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Daily Quercetin Supplementation Dose-Dependently Increases Plasma Quercetin Concentrations in Healthy Humans[1,2]

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Abstract

Our aim was to investigate the effects of an oral supplementation of quercetin at 3 different doses on plasma concentrations of quercetin, parameters of oxidant/antioxidant status, inflammation, and metabolism. To this end, 35 healthy volunteers were randomly assigned to take 50, 100, or 150 mg/d (group Q50–Q150) quercetin for 2 wk. Fasting blood samples were collected at the beginning and end of the supplementation period. Compared with baseline, quercetin supplementation significantly increased plasma concentrations of quercetin by 178% (Q50), 359% (Q100), and 570% (Q150; $P < 0.01$ for all). High interindividual variation was found for plasma quercetin concentrations (36–57%). Quercetin did not affect concentrations of serum uric acid or plasma α- and γ-tocopherols, oxidized LDL, and tumor necrosis factor-α, or plasma antioxidative capacity as assessed by the ferric-reducing antioxidant potential and oxygen radical absorbance capacity assays. In addition, serum lipids and lipoproteins, body composition, and resting energy expenditure did not significantly change during quercetin supplementation. Pharmacokinetics of quercetin were investigated in a subgroup of 15 volunteers. The areas under the plasma concentration-time curves ranged from 76.1 μmol·min·L^{-1} to 305.8 μmol·min·L^{-1} (50- and 150-mg dosages, respectively). Median maximum plasma concentrations of quercetin (431 nmol/L) were observed 360 min after intake of 150 mg quercetin. In conclusion, daily supplementation of healthy humans with graded concentrations of quercetin for 2 wk dose-dependently increased plasma quercetin concentrations but did not affect antioxidant status, oxidized LDL, inflammation, or metabolism. J. Nutr. 138: 1615–1621, 2008.

Introduction

Quercetin is one of the major flavonoids, ubiquitously distributed in (edible) plants, and one of the most potent antioxidants of plant origin (1). Consumption of flavonoids in general and quercetin in particular may be associated with a decreased risk of coronary heart disease and other degenerative diseases (1). The daily intake of quercetin with a typical Western diet was estimated to range between 0 and 30 mg, with a median of 10 mg. The primary dietary sources of quercetin in Western populations are tea, red wine, fruits, and vegetables (2,3). In some countries, quercetin is available as a dietary supplement with daily doses of 200–1200 mg quercetin (manufacturers' recommendations). In addition, quercetin may be used as a nutraceutical for functional foods within a concentration range of 0.008–0.5% or 10–125 mg/serving (4). Recent reviews of the data related to the potential toxicity of quercetin support its safety as a dietary supplement and for the addition to foods (4,5).

Potential health effects of bioactive compounds depend on their bioavailability following oral administration. In plant foods, quercetin is mainly present as various glycosides. It has been repeatedly shown that the sugar moiety is a major determinant of the rate of intestinal absorption of quercetin (6). In addition, matrix effects (i.e. the vehicle used for quercetin administration or the composition of the diet) seem to be important for quercetin bioavailability (6). In all species investigated so far, including humans, pigs, and rats, quercetin and its methylated derivatives with an intact flavonol structure (isorhamnetin, tamarixetin) are not present as free aglyca but only in the conjugated form (mainly glucuronide and sulfate conjugates). Data on bioavailability of quercetin aglycone or quercetin glycosides vary substantially between studies, according to the methods applied for quercetin measurement, and in different species (6). In humans, absorption after oral supplementation ranged from 0 to over 50% of the administered dose (7–9). In pigs, after intake of 50 mg quercetin aglycone · kg body weight^{-1}, Ader et al. (10) calculated a total oral bioavailability of 17% of the applied dose compared with i.v. application.

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As to the biological activities of quercetin, in vitro studies indicate antioxidant, antiinflammatory, antithrombotic, anticarcinogenic, and vasodilatory actions (1). However, quercetin intervention trials in humans have so far shown inconclusive and even conflicting results (1,11). Quercetin had favorable effects on a variety of antioxidant biomarkers, such as antioxidant enzymes, plasma antioxidant capacity, resistance to LDL oxidation, reduced lymphocyte DNA damage, and reduced urinary 8-OH-2′-deoxyguanosine. However, other studies did not support these data (12–17). The majority of human studies used rather high doses of quercetin (and mostly only 1 concentration) and/or quercetin was not administered as a pure compound but via quercetin-rich foods/meals (e.g. white onions, onion soup) containing further phytochemicals, which may have influenced the results.

The aim of this intervention study was to systematically investigate the bioavailability of quercetin from 3 different quercetin doses and its potential effects on parameters of the oxidant/antioxidant status, inflammation, and metabolism in humans. We used moderate supranutritional but not pharmacological doses, because there has been an interest in developing quercetin-rich functional foods that may be beneficial in the prevention of coronary heart disease. Functional foods, by law, can only contain nutritionally relevant concentrations of quercetin (vide infra). However, data regarding the bioavailability of dietary quercetin at nutritionally relevant concentrations and its effects on risk markers for cardiovascular disease are scare.

Methods

Subjects

Thirty-six volunteers (18 women, 18 men) were recruited among university students and former participants of nutritional trials at Kiel University, Germany. Inclusion criteria were: nonsmoking status, a BMI between 19 and 25 kg/m^2, and between 19 and 40 y of age. Exclusion criteria were: metabolic and endocrine diseases, malabsorption syndromes, smoking, overweight, pregnancy/lactation, alcohol abuse, vegetarian or other restrictive dietary requirements, and use of dietary supplements or any form of medication (with the exception of oral contraceptives). All women took oral contraceptives and were instructed not to discontinue their use or change the form of contraception. Each subject underwent a basic examination (body weight and height, blood pressure/pulse, medical anamneses and dietary questionnaire) before the study. All subjects were asked to maintain their regular lifestyles and usual extent of physical activities throughout the study.

The protocol and the objectives of the study were explained to the subjects in detail, who gave their written consent. The study protocol was approved by the ethical committee of the Medical Faculty of the Christian-Albrechts-University of Kiel, Germany and was in accordance with the Helsinki Declaration of 1975, as revised in 1983 and 1989.

All 36 subjects successfully completed the study. One woman was excluded retrospectively because of an unexplained high fasting serum triacylglycerol concentration (5.57 mmol/L) at the end of the study.

Study design

The study was conducted in a double-blinded parallel design and consisted of a 2-wk wash-out period followed by a 2-wk supplementation period. During the wash-out period, all subjects ingested a quercetin-low diet to limit the influence of dietary quercetin on the results of the study. Subjects were given a list of foods and beverages rich in quercetin (such as unpeeled apples, grape products, fruit juice, citrus fruits, berries, onions, broccoli, French beans, kale, tea, and red wine). Because dietary fatty acids, especially (n-3) fatty acids, and tocopherols affect antioxidant status and metabolic parameters (18,19), subjects were requested to exclusively use 1 particular commercial rapeseed oil and 1 margarine, respectively, which were rich in monounsaturated fatty acids. Other dietary sources of fatty acids and/or tocopherols such as nuts, seeds, and fatty fish were not permitted.

After the wash-out period, subjects were randomly assigned to 1 of 3 supplementation groups: group A (50 mg/d quercetin), group B (100 mg/d quercetin), and group C (150 mg/d quercetin). Subjects were instructed to take a total of 6 quercetin capsules per day, 2 capsules with each principal meal. Capsules were distributed weekly with a surplus of 10%.

The hard gelatin capsules contained quercetin dihydrate (Voigt Global Distribution), mannitol, and the flow-regulating excipient silicium dioxide. Quercetin dosages were selected to represent ~5- (50 mg/d), 10- (100 mg/d), or 15-fold (150 mg/d) of the estimated daily dietary quercetin intake in Germany of ~10 mg (2,3).

Compliance was monitored by capsule count at the end of the study and by instructing subjects to document capsule consumption, observed side-effects, deviations from their normal physical activity, or any other observations considered relevant in a study diary.

Dietary analysis

At the beginning and end of the supplementation period, all participants completed a 3-d dietary record (completed on 2 weekdays and 1 weekend day). All entries were analyzed using the computer-based nutrient calculation program EBISpro (E&D Partner) based on the German Nutrient Database Bundeslebensmittelschlüssel, version II.3 (BgVV).

Nutritional status

Body height was measured to the nearest 0.5 cm with a stadiometer during the basic examination. Body composition and body weight were measured at d 0, 7, and 14 of the supplementation period after an overnight fast. Body composition [fat mass (FM)[5] and fat-free mass (FFM)] was determined by air-displacement plethysmography using the BOD POD Body Composition system (Life Measurements Instruments), which is composed of a plethysmograph, an electronic scale, and a computer (software version 1.69). A detailed description of the measurement is given elsewhere (20).

Resting energy expenditure

In a subgroup of 12 volunteers ($n = 4$ per group; 6 females and 6 males), we measured resting energy expenditure (REE) at d 0 and d 14 after an overnight fast by the ventilated hood system (V_{max} model 29n, Sensor Medics; Viasys Healthcare) for 30 min after resting for 10 min during calibration of the system in a metabolic ward as described previously (21). VO_2 and CO_2 production were converted to REE using the abbreviated Weir equation (22). Data for 1 woman were excluded because of hypertriglyceridemia (for details, see Subjects).

Pharmacokinetic study

In a subgroup of 15 volunteers ($n = 5$ per dosage group; 7 females and 8 males), the pharmacokinetics of quercetin were examined at d 7 (halftime of the supplementation period). After an overnight fast, subjects were cannulated and a baseline blood sample was taken. The subjects then ingested their total daily quercetin dose (6 capsules) with a glass of water (200 mL) as part of a standardized breakfast (composition: 2.2 MJ, 24 g protein, 46 g carbohydrates, 27 g fat). Additional blood samples for the determination of plasma concentrations of quercetin and its metabolites were taken at 30, 60, 90, 120, 180, 240, 360, 480, and 1440 min after quercetin ingestion. Lunch was served 4 h after quercetin administration, dinner at 10 h, and snacks at 2 h and 6 h after quercetin administration. All foods contained only minor amounts of flavonoids and were provided at the study site. Subjects were allowed to drink only water. Food and water intakes were documented in a dietary record.

Laboratory measurements

Blood sampling. For the main study, venous blood samples were obtained at the end of the 2-wk wash-out period (d 0, identified as the

[5] Abbreviations used: AUC, area under the plasma concentration-time curve; c_{max}, maximum plasma concentration of quercetin; FFM, fat-free mass; FM, fat mass; FRAP, ferric-reducing antioxidant potential; k, elimination constant; ORAC, oxygen radical absorbance capacity; Q50, Q100, Q150, group designations; REE, resting energy expenditure; $t_{1/2}$, elimination half-life; t_{max}, time between oral administration of quercetin and the appearance of c_{max}; TNF, tumor necrosis factor.

study baseline) and the end of the supplementation period (d 14). All samples were taken between 0730 and 0845 after an overnight fast under standardized conditions. The last 2 quercetin capsules were taken ~12 h before blood sampling. Blood was drawn into tubes containing EDTA, lithium heparin, or no additives (Sarstedt). Plasma and serum were obtained by centrifugation at 2000 × *g*; 15 min at 4°C. Aliquots of plasma and serum were immediately frozen and stored in gas-tight cryovials at −75°C until analysis.

Plasma quercetin and its metabolites. Analyses of plasma concentrations of quercetin and the monomethylated derivatives isorhamnetin (3′-*O*-methyl quercetin) and tamarixetin (4′-*O*-methyl quercetin) were performed by HPLC with fluorescence detection as described previously (10). All samples were treated enzymatically with β-glucuronidase/sulfatase type H-2 (crude enzyme extract from *Helix pomatia*; Sigma-Aldrich AG) prior to the extraction of the flavonols. Authentic flavonols (Carl Roth) were used as external standards.

Plasma α- and γ-tocopherol and tumor necrosis factor-α. Concentrations of α- and γ-tocopherol in plasma were determined by HPLC with fluorescence detection (Jasco) as previously described (23). The concentrations of α- and γ-tocopherol were quantified using authentic tocopherols (Calbiochem) as external standards. Plasma concentrations of tumor necrosis factor (TNF)-α were determined with a high-sensitivity ELISA kit (R&D Systems, Europe) according to the manufacturer's protocol.

Plasma antioxidant capacity and ox-LDL concentrations. The antioxidant capacity of plasma was assessed by measuring the ferric-reducing antioxidant potential (FRAP) and the oxygen radical absorbance capacity (ORAC). FRAP was determined according to the method of Benzie and Strain (24). Briefly, plasma samples were mixed with FRAP reagent, incubated for 15 min at 37°C, and the absorbance at 593 nm recorded in a spectrophotometer (DU800; Beckmann Coulter).

ORAC was determined according to the method of Cao et al. (25). 2,2′-Azobis(2-amidinopropan) was used to generate peroxyl radicals, trolox served as control and sodium fluorescein as fluorescent probe. In brief, 25 μL of blank, trolox standard or acetone precipitated plasma samples were mixed with sodium flourescein and incubated at 37°C for 10 min. Subsequently, 25 μL 2,2′-azobis(2-amidinopropan) solution was added and fluorescence was measured at an excitation wavelength of 485 nm and an emission wavelength of 520 nm at 60-s intervals for 50 min. Plasma ox-LDL concentrations were measured using a commercial ELISA kit (Immundiagnostik AG) according to the manufacturer's protocol.

Serum uric acid and lipid concentrations. Fasting serum concentrations of uric acid, total cholesterol, and triacylglycerols were measured in series on a VITROS 950 Chemistry System autoanalyzer (Ortho-Clinical Diagnostics) with the manufacturer's assay kits, quality controls, and reagents. LDL-cholesterol was calculated by use of the Friedewald equation.

Data analysis

REE was adjusted for FFM by use of a linear regression analysis according to Ravussin and Bogardus (26). Pharmacokinetic parameters were calculated using the computer program Origin (version 7, OriginLab). Plasma quercetin concentrations obtained at the different time points (T30–T1440) of the pharmacokinetic study were corrected for individual baseline quercetin concentrations (T0). The maximum observed plasma concentration (c_{max}) and the time to reach c_{max} (t_{max}) were determined by visual inspection of each subject's plasma concentration-time profile. The rate of absorption and rate of elimination was calculated by linear regression. The area under the plasma concentration-time curve (AUC) was determined according to the linear trapezoidal rule. The slope of the terminal log-linear portion of the concentration-time profile was determined by least-squares regression analysis and used as the elimination rate constant (k). The terminal elimination half-life ($t_{1/2}$) was calculated as ln2/k.

Rate of elimination, k, and $t_{1/2}$ values of plasma quercetin could not be determined for individual concentration time-curves, because too few time points were available during the elimination phase. Therefore, these kinetic parameters were calculated only from the mean value curves obtained in response to the 3 different quercetin doses.

Statistical analysis

All statistical analyses were performed using the SPSS statistical software package (version 13). Distribution of the data was checked using normal plots and histograms of the data and by performing Kolmogorov-Smirnov tests and Shapiro-Wilk tests. Between-group comparisons were analyzed by ANOVA followed by post hoc Tukey test, if data were normally distributed, and by Kruskal-Wallis H-test und Mann-Whitney U-test if not. Within-group comparisons were performed using the paired *t* tests if data were normally distributed and analyzed by the Wilcoxon's matched-pairs signed-ranks test if not. Unless otherwise indicated, results are expressed as means ± SD or for skewed data as median and 25th, 75th percentiles. All tests were 2-tailed and a $P < 0.05$ level of significance was used to assess significance. Spearman rank correlation coefficient was used for relationships between different parameters.

Results

Baseline characteristics, compliance, adverse effects, and nutrition. Baseline characteristics of the study subjects are presented in **Table 1**. Subjects took 97.2% of the provided capsules with no differences among the groups. No adverse effects of quercetin intake were reported. Three-day dietary records showed no significant differences between groups and within groups (comparing baseline and end of the supplementation period) in mean daily intakes of energy (10.3 MJ/d), protein (15.3% of energy intake), carbohydrates (45.0% of energy intake), fat (36.5% of energy intake), fatty acids, cholesterol, antioxidants (vitamin E, 1.3 mg/MJ; vitamin C, 10.0 mg/MJ), or dietary fiber (data not shown).

Plasma concentrations of quercetin, isorhamnetin, and tamarixetin. Supplementation with 50, 100, or 150 mg/d quercetin (Q50, Q100, or Q150, respectively) increased plasma quercetin concentrations by 178% (median change: 92.2 nmol/L; $P < 0.01$), 359% (median change: 171.8 nmol/L; $P < 0.01$), or

TABLE 1 Baseline characteristics of the subjects[1]

Variable	Total group	Men	Women
n	35	18	17
Age, *y*	26.2 ± 3.7	27.0 ± 4.6	25.4 ± 2.0
Body height, *m*	1.75 ± 0.11	1.82 ± 0.09	1.67 ± 0.06*
Body weight, *kg*	68.1 ± 12.6	77.1 ± 9.5	58.7 ± 7.3*
BMI, *kg/m²*	22.1 ± 2.2	23.2 ± 1.9	20.9 ± 1.7*
FM, *kg*	11.8 ± 4.7	9.4 ± 4.6	14.3 ± 3.4*
FM, *%*	18.0 ± 7.9	12.1 ± 5.6	24.2 ± 4.6*
FFM, *kg*	56.4 ± 14.0	67.7 ± 9.3	44.4 ± 5.5*
Blood pressure, *mm Hg*			
Systolic	122.6 ± 8.5	125.6 ± 10.4	119.4 ± 4.3
Diastolic	76.0 ± 4.8	74.7 ± 4.7	77.4 ± 4.7
Plasma glucose, *mmol/L*	4.6 ± 0.4	4.8 ± 0.4	4.4 ± 0.3*
Serum total cholesterol, *mmol/L*	4.52 ± 0.94	4.13 ± 0.70	4.93 ± 1.01*
Serum LDL cholesterol, *mmol/L*	2.29 ± 0.65	2.26 ± 0.61	2.33 ± 0.71
Serum HDL cholesterol, *mmol/L*	1.73 ± 0.45	1.43 ± 0.26	2.04 ± 0.39*
Serum triacylglycerols, *mmol/L*	1.06 (0.78, 1.26)	0.95 (0.66, 1.14)	1.20 (0.82, 1.52)
Plasma α- + γ-tocopherol, *μmol/L*	31.8 (27.4, 36.6)	29.0 (26.7, 33.8)	34.8 (28.9, 38.3)*

[1] Values are means ± SD or medians (25th, 75th percentile). *Different from men, $P < 0.05$.

570% (median change: 316.2 nmol/L; $P < 0.01$), respectively (**Table 2**). There was a high interindividual variation in plasma quercetin concentrations at baseline (range for all study subjects: 30.0–163 nmol/L) and after quercetin supplementation (Q50, 95.9–255 nmol/L; Q100, 164–497 nmol/L; Q150, 240–1292 nmol/L). Interindividual variances of plasma quercetin levels at the end of the supplementation period were: Q50, 36%; Q100, 40%; and Q150, 57%. In addition, there was a high interindividual variation in the increases of plasma quercetin concentrations within the 3 supplementation groups [Q50 (min-max), 38–194 nmol/L or 64–370%; Q100, 90–462 nmol/L or 117–1304%; Q150, 177–1239 nmol/L or 139–2343%].

There were no significant gender-specific differences in quercetin concentrations at baseline and after quercetin supplementation and also no significant gender-specific differences in changes of quercetin levels (d 0 compared with d 14).

All 3 dosages of quercetin significantly increased plasma concentrations of isorhamnetin (median changes: Q50, 5.2 nmol/L or 130%; Q100, 11.8 nmol/L or 303%, Q150, 22.2 nmol/L or 2018%; Table 2). Significant increases of plasma concentrations of tamarixetin occurred during the 2-wk supplementation of 50 mg/d and 150 mg/d quercetin. Isorhamnetin and tamarixetin were not detected in the fasting plasma samples of any subjects (Table 2). During the supplementation period, the methylated derivatives of quercetin accounted for only 7–8% of total plasma flavonoids. Therefore, pharmacokinetic evaluations were performed using only data for quercetin.

Pharmacokinetic study. After ingestion of 150 mg quercetin aglycone, maximal plasma quercetin concentrations were significantly higher than after ingestion of 50 mg quercetin (**Table 3**). However, the rate of absorption did not differ significantly between the 3 quercetin dosages (Table 3). The median AUC from 0 to 1440 min was 4 times higher after administration of 150 mg than after 50 mg (Table 3). The elimination rates were: Q50, 0.09 $nmol \cdot min^{-1} \cdot L^{-1}$; Q100, 0.15 $nmol \cdot min^{-1} \cdot L^{-1}$; and Q150, 0.29 $nmol \cdot min^{-1} \cdot L^{-1}$. The constants of quercetin elimination were: Q50, $7 \cdot 10^{-4}$ min^{-1}; Q100, $8 \cdot 10^{-4}$ min^{-1}; and Q150, $7 \cdot 10^{-4}$ min^{-1}. The $t_{1/2}$ were Q50, 16.5 h; Q100, 14.1 h; and Q150, 16.8 h.

Pharmacokinetic parameters and individual time courses of the quercetin plasma concentrations (**Fig. 1**) showed a high interindividual variance. No association was found between body weight (mg oral dose quercetin · kg body $weight^{-1}$) and t_{max} values. No significant differences were observed between women and men.

Antioxidant capacity of plasma and concentrations of antioxidants, oxidized LDL, and TNFα. Quercetin supplementation did not significantly affect serum concentrations of uric acid, plasma concentrations of α- and γ-tocopherol, ox-LDL, high-sensitive TNFα, or plasma antioxidant capacity (FRAP and ORAC) (data not shown). There were no significant associations between ox-LDL concentrations and lipoprotein-lipid profile variables (total cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerols) and also no significant associations between ox-LDL concentrations and TNFα (data not shown).

Serum lipids and energy expenditure. Irrespective of the dose applied, quercetin supplementation did not significantly affect fasting serum concentrations of triacylglycerols or total, LDL, or HDL cholesterol. The total cholesterol:HDL cholesterol ratio was unchanged (data not shown). In addition, there were no significant effects on REE, REE_{adj}, or respiratory quotient (data not shown). Body weight and body composition (FM, FFM) did not change significantly throughout the trial (data not shown).

Discussion

In healthy volunteers, supplementation of diets with 50, 100, or 150 mg/d quercetin aglycone for 2 wk resulted in a significant, dose-dependent accumulation of quercetin in plasma. The pharmacokinetics of plasma quercetin from quercetin aglycone differed depending on the quercetin dosages given. The AUC as a measure of bioavailability ranged from 76.1 $\mu mol \cdot min \cdot L^{-1}$ (50 mg dosage) to 305.8 $\mu mol \cdot min \cdot L^{-1}$ (150 mg dosage). Plasma tocopherol levels, serum uric acid, parameters of oxidative stress, inflammation, and metabolism remained unaffected by daily quercetin supplementation.

Bioavailability and pharmacokinetics of quercetin. The plasma concentrations of (total) quercetin measured represent all unconjugated plus conjugated forms of quercetin, because plasma samples were enzymatically treated to hydrolyze conjugated quercetin. The analytical method applied does, however, not allow us to differentiate between different conjugates, such as glucuronides and sulfates. Our results agree with other human studies that suggest that quercetin is absorbed after oral supplementation of quercetin aglycone (6,27). Hollman et al. (7) reported that 24% of quercetin is absorbed after consumption of quercetin aglycone (100 mg/d) in ileostomized but otherwise healthy volunteers. This was confirmed in several additional studies in subjects with an intact colon (28,29).

In accordance with previous human studies (13,30–33), we observed a relatively high interindividual variability in plasma quercetin concentrations in our relatively homogeneous study population. Before and during the study, a low quercetin diet was

TABLE 2 Plasma concentrations of quercetin, isorhamnetin, and tamarixetin in men and women supplemented for 2 wk with 50, 100, or 150 mg/d quercetin[1–3]

Variable	Time	Q50		Q100		Q150	
n		11		12		12	
Quercetin,	Baseline	52.5 (44.4, 58.9)	[11]	44.9 (34.4, 74.9)	[12]	64.2 (51.63, 70.88)	[12]
nmol/L	End	145 (110, 190)*[a]	[11]	217 (176, 252)*[b]	[12]	380 (331.4, 636.3)*[c]	[12]
Isorhamnetin,	Baseline	4.0 (3.0, 6.0)	[11]	3.9 (1.5, 6.0)	[11]	1.1 (0.0, 7.1)	[6]
nmol/L	End	9.2 (6.8, 10.0)*[a]	[11]	15.7 (13.2, 20.7)*[b]	[12]	23.3 (15.6, 33.6)*[b]	[12]
Tamarixetin,	Baseline	0.0 (0.0, 0.0)	[0]	0.0 (0.0, 0.0)	[0]	0.0 (0.0, 0.0)	[1]
nmol/L	End	3.4 (0.0, 5.1)*[a]	[7]	0.0 (0.0, 0.0)[b]	[2]	6.2 (0.3, 17.2)*[ac]	[10]

[1] Values are medians (25th, 75th percentile). *Different from baseline, $P < 0.05$. Medians in a row with superscripts without a common letter differ, $P < 0.05$.
[2] Results below the limit of detection have been denoted the value 0.
[3] Values in angled parentheses indicate the number of subjects of all subjects having plasma concentrations above the limit of detection.

TABLE 3 Plasma pharmacokinetic parameters of quercetin after oral intake of 50, 100, or 150 mg quercetin in a subgroup of 15 volunteers[1]

Variable	Q50	Q100	Q150
n	5	5	5
c_{max}, *nmol/L*	189 (141, 250)[a]	295 (188, 459)[ab]	431 (242, 529)[b]
t_{max}, *min*	120 (120, 330)	180 (105, 420)	360 (210, 420)
AUC_{0-1440}, $\mu mol \cdot min \cdot L^{-1}$	76.1 (60.4, 135.0)	108.0 (77.6, 194.0)	305.8 (63.4, 382.0)
AUC_{0-480}, $\mu mol \cdot min \cdot L^{-1}$	49.3 (39.9, 55.4)[a]	65.7 (56.7, 104.6)[b]	99.2 (57.7, 161.9)[b]
Rate of absorption, $nmol \cdot min^{-1} \cdot L^{-1}$	1.2 (0.6, 1.8)	1.3 (0.9, 2.2)	1.1 (0.8, 1.9)

[1] Values are median (25th, 75th percentile). Medians in a row with superscripts without a common letter differ, $P < 0.05$.

eaten, suggesting that changes in plasma quercetin levels were almost exclusively due to quercetin intake from capsules. The variability in plasma quercetin concentrations was higher at the end of the supplementation period than at baseline. Thus, it is possible that some individuals may be better absorbers than others, possibly because of particular polymorphisms for intestinal enzymes or transporters (27). However, other explanations, such as meal composition and/or matrix effects, should be also taken into account. In particular, alcohol and the macronutrients fat, protein, and carbohydrates have been proposed to affect the bioavailability of flavonoids (6). However, human studies examining the influence of dietary factors on the bioavailability of quercetin are sparse. Graefe et al. (31) conducted a 4-way crossover study in 12 healthy volunteers to determine the influence of food matrix on absorption of quercetin. Each subject received an onion supplement or quercetin-4′-*O*-glucoside (both equivalent to 200 mg quercetin) as well as quercetin-3-glucorhamnosid (rutin) and buckwheat tea (both equivalent to 200 mg quercetin). The bioavailability and pharmacokinetic parameters did not differ between the onion supplement or quercetin-4′-*O*-glucoside. To a minor extent, the plant matrix appeared to influence the rate and extend of absorption of quercetin from buckwheat tea compared with the isolated compound. In pigs, Lesser et al. (34) showed that the oral bioavailability of quercetin aglycone from a test meal was enhanced by 57% by the addition of 15% (wt:wt) fat to a low-fat standard pig diet. It was speculated that the enhancement of quercetin bioavailability may have been the result of improved solubility of the lipophilic quercetin aglycone in the intestinal tract and/or additional absorption of quercetin via the lymphatic system in the presence of fat. In humans, however, a typical Western-type diet is likely to contain sufficient dietary fat to facilitate the absorption of quercetin (34). In subjects participating in our study, mean daily energy intake from dietary fat was 36% of total energy intake (96 g/d fat).

Intersubject variability was also demonstrated by the data of our pharmacokinetic study. In some subjects, the individual plasma appearance-time curve of quercetin exhibited reentry peaks (Fig. 1). This is accordance with results from Erlund et al. (29), who compared the pharmacokinetics of quercetin from aglycone and rutin in humans, and Moon et al. (33), who examined the pharmacokinetics of quercetin aglycone, as well as its conjugated metabolites.

After oral ingestion of quercetin aglycone, the median plasma concentrations of total quercetin peaked at 120–360 min in our volunteers (Table 3). Similar findings have been reported by Erlund et al. (29) and Moon et al. (33). In accordance with Erlund et al.

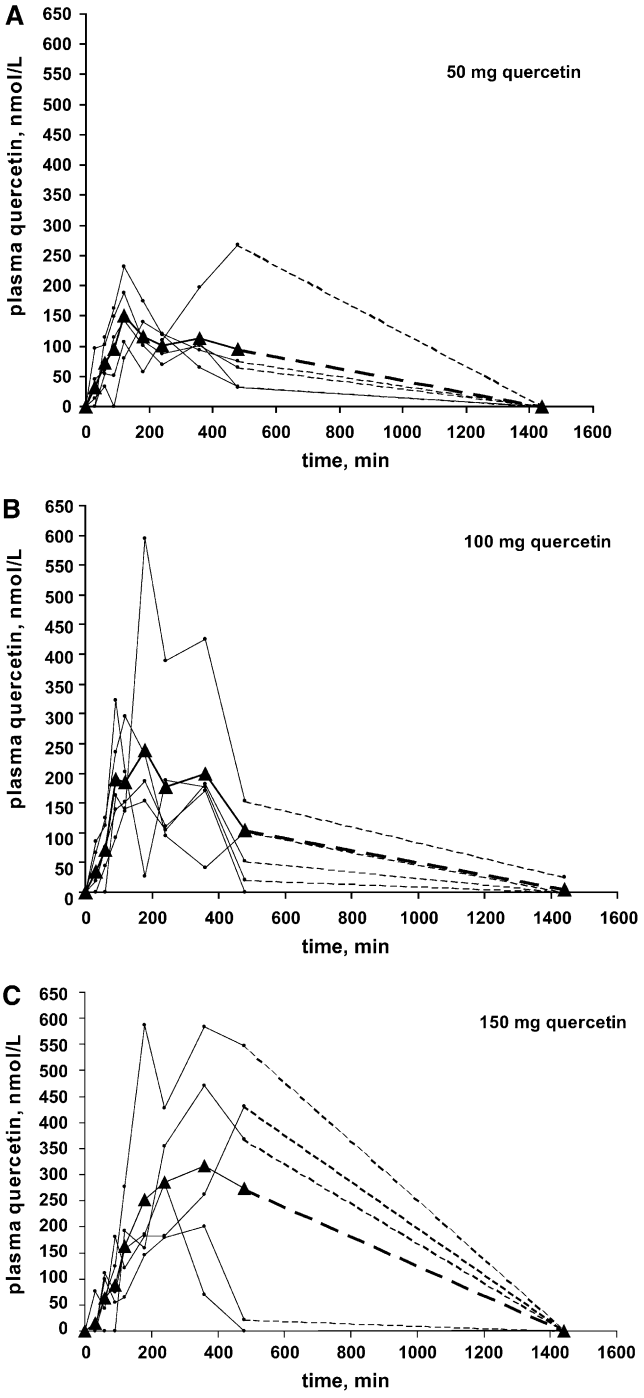

FIGURE 1 Mean (bold line) and individual (fine line) plasma concentration-time curves of quercetin after oral intake of 50 (*A*), 100 (*B*), or 150 mg (*C*) quercetin to a subgroup of 15 volunteers ($n = 5$ per dosage group). The last 2 data points are connected by a dashed line, because elimination processes during this time cannot be reliably determined.

(29), we found a linear trend for the median c_{max} and AUC values after intakes of all doses of quercetin aglycone. Both c_{max} and AUC values increased with increasing doses. In contrast, the rates of absorption as well as the parameters of elimination, such as the rate of elimination and $t_{1/2}$, appeared to be similar in all 3 dosage groups. However, a correction for the quercetin dose given (division by dose) revealed that the rates of absorption and elimination appeared to decrease with increasing quercetin intake. Our mean elimination $t_{1/2}$ values of ~16 h are in accordance with

previous estimations of 11–28 h in humans (27), suggesting that the maintenance of high plasma concentrations of quercetin metabolites could be achieved with regular and frequent consumption of quercetin-containing natural and/or functional foods.

Effects on markers of oxidative stress. Although we observed elevated concentrations of quercetin in the plasma of quercetin-supplemented subjects, we could not measure quercetin-evoked changes in the oxidant/antioxidant status using plasma FRAP, ORAC, plasma levels of ox-LDL, serum uric acid, and plasma tocopherols in the study subjects. This finding is in accordance with data obtained in other human intervention trials examining the potential effects of quercetin supplementation (quercetin aglycone or quercetin glycosides) on antioxidant biomarkers (15,17,35). Thus, the in vivo findings in humans are in contrast to results obtained from in vitro experiments and animal studies indicating a strong antioxidant potential of quercetin (30,36–38). There are several reasons that may explain the lack of effects on parameters of oxidant/antioxidant status. First, in our protocol, plasma concentrations of quercetin (Q150, 380 nmol/L) may have been too low to improve the plasma antioxidant status. In fact, quercetin concentrations were below those shown to be effective in in vitro assays (39). Second, our subjects were young, healthy, and had an adequate dietary intake of essential antioxidants (vitamins E, C, β-carotene). In addition, they did not smoke or perform excessive physical exercise. Accordingly, plasma tocopherol and serum concentrations of cardioprotective HDL cholesterol were high in our volunteers (Table 1). Hence, an effect of quercetin to improve biomarkers of oxidative stress in our subjects was unlikely to occur. Third, flavonoids, such as quercetin, are carried in rat and human blood by serum albumin in the form of complexes between albumin and quercetin 3-*O* conjugates (mainly glucuronides and sulfates) (40,41). At present, antioxidant activity of quercetin metabolites bound to albumin is unclear.

Quercetin, oxidative stress, and inflammation. Experimental studies showed that quercetin inhibits the gene expression and production of TNFα by peripheral blood mononuclear cells via modulation of the nuclear factor-$\kappa\beta$ signal transduction cascade (42). Zern et al. (43) recently found that dietary supplementation with grape powder containing quercetin in pre- and postmenopausal women significantly decreased oxidative stress as measured by F2-isoprostanes and also decreased the levels of plasma TNFα. As previously demonstrated, TNFα and other cytokines and growth factors are stimulated by reactive oxygen species and ox-LDL (44,45). Our finding that quercetin supplementation did not decrease the concentrations of the pro-inflammatory cytokine TNFα might be explained by a general lack of quercetin to reduce TNFα under the conditions prevailing in the present study or could be due to the lack of oxidative stress in our study subjects.

Effects of quercetin on metabolism. Recent studies provide evidence that some flavonoids, particularly green-tea catechins such as epigallocatechin gallate, may have metabolic effects in animals and humans. For example, in healthy normal-weight humans, consumption of green tea extracts has been shown to increase fat oxidation and energy expenditure, particularly if combined with a metabolic stimulant such as caffeine (46–48), and to reduce total and abdominal fat in subjects with visceral fat-type obesity (49). To our knowledge, no previous study has evaluated whether quercetin supplementation had similar effects. In our study, quercetin supplementation did not affect energy expenditure and body composition. Contrary to animal studies, where flavonoids reduced triacylglycerol and total cholesterol concentrations (50,51), we found no significant effects on serum lipids and lipoproteins. This is in accordance with other human studies (15,17).

In conclusion, moderate daily supplementation of healthy young humans with graded concentrations of quercetin for 2 wk dose-dependently increased plasma quercetin concentrations but did not affect parameters of antioxidant status, inflammation, and metabolism.

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