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Antioxidant activity and metabolite profile of quercetin in vitamin-E-depleted rats

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Abstract

Dietary antioxidants interact in a dynamic fashion, including recycling and sparing one another, to decrease oxidative stress. Limited information is available regarding the interrelationships in vivo between quercetin and vitamin E. We investigated the antioxidant activity and metabolism of quercetin (Q) in 65 F-344 rats (n=13 per group) randomly assigned to the following vitamin E (VE)-replete and -deficient diets: (a) VE replete (30 mg α-tocopherol acetate/kg diet) control ad libitum (C-AL), (b) VE replete pair fed (C-PF), (c) VE replete+5.0 g Q/kg diet (R-VE+5Q), (d) VE deplete (<1 mg/kg total tocopherols)+5.0 g Q/kg diet (D-VE+5Q) and (e) D-VE. After 12 weeks, blood and tissue were collected for measurement of plasma vitamin E, quercetin and its metabolites, serum pyruvate kinase (PK), plasma protein carbonyls, malondialdehyde (MDA) and oxygen radical absorbance capacity. D-VE diets decreased serum α-tocopherol and increased PK activity in a time-dependent manner. The D-VE diet increased plasma protein carbonyls but did not affect MDA. Dietary quercetin supplementation increased quercetin and its metabolites in plasma and liver but did not affect D-VE-induced changes in plasma α-tocopherol, PK or protein carbonyls. Plasma isorhamnetin and its disposition in muscle were enhanced by the D-VE diet, as compared to the R-VE diet. Conversely, tamarixetin disposition in muscle was decreased by the D-VE diet. Thus, quercetin did not slow vitamin E decline in vivo; neither did it provide antioxidant activity in vitamin-E-depleted rats. However, vitamin E status appears to enhance the distribution of isorhamnetin into the circulation and its disposition in muscle.

Keywords: Antioxidants; Metabolism; Quercetin; Rats; α-Tocopherol

1. Introduction

Observational studies suggest that the consumption of dietary flavonoids is associated with a decreased risk of some cancers and cardiovascular disease [1,2]. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a ubiquitous flavonol in human diets found especially in fruits, vegetables, tea and red wine [3]. In addition to its antioxidant activity, the putative health effects of quercetin have been attributed to bioactivities such as the induction of apoptosis, antimutagenic actions, modulation of the cell cycle and inhibition of angiogenesis and angiotensin-converting enzyme II [3–8]. However, it is important to note that the effects of quercetin may be mediated substantially by its metabolites [9].

Upon absorption in the gastrointestinal tract, quercetin is metabolized by Phase II enzymes in gastric and intestinal epithelial cells, and conjugated metabolites are further metabolized in the liver and kidney [9,10]. The B-ring catechol structure is methylated at the 3′ or 4′ hydroxyl site by catechol-*O*-methyl transferase (COMT), resulting in the formation of isorhamnetin and tamarixetin, respectively [11]. Quercetin metabolites appear to accumulate in tissues even after short-term ingestion of quercetin-rich vegetables [12]. In vitro, quercetin metabolites generated in enterocytes and

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liver have been found to serve as antioxidants by increasing the resistance of low-density lipoprotein (LDL) cholesterol to oxidation [13]. Flavonoids have also been found to interact in synergy with α-tocopherol in protecting LDL against oxidation [14].

Reports on interactions between quercetin and vitamin E within the antioxidant defense network are mixed. For example, Choi et al. [15] reported that quercetin administration elevated both serum and liver α-tocopherol concentrations, with the greatest increases occurring in rats fed vitamin-E-deplete diets, as compared to rats on vitamin-E-replete diets. Similarly, Frank et al. [16] reported that dietary flavonoids, quercetin and catechins increase serum and tissue α-tocopherol. In contrast, Benito et al. [17] and Fremont et al. [18] found no effect of quercetin administration in rats on LDL α-tocopherol or total plasma and HDL α-tocopherol, respectively.

To investigate this relationship further, we fed quercetin to vitamin-E-replete or vitamin-E-deficient rats for 12 weeks and measured the status of α-tocopherol, quercetin and its principal metabolites, as well as relevant biomarkers of antioxidant action.

2. Materials and methods

2.1. Chemicals

The following reagents were obtained from Sigma (St. Louis, MO): adenosine diphosphate sodium salt (Na-ADP), phosphoenolpyruvate (PEP), lactic dehydrogenase (LDH; EC 1.1.1.27), reduced β-nicotinamide adenine dinucleotide phosphate (β-NADH) and pyruvate kinase (PK; EC 2.7.1.40).

2.2. Animals and diets

Sixty-five male weanling (3 weeks old) F-344 rats with a mean body weight of 80.8±0.6 g were obtained from Harlan (Dublin, VA) and individually housed in wire cages at 25°C with a 12-h light–dark cycle throughout the study in the Comparative Biology Unit of the Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University. This study was approved by the Tufts HNRCA Animal Care and Use Committee.

A modified vitamin-E-deficient AIN-93G basal mix with vitamin-E-stripped soybean oil (<10 mg/kg total tocopherol) was obtained from Dyets Inc. (Bethlehem, PA). Vitamin-E-deficient and adequate vitamin mixes were obtained from Harlan Teklad (Madison, WI). The tocopherol content was analyzed in each set of ingredients and in the final diets. Quercetin dihydrate (98% purity) was purchased from Alfa Aesar (Ward Hill, MA). The diets were prepared according to AIN-93G guidelines [19], with the replete diet containing 30 mg α-tocopherol acetate/kg to meet the vitamin E allowance for rats [20] and the deficient diet containing <1 mg α-tocopherol/kg. Quercetin (Q) was supplemented at 5.0 g/kg diet. This dose was derived from the results of a preliminary study indicating 5-, 11-, 12- and 14-fold increases in plasma quercetin after feeding doses of 0.5, 2.5, 5 and 10 mg Q/kg diet for 14 days. With a paucity of reports on the potential toxicity of quercetin, we chose the 5-g/kg dose to increase quercetin status and to minimize the risk of possible untoward events over the longer period of the full study (12 weeks). The diets were stored at −20°C under N_2.

2.3. Experimental design

All rats were fed the vitamin-E-replete diet for 1 week while acclimating to the facility. The animals were weight matched and divided into five groups (n=13/group), as shown in Table 1. Prior to the initiation of the dietary intervention, plasma and serum were prepared from orbital bleeding to assess the baseline concentrations of plasma quercetin, serum vitamin E and PK. Except for animals in the control ad libitum (C-AL) group, all rats were pair fed their respective diets with the lowest daily feed intake of all rats in other groups for 12 weeks. Plasma α-tocopherol and PK were monitored every 2 weeks. At the end of the feeding period, rats were fasted for 2 h, blood was collected via orbital bleeding under Aerrane (Barter, Deerfield, IL) anesthesia and tissues were collected after carbon dioxide asphyxiation. All samples were immediately frozen in liquid nitrogen and stored at −80°C until analyzed. Tissues were pulverized under liquid N_2, and the powder was thoroughly mixed prior to analysis.

2.4. α- and γ-tocopherol

α- and γ-tocopherol in plasma and tissues were measured according to Martin et al. [21], using tocol as an internal standard. α- and γ-tocopherol were determined after separation by HPLC using a 3-mm C18 reverse-phase column (Perkin-Elmer, West Lafayette, IN) with the mobile phase (1% H_2O in methanol with 10 mM lithium perchlorate) delivered at 1.0 ml/min. Eluted peaks were detected at an applied potential of +0.6 V by an LC 4B amperometric electrochemical detector (Bioanalytical Systems, Austin, TX).

2.5. Pyruvate kinase

Serum PK activity was measured according to Dhahbi et al. [22] and Beutler et al. [23]. Briefly, 100 μl diluted serum was added to 880 μl of the reaction cocktail [100 mmol/L

Table 1
Designation of dietary groups

Dietary group	α-Tocopheryl acetate (mg/kg)	Quercetin (g/kg)
Control diet, ad libitum (C-AL)	30	0
Control diet, pair fed (C-PF)	30	0
Quercetin, pair fed (R-VE+5Q)	30	5
VE depletion+quercetin, pair fed (D-VE+5Q)	<1	5
VE depletion, pair fed (D-VE)	<1	0

Tris–HCl (pH 8), 100 mmol/L KCl, 10 mmol/L $MgCl_2$, 0.2 mmol/L NADH, 6 mmol/L ADP and 350 U/L LDH] and incubated for 10 min at 37°C. After addition of 20 μl PEP (0.3 mol/L), the decrease in NADH absorbance at 340 nm was recorded until extinction. Enzyme activity was calculated from a calibration curve constructed with a PK standard.

2.6. Quercetin, isorhamnetin and tamarixetin

Quercetin, isorhamnetin and tamarixetin in plasma and tissues were analyzed according to Chen et al. [14]. Briefly, 200 μl plasma was incubated with 20 μl vitamin C–EDTA [200 mg ascorbic acid plus 1 mg EDTA in 1.0 ml of 0.4 mol/L NaH_2PO_4 (pH 3.6)] and 20 μl β-glucuronidase (98,000 U/L β-glucuronidase and 2400 U/L sulfatase) at 37°C for 45 min. Hydrolyzed metabolites were extracted with acetonitrile, dried under purified N_2 and reconstituted in 200 μl of the aqueous HPLC mobile phase. Aliquots of the supernatants were analyzed by HPLC after centrifugation at 14,000×*g* for 5 min. Tissue samples (0.5 g) were first homogenized in 5 ml acetonitrile containing 0.01% BHT. The supernatant was removed after centrifugation, dried under purified N_2, reconstituted in 500 μl sodium acetate buffer, incubated with β-glucuronidase at 37°C overnight and then treated the same as plasma samples. All HPLC procedures were performed with ESA instruments, including two pumps (Model 582), an autosampler (Model 542) and a Coularray 5600 A detector. Analyte separation was achieved using a Zorbax ODS C18 column (4.6×150 mm, 3.5 μm) with a 0.6-ml/min flow rate and mobile phase gradient from Phase A (75 mmol/L citric acid and 25 mmol/L ammonium acetate in 90% H_2O and 10% acetonitrile) to Phase B (75 mmol/L citric acid and 25 mmol/L ammonium acetate in 50% H_2O and 50% acetonitrile) for 68 min. The following mobile phase gradient profile was used (% Solvent B): 1% (0–15 min), 1–10% (15–25 min), 10–80% (25–60 min), 80–10% (60–65 min) and 10–1% (65–68 min). Detection was achieved with potentials applied from 60 to 720 mV with 60-mV increments. The recovery rate of internal standard 2′,3′,4′-trihydroxyacetophenone, spiked in the sample before extraction and enzyme digestion, was in the range of 75–80%. Detection limit on the column was 2.5 ng for quercetin, isorhamnetin and tamarixetin. Flavonoid concentrations were calculated from calibration curves constructed from authenticated standards purchased from Sigma. The linearity of the standard curves for quercetin, isorhamnetin and tamarixetin is reflected by *r* values of .998, .995 and .997, respectively.

2.7. Oxygen radical absorbance capacity

Plasma antioxidant capacity was determined by the modified oxygen radical absorbance capacity ($ORAC_{total}$ and $ORAC_{pca}$) assays described by Huang et al. [24] on a microplate reader (FLUOstar OPTIMA, BMG LABTECH GmbH, Germany). $ORAC_{total}$ reflects the total antioxidant capacity due to all hydrophilic plasma antioxidant components. $ORAC_{pca}$ measures the antioxidant capacity of the nonprotein plasma antioxidants after protein precipitation by perchloric acid (PCA).

2.8. Malondialdehyde

Plasma malondialdehyde (MDA) was determined by reverse-phase HPLC with the thiobarbituric acid–MDA conjugate injected into a C18 column and quantified with fluorescence detection (excitation, 515 nm; emission, 553 nm) according to Volpi and Tarugi [25].

2.9. Protein carbonyls

Protein carbonyls in plasma and tissues were determined by the method of Reznick and Packer [26]. Plasma samples were derivatized with dinitrophenyl hydrazine (10 mmol/L) in HCl (2.5 mol/L) and incubated for 1 h in the dark with intermittent vortexing. After sequential treatment with trichloroacetic acid (20%) and ethanol:acetate (1:1, v:v) mixture, the precipitate was dissolved in 2 ml guanidine hydrochloride (6 mol/L) and incubated at 37°C for 10 min. Spectra were obtained from 390 to 355 nm against corresponding HCl-treated samples as blanks. Tissue samples (200 mg) were first homogenized on ice in phosphate buffer (50 mmol/L, pH 7.4) containing digitonin (0.1%, w/v), EDTA (1 mmol/L), streptomycin sulfate (1%, w/v) and the following protease inhibitors: phenylmethylsulfonyl fluoride (40 μg/ml), leupeptin (5 μg/ml), pepstatin (7 μg/ml) and aprotinin (5 μg/ml). After centrifugation at 8000×*g* for 10 min at room temperature, the supernatant was analyzed in the same manner as plasma samples.

2.10. Statistical analysis

Data are reported as mean±S.E.M. The Tukey–Kramer honestly significant difference (HSD) test was used to evaluate the significance among dietary treatments after determining the difference via one-way ANOVA followed by a Bonferonni adjustment. Differences in quercetin and its metabolites between the vitamin E (VE) deplete+5.0 g Q/kg (D-VE+5Q) diet and the VE replete+5.0 g Q/kg (R-VE+5Q) diet, as well as percentage of total quercetin, were analyzed via Student's *t* test. Differences with $P \leq .05$ were considered significant. The JMP IN 4 statistical software package (SAS Institute Inc., Cary, NC) was used to perform these analyses.

3. Results

3.1. Dietary intakes and body weight

Dietary intake was monitored daily to assess potential differences in food intake due to vitamin E deficiency or palatability of the diets. Except for rats in the C-AL group, all animals were fed the same amount of diet daily, adjusted accordingly to those with the lowest intake. However, no differences in food intake were found between C-AL and

pair-fed groups. At 12 weeks, mean body weight did not differ between groups at 280.0±6.1, 276.4±6.4, 274.6±4.0, 290±9.6 and 275±6.5 in the C-AL, control pair fed (C-PF), R-VE+5Q, D-VE+5Q and D-VE groups, respectively. Based on the results of daily feed consumption, the average daily quercetin intake in rats fed the 5.0-g/kg quercetin diet was 120.2±1.73 mg.

3.2. Biomarkers of vitamin E deficiency

Quercetin administration did not affect plasma and tissue α-tocopherol concentrations or PK activities in rats fed either R-VE or D-VE diets. Thus, the group averages for plasma α-tocopherol and PK are shown in Fig. 1A and B, respectively. In the D-VE group, α-tocopherol status decreased from 21.0±2.6 to 5.6±0.2 μmol/L after 2 weeks ($P \leq .05$) and continued to fall until 8 weeks. α-Tocopherol concentrations in the liver, heart, muscle and testis at 12 weeks were comparable in rats fed the R-VE diets and higher than those fed the D-VE diets (Fig. 2). Plasma and tissue γ-tocopherol were negligible in both R-VE and D-VE groups (data not shown).

Elevated serum PK activity is a biomarker of vitamin E deficiency [27]. Increases in PK activity were apparent after 6 weeks of feeding the D-VE diets (Fig. 1B). After 12 weeks, PK activity was 11.0±0.6 U/ml in rats from the D-VE groups and 1.3±0.1 U/ml in rats from the R-VE groups ($P \leq .05$). Quercetin supplementation did not alter serum PK activity.

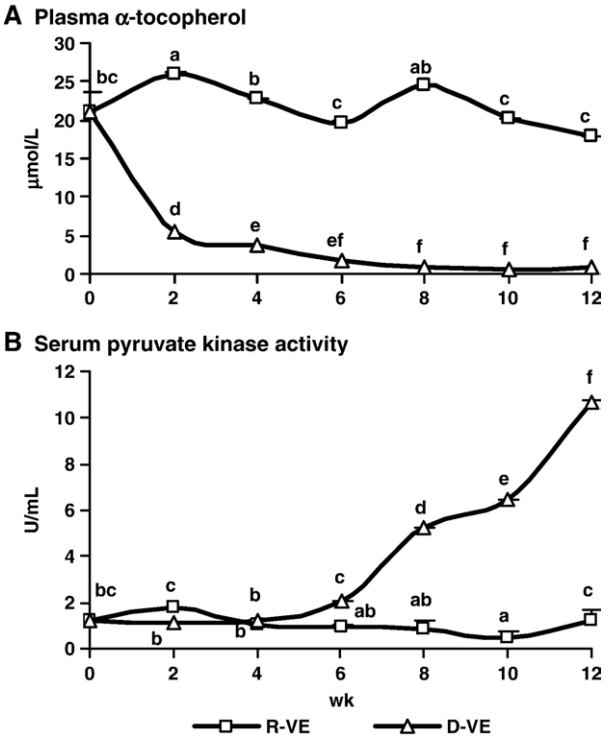

Fig. 1. Group average changes in plasma α-tocopherol (A) and serum PK activity (B) in rats fed either vitamin-E-replete diets (C-AL, C-PF, R-VE+5Q) or vitamin-E-deplete diets (D-VE+5Q, D-VE) for 12 weeks. Means with different letters differ at $P \leq .05$, using a Tukey HSD test.

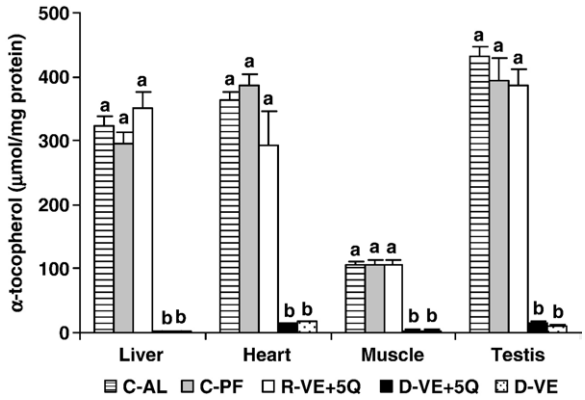

Fig. 2. α-Tocopherol concentrations in the liver, heart, skeletal muscle and testis of rats at 12 weeks. Means in the same tissue without the same letter differ at $P \leq .05$, using a Tukey HSD test.

3.3. Biomarkers of oxidative stress

Neither D-VE nor 5Q diets affected plasma MDA, a biomarker of lipid peroxidation, with concentrations, at 12 weeks, of 1.76±0.54, 1.7±0.43, 1.80±0.30, 2.24±0.46, and 2.19±0.3 μmol/L in the C-AL, C-PF, R-VE+5Q, D-VE+5Q and D-VE groups, respectively. Tissue MDA content was also unaffected in the following (values correspond to the C-AL, C-PF, R-VE+5Q, D-VE+5Q and D-VE groups, respectively): heart: 0.11±0.01, 0.14±0.04, 0.11±0.01, 0.1±0.12 and 0.13±0.02 nmol/mg protein; liver: 2.26±0.16, 2.00±0.19, 2.01±0.15, 1.92±0.18 and 2.03±0.13 nmol/mg protein; muscle: 1.67±0.21, 1.70±0.13, 2.30±0.36, 1.70±0.19 and 2.06±0.27 nmol/mg protein.

In contrast, plasma protein carbonyl, a biomarker of protein oxidation, was higher in rats fed D-VE than R-VE diets ($P \leq .05$; Fig. 3). Quercetin supplementation did not

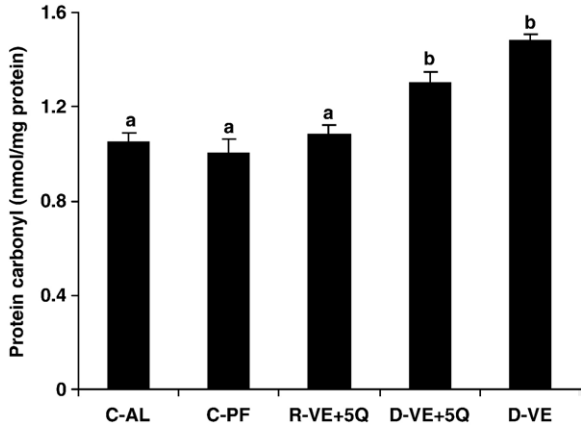

Fig. 3. Plasma protein carbonyls in rats at 12 weeks. Means with different letters differ at $P \leq .05$, using a Tukey HSD test.

prevent the D-VE-induced increase in plasma protein carbonyls. Protein carbonyl concentrations in the liver (1.92–2.26 nmol/mg protein) and muscle (1.67–2.30 nmol/mg protein) were not affected by either D-VE diets or quercetin supplementation.

Neither vitamin E depletion nor quercetin supplementation affected $ORAC_{total}$ or $ORAC_{pca}$ values. Mean plasma $ORAC_{total}$ for the R-VE and D-VE groups was 16,448±588 and 14,390±1003 μmol/L, respectively, and mean $ORAC_{pca}$ was 1901±185 and 2085±204 μmol/L, respectively.

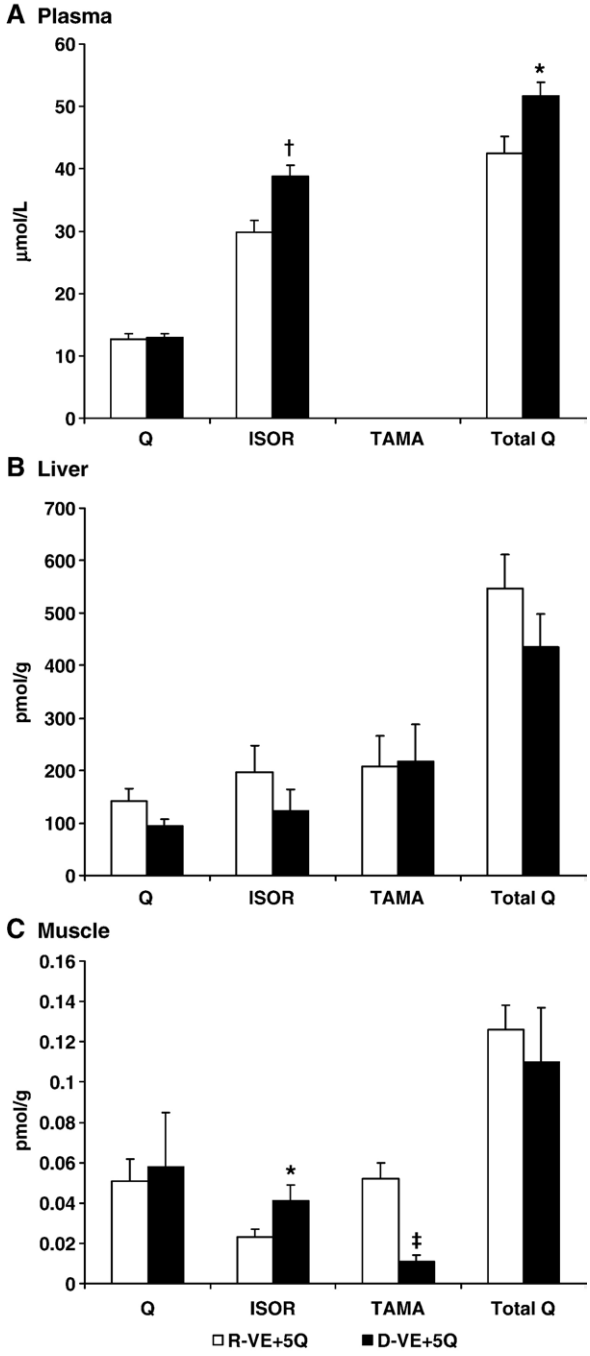

Fig. 4. Quercetin and its metabolites in plasma (A), liver (B) and muscle (C) of rats at 12 weeks. Means of the same compound differ at *$P \leq .05$, †$P \leq .005$ and ‡$P \leq .001$, respectively, using Student's t test.

Table 2
Percentage disposition of quercetin and its methylated metabolites, isorhamnetin and tamarixetin in rat plasma, liver and muscle

	Percentage of total quercetin	
	R-VE+5Q	D-VE+5Q
Plasma		
Quercetin	29.8±0.6†	25.1±1.2
Isorhamnetin	70.2±0.6†	74.9±1.2
Tamarixetin	0	0
Liver		
Quercetin	25.3±2.2	22.6±2.3
Isorhamnetin	33.0±7.2	32.9±9.6
Tamarixetin	41.7±9.1*	44.5±11.4
Muscle		
Quercetin	38.2±4.3	39.8±6.1
Isorhamnetin	21.2±4.7†	47.9±6.9
Tamarixetin	40.6±5.2‡	12.3±3.0

Percentages of compounds are expressed relative to the total amount of quercetin and its metabolites. Data are expressed as mean±S.E.M.
Means in the same row differ at *$P \leq .05$, †$P \leq .005$ and ‡$P \leq .001$, using Student's t test.

3.4. Quercetin and its methylated metabolites in plasma and tissues

While plasma quercetin concentrations did not differ in rats fed the 5Q diet with either the R-VE or D-VE diets (Fig. 4A), isorhamnetin was higher in rats fed the D-VE diet than in those fed the R-VE diet ($P \leq .005$). Total plasma quercetin was 21.6% higher in rats fed the D-VE+5Q diet than in those fed the R-VE+5Q diet ($P \leq .05$). The percentage of isorhamnetin in total plasma quercetin was increased by 4.7% in the D-VE diet (Table 2). Unlike the liver where a significant amount of tamarixetin was detected, plasma did not have detectable concentrations of this metabolite. Liver concentrations of quercetin and its methylated metabolites were not affected by vitamin E status (Fig. 4B). Dietary vitamin E did not alter percentage distributions of quercetin and isorhamnetin in the liver but enhanced percentage of tamarixetin (Table 2). Further, tamarixetin in the liver was apparently predominant in comparison to isorhamnetin and quercetin (Table 2).

Lower concentrations of quercetin and its methylated metabolites were detected in the gastrocnemius muscle than in the liver. Muscle concentrations of quercetin were not affected by vitamin E, while isorhamnetin was larger and tamarixetin was lower in rats fed the D-VE+5Q diet than in those fed the R-VE+5Q diet ($P \leq .05$ and $P \leq .001$, respectively; Fig. 4C). The proportion of quercetin metabolites in total muscle quercetin was not altered by dietary vitamin E (Table 2). The percentage of isorhamnetin was 21.2% in the R-VE+5Q group and 47.9% in the D-VE+5Q group, while percentage of tamarixetin was 40.6% and 12.2%, respectively ($P \leq .001$).

4. Discussion

The antioxidant defense network is composed of endogenous and dietary factors that act in a dynamic interrelationship, including complex sparing and recycling reactions that allow for quenching a variety of reactive species and also conserving elements of the network itself [28]. While several studies describe this relationship between vitamins C and E, less information is available regarding the role of flavonoids in the network. Pedrielli and Skibsted [5] described in vitro synergistic and regenerating reactions between quercetin and vitamin E in homogeneous solutions of peroxidating methyl linoleate as determined by the extended delay in oxygen consumption by a mixture of both quercetin and α-tocopherol compared to α-tocopherol alone. Similar relationships were found for catechins in vitro [5] and, in vivo, Frank et al. [29] found that dietary catechin enhanced serum and liver α-tocopherol status in vitamin-E-replete rats. Our results in rats, however, do not suggest any interaction between α-tocopherol and quercetin in rats fed with diets either replete or deficient in vitamin E. While quercetin did not alter vitamin E status, α-tocopherol deficiency modified quercetin metabolism.

Previous studies that examined the effect of quercetin on vitamin E status are difficult to reconcile because of their different experimental designs. Kumar et al. [30] found that 1 mg/day quercetin for 5 days did not alter vitamin E status in the lung. However, Choi et al. [15] reported that 2 and 20 mg/day quercetin fed to vitamin-E-deficient rats increased α-tocopherol concentration in serum and liver in a dose-dependent manner. In contrast, we found that the 5Q diet did not slow the rate of α-tocopherol depletion in serum; neither did it maintain concentrations in the liver, muscle or heart of vitamin-E-deficient rats, although their plasma quercetin concentrations were comparable to those reported by Choi et al. [15]. Some of the differences between our findings and those of Choi et al. [15] may result from differences in the daily dose of vitamin E in the replete diets (30 IU vs. 75 IU, respectively) and the study duration (12 weeks vs. 4 weeks, respectively). Further, it is also important to note that the bioavailability of quercetin may be dependent on the source and type of quercetin ingested, for example, with quercetin glucosides being more bioavailable than the aglycone form and rutin [31–33], as well as interactions with protein [34,35], lipid [36] and other dietary components. Low vitamin E status is generally associated with oxidative stress, principally indicated by increases in products of lipid peroxidation [37,38]. In contrast, we found no difference in MDA in plasma, liver or muscle from rats fed diets depleted or replete in vitamin E. In addition to differences in study design and factors such as rat strain and age, other possible factors confounding the reported simple inverse relationships between vitamin E status and biomarkers of oxidative stress are complex compensatory mechanisms, for example, induction of ubiquinone-dependent systems by deficiencies of vitamin E and selenium [39]. While D-VE diets did not increase MDA, significant increases in plasma protein carbonyls were found, suggesting a differential responsiveness between lipids and proteins to the degree of vitamin-E-deficiency-induced oxidative stress in this study.

While the antioxidant capacity of quercetin is readily apparent in vitro [40–42] and ex vivo [43–45], there are limited reports of its efficacy in vivo. A small number of in vivo studies indicate that quercetin decreases hepatic lipid peroxidation in rats [15,46], scavenges superoxide generation in rabbit hind-limb ischemic–reperfusion injury [47], decreases UV-light-induced increases in plasma MDA while increasing the levels of reduced glutathione [48], decreases plasma thiobarbituric acid reactive substances and hydroperoxides and restores the activities of superoxide dismutase and catalase to near-normal levels in streptozotocin-induced diabetic rats [49]. In contrast and similar to our results, Willcox et al. [50] found that quercetin at 5.0 g/kg diet did not prevent increases in F_2-isoprostanes, a product of lipid peroxidation, in plasma or hearts of vitamin-E-deficient rats. Consistently, we did not find that quercetin prevented increases in plasma protein carbonyls in vitamin-E-deficient rats, although it has been found to protect apolipoprotein B100 in LDL in vitro against bromine radical-induced oxidation [51].

Similar to reports showing that antioxidants exhibit interactive mechanisms in protecting against reactive oxygen/nitrogen species, we have previously observed that polyphenolic mixtures and vitamin E or C work in a synergistic manner to protect LDL against oxidation [14]. However, in this study, results from the ORAC assay did not reveal any impact of quercetin and vitamin E on total antioxidant capacity in plasma. Although some flavonoids have been shown to increase ORAC values [52], this assay may not be readily responsive to the changes in plasma concentrations of vitamin E and quercetin against a background antioxidant capacity contributed by protein, uric acid and other redox constituents in plasma [53].

Quercetin metabolism has been investigated in numerous animal models [9,10]. We have recently shown that quercetin is extensively metabolized in the gastrointestinal tract and liver and is present in tissues as sulfated, glucuronated and methylated metabolites [9]. Similar to the results of Manach et al. [12], tamarixetin was not detected in the plasma while isorhamnetin, another methylated quercetin, was preferentially secreted from the liver into the circulation. However, to our knowledge, the influence of other nutrients on quercetin metabolism has not been reported. We found that vitamin E deficiency preferentially affects the methylation of quercetin, reflected by the increase in plasma isorhamnetin and percentage disposition of isorhamnetin in total quercetin, as well as the increase in percentage disposition of hepatic tamarixetin in total quercetin, as compared to rats fed a diet replete in this vitamin. Further, muscle low in α-tocopherol had both lower concentrations and percentage disposition of tamarixetin in total quercetin but showed an increase in percentage disposition of isorhamnetin in total quercetin.

These effects might result from an alteration of the transport of isorhamnetin from tissue(s) into the circulation, a modification of the affinity of COMT for site-specific O-methylation of flavonol hydroxyl groups and/or an alteration in tamarixetin excretion from the liver into bile. An interaction between vitamin E status and COMT activity was recently suggested by a study in which vitamin E plus selenium prevented an increase in COMT activity induced by cigarette smoke in mice [54]. However, the mechanisms by which vitamin E status alters quercetin metabolism remain to be elucidated.

In conclusion, we found that dietary quercetin neither slowed the decline in vitamin E status nor protected proteins from oxidation in vitamin-E-depleted rats. Further, α-tocopherol status appears to affect quercetin metabolism as vitamin E deficiency is associated with changes in COMT-mediated O-methylation to isorhamnetin and tamarixetin.

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