The variable plasma quercetin response to 12-week quercetin supplementation in humans

Authors:
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Abstract

Background/Objectives: Quercetin supplementation results in a variable plasma quercetin response in humans. The purpose of this study was to determine whether this variance is related to gender, age, body mass index (BMI), and other demographic and lifestyle factors.

Subjects/Methods: Subjects (N ¼ 1002, ages 18–85 years, 60% female and 40% male) were recruited from the community and randomized to one of three groups, with supplements administered using double-blinded procedures: Q-500 (500 mg/day), Q-1000 (1000 mg/day), or placebo. Subjects ingested two soft chew supplements twice daily during the 12-week study. Fasting blood samples were obtained pre- and post-study, analyzed for plasma quercetin, and then compared between and within groups by gender, age group (o40, 40–59, and X60 years), BMI (o25, 25–29.9, and X30 kg/m2), self-reported physical fitness level, and diet intake (food group servings).

Results: Quercetin supplementation over 12 weeks caused a significant increase in overnight-fasted plasma quercetin, with a net increase of 332±21.0 and 516±30.8 mg/l for Q-500 and Q-1000 compared with 53.6±6.4 mg/l for placebo (interaction effect, Po0.001). The increase in plasma quercetin was highly variable within each quercetin supplementation group, but was unrelated to age, gender, BMI, fitness levels, or diet intake.

Conclusions: In summary, quercetin supplementation in doses of 500 and 1000 mg/day caused large but highly variable increases in plasma quercetin that were unrelated to demographic or lifestyle factors.

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The variable plasma quercetin response to 12-week quercetin supplementation in humans

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Keywords: quercetin; flavonoid; human; plasma; supplementation; variation

Introduction

In vitro, animal and epidemiologic studies support multiple bioactive effects for quercetin (3,3^0,4^0,5,7-pentahydroxyflavone), including anti-oxidative (Hanasaki *et al*., 1994), anti-inflammatory (Read, 1995), anti-fibrogenic (Lee *et al*., 2003), anti-hypertensive (Duarte *et al*., 2001), anti-pathogenic (Gulati *et al*., 2006), and cardioprotective benefits (Boots *et al*., 2008a). The *in vivo* health benefit of quercetin seems limited, however, because of low bioavailability and extensive metabolic transformation (Boyle *et al*., 2000; Lotito and Frei, 2006; Loke *et al*., 2008; Boots *et al*., 2008b). Quercetin and its methylated derivatives (isorhamnetin, tamarixetin) are not present in aglycone form in human blood because of extensive glucuronide and sulfate conjugation processes (Manach *et al*., 1998; Loke *et al*., 2008). The few available studies in humans show little if any quercetin supplementation-related decrease in oxidative stress and inflammation, a finding that is in contrast to *in vitro* and animal-based experiments (Boyle *et al*., 2000; Lotito and Frei, 2006; Egert *et al*., 2008).

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 h (Manach *et al*., 1998; Erlund *et al*., 2000; Goldberg *et al*., 2003; Egert *et al*., 2008). Long-term quercetin supplementation, however, results in a highly variable plasma quercetin response, and little is known regarding the factors that explain this variance (Conquer *et al*., 1998; Erlund *et al*., 2000; Goldberg *et al*., 2003; Egert *et al*., 2008). In four different human trials, quercetin supplementation with

doses of 8–1000 mg/day for 2–6 weeks resulted in overnight-fasted plasma quercetin values with an unexpectedly wide inter-individual variation (Conquer *et al*., 1998; Erlund *et al*., 2000; Edwards *et al*., 2007; Egert *et al*., 2008). Edwards *et al*., for example, reported a 13-fold range in overnight-fasted plasma quercetin for two-thirds of 41 subjects after 4 weeks of supplementation with 730 mg quercetin per day. Egert *et al*. have speculated that the variation in plasma quercetin response to quercetin supplementation may be explained by differences in absorption rates because of polymorphisms for intestinal enzymes and transporters. Support for this hypothesis comes from Hollman's study (Hollman *et al*., 1995) of ileostomy subjects, in which a single dose of 100 mg quercetin had a measured absorption rate of 24±9% (mean±s.d.) with urine accumulation rates for quercetin that varied more than ninefold.

We hypothesize that the variance in plasma quercetin response to long-term supplementation may be related at least in part to basic demographic and lifestyle factors. The primary objective of this study was to examine the influence of multiple factors including age, gender, body mass index (BMI), physical fitness level, chronic disease status, and habitual diet intake on plasma quercetin responses to 12-weeks supplementation with 500 or 1000 mg quercetin per day in a large and disparate group of community-dwelling adults.

Materials and methods

Subjects

Subjects (*N* ¼ 1023), 18–85 years of age, were recruited through mass advertising from the community. Half of the subjects were studied during a 12-week period from January to April 2008, and the second half from August to November 2008. Subjects had to be noninstitutionalized, and women were excluded if pregnant or lactating. No other exclusion criteria were used. During recruitment, subjects were stratified by gender (B40% male, 60% female), age (40% young adult 18–40 years of age, 40% middle-aged 41–59, and 20% 60 and over), and BMI groups (33% normal 18.5–24.9, 33% overweight 25–29.9, and 33% obese X30 kg/m^2) to ensure uniform representation of these various subgroups in placebo, Q-500, and Q-1000. Subjects agreed to avoid any other supplements containing quercetin; no other restrictions were placed on diet, supplement usage, or medications. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the institutional review board of Appalachian State University. Written informed consent was obtained from all subjects.

Research design

Subjects were randomized to one of three groups: Q-500 (500 mg/d quercetin), Q-1000 (1000 mg/day quercetin), or placebo (PL). These doses were based on earlier animal studies (Davis *et al*., 2009) and human studies conducted by our research team (Nieman *et al*., 2007a, b, c, 2009). Supplements were administered using double-blinded procedures. Subjects ingested two soft chew supplements twice daily (on awakening, and between 1400 hours and the last meal of the day) during the 12-week study period. The duration of the study period (12 weeks) was based on our desire to measure plasma quercetin responses to supplementation over a longer period than earlier studied in humans (2–6 weeks).

Quercetin supplements were provided by Quercegen Pharma (Newton, MA, USA) and were soft, individually wrapped chews (5.3 g/piece) that contained either 125 or 250 mg quercetin aglycone, 125 or 250 mg vitamin C (ascorbic acid and sodium ascorbate), 5 or 10 mg niacin, and 20 kcal of sugars in a carnauba wax, soy lecithin, corn starch, glycerine, and palm oil base colored with FD&C yellow 5 and 6. Data from Quercegen Pharma indicate that bioavailability of quercetin is enhanced with ascorbic acid and niacin (unpublished data). Thus, this study tested whether or not soft chews with or without the combination of quercetin, ascorbic acid, and niacin had an influence on the plasma quercetin responses. Thus, placebo supplements were prepared in the same way minus the quercetin, vitamin C, and niacin. Earlier studies performed by our laboratory used these supplement and placebo formulas, and this choice was repeated in this study to allow for comparison of results (Nieman *et al*., 2007a, b, c, 2009). Subjects started supplementing after the first blood sample and continued for 12 weeks.

Lifestyle habit information was obtained by subjects' self-report through an Internet-based site (SurveyMonkey.com, Portland, OR, USA) 2 weeks before the first laboratory visit for the study. A food frequency questionnaire was used to obtain typical daily consumption of fruits, vegetables, and red meat. Subjects were asked 'On average, how many servings ofy.do you eat per day?' Serving size information was provided for each food group, and then subjects checked a box representing how many servings they consumed on an average day (Lee and Nieman, 2010). Self-reported physical fitness level was assessed on a 10-point Likert scale, with subjects asked to compare their level with other persons of the same age, with 1 corresponding to low fitness and 10 to high fitness. Subjects' height was measured with a stadiometer, and body mass determined using a Tanita scale (Tanita, Arlington Heights, IL, USA). Subjects completed a monthly electronic log to verify adherence to the supplementation regimen. If a subject indicated difficulty in adhering to the supplementation schedule, they were removed from the study.

Blood sample

Blood samples were drawn into heparin tubes after an overnight fast in the morning (7–9 a.m.) before and after the 12-week supplementation period. A subgroup of 170 subjects

had their blood drawn monthly after the starting point of supplementation. Blood was immediately centrifuged at 4000 *g* at 4 1C and aliquots of plasma samples were snap frozen to store at -80 1C.

Enzymatic hydrolysis

Quercetin and its conjugates were measured as described earlier (Nieman *et al*., 2007a, b, c). To each 500 ml human plasma, 10% DL-dithiothreitol solution (10 ml) was added before the addition of 50 ml of 0.58 mol/l acetic acid. The mixture was then spiked with 10 ml of 0.356 mmol/l fisetin internal standard. To this, a mixture of 50 ml enzyme b-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 1C. Then 500 ml of 0.01 mol/l oxalic acid was added to stop the enzymatic reactions. The microplate was vortexed for another 1 min before centrifugation at 3000 r.p.m. for 17 min (Allegra X-22R centrifuge; Beckman Coulter, Fullerton, CA, USA).

Solid phase extraction

Waters (Milford, MA, USA) Oasis HLB 96-well sample extraction plates (30 mg, 30 mm) 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 o0.2 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 o0.2 ml/min. A total of 10% DL-dithiothreitol solution (10 ml) was added before solvent evaporation at 30 1C with N_2 blowing through. The dried samples were reconstituted into 50/50 methanol/water for HPLC analysis.

HPLC-PDA analysis

Chromatographic analysis was performed using the Ultimate 3000 HPLC-PDA system (Dionex Corporation, Sunnyvale, CA, USA) using a Gemini C18 column (150 x 4.6 mm, 100A, 5 mm) with Gemini C18 4 x 3.0 mm SecurityGuard cartridges (Phenomenex, Torrance, CA, USA). The column was kept at 30 1C and the flow rate was 1.0 ml/min. Separation was carried out using a 15 min gradient of acetonitrile containing 0.1% formic acid and water containing 0.1% formic acid. The PDA detector was set to monitor at 375 nm as well as scanning from 250 to 700 nm, and the injection volume was kept at 50 ml.

Statistical procedures

Data in Table 2 and Figure 2 were analyzed using a 3 (group) x 2 (time) repeated measures ANOVA, between groups design, with *post hoc* analysis using Bonferroni's adjusted independent *t*-tests that contrasted pre- to post-supplementation changes of Q-500 and Q-1000 with placebo (*P*o0.0125). 2 presents these data by gender, BMI, and age groups. Data in Figure 1 were analyzed using a 3 (group) x 4 (time) repeated measures ANOVA. Data are expressed as means±s.e. We used a general linear model to predict change in plasma quercetin levels (Figure 3) (dependent factor) with all of the following independent factors: quercetin intake (placebo, Q-500, and Q-1000 groups), gender (male, female), BMI, age, self-reported physical fitness level, cigarette smoking habit, self-reported diet intake by food groups (tertiles of fruit, vegetable, and red meat food groups), and chronic disease status. Change in plasma quercetin was calculated by subtracting pre-supplementation from post-supplementation levels, and then was log transformed to meet the normality assumption of the general linear model.

Results

Table 1 summarizes subject characteristics for each group. No significant group differences were found on age, weight, height, and BMI. For all subjects combined, 60% were female and 40% were male with a wide range in age and BMI. Of the 1023 subjects recruited into the study, 1002 completed all phases of the study. Among the 21 dropouts (7 from the placebo group, 6 from Q-500, and 8 from Q-1000), 12 failed to take the supplement and/or adhere to testing procedures, and 9 reported adverse symptoms from taking the supplement. The adverse symptoms varied widely, and follow-up revealed no consistent pattern of symptoms that could be

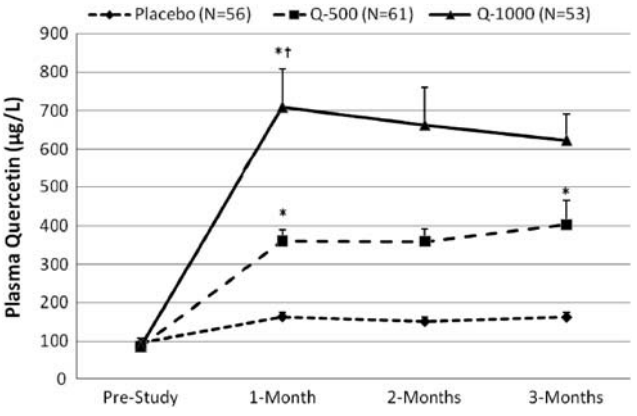

Figure 1 Overnight-fasted plasma quercetin concentration for subjects tested pre-study and monthly during the study. Values are mean±s.e. (mg/l). *Mean values were significantly different from that at pre-study (*P*o0.001). †Mean values were significantly different from that of Q-500 (*P*o0.001).

Table 1 Subject characteristics

Variable	Gender	Placebo	Q-500	Q-1000	Total group
N		335	334	333	1002
Age (years)	Males	43.8±1.5	45.3±1.2	45.5±1.4	46.0±0.5
	Females	47.4±1.1	47.2±1.1	45.2±1.1	(range 18–85)
Weight (kg)	Males	84.8±1.4	85.7±1.2	88.1±1.5	77.2±0.6
	Females	71.2±1.1	71.6±1.2	71.4±1.3	(42.7–157.5)
Height (m)	Males	1.77±0.06	1.78±0.04	1.77±0.06	1.70±0.03
	Females	1.64±0.05	1.65±0.05	1.64±0.04	(1.39–2.02)
BMI (kg/m^2)	Males	27.0±0.4	26.9±0.4	28.1±0.4	26.7±0.2
	Females	26.4±0.4	26.2±0.4	26.4±0.5	(16.7–52.7)

Abbreviation: BMI, body mass index.
Values are mean±s.e.
No statistical differences between groups were found for any of the variables.

Table 2 Plasma quercetin concentration pre- and post-12-week study

Variable	Time	Placebo	Q-500	Q-1000
N		333	332	329
Quercetin (㎎/l)	Pre-study	102±4.7	86.2±4.2	89.6±4.1
	Post-study	156±5.8	418±21.3*	605±30.6*ᵂ
Gender				
Males, n		122	136	131
Quercetin (㎎/l)	Pre-study	112±8.4	86.9±5.9	86.5±5.9
	Post-study	163±10.7	414±37.4*	655±57.5*ᵂ
Females, n		211	195	199
Quercetin (㎎/l)	Pre-study	96.8±5.5	85.3±5.8	91.7±5.6
	Post-study	152±6.9	418±25.0*	573±33.9*ᵂ
Age (years)				
o40 years, n		112	107	121
Quercetin (㎎/l)	Pre-study	118±9.0	86.0±8.0	86.1±6.5
	Post-study	160±10.7	433±36.1*	674±64.9*ᵂ
40–59 years, n		140	141	145
Quercetin (㎎/l)	Pre-study	91.7±6.4	84.7±6.0	94.6±6.2
	Post-study	150±8.6	394±26.4*	533±28.5*ᵂ
60–85 years, n		81	84	64
Quercetin (㎎/l)	Pre-study	98.5±9.3	89.1±8.2	85.2±34.4
	Post-study	161±11.9	439±55.0*	640±74.8*ᵂ
BMI (kg/m^2)				
o25, n		152	157	138
Quercetin (㎎/l)	Pre-study	105±6.6	88.7±6.3	105±6.8
	Post-study	153±8.5	439±33.8*	593±40.7*ᵂ
25–29.9, n		95	94	109
Quercetin (㎎/l)	Pre-study	105±9.3	75.7±6.5	74.8±5.9
	Post-study	161±11.1	375±32.0*	611±59.6*ᵂ
X30, n		86	81	83
Quercetin (㎎/l)	Pre-study	95.4±9.4	93.6±9.3	83.9±8.5
	Post-study	155±11.7	427±44.2*	617±65.1*ᵂ

Abbreviation: BMI, body mass index.
Values are mean±s.e.
*Po0.001, significant change from pre-study compared with placebo.
ᵂPo0.001, significant change from pre-study compared with Q-500.

ascribed to taking the quercetin supplements. 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%), diabetes (4%). Q-1000, Q-500, and placebo groups did not differ when compared for physical fitness level, food group intake, or chronic disease status (data not shown).

Plasma quercetin data before and after the 12-week study are shown in Table 2. As depicted in Figure 1, the dose–response effect occurred before the first month and was maintained through the rest of the study. Significant increases in plasma quercetin were measured for Q-500 and Q-1000 (group interaction factor Po0.001) (see Figure 2; Table 2). The increase in plasma quercetin for Q-1000 was also significantly greater than Q-500, indicating a dose–response effect. The increase in plasma quercetin for Q-500 and Q-1000 compared with placebo did not differ when comparing gender, age groups, or BMI (Table 2). Figure 3 compares scatter plots for the change in plasma quercetin in each group. Using the general linear model and P-value cutoff 0.05, we found that change in plasma quercetin levels was significantly predicted by quercetin intake (placebo, Q-500, Q-1000 groups), but was not predicted by gender, age, BMI, chronic disease status, self-reported diet intake, smoking, and physical fitness levels.

Discussion

Quercetin supplementation over 12 weeks in this large and disparate group of community-dwelling adults caused a significant increase in overnight-fasted plasma quercetin concentration, with a percentage increase of 385 and 575% for Q-500 and Q-1000, respectively. Using data from a subgroup of our study population, the dose–response increase in plasma quercetin for Q-500 and Q-1000 was achieved within the first month and maintained for the rest of the study. High inter-subject variation was unrelated to demographic or lifestyle factors, including age, gender, BMI, self-reported physical fitness levels, chronic disease status, or habitual dietary intake.

Earlier quercetin supplementation studies in humans have also reported high inter-individual variation in the plasma quercetin response. These studies have had several design limitations including small subject numbers with widely varying dosing regimens, making it difficult to draw conclusions regarding the underlying causes for the wide variation in plasma quercetin response. Conquer *et al*. (1998) provided 13 healthy subjects with 1000 mg/day quercetin for 28 days. Post-supplementation overnight-fasted plasma quercetin levels ranged between 0 and 899 ㎎/l for 95% of subjects, but no rationale was provided for this variation. Another human study (N ¼ 41) using quercetin aglycone supplements (730 mg quercetin/day for 28 days) (Edwards *et al*., 2007) showed that post-supplementation plasma quercetin concentrations (mean±s.d.) for the placebo

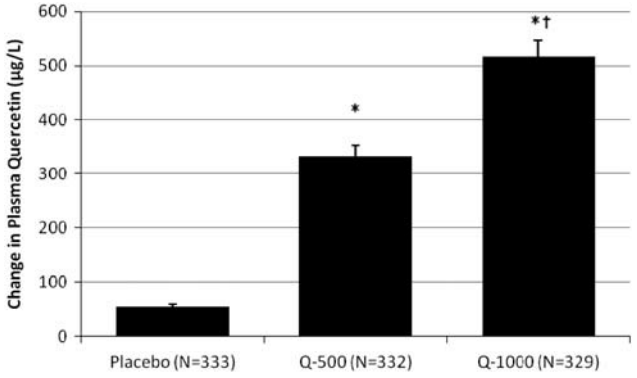

Figure 2 The change in overnight-fasted plasma quercetin concentration pre- and post-study. Values are mean±s.e. (㎎/l). *Mean value was significantly different from that of placebo (*P*o0.001). ᵂMean value was significantly different from that of Q-500 (*P*o0.001).

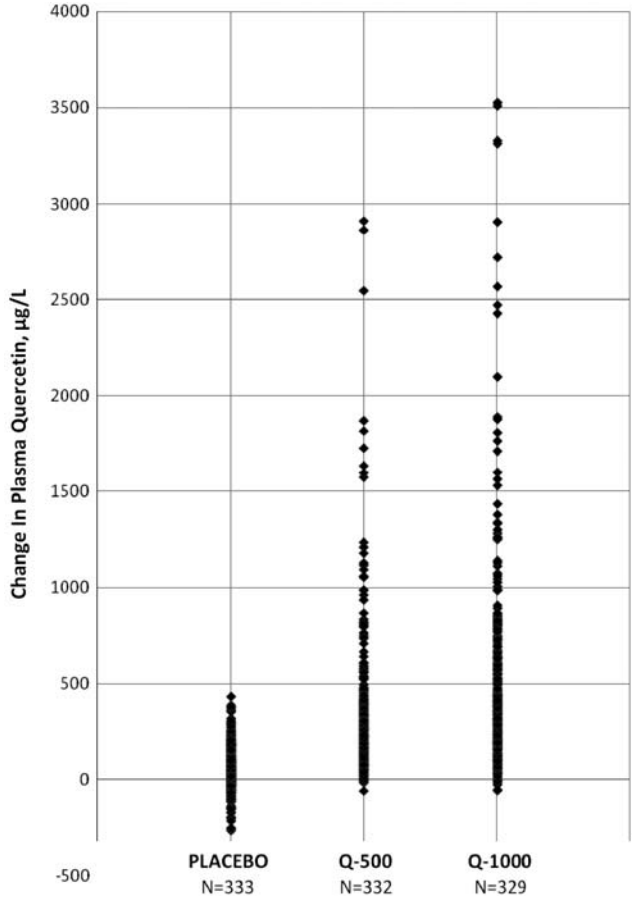

Figure 3 Scatter plot in the change in overnight-fasted plasma quercetin concentration pre- and post-study.

and treatment groups were 210±198 and 429±365 ㎎/l, respectively. The s.d. indicates a wide range of post-supplementation plasma quercetin concentrations.

Egert *et al*. (2008) provided 35 healthy subjects with 50, 100, or 150 mg quercetin/day for 2 weeks. A high inter-individual variation in the increase of plasma quercetin concentrations compared with baseline was reported within each of the three quercetin supplementation groups: 64–370%, 117–1304%, and 139–2343% for the 50, 100, and 150 mg/day groups, respectively. In a subgroup of 15 subjects, plasma pharmacokinetic responses after oral intakes of 50, 100, or 150 mg quercetin varied widely, but no association was found with gender or body weight (mg quercetin per kilogram body weight) and t_{max} values. The variability in plasma quercetin concentrations was higher post-supplementation than at baseline. The authors speculated that some individuals may be better absorbers of oral quercetin supplements because of polymorphisms for intestinal enzymes or transporters (Manach *et al*., 2005; Egert *et al*., 2008). Egert *et al*. suggested that another factor explaining the variant plasma response to quercetin supplementation may include the composition of meals taken around the time of supplementation. Our data agrees that variation in plasma quercetin response with supplementation is unrelated to gender or BMI.

Most recently, a group of 93 overweight subjects with a high-cardiovascular disease risk phenotype completed a double-blinded, placebo-controlled, cross-over study. Subjects ingested 150 mg/day quercetin or placebo over 6-week periods separated by a 5-week washout period (Egert *et al*., 2009). High inter-individual variations were observed in plasma quercetin concentrations post-supplementation, with a range of 13–224 ㎎/l. This variation was unrelated to gender status, as confirmed in our study.

Thus, using data from multiple studies including our own, human subjects can absorb about one-fourth of quercetin ingested from food or supplements, with a resulting plasma concentration that varies widely both acutely and chronically (Erlund *et al*., 2000; Egert *et al*., 2008, 2009). The acute variation in individual dose–response curves after quercetin supplementation seems to increase in parallel with the amount ingested, as inferred by our data. According to Barnes (2008), solving the issue of inter-subject variation in flavonoid bioavailability could involve a coordinated research design using the tools of genomics, transcriptomics, proteomics, and metabolomics. The discovery of specific alterations in single nucleotide polymorphisms in genes that interact with nutrients and other bioactive foods in humans may help explain differences in absorption, metabolism, and functional responses to flavonoid supplementation.

We acknowledge several limitations in the design of our investigation. In particular, we relied on subject recall for lifestyle information including diet intake and physical fitness level. Subjects used a semi-quantitative food frequency questionnaire to indicate typical daily intake for three food groups (fruits, vegetables, and red meats). This approach is often used to divide groups into high, medium, and low intake, but does not provide information

on the volume of specific foods or intake of fats and condiments that may influence quercetin absorption. As addressed in the Materials and methods section of this paper, unpublished animal data support the addition of vitamin C and niacin to quercetin supplements to enhance bioavailability. Thus, we tested the combination of quercetin, vitamin C, and niacin compared with a blank placebo on the plasma quercetin response, and the reader should keep this context in mind.

In summary, a large group of male and female adults varying widely in age and BMI ingested high amounts of quercetin or placebo for 12 weeks. As found in earlier studies, overnight-fasted plasma quercetin levels after supplementation varied widely between subjects within each group. This variation was unrelated to age, gender, BMI, self-reported physical fitness levels, smoking habit, chronic disease status, or diet intake as assessed through a semi-quantitative, food frequency approach. These data support earlier results that the variable plasma quercetin response to supplementation is not associated with demographic or lifestyle factors, but to other unmeasured variables that may include differences in absorption rates because of polymorphisms for intestinal enzymes and transporters.

Conflict of interest

DC Nieman holds a position on the science advisory board for Quercegen Pharma. The remaining authors declare no conflict of interest.

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