulter, Fullerton, CA). This allowed for erythrocyte lysis, as well as stabilizing and fixing of the blood cells in one step without the trauma of mixing, vortexing, and centrifuging of samples. Samples were analyzed on a Beckman Coulter Epics XL-MCL flow cytometer, using forward scatter and side scatter, and were gated on granulocytes. A total of 5000 cells were counted in each reaction tube. The mean channel number for the fluorescence peaks was determined, and the polymorphonuclear (PMN) oxidative-burst activity was calculated by subtracting the mean channel number of the endogenous respiratory-burst activity from the mean channel number of the stimulated respiratory-burst activity.

Plasma myeloperoxidase. An enzyme-linked immunoassay was used to measure plasma myeloperoxidase (MPO) protein levels in accordance with instructions from the manufacturer (Bioxytech, Oxis International, Inc., Portland, OR). CV intra and CV inter variations for MPO were 3.45 and 7.76%, respectively.

Salivary IgA. Unstimulated saliva was collected for four minutes by expectoration into 15-mL plastic, sterilized vials. Participants were urged to pass as much saliva as possible into the vials during the 4-min timed session. The saliva

TABLE 1. Subject characteristics (mean \pm SE).

	Cyclers (<i>N</i> = 20) Quercetin	Cyclers (<i>N</i> = 20) Placebo	<i>P</i> Value
Age (yr)	26.1 \pm 1.8	29.1 \pm 2.4	0.321
Body mass (kg)	74.7 \pm 0.2	74.2 \pm 1.4	0.846
Body fat (%)	13.8 \pm 1.2	11.5 \pm 0.6	0.094
Training ($\text{km}\cdot\text{wk}^{-1}$)	242 \pm 27	270 \pm 29	0.493
$\dot{V}\text{O}_{2\text{max}}$ ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	53.2 \pm 1.2	54.7 \pm 1.1	0.365
Maximal power (W)	314 \pm 9	320 \pm 69	0.554
HR _{max} (bpm)	188 \pm 1	190 \pm 2	0.328

$\dot{V}\text{O}_2$, volume of oxygen consumption; HR, heart rate.

TABLE 2. Performance data—average of three 3-h cycling bouts (mean ± SE).

	Quercetin (N = 20)	Placebo (N = 20)	P Value
Power (W)	178 ± 5	181 ± 4	0.626
Power (% W_{max})	56.8 ± 0.2	56.5 ± 0.3	0.486
Heart rate (bpm)	149 ± 2	149 ± 3	0.897
Heart rate (% HR_{max})	79.4 ± 0.8	78.1 ± 1.0	0.300
$\dot{V}O_{2max}$ (mL·kg ⁻¹ ·min ⁻¹)	36.0 ± 0.9	36.9 ± 0.8	0.458
$\dot{V}O_2$ (% $\dot{V}O_{2max}$)	67.8 ± 0.8	67.6 ± 1.1	0.919
Cadence (rpm)	80.8 ± 2.2	83.7 ± 2.2	0.349

samples were centrifuged for 5 min at 200g, then frozen in dry ice after collection and stored at -80°C until analysis. Saliva volume was measured to the nearest 0.1 mL, and saliva total protein was quantified using the Coomassie protein assay reagent, a modification of the Bradford Coomassie dye binding colorimetric method. Salivary IgA (sIgA) was measured by enzyme linked immunosorbent assay according to the procedures of the Hunter Immunology Unit (Royal Newcastle Hospital, Newcastle, Australia) (24). The data were expressed as sIgA secretion rate ($\mu\text{g}\cdot\text{min}^{-1}$). CV intra and CV inter variation for sIgA concentration were 4.92 and 9.26%, respectively.

Illness logs. Subjects filled in a health log during the 5-wk supplementation period, listing codes for symptoms of upper-respiratory tract infection (URTI). The following health codes were recorded daily, in accordance with previous investigations by our research team (24): 1) no health problems today; 2) sick with cold symptoms (runny, stuffy nose, sore throat, coughing, sneezing, colored discharge); 3) sick with flu symptoms (fever, headache, general aches and pains, fatigue and weakness, chest discomfort, cough); 4) sick, with nausea, vomiting, and/or diarrhea; 5) muscle, joint, or bone problems/injury; 6) allergy symptoms; 7) other health problems (describe). An URTI episode was recorded if cold or flu symptoms persisted for 2 d or longer.

Statistical analysis. Data are expressed as mean ± SE. Data in Tables 1 and 2 were compared between groups using Student's *t*-tests. Data in Tables 3 and 4, and Figures

1 through 5, were analyzed using a 2 (groups) × 6 (time points) repeated-measures ANOVA. For certain variables of interest, an overall *day* effect was calculated that compared changes across the 3 d of measurement. When Box's *M* suggested that the assumptions necessary for the univariate approach were not tenable, the multivariate approach to repeated-measures ANOVA was used (Pillai's trace). When interaction effects were significant ($P \leq 0.05$), pre- to postexercise changes were calculated and compared between quercetin and placebo groups using Student's *t*-tests, with significance set at a $P \leq 0.05$. Upper-respiratory tract infection incidence rates (Fig. 6) were compared between groups using a Kaplan-Meier analysis.

RESULTS

Subject characteristics for the 40 cyclists randomized to quercetin and placebo groups are summarized in Table 1. No significant differences were found between groups for age, body composition, training history, or maximal performance measures. Subjects in the quercetin and placebo groups came into the study averaging approximately 1.5 ± 0.2 and 1.6 ± 0.2 h of cycling per training bout. Thus, the 3-d intensified exercise period (9 h of exercise) represented nearly a doubling of their normal exercise workload. Three-day food records before the 3-d exercise period revealed no significant group differences in energy or macronutrient intake (data not shown), and for all subjects combined, energy intake was 2684 ± 117 kcal·d⁻¹, with carbohydrate representing 56.6 ± 2.5%, protein 16.2 ± 0.8%, and fat 27.2 ± 1.8%. A poststudy questionnaire revealed that nine subjects in each group guessed they were on quercetin, four in each group guessed placebo, and seven in each group were uncertain (thus, there were no significant group differences).

Performance data in Table 2 reveal that subjects in quercetin and placebo groups were able to maintain a mean power output of approximately 57% W_{max} at an oxygen

TABLE 3. Pre- to postexercise (3-h cycling bouts at approximately 57% W_{max}) changes in blood leukocyte subset counts in quercetin compared with placebo groups during a 3-d period (mean ± SE).

	Quercetin (N = 20)		Placebo (N = 20)		P Values: Time; 2 × 6 Interaction
	Pre	Post	Pre	Post	
Granulocytes (10 ⁹ L ⁻¹)					
Day 1	3.40 ± 0.22	12.2 ± 0.8	3.17 ± 0.21	11.4 ± 1.0	< 0.001; 0.870
Day 2	3.80 ± 0.27	9.82 ± 0.59	3.18 ± 0.16	8.99 ± 0.57	
Day 3	3.19 ± 0.22	8.22 ± 0.53	2.79 ± 0.17	7.02 ± 0.40	
Total lymphocytes (10 ⁹ L ⁻¹)					
Day 1	1.89 ± 0.15	2.52 ± 0.23	1.80 ± 0.11	2.16 ± 0.15	< 0.001; 0.561
Day 2	1.90 ± 0.13	2.59 ± 0.22	1.79 ± 0.13	2.36 ± 0.21	
Day 3	1.89 ± 0.13	2.38 ± 0.16	1.72 ± 0.10	2.09 ± 0.13	
T cells (10 ⁹ L ⁻¹)					
Day 1	1.08 ± 0.09	1.52 ± 0.13	1.14 ± 0.07	1.38 ± 0.09	< 0.001; 0.365
Day 2	1.20 ± 0.10	1.62 ± 0.15	1.20 ± 0.09	1.54 ± 0.12	
Day 3	1.19 ± 0.10	1.51 ± 0.11	1.12 ± 0.07	1.37 ± 0.09	
Natural killer cells (10 ⁹ L ⁻¹)					
Day 1	0.38 ± 0.09	0.37 ± 0.07	0.29 ± 0.04	0.27 ± 0.04	< 0.001; 0.794
Day 2	0.22 ± 0.04	0.24 ± 0.04	0.18 ± 0.02	0.21 ± 0.03	
Day 3	0.28 ± 0.05	0.24 ± 0.04	0.21 ± 0.02	0.17 ± 0.02	

TABLE 4. Pre- to postexercise (3-h cycling bouts at approximately 57% $\dot{V}O_{2\max}$) changes in plasma cortisol, epinephrine, and norepinephrine in quercetin compared with placebo groups during a 3-d period (mean \pm SE).

	Quercetin (N = 20)		Placebo (N = 20)		P Values: Time; 2 \times 6 Interaction
	Pre	Post	Pre	Post	
Cortisol (nM)					
Day 1	139 \pm 10	230 \pm 19	113 \pm 8	253 \pm 22	< 0.001; 0.151
Day 2	100 \pm 8	217 \pm 17	82 \pm 5	185 \pm 19	
Day 3	96 \pm 7	190 \pm 17	91 \pm 6	158 \pm 16	
Epinephrine (nM)					
Day 1	0.41 \pm 0.05	0.91 \pm 0.12	0.37 \pm 0.07	0.84 \pm 0.16	< 0.001; 0.899
Day 2	0.25 \pm 0.04	0.50 \pm 0.07	0.19 \pm 0.03	0.54 \pm 0.11	
Day 3	0.28 \pm 0.04	0.55 \pm 0.06	0.19 \pm 0.05	0.44 \pm 0.07	
Norepinephrine (nM)					
Day 1	2.70 \pm 0.22	6.69 \pm 0.58	2.70 \pm 0.25	5.85 \pm 0.39	< 0.001; 0.110
Day 2	2.83 \pm 0.23	5.99 \pm 0.40	3.02 \pm 0.25	5.90 \pm 0.65	
Day 3	2.99 \pm 0.21	5.85 \pm 0.46	3.28 \pm 0.30	4.79 \pm 0.48	

consumption of approximately 68% $\dot{V}O_{2\max}$ and a cadence slightly above 80 rpm. Plasma volume change using hemoglobin and hematocrit data did not differ between groups during the three exercise bouts and averaged less than 1% because of ingestion of 0.5–1.0 L water per hour of exercise (data not shown). After 3 wk of supplementation, plasma quercetin levels for the quercetin and placebo groups were 1158 \pm 218 and 113 \pm 30 $\mu\text{g}\cdot\text{L}^{-1}$, respectively ($P < 0.001$).

Group comparisons of all preexercise data on day 1 (i.e., resting-state values after 3 wk of supplementation), shown in Tables 3 and 4 and Figures 1–5, show no significant differences. Significant main time effects were measured for blood leukocyte subset counts, and plasma cortisol, epinephrine, and norepinephrine, with no quercetin compared with placebo group differences, as outlined in Tables 3 and 4. The magnitude of postexercise increases for blood granulocyte counts, total lymphocyte counts, NK cell counts, plasma cortisol, plasma epinephrine, and plasma norepinephrine diminished across the 3-d period (day effect for all variables, $P < 0.05$). NK cell lytic activity decreased from pre- to postexercise during each of the 3-h cycling bouts (Fig. 1) (and across the 3-d period, $P < 0.001$), with no group differences [time effect, $P < 0.001$; 2 (group) \times 6 (time) interaction effect, $P = 0.632$]. PHA-stimulated lymphocyte proliferation increased (in parallel with the

increase in blood T cell counts) after exercise during each cycling bout (Fig. 2), with no group differences (time effect, $P < 0.001$; interaction effect, $P = 0.804$). Polymorphonuclear respiratory-burst activity decreased after each cycling bout (and across the 3-d period, $P < 0.001$) (Fig. 3), with no group differences (time effect, $P < 0.001$; interaction effect, $P = 0.938$). Plasma myeloperoxidase increased after each cycling bout (Fig. 4), with no group differences (time effect, $P < 0.001$; interaction effect, $P = 0.328$). Salivary IgA output tended to decrease over time ($P = 0.044$), but not after exercise (Fig. 5), with no group differences (interaction effect, $P = 0.920$).

Figure 6 summarizes the upper-respiratory tract infection data during the entire supplementation period (5 wk and 3 d). Infection rates did not differ during the initial 21-d period, but they were significantly different during the 14-d period after the 3 d of intensified exercise (quercetin group incidence = 1/20; placebo group incidence = 9/20; Kaplan–Meier analysis, log-rank test statistic = 8.31, $P = 0.004$).

DISCUSSION

Cyclists randomized to 1000 $\text{mg}\cdot\text{d}^{-1}$ of pure quercetin compared with placebo under double-blinded methods had higher plasma quercetin levels after 3 wk of supplementation (9.2-fold difference) and experienced a markedly lower incidence of URTI symptoms during the 2-wk period after

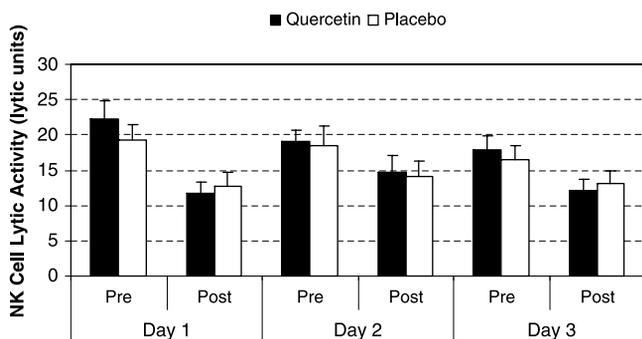


FIGURE 1—Natural killer (NK) cell lytic activity before and after 3 h of cycling at 57% $\dot{V}O_{2\max}$ in quercetin ($N = 20$) and placebo ($N = 20$) groups during three consecutive days. Main time effect, $P < 0.001$; group \times time interaction effect, $P = 0.632$.

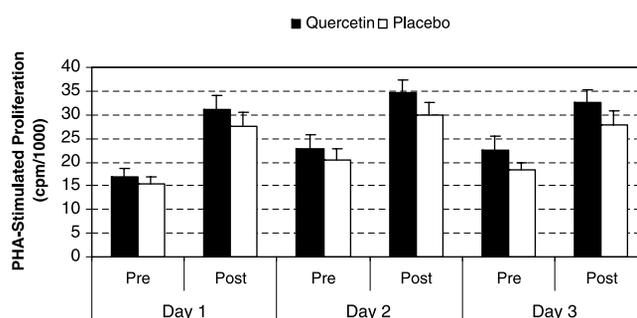


FIGURE 2—PHA-stimulated lymphocyte proliferation before and after 3 h of cycling at 57% $\dot{V}O_{2\max}$ in quercetin ($N = 20$) and placebo ($N = 20$) groups during three consecutive days. Main time effect, $P < 0.001$; group \times time interaction effect, $P = 0.804$.

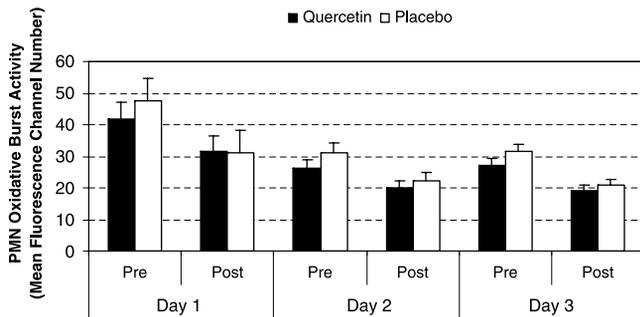


FIGURE 3—Polymorphonuclear respiratory-burst activity before and after 3 h of cycling at 57% W_{max} in quercetin ($N = 20$) and placebo ($N = 20$) groups during three consecutive days. Main time effect, $P < 0.001$; group \times time interaction effect, $P = 0.938$.

intensified exercise. Group differences in illness rates occurred despite comparable measures of immune function taken before and after exercise during a 3-d period of intensified exercise after 3 wk of supplementation. We collected data during February and March of 2006, a period when illness rates are typically high across university campuses. This time period, combined with the unusual physiologic stress the subjects experienced (9 h of intense exercise during a 3-d period, combined with nearly 1 L of blood sampling), help explain the high illness rate in the placebo group.

Thus, quercetin may have reduced URTI rates using direct antipathogenic pathways. A growing literature base supports the antipathogenic capacities of quercetin when cultured with target cells and a broad spectrum of pathogens including URTI-related rhinoviruses, adenoviruses, and coronaviruses (4,5,7,9). These studies indicate that quercetin blocks viral replication at an early stage of multiplication using several mechanisms, including inhibition of proteases by molecular docking, binding of viral capsid proteins, and suppression of virulence enzymes such as DNA gyrase and cellular lipase (4,5,7). We are unaware of any other human clinical trials demonstrating the efficacy of quercetin compared with placebo in reducing URTI incidence, and this is the first investigation to show that quercetin supplements lower illness rates in athletes during periods of

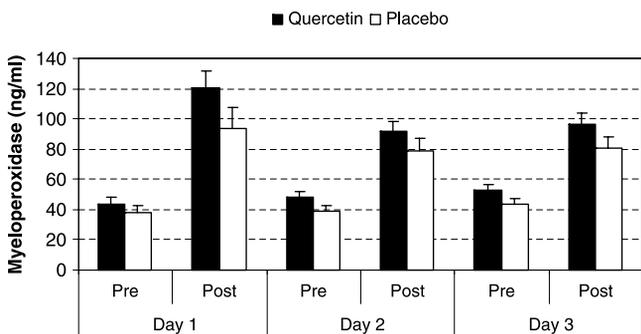


FIGURE 4—Plasma myeloperoxidase concentrations before and after 3 h of cycling at 57% W_{max} in quercetin ($N = 20$) and placebo ($N = 20$) groups during three consecutive days. Main time effect, $P < 0.001$; group \times time interaction effect, $P = 0.328$.

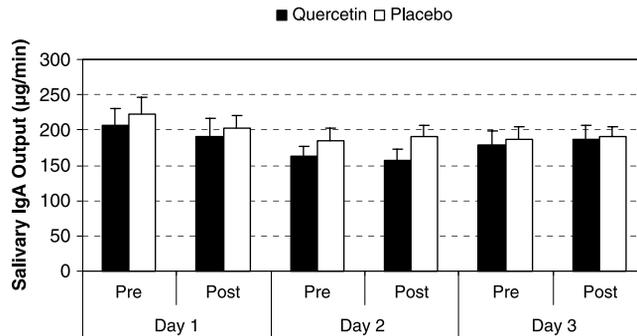


FIGURE 5—Salivary IgA output before and after 3 h of cycling at 57% W_{max} in quercetin ($N = 20$) and placebo ($N = 20$) groups during three consecutive days. Main time effect, $P < 0.001$; group \times time interaction effect, $P = 0.920$.

intensified training and elevated URTI risk. Thus, further research is warranted to explore quercetin's antipathogenic capacities under varying human conditions.

Acute changes in immune measures experienced by the cyclists included previously reported increases in neutrophil and T cell counts, MPO, and mitogen-stimulated lymphocyte proliferation, and decreases in NK cell lytic activity and PMN respiratory-burst activity (12,22–26,28,31). The magnitude of postexercise increases for blood granulocyte, total lymphocyte, and NK cell counts, plasma stress hormone levels, NK cell lytic activity, and PMN respiratory-burst activity diminished significantly across the 3-d period. This occurred despite maintenance of the same exercise workload and intensity for each 3-h bout of cycling. Suzuki et al. (30) also have noted some attenuation of exercise-induced neutrophilia and neutrophil oxidative activity in subjects cycling at 90 W for 90 min, 3 d in a row. Our data suggest that trained cyclists adapt quickly to the physiologic stress of repeated bouts of intense and prolonged exercise, and

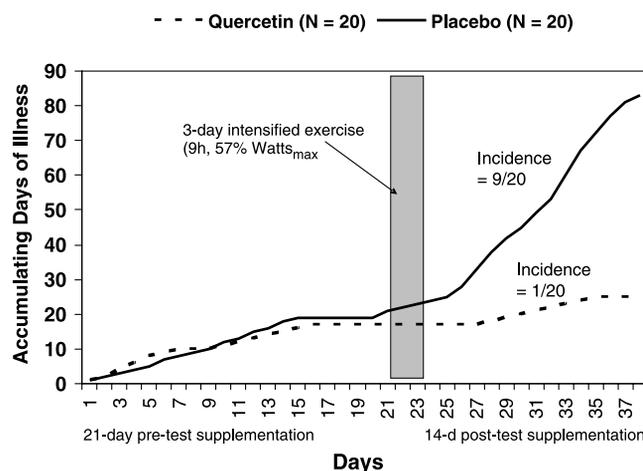


FIGURE 6—Upper-respiratory tract infection rates did not differ during the initial 21-d period between groups, but they were significantly different during the 14-d period after the 3 d of intensified exercise (quercetin group incidence = 1/20; placebo group incidence = 9/20; Kaplan–Meier analysis, log-rank test statistic = 8.31, $P = 0.004$).

by the second and third bout they experience lower levels of stress hormones and diminished immune perturbations.

We and others have previously shown that carbohydrate compared with placebo beverage ingestion during prolonged and intensive exertion attenuates increases in neutrophil counts, but it has little or no effect on the other immune measures included in this study (for review, see Nieman and Bishop (22)). Other nutritional interventions with athletes have had inconsistent or null effects (22,25,26,28,31).

Published reports of quercetin's effects on immune function, oxidative stress, and inflammation led us to hypothesize that this flavonol would attenuate exercise-induced immune dysfunction (6,11,14,20,21), but our data do not support this hypothesis, at least within the context of this study. Prior studies, however, have used *in vitro* and animal models to test quercetin's influence on immune function, some with unusually high levels of quercetin. For example, rats fed 100 mg·kg⁻¹ of quercetin daily for 7 wk had significantly enhanced NK cell activity compared with controls (11). Human peripheral blood mononuclear cells cultured with quercetin at concentrations ranging between 1 and 50 μM for 24–96 h significantly inhibited TNF-α production and gene expression in T cells and monocytes

(21). Consumption of polyphenol-rich cereals by prematurely aging mice significantly enhanced macrophage phagocytosis, microbicidal activity, NKCA, and lymphocyte proliferation compared with controls, with most of these immune benefits linked by the investigators to quercetin (1). Our data indicate that quercetin did not alter resting immunity or act as a countermeasure to exercise-induced immune changes, underscoring the difficulty and, hence, caution that should be used in applying *in vitro* and animal data to human investigations.

In summary, 3 wk of 1000 mg·d⁻¹ of pure quercetin compared with placebo ingestion in cyclists led to marked differences in rates of illness (5 vs 45%, respectively) during the 2-wk period after intensified exercise. URTI incidence differed between groups despite no measurable influence of quercetin on multiple measures of immune function taken before and after 3 h of exercise. These data support *in vitro* observations that quercetin supplements may reduce illness rates after periods of heavy training by athletes through direct antipathogenic mechanisms.

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REFERENCES

- ÁLVAREZ, P., C. ALVARADO, M. PUERTO, A. SCHLUMBERGER, L. JIMÉNEZ, and M. DE LA FUENTE. Improvement of leukocyte functions in prematurely aging mice after five weeks of diet supplementation with polyphenol-rich cereals. *Nutrition* 22: 913–921, 2006.
- CHANG, L., G. A. GUSEWITCH, D. B. W. CHRITTON, J. C. FOLZ, L. K. LEBECK, and S. L. NEHLSSEN-CANNARELLA. Rapid flow cytometric assay for the assessment of natural killer activity. *J. Immunol. Methods* 166:45–54, 1993.
- CHEN, J.-C., P.-D. L. CHAO, C.-P. CHEN, et al. Inhibition of iNOS gene expression by quercetin is mediated by the inhibition of IκB kinase, nuclear factor-kappa B and STAT1, and depends on heme oxygenase-1 induction in mouse BV-2 microglia. *Eur. J. Pharmacol.* 521:9–20, 2005.
- CHEN, L., J. LI, C. LUO, et al. Binding interaction of quercetin-3-β-galactoside and its synthetic derivatives with SARS-CoV 3CLpro: structure-activity relationship studies reveal salient pharmacophore features. *Bioorg. Med. Chem.* 14:8295–8306, 2006.
- CHIANG, L. C., W. CHIANG, M. C. LIU, and C. C. LIN. In vitro antiviral activities of *Caesalpinia pulcherrima* and its related flavonoids. *J. Antimicrob. Chemother.* 52:194–198, 2003.
- COMALADA, M., I. BALLESTER, E. BAILÓN, et al. Inhibition of pro-inflammatory markers in primary bone marrow-derived mouse macrophages by naturally occurring flavonoids: analysis of the structure-activity relationship. *Biochem. Pharmacol.* 72:1010–1021, 2006.
- CUSHNIE, T. P., and A. J. LAMB. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* 26:343–356, 2005.
- DE BOER, V. C. J., A. A. DIHAL, H. VAN DER WOUDE, et al. Tissue distribution of quercetin in rats and pigs. *J. Nutr.* 135:1718–1725, 2005.
- DIMOVA, S., R. MUGABOWINDEKWE, T. WILLEMS, et al. Safety-assessment of 3-methoxyquercetin as an antirhinoviral compound for nasal application: effect on ciliary beat frequency. *Int. J. Pharm.* 263:95–103, 2003.
- ERLUND, I., R. FREESE, J. MARNIEMI, P. HAKALA, and G. ALFTHAN. Bioavailability of quercetin from berries and the diet. *Nutr. Cancer* 54:13–17, 2006.
- EXON, J. H., B. A. MAGNUSON, E. H. SOUTH, and K. HENDRIX. Dietary quercetin, immune functions and colonic carcinogenesis in rats. *Immunopharmacol. Immunotoxicol.* 20:173–190, 1998.
- GLEESON, M. Immune system adaptation in elite athletes. *Curr. Opin. Clin. Nutr. Metab. Care* 9:659–665, 2006.
- ISHII, K., T. FURUTA, and Y. KASUYA. High-performance liquid chromatographic determination of quercetin in human plasma and urine utilizing solid-phase extraction and ultraviolet detection. *J. Chromatography* 794:49–56, 2003.
- KEMPURAJ, D., M. L. CASTELLANI, C. PETRARCA, et al. Inhibitory effect of quercetin on tryptase and interleukin-6 release, and histidine decarboxylase mRNA transcription by human mast cell-1 cell line. *Clin. Exp. Med.* 6:150–156, 2006.
- MACRAE, H. S. H., and K. M. MEFFERD. Dietary antioxidant supplementation combined with quercetin improves cycling time trial performance. *Int. J. Sport Nutr. Exerc. Metab.* 16:405–419, 2006.
- MANACH, C., C. MORAND, V. CRESPI, et al. Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. *FEBS Lett.* 426:331–336, 1998.
- MANACH, C., A. SCALBERT, C. MORAND, C. RÉMÉSY, and L. JIMÉNEZ. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* 79:727–747, 2004.
- MANACH, C., G. WILLIAMSON, C. MORAND, A. SCALBERT, and C. RÉMÉSY. Bioavailability and bioefficiency of polyphenols in humans. 1. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* 81(Suppl):230S–242S, 2005.
- MINK, P. J., C. G. SCRAFFORD, L. M. BARRAJ, et al. Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. *Am. J. Clin. Nutr.* 85:895–909, 2007.
- NAIR, M. P. N., C. KANDASWAMI, S. MAHAJAN, et al. The flavonoid, quercetin, differentially regulates Th-1 (IFN-γ) and Th-2 (IL4)

- cytokine gene expression by normal peripheral blood mononuclear cells. *Biochim. Biophys. Acta* 1593:29–36, 2002.
21. NAIR, M. P., S. MAHAJAN, J. L. REYNOLDS, et al. The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells via modulation of the NF- κ B system. *Clin. Vaccine Immunol.* 13:319–328, 2006.
 22. NIEMAN, D. C., and N. C. BISHOP. Nutritional strategies to counter stress to the immune system in athletes, with special reference to football. *J. Sports Sci.* 24:763–772, 2006.
 23. NIEMAN, D. C., C. L. DUMKE, D. A. HENSON, S. R. MCANULTY, S. J. GROSS, and R. H. LIND. Muscle damage is linked to cytokine changes following a 160-km race. *Brain Behav. Immun.* 19:398–403, 2005.
 24. NIEMAN, D. C., D. A. HENSON, C. L. DUMKE, R. H. LIND, L. R. SHOOTER, and S. J. GROSS. Relationship between salivary IgA secretion and upper respiratory tract infection following a 160-km race. *J. Sports. Med. Phys. Fitness* 46:158–162, 2006.
 25. NIEMAN, D. C., D. A. HENSON, S. R. MCANULTY, et al. Vitamin E and immunity after the Kona Triathlon World Championship. *Med. Sci. Sports Exerc.* 36:1328–1335, 2004.
 26. NIEMAN, D. C., D. A. HENSON, S. R. MCANULTY, et al. Influence of vitamin C supplementation on oxidative and immune changes following an ultramarathon. *J. Appl. Physiol.* 92:1970–1977, 2002.
 27. SAMPSON, L., E. RIMM, P. C. HOLLMAN, J. H. DE VRIES, and M. B. KATAN. Flavonol and flavone intakes in US health professionals. *J. Am. Diet. Assoc.* 102:1414–1420, 2002.
 28. SHING, C. M., J. PEAKE, K. SUZUKI, et al. Effects of bovine colostrum supplementation on immune variables in highly trained cyclists. *J. Appl. Physiol.* 102:1113–1122, 2007.
 29. SIRI, W. E. Body composition from fluid spaces and density: analysis of methods. In: *Techniques for Measuring Body Composition*, J. Brozek and A. Henschel (Eds.). Washington, DC: National Academy of Science, pp. 223–244, 1961.
 30. SUZUKI, K., M. TOTSUKA, S. NAKAJI, et al. Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage. *J. Appl. Physiol.* 87:1360–1367, 1999.
 31. TOFT, A. D., M. THORN, K. OSTROWSKI, et al. N-3 polyunsaturated fatty acids do not affect cytokine response to strenuous exercise. *J. Appl. Physiol.* 89:2401–2406, 2000.
 32. WALLE, T., U. K. WALLE, and P. V. HALUSHKA. Carbon dioxide is the major metabolite of quercetin in humans. *J. Nutr.* 131:2648–2652, 2001.