Influence of Quercetin Supplementation on Disease Risk Factors in Community-Dwelling Adults

Authors
AMY M. KNAB; **ANDREW SHANELY**; DRU A. HENSON ; FUXIA JIN ; SERENA A. HEINZ; MELANIE D. AUSTIN ; DAVID C. NIEMAN

Abstract

Background: In vitro data indicate quercetin has anti-oxidative and anti-inflammatory functions with the potential to lower disease risk factors, but data in human beings are limited.

Objective The objective of this study was to investigate the effect of quercetin, vitamin C, and niacin supplements (500 mg quercetin, 125 mg vitamin C, and 5 mg niacin [Q-500]; 1,000 mg quercetin, 250 mg vitamin C, and 10 mg niacin [Q-1,000]), on disease risk factors in a large group of community adults (n=1,002, 60% women) vary- ing widely in age and body mass index. Design Subjects were randomized into one of three groups (placebo, Q-500, or Q-1,000) and ingested supplements for 12 weeks. Blood samples were taken pre- and post-supplementation, and plasma quercetin, inflammatory markers (ie, C-reactive protein and five cytokines), diagnostic blood chemistries, blood pressure, and blood lipid profiles were measured.

Results Plasma quercetin increased in the Q-500 and Q-1,000 groups. No differences in blood chemistries were found except for a small decrease in serum creatinine and increase in glomerular filtration rate in Q-500 and Q- 1,000 groups. A small decrease in mean arterial blood pressure was measured for Q-500 and Q-1,000 groups compared to placebo. A difference in serum total cholesterol was measured between Q-500 and placebo groups, and there was small decrease in high-density lipoprotein cholesterol levels in the Q-1,000 group. Change in inflammatory measures did not differ between groups except for a slight decrease in interleukin-6 for the Q-1,000 group. Conclusions Q-500 or Q-1,000 supplementation for 12 weeks had a negligible influence on disease risk factors.

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AMY M. KNAB, PhD; R. ANDREW SHANELY, PhD; DRU A. HENSON, PhD; FUXIA JIN, PhD; SERENA A. HEINZ; MELANIE D. AUSTIN, MS; DAVID C. NIEMAN, DrPH

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Background In vitro data indicate quercetin has antioxidative and anti-inflammatory functions with the potential to lower disease risk factors, but data in human beings are limited.

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Results Plasma quercetin increased in the Q-500 and Q-1,000 groups. No differences in blood chemistries were found except for a small decrease in serum creatinine and increase in glomerular filtration rate in Q-500 and Q-1,000 groups. A small decrease in mean arterial blood pressure was measured for Q-500 and Q-1,000 groups compared to placebo. A difference in serum total cholesterol was measured between Q-500 and placebo groups, and there was small decrease in high-density lipoprotein cholesterol levels in the Q-1,000 group. Change in inflammatory measures did not differ between groups except for a slight decrease in interleukin-6 for the Q-1,000 group. **Conclusions** Q-500 or Q-1,000 supplementation for 12 weeks had a negligible influence on disease risk factors.

Flavonoids, a group of naturally occurring polyphenolic compounds found in plants, are associated with antioxidative (1), anti-inflammatory (2), antipathogenic (3,4), cardioprotective (5), and anticarcinogenic (6) activities. Inflammation and oxidative stress are key mechanisms in the pathogenesis of certain disease states, supporting the strategy of increased flavonoid intake either through diet or supplementation for prevention of cardiovascular disease (CVD). Indeed, epidemiologic studies indicate that high consumption of foods rich in flavonoids is associated with a decrease in CVD risk factors (7-9), and risk of CVD (5). Flavonoid supplementation as a therapeutic method for reducing disease risk factors is receiving increasing attention.

In vitro and animal studies support the notion that quercetin, the major flavonoid consumed by human beings, reduces disease risk factors (10). For example, in mice fed a high-fat diet, quercetin lowered circulating plasma cytokines (interferon-, interleukin [IL]-1, and IL-4), without a change in body composition or weight (11). In addition, quercetin significantly decreased serum total cholesterol and phospholipid levels, and liver enzyme activity involved with fatty acid synthesis in mice fed quercetin for 15 days (12). In rats, quercetin inhibits nuclear factor-kappaB, cytokine expression, and cytokine-inducible nitric oxide synthase expression (13,14), and decreases blood pressure (15,16).

Data on the effects of supplemental isolated flavonoids, specifically quercetin, on lowering disease risk factors in humans is limited with conflicting results (17). Egert and colleagues (18) reported no effect of 2 weeks of quercetin supplementation (50, 100, or 150 mg/day) on inflammatory markers, lipid profile, or body composition in 35 healthy subjects. In a subsequent crossover trial of 93 overweight subjects, Egert and colleagues (19) reported a decrease in both systolic blood pressure and high-density lipoprotein (HDL) cholesterol after 6 weeks of ingesting

quercetin at a dose of 150 mg/day. Conquer and colleagues (20) reported no change in serum lipoproteins or blood pressure in 27 healthy subjects ingesting 1,000 mg/day quercetin for 1 month. Another study found that 730 mg quercetin/day for 1 month decreased blood pressure in overweight subjects with mild hypertension (21). Egert and colleagues (19) proposed that quercetin may provide protection against CVD, especially in those at high risk.

Quercetin-related benefits on CVD risk factors are unclear given inconsistencies in dosing regimens and low subject numbers in previous trials in human beings. Our study investigated two doses of quercetin combined with vitamin C and niacin (500 mg quercetin, 125 mg vitamin C, and 5 mg niacin [Q-500]) or 1,000 mg quercetin, 250 mg vitamin C, and 10 mg niacin [Q-1,000], for 12 weeks on disease risk factors (eg, blood pressure, blood lipid levels, and inflammatory cytokines) in a large community group (n=1,002) varying widely in age and body mass index (BMI). Doses were based on prior studies of human beings conducted in our lab (22). The mixture was based on unpublished animal studies showing increased bioavailability of quercetin when mixed with vitamin C (1:1 ratio) and niacin (Quercegen Pharma, personal communication, September 2006). Our hypothesis was that a quercetin-vitamin C-niacin supplement (Q-500 or Q-1000) would improve disease risk factors compared to placebo.

METHODS

Subjects

Male and female noninstitutionalized subjects (N=1,023), 18 to 85 years of age, were recruited via mass advertising from the community. Approximately half of subjects were studied during a 12-week period from January to April 2008, and the second half from August to November 2008. Women who were pregnant or lactating were excluded from the study, but no other exclusion criteria were employed. Both diseased and non-diseased subjects were allowed to participate, and during recruitment, subjects were strati- fi by sex (40% men), age (40% young adults aged 18 to 40 years, 40% middle-aged adults aged 41 to 65, and 20% elderly adults, aged 65 and older), and BMI (45% normal or
18.5 to 24.9, 30% overweight or 25 to 29.9, and 25% obese or 2:30) to ensure representation of these various subgroups. Thirty-seven percent of subjects reported past or current history for one or more chronic diseases: hypertension (19%), arthritis (16%), cancer (6%), cardiovascular disease (4%), and/or diabetes (4%). Where noted, subjects taking medication for a chronic disease were excluded from analysis. During the study, subjects agreed to avoid any other supplements containing quercetin; no other restrictions were placed on diet, supplement use, or medications. All experimental procedures were approved by the Appalachian State University Institutional Review Board, and written informed consent was obtained from each subject.

Research Design

Subjects were randomized to one of three groups: Q-500 (500 mg quercetin, 125 mg vitamin C, 5 mg niacin/day), Q-1,000 (1,000 mg quercetin, 250 mg vitamin C, 10 mg niacin/day), or placebo. Supplements were administered utilizing double-blind procedures. Subjects ingested two soft chew supplements twice daily (upon awakening, and between 2 PM and the last meal of the day) during the 12-week study period. Supplements were prepared by Nutravail Technologies (Chantilly, VA) with Quercegen Pharma (Newton, MA), and were soft, individually wrapped chews (5.3 g/piece) that contained either 125 or 250 mg quercetin, 125 or 250 mg vitamin C (ascorbic acid and sodium ascorbate), 5 or 10 mg niacin, and 20 kcal sugars in a carnauba wax, soy lecithin, corn starch, glycerin, and palm oil base colored with FD&C Yellow No. 5 and No. 6. Placebo supplements were prepared exactly the same way minus the quercetin, ascorbic acid and sodium ascorbate, and niacin. Data from Quercegen Pharma (unpublished data, personal communication, September 2006) indicate that the bioavailability of quercetin is enhanced with vitamin C and niacin, and thus this study tested whether the combination of quercetin, vitamin C, and niacin had an influence on the outcome measures.

Following the first blood sample, subjects began supplementing with the chews and continued this for 12 weeks. The following information was also reported via monthly logs by each subject: adherence to the supple- mentation regimen; physical activity and diet status; change in disease status and medication use; and gastro- intestinal (constipation, heartburn, bloating, diarrhea, nausea, and vomiting), skin (rash, dryness, and flushing), allergy, and mental (energy, headache, stress, and focus/ concentration) symptoms.

Outcome Measures

To obtain lifestyle habit information, subjects were asked to complete a lifestyle habit survey 2 weeks before the first lab visit for the study. Height was measured using a stadiometer, whereas body mass and body composition were measured using a Tanita bioelectrical impedance scale (Tanita, Arlington Heights, IL). Following an overnight fast, and a 15-minute seated rest, resting blood pressure was measured. Blood samples were obtained following the overnight fast (between 7 and 9 AM) before and after the 12-week supplementation period. These blood samples were then analyzed for outcome measures as described below. Unless otherwise specified all chemicals were purchased from Sigma Aldrich (St Louis, MO).

Plasma Quercetin. Plasma quercetin was measured as previously described (22). Briefly, total plasma quercetin (quercetin and its primary conjugates) from heparin-treated blood was measured following solid-phase extraction via reversed-phase high-performance liquid chromatography with ultraviolet detection. Quercetin conjugates were hydrolyzed by incubating 500-L plasma aliquots with 10 L 10% DL-dithiothreitol solution, 50 L 0.58 mol/L acetic acid, 50 L of a mixture of -glucuronidase/arylsulfatase, and crude extract from Helix pomatia (Roche Diagnostics Corporation, Indianapolis, IN) for 2 hours at 37°C. Chromatographic analysis was performed using the Ultimate 3000 HPLC-PDA system (Dionex Corporation, Sunnyvale, CA) with a Gemini C18 column (Phenomenex, Torrance, CA).

Table 1. Subject characteristics (n=1,002) for placebo, Q-500[a], and Q-1,000[b] groups

Variable	Placebo (n=335; 122 men, 213 women)	Q-500 (n=334; 138 men, 196 women)	Q-1,000 (n=333; 134 men, 199 women)
	← mean±standard error →		
Age (y)			
Men	43.8±1.6	45.3±1.5	45.5±1.4
Women	47.4±1.1	47.2±1.1	45.2±1.1
Weight (kg)			
Men	85.2±1.3	86.0±1.6	88.4±1.5
Women	71.4±1.1	71.9±1.2	71.5±1.3
Height (m)			
Men	1.77±0.01	1.78±0.01	1.77±0.01
Women	1.64±0.01	1.65±0.01	1.64±0.01
Body mass index			
Men	27.1±0.4	27.1±0.5	28.2±0.4
Women	26.4±0.4	26.3±0.4	26.4±0.5
% Body fat			
Men	22.2±0.8	22.3±0.8	25.2±0.8*
Women	34.7±0.7	34.8±0.7	33.9±0.7
Sex (%)			
Men	37	43	39
Women	63	57	61
Education (y)	15.6±0.1	15.6±0.2	15.6±0.2
Marital status (%)			
Single	34	32	33
Married	53	60	55
Other	13	8	12
Race (%)			
White	93	93	92
Black	2	2	4
Other	5	5	4

[a]Q-500 supplement included 500 mg quercetin, 125 mg vitamin C, and 5 mg niacin.
[b]Q-1,000 supplement included 1,000 mg quercetin, 250 mg vitamin C, and 10 mg niacin.
*Significantly different than placebo and Q-500 (P<0.025).

Blood Pressure Measurements. Blood pressure was measured using an aneroid sphygmomanometer on all sub- jects in an overnight fasted state. Subjects rested in a seated position with arm supported at heart level in a quiet room for 15 minutes before blood pressure measurements. At least two measurements were obtained until a consistent reading within ±5 mm Hg was obtained for both systolic (SBP) and diastolic blood pressures (DBP). Mean arterial blood pressure (MAP) was calculated using the following equation: MAP=DBP+1/3(SBP-DBP).

Inflammatory Markers. Enzyme-linked immunosorbant assays (R&D Systems, Inc, Minneapolis, MN) were used to measure total plasma concentrations of IL-6 (high sensitivity), IL-10, granulocyte colony-stimulating factor (high sensitivity), monocyte chemoattractant protein 1, and tumor necrosis factor-, in accordance with the manufacturer protocol, as described in detail elsewhere (23). All samples and provided standards were analyzed in duplicate. Serum C-reactive protein (high sensitivity) was measured using an LX-20 clinical analyzer (Beckman, Brea, CA).

Blood Lipids, Diagnostic Chemistry Panel, Complete Blood Count, Leukocyte Differential. Serum total cholesterol, low-density lipoprotein cholesterol, HDL cholesterol, triglycerides, glucose, diagnostic chemistries, and a complete blood count with leukocyte differential were analyzed in the clinical lab of the Watauga Medical Center (Boone, NC) using standard clinical laboratory equipment and quality standards. Glomerular filtration rate was estimated using the Cockcroft & Gault equation (24).

Statistical Procedures

Data were analyzed using a 3 (group)X2 (time) repeated measures analysis of variance (ANOVA), between groups design, with post hoc analysis using Bonferroni adjusted independent t tests that contrasted pre- to post-supplementation changes of Q-500 and Q-1,000 with placebo (P<0.025). 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 (Pillais trace). When interaction effects were significant (P:S0.05), post hoc analysis was con- ducted using Bonferroni adjusted independent t tests that contrasted pre- to post-supplementation changes of Q-500 and Q-1,000 with placebo (P<0.025). Additional repeated measures ANOVAs were conducted by adding categorical covariates to the model to test for the influence of sex (man or woman), BMI (normal <25, over-

Table 2. Diagnostic chemistry values pre- and post-study for participants in placebo, Q-500[a], and Q-1,000[b] groups

Variable	Placebo (n=335)	Q-500 (n=333)	Q-1,000 (n=332)	Interaction *P* value
	← mean±standard error →			
Hemoglobin (g/dL)[c]				
Pre-study	14.9±1.3	14.9±1.4	14.9±1.4	
Post-study	14.8±1.4	14.8±1.3	14.8±1.3	0.999
Hematocrit (%)				
Pre-study	44.3±3.8	44.6±3.8	44.4±4.0	
Post-study	44.0±3.8	44.1±3.6	44.1±3.7	0.487
Creatinine (mg/dL)[d]				
Pre-study	0.881±0.161	0.893±0.159	0.877±0.176	
Post-study	0.892±0.160	0.887±0.157*	0.873±0.167*	0.014
Glomerular filtration rate[e] (mL/min)				0.006
Pre-study	104.4±32.8	104.6±33.1	109.2±36.5	
Post-study	103.6±32.4	106.2±33.7*	110.2±36.8*	
Glucose (mg/dL)[f]				
Pre-study	89.3±11.4	89.7±12.6	90.5±17.3	
Post-study	91.1±11.5	91.8±13.8	92.1±17.7	0.751

[a]Q-500 supplement included 500 mg quercetin, 125 mg vitamin C, and 5 mg niacin.
[b]Q-1,000 supplement included 1,000 mg quercetin, 250 mg vitamin C, and 10 mg niacin.
[c]To convert g/dL hemoglobin to g/L, multiply g/dL by 10. To convert g/L hemoglobin to g/dL, multiply g/L by 0.1. Hemoglobin of 14.9 g/dL=149 g/L.
[d]To convert mg/dL creatinine to mol/L, multiply mg/dL by 88.4. To convert mol/L creatinine to mg/dL, multiply mol/L by 0.011. Creatinine of 0.881 mg/dL=78 mol/L.
[e]Estimated glomerular filtration rate (24).
[f]To convert mg/dL glucose to mmol/L, multiply mg/dL by 0.0555. To convert mmol/L glucose to mg/dL, multiply mmol/L by 18.0. Glucose of 89.3 mg/dL=5.0 mmol/L.
*Significant change from presupplementation compared to placebo ($P<0.025$).

weight 25 to 29.9, and obese 2:30), and age (<40, 40 to 59, and 2:60 years). Subjects reporting current use of cholesterol or blood pressure medications were excluded from the analysis for lipid profile and MAP, respectively. Data are expressed as means±standard error.

RESULTS

Table 1 summarizes subject characteristics for those who completed this study. Subjects varied widely in age (18 to 85 years), BMI (16.7 to 52.7), and percent body fat (3.4% to 59.5%), and were 60% women and 40% men. No differences in any of the characteristics were found between groups except the men in the Q-1,000 group had significantly higher body fat percentage compared to those in the placebo group (Table 1). Of the 1,023 subjects recruited into the study, 1,002 completed all phases of the study. Among the 21 dropouts (seven from the placebo group, six from Q-500, and eight from Q-1,000), 12 failed to take the supplement and/or adhere to testing procedures, and nine reported adverse symptoms from taking the supplement. Follow-up revealed no consistent pattern of symptoms that could be ascribed to taking the quercetin supplements. Monthly symptom logs revealed no group differences over time for gastrointestinal, skin, allergy, or mental symptoms (data not shown). In addition, change in BMI and percent body fat did not differ significantly between groups during the 12-week study even after adjustment for sex (data not shown).

Results of blood chemistry measurements are reported in Table 2. No significant differences were found for outcome measures except for small but significant decreases in serum creatinine in the Q-500 and Q-1,000 groups compared to placebo, and increases in glomerular filtration rate for both Q-500 and Q-1,000 groups compared to placebo (Table 2). Hemoglobin and hematocrit also did not differ over time. Plasma quercetin increased in a dose-responsive manner in both Q-500 and Q-1,000 groups compared to placebo (interaction effect, $P<0.001$) (Figure). Although there was individual variation in the plasma quercetin response to supplementation, increases in plasma quercetin were not related to sex, age, or BMI (data not shown).

After excluding subjects on blood pressure medication, a small but significant decrease in MAP was measured for Q-500 and Q-1,000 compared to placebo (interaction $P=0.046$) and this effect was not related to age, sex, or BMI. Table 3 shows that the drop in MAP occurred through a combined but nonsignificant reduction in both systolic and diastolic blood pressures.

For all subjects not on cholesterol medication, the pattern of change in serum total cholesterol over time differed in Q-500 compared to placebo ($P<0.025$), but not between Q-1,000 and placebo (Table 4). A significant interaction effect was measured for low-density lipoprotein cholesterol without specific group differences (Table 4). A small decrease in HDL cholesterol was measured in the Q-1,000 group compared to the placebo group. No significant interaction effect was seen for triglycerides. These effects did not differ after adjustment for age or sex. After covariance with BMI (interaction *P* value 0.036) the quercetin-related decrease in total cholesterol was slightly larger in those with the highest BMI.

The pre- to postsupplementation change in plasma IL-6 for Q-1,000 compared to placebo was statistically differ-

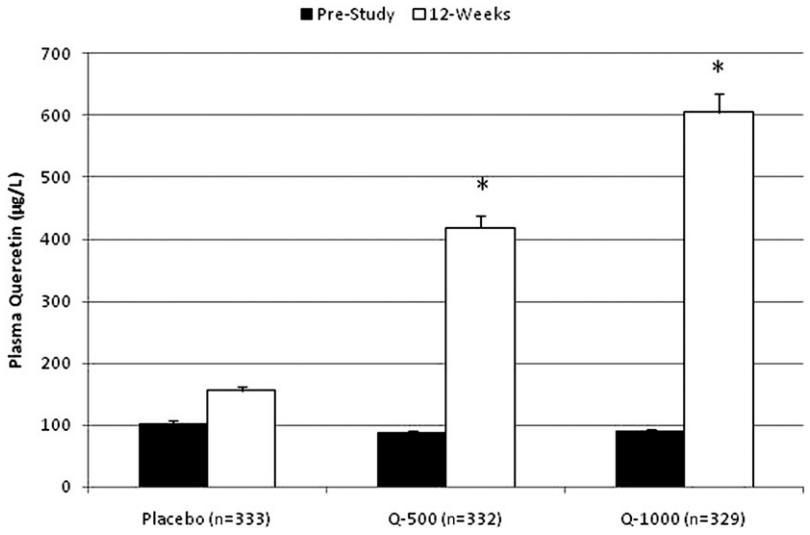

Figure. Plasma quercetin significantly increased in a dose-dependent manner following 12 weeks of supplementation. *Denotes change from pre-study to post-study significantly different from placebo (P<0.001).

Table 3. Pre- and post-study measurements of systolic, diastolic, and mean arterial blood pressure (BP) for participants receiving placebo, Q-500[a] supplement, or Q-1,000[b] supplement who were not on blood pressure–lowering medication

Variable	Placebo (n=265)	Q-500 (n=271)	Q-1,000 (n=257)	Interaction *P* value
	← mean ± standard error →			
Systolic BP (mm Hg)				
Pre-study	118±0.8	118±0.8	117±0.9	
Post-study	117±0.8	115±0.8	115±0.9	0.193
Diastolic BP (mm Hg)				
Pre-study	75.2±0.5	74.9±0.6	75.3±0.6	
Post-study	75.6±0.5	74.1±0.6	74.5±0.6	0.094
Mean arterial BP (mm Hg)				
Pre-study	89.5±0.6	89.2±0.6	89.2±0.6	0.046
Post-study	89.4±0.6	87.8±0.6*	87.9±0.6*	

[a]Q-500 supplement included 500 mg quercetin, 125 mg vitamin C, and 5 mg niacin.
[b]Q-1,000 supplement included 1,000 mg quercetin, 250 mg vitamin C, and 10 mg niacin.
*Pre- to post-study change differed significantly from placebo group (P<0.025).

ent (P<0.025), and this effect was not related to age, sex, or BMI. Pre- to post-study changes in serum C-reactive protein and plasma cytokines (monocyte chemoattractant protein 1, granulocyte colony stimulating factor, IL-10, and tumor necrosis factor), did not differ between groups (Table 5).

DISCUSSION

These data indicate that 12-week supplementation with either Q-500 or Q-1,000 increases plasma quercetin levels in a dose-responsive manner and is not associated with adverse symptoms or detrimental changes in diagnostic chemistries. Q-500 or Q-1,000 supplementation did not affect plasma markers of inflammation, except for a small statistical difference in IL-6 in the Q-1,000 group compared to placebo. Only small differences were seen in lipid measures, including a significant decrease in total cholesterol only in the Q-500 group, with a decrease in HDL cholesterol in the Q-1,000 group when compared with placebo. A small but significant difference in MAP was seen in both Q-500 and Q-1,000 groups compared to placebo (however, no difference for SBP or DBP), for subjects not taking medication.

Both the Q-500 and Q-1,000 groups experienced a small decrease in serum creatinine and an increase in glomerular filtration rate, which led to an increase in urine output of approximately 30 mL/day (24). The decrease in MAP with quercetin has been reported previously in animal models, and increased urine output is one proposed mechanism (15). Other quercetin-related effects on MAP may be due to a down-regulation of kidney epithelial sodium channel mRNA expression (thus less sodium re- absorption), and increased nitric oxide availability through elevation of nitric acid-synthase activity (15,16,25).

Table 4. Pre- and post-study total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels of participants in the placebo, Q-500[a], and Q-1,000[b] groups who were not on cholesterol-lowering medication

Variable	Placebo (n=288)	Q-500 (n=277)	Q-1,000 (n=271)	Interaction *P* value
Total cholesterol (mmol/L)[c]				
Pre-study	5.12±0.06	5.15±0.06	5.04±0.06	0.049
Post-study	5.10±0.05	5.02±0.06*	4.99±0.06	
LDL cholesterol (mmol/L)[c]				
Pre-study	2.96±0.05	3.07±0.05	2.94±0.05	0.017
Post-study	2.83±0.05	2.87±0.05	2.86±0.05	
HDL cholesterol (mmol/L)[c]				
Pre-study	1.52±0.03	1.44±0.03	1.48±0.03	0.019
Post-study	1.53±0.03	1.42±0.03	1.45±0.03*	
Triglyceride (mmol/L)[d]				
Pre-study	1.30±0.05	1.30±0.05	1.23±0.05	0.366
Post-study	1.35±0.08	1.32±0.05	1.27±0.05	

[a]Q-500 supplement included 500 mg quercetin, 125 mg vitamin C, and 5 mg niacin.
[b]Q-1,000 supplement included 1,000 mg quercetin, 250 mg vitamin C, and 10 mg niacin.
[c]To convert mmol/L cholesterol to mg/dL, multiply mmol/L by 38.6. To convert mg/dL cholesterol to mmol/L, multiply mg/dL by 0.026. Cholesterol of 5.00 mmol/L=193
[d]To convert mmol/L triglyceride to mg/dL, multiply mmol/L by 88.6. To convert mg/dL triglyceride to mmol/L, multiply mg/dL by 0.0113. Triglyceride of 1.30
*Pre- to post-study change differed significantly from placebo group (*P*<0.025).

Table 5. Pre- and post-study measurements of inflammatory markers C-reactive protein (CRP), tumor necrosis factor- (TNF), monocyte chemoattractant protein 1 (MCP-1), granulocyte colony-stimulating factor (GCSF), interleukin-6 (IL-6), and interleukin-10 (IL-10)[a] for placebo, Q-500[b], and Q-1,000[c] groups

Variable	Placebo (n=312)	Q-500 (n=316)	Q-1,000 (n=309)	Interaction *P* value
	← mean± standard error →			
CRP (mg/L)[d]				
Pre-study	3.30±0.25	3.00±0.24	3.12±0.25	
Post-study	3.53±0.27	3.37±0.35	3.07±0.26	0.439
TNF-o: **(pg/mL)**				
Pre-study	2.16±0.10	2.05±0.09	1.96±0.08	
Post-study	2.05±0.09	1.95±0.08	1.91±0.07	0.542
MCP-1 (pg/mL)				
Pre-study	167±3.1	165±3.0	168±3.0	
Post-study	165±2.8	165±2.9	163±3.0	0.311
GCSF (pg/mL)				
Pre-study	31.5±0.8	31.2±0.8	31.3±0.8	
Post-study	32.6±0.81	31.4±0.8	32.4±0.8	0.654
IL-6 (pg/mL)				
Pre-study	1.72±0.07	1.68±0.07	1.88±0.08	
Post-study	1.90±0.07	1.91±0.08	1.87±0.08*	0.021
IL-10 (pg/mL)	(n=160)	(n=167)	(n=166)	
Pre-study	1.85±0.14	1.68±0.09	1.93±0.26	
Post-study	1.91±0.14	1.80±0.19	2.03±0.34	0.864

[a]Only measured in subgroup of subjects.
[b]Q-500 supplement included 500 mg quercetin, 125 mg vitamin C, and 5 mg niacin.
[c]Q-1,000 supplement included 1,000 mg quercetin, 250 mg vitamin C, and 10 mg niacin.
[d]To convert mg/L CRP to nmol/L, multiply mg/L by 9.524. To convert nmol/L CRP to mg/L, multiply nmol/L by 0.105. CRP of 3.30 mg/L=31.43 nmol/L.
*Pre- to post-study change differed significantly from placebo group (*P*<0.025).

Two of three previous studies (18,20,21) with human subjects also report changes in blood pressure measures following quercetin supplementation. A modest reduction in blood pressure was found among mildly hypertensive subjects (N=22) after 28 days of 730 mg quercetin daily (21). Egert and colleagues (19) found a drop in MAP of 0.5 mm Hg in overweight subjects (aged 25 to 65 years). The drop in MAP observed in our study was slightly greater

than observed by Egert and colleagues, possibly due to the supplementation dosing regimen and population differences. Egert and colleagues (19) speculated that improved endothelial function was the underlying mechanism explaining the quercetin related blood pressure lowering effect, especially in younger and middle-aged individuals. The third human trial was unable to mea- sure a blood pressure lowering effect among 27 healthy subjects after 28 days of 1,000 mg quercetin a day (20).

A modest decrease in serum cholesterol levels was measured in men and women not on cholesterol medications in the Q-500 group but not the Q-1,000 group compared to placebo. Three previous human trials found no effect of short-term quercetin ingestion on blood total cholesterol, triglycerides, or low-density lipoprotein cholesterol (18- 20). Egert and colleagues (19) reported a decrease in HDL cholesterol in 93 subjects ingesting 150 mg of quercetin/ day for 6 weeks. The change in HDL cholesterol following quercetin supplementation reported by Egert and col- leagues (19) is similar in magnitude to our data. It is noted that a decrease in HDL cholesterol is detrimental to health. Thus, it appears that high doses of quercetin, in our study 1,000 mg/day (when combined with vitamin C and niacin), have detrimental effects on HDL cholesterol levels in human beings. Contrary to our findings, some but not all animal-based studies support improvements in the lipid profile following quercetin supplementation (12,26-28). Quercetin supplementation significantly reduced total cholesterol in rats fed a high-cholesterol diet (26), but not when placed on high-fat diets (28). In animals, quercetin may decrease serum total cholesterol via an increased fecal excretion of cholesterol and bile acids through binding and thus inhibition of enterohepatic re- circulation (27,29). Quercetin may also decrease hydroxy-methylglutaryl coenzyme A-reductase activity in the liver (12). Although the mechanism is not clear in humans, the lack of dose response in our study indicates higher doses of quercetin do not yield better cholesterol lowering effects. Interestingly, studies in human beings using food products (such as concentrated grape juice) report an improvement in cholesterol profile(30,31), indicating that a mixed fla vonoid approach may be more efficient than the one used in this study.

Q-500 and Q-1,000 supplementation effects on inflammation were limited to a small reduction of IL-6 in the Q-1,000 compared to placebo group, but the importance of this finding is weakened due to group variance in pre- study IL-6 levels. No effects of Q-500 or Q-1,000 were measured for C-reactive protein or four other cytokines, which is consistent with three other studies in human subjects (18,19,32). Egert and colleagues. (19) suggested the dose of 150 mg/day was too small to elicit an anti- inflammatory effect; however, the doses used in our study were substantially larger and still did not elicit any significant effect on overall inflammatory markers. Quercetin's anti-inflammatory effects are evident with in vitro (2,13,32-34) and animal-based models (13,33). However, the anti-inflammatory effect of in vivo quercetin conjugates is attenuated (32,35). We recently showed that ingestion of quercetin supplements combined with other flavonoids, fish oil, and antioxidants decreased postexercise inflammation in cyclists following heavy exertion (23). This finding supports the notion of a mixed flavonoid approach to improve the anti-inflammatory effects of quercetin.

CONCLUSIONS

Supplementation with either Q-500 or Q-1,000 was safe but had a negligible influence on disease risk factors. A large apple provides 8 to 10 mg quercetin; thus, the supplement levels used in this study are not attainable through the diet alone. Quercetin was combined with vitamin C and niacin into one supplement, and thus it is not possible to entirely attribute effects reported in our study to quercetin alone. No effects were seen on most measures of inflammation. The cholesterol lowering effect was small, and limited to the Q-500 group, while a decrease in HDL cholesterol was measured in the Q-1,000 group. The lack of effect of long-term quercetin-vitamin C-niacin supplementation on the disease risk factors measured in this study is in agreement with the findings reported by other investigators. Future research will determine if the bioactive effects of quercetin measured in vitro can be replicated in humans by adding other synergistic food components such as isoquercetin, green tea extract, fish oil, antioxidants, and other types of polyphenols (36-38).

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