A Quercetin Supplemented Diet Does Not Prevent Cardiovascular Complications in Spontaneously Hypertensive Rats

Justin Carlstrom, J. David Symons, Tzu Ching Wu, Richard S. Bruno, Sheldon E. Litwin, and Thunder Jalili*

Abstract

Diets high in quercetin may decrease the risk of developing cardiovascular disease. We tested whether quercetin delays or reduces the severity of hypertension, vascular dysfunction, or cardiac hypertrophy in the spontaneously hypertensive rat (SHR). Normotensive, 5-wk-old SHR consumed standard (n = 18) or quercetin-supplemented diet (1.5 g quercetin/kg diet, n = 22, SHR-Q) for 5 or 11 wk. Wistar Kyoto rats (WKY, n = 19), fed a standard diet, served as controls. At 16 wk, plasma quercetin, measured by HPLC, was 2.09 ± 0.33 μmol/L in SHR-Q and below assay detection limits in SHR and WKY rats. At 10 and 16 wk of age, arterial blood pressure and heart weight:body weight were not different between SHR and SHR-Q. At 16 wk, cardiac function (echocardiography), vascular morphology (hematoxylin and eosin staining of aortae), and resistance and conductance vessel reactivity (wire myography) was unchanged in SHR vs. SHR-Q. Thus, a quercetin-supplemented diet does not delay the onset or lessen the severity of cardiovascular complications that develop in SHR. These findings contrast with previous reports of cardiovascular protection when quercetin was delivered via oral gavage. To determine whether the efficacy of quercetin depends on its method of delivery, 15-wk-old SHR were given quercetin (10 mg/kg) once daily via oral gavage for 4 consecutive days. Arterial blood pressure (mm Hg) was lower in gavaged SHR than in SHR-Q (148 ± 6) than in SHR-Q (162 ± 2, P < 0.02) and SHR (168 ± 3, P < 0.001). These data suggest that mode of delivery is a critical determinant in whether quercetin provides cardiovascular benefits.

Introduction

Hypertension is a strong risk factor for the development of heart failure, myocardial infarction, kidney failure, stroke, and death (1). Although effective pharmacological strategies for the treatment of hypertension exist, there is a great deal of interest in using dietary agents (e.g., phytochemicals) to prevent or reduce hypertension. Epidemiological studies indicate that quercetin consumption is inversely related to the risk for cardiovascular disease (2–8). In addition, laboratory investigations using several experimental animal models suggest that quercetin is beneficial in this regard. For example, we showed that hypertension was normalized and cardiac hypertrophy was attenuated in rats that consumed quercetin-supplemented vs. a standard diet following abdominal aortic banding to produce pressure overload (9). Two additional studies reported protective effects of quercetin when administered once daily via oral gavage (10,11). In the first, quercetin treatment regressed established hypertension and cardiac hypertrophy in the adult spontaneously hypertensive rat (SHR) (11). In the second, quercetin treatment delayed the onset and attenuated the severity of hypertension produced by 6 wk of nitric oxide synthase inhibition using Nω-nitro-L-arginine methyl ester (L-NAME) (10). Given that quercetin lowered indices of oxidative stress in both studies, the antioxidant potential of quercetin was hypothesized to have contributed to the protective effects.

SHR are normotensive at ~6 wk of age and develop hypertension, cardiac hypertrophy, vascular dysfunction, and increased oxidative stress by ~12 wk (12,13) and progress to heart failure by 18–24 mo (14). In this study we sought to determine whether a quercetin-enriched diet delays the onset of hypertension and cardiac growth in SHR. Furthermore, we tested whether a quercetin-enriched diet attenuates the severity of hypertension, cardiac growth, vascular remodeling, vascular dysfunction, and oxidant stress that develops in the adult SHR.

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1 Supported by the University of Utah Research Council (T.J.), and AHA, National Affiliate, Scientist Development Grant (013009N) (J.D.S.).
2 Conflict of interest disclosure: Patent pending on the use of quercetin as an antihypertensive agent to T.J.
3 To whom correspondence should be addressed. E-mail: thunder.jalili@utah.edu or 00295675.acs.unc@hsc.utah.edu.

The Journal of Nutrition
Nutrition and Disease

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Hypertension is a strong risk factor for the development of heart failure, myocardial infarction, kidney failure, stroke, and death (1). Although effective pharmacological strategies for the treatment of hypertension exist, there is a great deal of interest in using dietary agents (e.g., phytochemicals) to prevent or reduce hypertension. Epidemiological studies indicate that quercetin consumption is inversely related to the risk for cardiovascular disease (2–8). In addition, laboratory investigations using several experimental animal models suggest that quercetin is beneficial in this regard. For example, we showed that hypertension was normalized and cardiac hypertrophy was attenuated in rats that consumed quercetin-supplemented vs. a standard diet following abdominal aortic banding to produce pressure overload (9). Two additional studies reported protective effects of quercetin when administered once daily via oral gavage (10,11). In the first, quercetin treatment regressed established hypertension and cardiac hypertrophy in the adult spontaneously hypertensive rat (SHR) (11). In the second, quercetin treatment delayed the onset and attenuated the severity of hypertension produced by 6 wk of nitric oxide synthase inhibition using Nω-nitro-L-arginine methyl ester (L-NAME) (10). Given that quercetin lowered indices of oxidative stress in both studies, the antioxidant potential of quercetin was hypothesized to have contributed to the protective effects.

SHR are normotensive at ~6 wk of age and develop hypertension, cardiac hypertrophy, vascular dysfunction, and increased oxidative stress by ~12 wk (12,13) and progress to heart failure by 18–24 mo (14). In this study we sought to determine whether a quercetin-enriched diet delays the onset of hypertension and cardiac growth in SHR. Furthermore, we tested whether a quercetin-enriched diet attenuates the severity of hypertension, cardiac growth, vascular remodeling, vascular dysfunction, and oxidant stress that develops in the adult SHR.
Materials and Methods

Animals and diets. All protocols were approved by the University of Utah Institutional Animal Care and Use Committee. Four-week-old male SHR and Wistar-Kyoto rats (WKY) were obtained from Harlan and acclimated for 1 wk. SHR were given free access to standard rodent diet (SDR) or diet enriched with purified quercetin aglycone (SHR-Q; 1.5 g quercetin/kg diet) for 5 (SHR, n = 6; SHR-Q, n = 6) or 11 wk (SHR, n = 12; SHR-Q, n = 16). WKY rats were allowed free access to standard rodent diet for 5 (n = 6) or 11 (n = 13) wk. Therefore, all rats were studied at 10 or 16 wk of age. Diets were prepared based on AIN-93 recommendations (Research Diets) (15). The quercetin dose was chosen because we previously demonstrated that it: 1) produces detectable plasma and liver levels, 2) does not affect blood pressure or myocardial function in normotensive rats, but 3) attenuates hypertension and cardiac hypertrophy in rats subjected to 14 d of pressure overload evoked by abdominal aortic constriction (9).

Myocardial function. Echocardiography was performed at 16 wk using methods we have described (9,16).

Arterial blood pressure and tissue sampling. Rats were evaluated at 10 and 16 wk of age. A fluid-filled catheter was placed into the caudal artery of rats anesthetized with 2–5% isoflurane, and, 60 min after regaining consciousness, arterial blood pressure and heart rate were measured over 20 cardiac cycles (Biopac Systems) (9,17–19). Next, rats were anesthetized, the chest opened, and were killed by removing the heart. Sections of heart, segments of thoracic aorta, and mesenteric arteries, which were removed for analysis as previously detailed (9).

Measurement of vascular reactivity. Segments of thoracic aorta, mesenteric arteries, and coronary arteries from 16-wk-old rats were mounted on wire-type myographs as described previously (9,18). For thoracic aorta and mesenteric arteries, concentration-response curves to acetylcholine (ACh, 10 nmol–1 mmol/L) and sodium nitroprusside (SNP, 10 nmol–10 mmol/L) were performed after vessels were precontracted with 1 μmol/L norepinephrine (NE) to estimate endothelium-dependent and independent vasorelaxation, respectively. Dose-response curves to NE (100 nmol–10 mmol/L) and potassium chloride (KCl, 20–100 mmol/L) were completed to assess receptor-mediated and nonreceptor-mediated vasorelaxation, respectively. In aortae, basal nitric oxide synthase activity was estimated by adding Nω-nitro-L-arginine (L-NMMA, 10 mmol/L) to vessels precontracted using 1 μmol/L NE. After the endothelial response to L-NMMA was recorded, the degree of nitric oxide synthase inhibition was quantified by adding 1 mmol/L ACh and noting the resultant vasorelaxation. In coronary arteries, vasorelaxation to ACh and SNP was evaluated on arteries precontracted with endothelin-1 (300 nmol/L). All protocols were separated by 30 min and standard time, volume, and vehicle controls were performed (9,18).

Vascular morphology. Four μm thick thoracic aortic sections from 16-wk-old rats were prepared as previously detailed (9,18). Images were taken with a microscope (Nikon E6000) equipped with a digital camera (Q imaging, Micro Publisher 5.0 RTV) using 2× objective.

Estimates of hepatic oxidant load. Lipid oxidation was determined in segments of liver by fluorescence detection of malondialdehyde (MDA) equivalents (nmol/mg protein) as previously described (18,20). Protein oxidation was estimated by quantifying protein carboxyls (nmol/mg protein) via spectrophotometric quantification of the dinitrophenylhydrazine adduct (18,20). For all assays, protein concentrations were determined using bovine serum albumin as the standard (21).

Plasma quercetin analysis. Plasma quercetin (unconjugated, β-glucuronidated, and sulfated forms) were extracted and measured by HPLC-Coularray as described (22), with modifications. Briefly, plasma (200 μL) was mixed with 375 U of β-glucuronidase and 10 U sulfatase in 0.4 mol/L NaH2PO4 (pH 5.0), 20 μL of 2% ascorbic acid (w/v) and 2.5 mmol/L DTPA in 0.4 mol/L NaH2PO4 (pH 5.0). Samples were incubated (37°C, 45 min) with gentle agitation and extracted with acetone by vigorous mixing, followed by centrifugation (14,000 × g, 4°C), for 5 min. Then, a portion of the supernatant was removed, dried under nitrogen, reconstituted with mobile phase A, and injected onto the HPLC.

The injected sample (50 μL) was separated (MD-150 column, ESA; 150 mm × 3.0 mm i.d., 3 μm) and detected using a binary gradient (0.6 ml/min) with Coularray detector settings of 100, 200, 300, and 400 mV. The following gradient profile was used: 0–35 min, linear gradient from 10–80% B; 35–37 min, 80–10% B; and 37–50 min, 10% B to allow for sufficient system equilibration prior to the next sample injection. The identification of quercetin (~17.5 min) was achieved by comparing their retention times and electrochemical responses to purified standards (Sigma).

Gavage protocol. To determine whether the method used to administer quercetin affects the cardiovascular outcomes, 16-wk-old male WKY rats (n = 3) and SHR (n = 3) were given quercetin aglycone (10 mg/kg) suspended in 1% methylcellulose (Sigma) once daily via oral gavage for 4 consecutive days as described (11). Twenty-four hours after the last gavage (i.e., d5), blood pressure was measured and arterial samples were taken for analysis of plasma quercetin concentrations.

Statistical analyses. A 1-way ANOVA was used to detect differences among groups at 10 and 16 wk of age, using SPSS, version 10. If a significant P-value was obtained, post-hoc (least significant differences) tests were performed to identify individual group differences. In case of unequal variances, Tamhane’s T2 test was performed. Significance was accepted at P < 0.05. Dose response curves generated from vascular function experiments were compared using 2-way (dose × experimental group) repeated measures ANOVA. Planned comparisons were made at each drug dose to determine whether differences existed among groups. Results are presented as means ± SEM. Vasorelaxation to ACh and SNP is expressed as the percentage of relaxation from precontraction tension. Vasocontractile responses to NE, L-NMMA, KCl, and endothelin-1 are presented as mg of developed tension or as the percentage of increase from precontraction tension (L-NMMA only).

Results

Plasma quercetin levels, blood pressure, cardiac hypertrophy, and aortic morphology. Plasma quercetin concentrations (unconjugated, β-glucuronidated, and sulfated forms of quercetin) were below assay detection limits in SHR and WKY rats fed control diets, but markedly elevated in SHR-Q (Table 1). To determine whether quercetin delayed the onset or lessened the severity of hypertension, we compared arterial blood pressure among groups at 10 and 16 wk. Systolic and diastolic blood pressures (Table 1), and mean arterial pressure were increased to a similar degree in SHR and SHR-Q compared with WKY rats at both time points. Heart rate was higher in SHR and SHR-Q than WKY rats at 16 wk (Table 1).

To assess whether quercetin delayed the onset or lessened the severity of cardiac hypertrophy, heart weight was normalized to body weight at both time points. Although heart weight:body weight was not different among groups at 10 wk, it was similarly increased in SHR and SHR-Q compared with WKY rats at 16 wk (Table 1).

Vascular remodeling resulting from chronic hypertension was assessed by examining thoracic aorta of 16-wk-old rats. Medial thickness, lumen diameter, and mean cross-sectional area were increased to the same extent in SHR and SHR-Q compared with WKY rats (Table 2, Fig. 1). Collectively, these data demonstrate that although plasma quercetin was elevated in SHR-Q, it did not delay the onset or lessen the severity of arterial hypertension, cardiac hypertrophy, or aortic remodeling.

In vivo myocardial function. Echocardiography was performed prior to the terminal experiments at 16 wk to determine the pattern of cardiac hypertrophy and to assess whether
myocardial function is improved in SHR-Q vs. SHR. The hearts of SHR and SHR-Q had a greater mean wall thickness (P < 0.01), and interventricular septum systolic dimension (P < 0.03) than WKY rats and tended to have a smaller left ventricular diastolic dimension (P = 0.09) (Fig. 2, Table 3). The posterior wall dimension and fractional shortening did not differ among the groups (Table 3). Taken together, these data show that concentric cardiac hypertrophy and myocardial function are similar in 16-wk-old SHR and SHR-Q.

Vascular reactivity. To determine whether quercetin could prevent or lessen vascular dysfunction, ex vivo examinations of aortic (conductance sized vessel), mesenteric (resistance sized vessel), and coronary (resistance sized vessel) arterial reactivity were performed.

Aortae. Maximal ACh-evoked vasorelaxation in aortae was reduced similarly in SHR and SHR-Q compared with WKY rats, indicating that quercetin did not prevent or lessen endothelium-dependent dysfunction (Fig. 3A, upper panel). Maximal endothelium-independent responses to SNP (1 mmol/L) did not differ among groups, indicating that vascular smooth muscle function was intact (Fig. 3A, lower panel). Aortae from SHR were more responsive to SNP than SHR-Q and WKY rats at 100 nmol/L. Nitric oxide synthase inhibition of precontracted segments of aortae produced a similar degree of tension development among groups, indicating basal activity of this enzyme was not affected by quercetin treatment. ACh-evoked relaxation in the presence of L-NMMA was performed to verify that the degree of nitric oxide synthase enzyme inhibition was similar among groups. Aortae were precontracted to the same degree among groups before adding ACh, SNP, or L-NMMA. Maximal responses to nonreceptor mediated vasoconstriction (i.e., KCl) and receptor-mediated vasoconstriction (i.e., NE) did not differ among groups (data not shown).

Mesenteric arteries. Maximal relaxation produced by ACh was less in mesenteric arteries from SHR and SHR-Q compared with WKY rats (Fig. 3B, upper panel). Maximal endothelium-independent responses to 1 mmol/L SNP did not differ among WKY rats, SHR, and SHR-Q. Mesenteric arteries were more responsive in SHR and SHR-Q than in WKY rats at 2 of 7 doses administered [i.e., 1 μmol/L and 10 μmol/L (Fig. 3B, lower panel)]. Mesenteric arteries were precontracted to the same degree among groups before adding ACh or SNP. Nonreceptor-mediated vasoconstriction (i.e., KCl) and receptor-mediated vasoconstriction (i.e., NE) did not differ among the groups (data not shown).

Coronary arteries. Vasorelaxation evoked by ACh was blunted in SHR and SHR-Q compared with WKY rats at concentrations between 100 nmol/L and 10 μmol/L (Fig. 3C, upper panel). Endothelium-independent vasorelaxation in response to SNP was similar among SHR, WKY, and SHR-Q (Fig. 3C, lower panel). Coronary arteries were precontracted to the same degree among groups before adding ACh or SNP. Taken together, dysfunction observed in resistance and conductance-sized vessels was not improved by quercetin treatment.

### TABLE 1: Morphological characteristics of rats fed control or quercetin supplemented diets beginning at 5 wk of age

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR</th>
<th>SHR-Q</th>
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<tbody>
<tr>
<td><strong>n</strong></td>
<td>6</td>
<td>6</td>
<td>6</td>
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<tr>
<td><strong>Age, wk</strong></td>
<td>9</td>
<td>11</td>
<td>10</td>
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<tr>
<td><strong>Heart wt, g</strong></td>
<td>0.97 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.18 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.03 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>Body wt, g</strong></td>
<td>245 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>297 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>263 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>Heart wt/body wt, mg/g</strong></td>
<td>3.96 ± 0.11</td>
<td>3.94 ± 0.10</td>
<td>4.03 ± 0.10</td>
</tr>
<tr>
<td><strong>Heart rate, beats/min</strong></td>
<td>398 ± 10</td>
<td>410 ± 8</td>
<td>436 ± 15</td>
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<tr>
<td><strong>Caudal blood pressure, mm Hg</strong></td>
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<tr>
<td>Systolic</td>
<td>127 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>162 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>155 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diastolic</td>
<td>111 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>148 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>142 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Arterial pressure, mm Hg</strong></td>
<td>115 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>152 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>147 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td><strong>Liver MDA, pmol/mg</strong></td>
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<td></td>
<td>168 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>168 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>162 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
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<td><strong>Liver protein carbonyls, nmol/mg</strong></td>
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<td></td>
<td>116 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>181 ± 48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>228 ± 17&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><strong>Plasma total quercetin, µmol/L</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>ND</td>
<td>ND</td>
<td>2.09 ± 0.33</td>
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<sup>1</sup> Values are means ± SEM. Means in a row with superscripts without a common letter differ, P < 0.05. MDA, malondialdehyde equivalents. Total plasma quercetin composed of unconjugated, β-glucuronidated and sulfated forms extracted from plasma. ND, not detectable.

### TABLE 2: Morphological parameters of aortae from 16-wk-old rats fed control or quercetin supplemented diets beginning at 5 wk of age

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR</th>
<th>SHR-Q</th>
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<tbody>
<tr>
<td><strong>MT, μm</strong></td>
<td>80 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>93 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>ED, μm</strong></td>
<td>1436 ± 12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1710 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1651 ± 47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>LD, μm</strong></td>
<td>1131 ± 15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1388 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1338 ± 34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>MCSA, μm²</strong></td>
<td>628,860 ± 13,402&lt;sup&gt;a&lt;/sup&gt;</td>
<td>782,305 ± 10,296&lt;sup&gt;b&lt;/sup&gt;</td>
<td>741,253 ± 59,848&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>M:L</strong></td>
<td>7.06 ± 0.14</td>
<td>6.73 ± 0.10</td>
<td>6.72 ± 0.21</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are means ± SEM. Means in a row with superscripts without a common letter differ, P < 0.05, n = 8.

<sup>2</sup> MT, medial thickness; ED, external diameter; LD, lumen diameter; MCSA, mean cross sectional area; M:L, media:lumen ratio.

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**Figure 1:** Hematoxylin and eosin stained aortic cross sections from 16-wk-old Wistar-Kyoto rats (WKY), spontaneously hypertensive rats that consumed standard rodent diet (SHR), and spontaneously hypertensive rats that consumed quercetin-supplemented (0.15%) diet (SHR-Q), n = 8.
Table 3  Echocardiographic assessment of cardiac structure and function from 16-wk–old rats fed control or quercetin supplemented diets beginning at 5 wk of age.

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR</th>
<th>SHR-Q</th>
</tr>
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<tbody>
<tr>
<td>FS, %</td>
<td>56 ± 4</td>
<td>49 ± 4</td>
<td>46 ± 2</td>
</tr>
<tr>
<td>LVDd, mm</td>
<td>7.2 ± 0.2</td>
<td>6.4 ± 0.2</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td>IVSd, mm</td>
<td>2.1 ± 0.1a</td>
<td>2.6 ± 0.1b</td>
<td>2.5 ± 0.1a</td>
</tr>
<tr>
<td>PWd, mm</td>
<td>1.9 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>MWT</td>
<td>0.20 ± 0.01a</td>
<td>0.25 ± 0.01b</td>
<td>0.24 ± 0.01b</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Means in a row with superscripts without a common letter differ, P < 0.05, n = 8.

Discussion

We hypothesized that if spontaneously hypertensive rats were fed quercetin-enriched diets prior to the onset of hypertension, i.e., at 5 wk, then the expected increases in arterial pressure and cardiac growth might be delayed, and/or the extent of these and related cardiovascular complications would be less severe. Rationale for our hypotheses was based on results from several investigations suggesting that quercetin: 1) attenuated the development of hypertension and cardiac hypertrophy in response to 14 d of pressure overload hypertrophy evoked by abdominal aortic constriction (9) or 6 wk of nitric oxide synthase inhibition (10); and 2) regressed established hypertension and cardiac hypertrophy in 19-wk–old SHR (11). Contrary to our hypotheses, arterial blood pressure and cardiac growth were similar between SHR-Q and SHR compared with WKY rats at 10 wk. Furthermore, the severity of hypertension, cardiac hypertrophy, vascular remodeling, vascular dysfunction, and oxidant stress that developed in 16-wk-old SHR vs. WKY rats was not attenuated in SHR-Q rats. Therefore, cardioprotection was not provided in 10 or 16-wk-old SHR that consumed quercetin enriched diet. In contrast to our results, a recent study by Sanchez et al. (24) demonstrated that 5-wk-old SHR treated by oral gavage with quercetin for 13 wk had lower blood pressure, heart rate, and improved endothelial dependent arterial relaxation compared with untreated SHR.

Possible reasons for the lack of efficacy of quercetin in the current study compared with others (10,11,24) could be related to: 1) the magnitude and severity of arterial hypertension and cardiac hypertrophy, 2) the particular form of quercetin administered, and/or 3) the method of quercetin delivery. First, regarding the magnitude of hypertension, this flavonoid was shown to attenuate systolic blood pressures of greater (i.e., ~200 mm Hg) (9,11) and lesser (~170 mm Hg) (10) severity than experienced by 16-wk–old SHR (i.e., ~180 mm Hg) in the present study. Based on these data, we believe the degree of hypertension in our rats was neither too severe nor insufficient for a beneficial effect of quercetin to be realized.

Second, efficacy of quercetin could depend on the particular form utilized (e.g., quercetin aglycone, rutin, or quercetin-3-glucoside). We enriched the rat diet with quercetin aglycone in a previous study (9) and in the present investigation. Because we (9) and others (10,11,24,25) demonstrated cardiovascular protection using quercetin aglycone, this choice of isoflavan is not likely responsible for the lack of effect observed in the present study.
Finally, the method of quercetin delivery may be an important consideration. In contrast to our method of incorporating quercetin directly into the diet and allowing ad libitum consumption, others administered quercetin once daily via oral gavage (10 mg/kg) (10,11,23,24). Although plasma and tissue quercetin concentrations were not reported in those studies, the protective effects were clearly evident. Specifically, when 14-wk-old SHR were treated for 1 wk with quercetin via oral gavage, systolic blood pressure was reduced (~218 mm Hg) compared with SHR treated with vehicle (~230 mm Hg)(11). Another study from the same laboratory showed that compared with vehicle administration, the onset of L-NAME-induced hypertension was delayed, and the severity of hypertension and cardiac growth were attenuated when quercetin was administered to rats via oral gavage. The protection afforded by quercetin when delivered in this manner, compared with the absence of similar effects in the present study, prompted us to test whether the route of quercetin delivery (i.e., once daily oral gavage vs. ad libitum consumption of quercetin supplemented diet) is a critical determinant of biological effect. Strikingly, we observed that mean arterial pressure in 16-wk-old gavaged SHR was significantly lower than 16-wk-old SHR and SHR-Q. The blood pressure reduction we observed is comparable to that reported by Duarte et al. (11) when 14-wk-old SHR were gavaged for 7 d with quercetin. In that study, however, more robust decreases in blood pressure were evident after 5 wk of quercetin treatment. Given these findings concerning blood pressure, we expected quercetin-gavaged SHR to exhibit higher plasma quercetin concentrations than SHR-Q. Surprisingly, plasma quercetin levels were lower in gavaged SHR (0.61 μmol/L) than in SHR-Q (2.09 μmol/L). One potential explanation is that SHR-Q consumed the quercetin-supplemented diet up to the time they were killed, whereas gavaged SHR were 24 h removed from the last dose, as done in previous studies (11). This time gap may have resulted in a gradual decrease in quercetin levels due to the ongoing metabolism in the liver. Indeed, previous studies reported that plasma total quercetin concentrations return to baseline 24 h after quercetin supplementation is terminated (26,27). Even though plasma quercetin concentrations obtained in this study were low in SHR 24 h after gavage, previous literature indicates that peak quercetin levels achieved after gavage are likely much higher than those achieved in diet-supplemented SHR-Q. In this regard, da Silva et al. (28) reported plasma quercetin concentrations of 9.6 μmol/L 6 h after 10 mg/kg quercetin was delivered via oral gavage to the rats. Unfortunately, the lack of a comprehensive evaluation regarding the time-related decay of plasma quercetin concentrations after the last gavage is a limitation of our study. However, the findings of da Silva et al. (28) as well as the cardiovascular protection observed in SHR after 5 wk of quercetin gavage by Duarte et al. (11), strongly suggest that the route of delivery is a critical determinant in producing efficacious concentrations of this flavonoid that may result in cardiovascular protection. As such, the route or method of compound delivery should be an important consideration when studies are designed to examine the biological effect(s) of phytochemicals.

In a previous study, we reported that quercetin-supplemented diets prevent increases in blood pressure and cardiac hypertrophy in rats with abdominal aortic constriction (AAC) (9). AAC is a surgical procedure that places a physical constriction, via ligature or hemoclip, on the abdominal aorta. This creates pressure overload in the heart and in the vasculature located proximal to the mechanical hindrance to blood flow, but not the rest of the body (9). It is not clear why a dietary approach identical to that described in the present study produced cardiovascular protection in the AAC rat but not the SHR. However, this discrepancy may be due to the local (AAC rat) vs. systemic (SHR) nature of hypertension in these models, the duration of hypertension (2 wk in AAC vs. 5–10 wk in SHR), or the genetic background of the rats (AAC Sprague Dawley background vs. SHR Wistar Kyoto background).

In summary, we show that arterial hypertension, cardiac hypertrophy, vascular dysfunction, vascular remodeling, and indices of oxidant stress are similar in SHR regardless of whether...
they consumed quercetin-supplemented or standard diet. These results did not support our original hypotheses, and are in contrast to previous reports wherein quercetin-induced cardioprotection was observed in models of pharmacologically induced hypertension (10) and SHR (11). We think that the mode of delivery, i.e., a dietary approach of enriching standard rodent diet with quercetin aglycone compared with administering a daily oral gavage (10,11), may be a critical element in building peak plasma concentrations that would result in beneficial effects of this and perhaps other phytochemicals.

**Literature Cited**


