Quercetin-Supplemented Diets Lower Blood Pressure and Attenuate Cardiac Hypertrophy in Rats With Aortic Constriction

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Abstract: Quercetin (Q), a flavonoid found in berries and onions, can reduce blood pressure in hypertensive animals and inhibit signal transduction pathways in vitro that regulate cardiac hypertrophy. We hypothesized that quercetin could prevent cardiovascular complications in rats with abdominal aortic constriction (AAC). Rats consumed standard or Q-supplemented chow (1.5 g Q/kg chow) for 7 days before AAC or sham surgery (SHAM, n = 15; AAC, n = 15; SHAMQ, n = 15; AACQ, n = 14). Fourteen days after surgery, plasma and liver Q concentrations were elevated ($P < 0.05$) and hepatic lipid oxidation was reduced ($P < 0.05$) in Q-treated versus untreated rats. Carotid arterial blood pressure and cardiac hypertrophy were attenuated ($P < 0.05$), and cardiac protein kinase C βII translocation was normalized ($P < 0.05$) in AACQ versus AAC. Expression of cardiac β-myosin heavy-chain mRNA was also reduced in AACQ versus AAC ($P < 0.05$). However, extracellular regulated kinase 1/2 phosphorylation was similar in AAC versus AACQ. The level of aortic endothelial dysfunction (wire myography) was also similar between AAC and AACQ, in spite of reduced aortic thickening in AACQ. Importantly, Q-treated rats did not show any deleterious changes in myocardial function (echocardiography). Our data supports an antihypertensive and antihypertrophic effect of Q in vivo in the absence of changes concerning vascular and myocardial function.

Key Words: flavonoid, blood pressure, cardiac hypertrophy, signal transduction, vascular function

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Cardiac hypertrophy has been associated with an increased risk of developing heart failure or death.[1] As such, there is interest in preventing and treating risk factors such as hypertension and myocardial infarction that contribute to the development of cardiac hypertrophy. Chronic high blood pressure increases the afterload experienced by the heart, which in turn increases wall stress and stimulates development of cardiac hypertrophy. The antioxidant quercetin (Q), a flavonoid found in onions, apples, red wine, and berries, may be an effective agent to reduce blood pressure and prevent cardiac hypertrophy. Spontaneously hypertensive rats with established high blood pressure that are treated daily with oral Q experience a drop in blood pressure, reduced cardiac hypertrophy, and improved vascular function.[2] Other studies using mice and guinea pigs with surgically induced pressure overload (aortic constriction) have reported that prior treatment with various antioxidants can actually prevent cardiac hypertrophy.[3,4] Taken together, these studies suggest that antioxidants such as Q may be efficacious in preventing/treating hypertension and cardiac hypertrophy in at least 2 unique models of cardiovascular disease, systemic hypertension and aortic constriction. Interestingly, epidemiological studies have also reported that Q intake is inversely related to ischemic heart disease and a number of other chronic diseases such as lung cancer, type 2 diabetes mellitus, and asthma.[5,6]

Cardiac hypertrophy stimulated by an increase in blood pressure and wall stress is thought to be regulated by a number of signal transduction pathways including protein kinase C (PKC), extracellular regulated kinase 1/2 (ERK1/2), and Akt.[7] Neurohormonal factors such as angiotensin II (a product of the rennin–angiotensin system), endothelin-1, and phenylephrine are activators of PKC, which can in turn activate ERK1/2 during conditions of pathological hypertrophy.[8–11] The role of Akt in cardiac hypertrophy is more complex because of the contrasting effects of its upstream activator, phosphoinositol 3 kinase (PI3K). Activation of Akt by the PI3Kα isoform is involved in physiological hypertrophy, whereas PI3Kγ-mediated Akt activation can be a regulator of pathological cardiac hypertrophy.[7,12–14]

The aim of the present study was to evaluate the mechanisms by which dietary Q could attenuate cardiac hypertrophy in the setting of a fixed aortic constriction.

We hypothesized that blood pressure and cardiac hypertrophy would be attenuated in rats that consume a Q-supplemented diet before a pressure overload challenge consisting of 14 days of abdominal aortic constriction (AAC). In addition, we hypothesized that signaling pathways regulating cardiac hypertrophy (eg, PKC, Akt, ERK1/2) would be less activated in Q-treated animals. To determine whether any reduction in cardiac hypertrophy and signal transduction activation was associated with a general reduction in oxidative stress, indices of hepatic lipid and protein oxidation were quantified. Finally, myocardial and vascular function were measured to assess the effect of Q consumption on structure and function of the heart and selected vessels.

MATERIALS AND METHODS

Animals and Diets

All protocols were approved by the University of Utah Institutional Animal Care and Use Committee. Adult, male Sprague-Dawley rats (250 g) were selected randomly and given free access to standard chow (AIN 93M, Research Diets, New Brunswick, NJ) or chow supplemented with Q aglycone (Q; AIN 93M + 1.5 g Q/kg chow; Sigma, St Louis, MO) for 7 days. This dose was chosen based on a previous study wherein cardiac hypertrophy was attenuated in pressure overloaded mice receiving 120 mg/kg Q intraperitoneally (IP).[15] Dietary administration was chosen to establish clinical relevance to human dietary habits. The estimated Q intake of rats fed diets with 1.5 g Q/kg chow was 130 mg/kg.[16] This estimate is based on an average consumption of 10 g chow/100 g body weight for a rat.[16] A recent study examining humans given a supplement of Q aglycone, the isoform used in the present study, reported the half-life of circulating metabolites to be 15 to 18 h.[17] In addition, the level of Q used in these studies (0.15% diet by weight) is ≈10 times lower than the levels at which the first signs of nephrotoxicity are observed in the male rat.[18] On day 8, suprarenal AAC using a hemoclip with an internal diameter of 0.63 mm or a sham operation (SHAM) was performed. Rats continued their respective diets for 14 more days, including the day of surgery. This duration of AAC produces 20% to 35% increases in heart weight:-body weight.[19] Data were compared among SHAM (n = 15), AAC (n = 15), SHAMQ (n = 15), and AACQ (n = 14) rats.

Myocardial Function

Cardiac function was determined in a subset of animals 12 to 13 days after surgery using echocardiography, as we have described.[20] Briefly, rats were anesthetized with ketamine (50–75 mg/kg, IP) and xylazine (10–15 mg/kg, IP) and 2D-guided M-mode images of the left ventricle were obtained using a General Electric Vivid 5 echocardiographic machine equipped with a 10-MHz transducer. Digital images were analyzed offline by a blinded observer.

Arterial Blood Pressure and Tissue Sampling

Fifteen days postsurgery, all rats were anesthetized using isoflurane (3%–5%) and fluid-filled catheters were inserted into the carotid and caudal arteries.[21] After animals regained consciousness and recovered for 60 min, arterial blood pressures and heart rate were measured over ≈20 cardiac cycles (Biopac Systems, Santa Barbara, CA). Next, an arterial blood sample was taken for Q analysis. Finally, rats were anesthetized deeply using 5% isoflurane, the chest opened, and heart, sections of liver, segments of thoracic aorta, and mesenteric arteries were removed. The heart was placed immediately in iced physiological saline solution, trimmed of adherent tissue, and weighed. The apical portion of the left ventricle (LV) was excised and used to analyze signaling proteins. Coronary arteries were dissected from the remaining portion of the LV and used to determine reactivity. Sections of liver were excised and prepared for quantification of quercetin, thiobarbituric acid reactive substances, and protein carbonyls. Segments of thoracic aorta were used to determine function and medial thickening, whereas mesenteric arteries were used to assess reactivity.

Tissue Homogenization

PKC

Cardiac lysates containing cytosolic and membrane proteins were prepared from the LV previously frozen at −80°C, as previously detailed.[22] All extraction procedures were performed at 4°C. Protein concentration was determined using a Bio-Rad Protein assay (Bio-Rad, Hercules, CA) with bovine serum albumin as a standard.

ERK1/2, Akt, Serine Phosphorylated PKC Substrates

All homogenization procedures were performed at 4°C. The LV was homogenized with a tissuemizer in 1 mL of ice-cold RIPA buffer (50 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 1% Nonidet P-40, 0.25% sodium deoxycholate, 1 mmol/L sodium orthovanadate, 1 mmol/L NaF, and 10 μL/mL Sigma protease inhibitor cocktail [Sigma, St Louis, MO]). After homogenization, the samples were sonicated twice on ice and centrifuged at 11,000*g* for 10 min at 4°C. Supernatants were recovered and stored at −80°C for subsequent immunoblotting. Protein concentration was determined using a Bio-Rad Protein assay with bovine serum albumin as a standard.

Western Blotting Analysis

Electrophoresis and transfer of proteins to polyvinylidene difluoride membranes were done as previously described.[22] Primary antibody directed against PKCα, βI, βII, ε, and δ (Santa Cruz Biotechnology, Santa Cruz, CA) were incubated overnight at 4°C in a 1:1000 dilution. Antibodies directed against total and phospho ERK1/2, Akt, and serine phosphorylated PKC substrates (Cell Signal Technology, Beverly, MA) were incubated at a 1:1000 dilution for 48 h at 4°C in 5% bovine serum albumin (phosphospecific antibodies) or 5% nonfat milk (nonphosphospecific antibodies) in Tris buffer with

0.05% Tween-20. Secondary antibody conjugated to horseradish peroxidase (goat anti-rabbit, Cell Signal Technology) was incubated for 1 h at 1:10,000 dilution. α-tubulin band density (Santa Cruz Biotechnology), previously shown to be unchanged in compensated cardiac hypertrophy,[23] was used as a loading control. Signals were visualized by enhanced chemiluminescence (Cell Signal Technology). Relative band density of immunoblots on film was measured with a scanner using NIH 1.63 image software (National Institutes of Health, Rockville, MD).

RNA Isolation and Real-Time Polymerase Chain Reaction

Total RNA was isolated from left ventricle using TRIZOL reagent (Invitrogen) according to the manufacturer's instructions. Reverse transcription was carried out with SuperScript II Reverse Transcriptase (Invitrogen) and random primers following the manufacturer's instructions. Forward and reverse primers used for amplification of fully processed cardiac β-myosin heavy chain (β-MHC) were based on those previously reported[24] [reverse primer β-MHC5869R (5′-CTCCAGGTCTCAGGGCTTCAC-3′), forward primer β-MHC5579F (5′-GACAGGAAGAACCTACTGCG-3′)]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control. GAPDH primers sequences were (Genbank accession no. NM 017008) forward (5′-GCCATCAACGACCCCTTCAT-3′) and reverse (5′-CCGCCTGCTTCACCACCTTC-3′). All real-time polymerase chain reaction experiments were done in triplicate using 2 ng of RNA and run for 25 to 26 cycles. Polymerase chain reaction products were resolved on 2% agarose gels with ethidium bromide and quantitated by densitometry using NIH Image v1.64 software.

Vascular Reactivity

Thoracic aortae (proximal to the site of aortic constriction and thus exposed to pressure overload), coronary arteries, and mesenteric arteries (distal to the aortic constriction and thus not exposed to pressure overload) were mounted on wire-type myographs[25] and warmed to 37°C for 30 min at zero tension. At 30 min, coronary (10 mg) and mesenteric (50 mg) arterial tension was increased manually. Next, a series of internal circumference-active tension curves were constructed to determine the vessel diameter that evoked the greatest tension development (L_{max}) to 100 mmol/L potassium chloride (KCl). L_{max} tension did not differ among groups and was 492 ± 48 and 803 ± 56 mg for coronary and mesenteric arteries, respectively. Tension on the aortas was increased manually over 60 min to ≈2 g and did not differ among groups (1979 ± 14 mg). The vessel bathing medium was exchanged at ≈15 min intervals throughout the experiment with physiological saline solution (pH 7.35–7.45). Vasorelaxation responses to acetylcholine (ACh, 10^{-8}–10^{-4} mmol/L) and sodium nitroprusside (SNP, 10^{-9}–10^{-4} mmol/L), and vasocontraction produced by N^G-monomethyl-L-arginine (L-NMMA, 10^{-3} mmol/L), were assessed on vessels precontracted with norepinephrine (NE, 10^{-7} mmol/L; aorta, mesentery)[26] or endothelin-1 (ET-1; $\approx 3 \times 10^{-8}$ mmol/L, coronary). Vasocontractile responses to KCl (10–100 mmol/L; all vessels) and NE (10^{-8}–10^{-4} mmol/L; aorta and mesentery only) also were assessed. Protocols were separated by 45 to 60 min. For all vessels studied: vasorelaxation is expressed as percent relaxation from precontraction tension; and vasocontraction is presented as milligrams of developed tension (NE, KCl, ET-1) or as percent increase from precontraction tension (L-NMMA). We have used these procedures previously.[27,28]

Plasma and Tissue Q Analyses

Samples were analyzed for free Q, conjugated Q, and 3-*O*-methyl Q (a metabolite). Approximately 30 mg of tissue was placed in a 12- × 75-mm glass test tube along with 200 mL KH_2PO_4 (pH 4.5) and glass beads. Samples were homogenized by inserting a 10- × 75-mm tube into the 12- × 75-mm tube, then rinsing the outside of the 10- × 75-mm tube with an additional 200 mL KH_2PO_4 (pH 4.5). The homogenate was then incubated with 20 mL of β-glucuronidase at 40°C for 2 h. Samples were acidified with 40 mL of dilute phosphoric acid (1:10) to inhibit protein binding and gently mixed with 2 mL of hexane/acetic acid (1:1). Samples were cooled at −60°C for 10 min and the solvent decanted from the frozen aqueous layer into clean 12- × 75-mm tubes. The extraction process was repeated with 2-mL hexane/acetic acid (1:1) and the organic phases combined. The solvent was then dried under N_2 at 40°C, and the residue reconstituted in 100-mL mobile phase for HPLC analysis.

Estimates of Liver Oxidant Load

Lipid oxidation was determined by fluorescence detection of malondialdehyde equivalents (nmol/mg protein) as previously described.[29] Protein oxidation was estimated by quantifying protein carbonyls (nmol/mg protein) via spectrophotometric quantification of the dinitrophenylhydrazine adduct.[29] For all assays, protein concentrations were determined using bovine serum albumin as the standard.[30]

Vascular Morphology

Thoracic aortas were fixed in formalin (24 h) and embedded in paraffin before three 4-μmol/L cross-sections were mounted on slides and stained with hematoxylin and eosin. Images were taken with a microscope (Nikon E6000) equipped with a digital camera (Q imaging, Micro Publisher 5.0 RTV) using 2 × objective. Final focus (microscope objective and digital camera optical zoom) used for capturing digital images was 4 ×. Software was calibrated using a micrometer photographed at the same focus and zoom so that pixels were expressed as microns. Tunica media thickness was measured from calibrated digital photographs using NIH Image 1.32 software.[28]

Statistical Analyses

A two-way analysis of variance was used to detect differences among groups using SPSS v10 (SPSS, Chicago, IL). When a significant P value was obtained ($P < 0.05$), post hoc procedures were performed using least-squares difference analyses to identify individual group differences. Results are presented as mean ± standard error.

RESULTS

General Animal Characteristics

Body weight (g) was not different among SHAM (335 ± 7), AAC (321 ± 6), SHAMQ (342 ± 10), and AAC-Q (342 ± 7) animals. Plasma (μg/mL) and liver (ng/mg protein) concentrations of Q isoforms (free Q, conjugated Q, free 3-*O*-methyl Q, conjugated 3-*O*-methyl Q) were similar in Q-fed groups and undetectable in control-fed groups (Table 1).

Cardiac Hypertrophy, Arterial Pressure, and Aortic Medial Thickening

AAC evoked greater heart:body weight compared to SHAM animals. This hypertrophic response was attenuated in AACQ rats (Fig. 1A). Q treatment had no effect on cardiac size in SHAMQ animals (Fig. 1A). Lung and liver weights were similar among groups, verifying the absence of pulmonary and hepatic congestion. AAC increased the carotid arterial systolic, diastolic, and mean blood pressures (Fig. 1B) and elevated the carotid-caudal arterial blood pressure gradient relative to SHAM and SHAMQ. Carotid systolic pressure was attenuated in AACQ versus AAC, but carotid diastolic and mean arterial pressures were normal compared to SHAM and SHAMQ (Fig. 1B). The carotid-caudal gradient was also lower in the AACQ versus AAC animals (Fig. 1C). Systolic, diastolic, and mean caudal pressures (ie, distal to the aortic constriction) were not different among groups (data not shown). Medial thickness of aortas was greater in AAC compared with all other groups, including AACQ (Fig. 1D). Finally, there was a strong correlation ($r = 0.804$, $r^2 = 0.647$, $P < 0.001$) between mean arterial blood pressure and cardiac hypertrophy as determined by heart weight:body weight, which emphasized the central role of blood pressure in determining cardiac mass in these animals (Fig. 1F). β-MHC mRNA expression normalized to GAPDH expression (determined by quantitative densitometry) was increased in left ventricles of AAC versus AACQ, SHAM, and SHAMQ (Fig. 1G). These data indicate that Q treatment prevented the increase in cardiac β-MHC expression typically observed in AAC.

TABLE 1. Concentrations of Quercetin (Q) Isoforms in Livers (ng/mg protein) and Plasma (μg/mL) of Rats Fed Q-Supplemented Diets

	SHAMQ (n = 5)	AACQ (n = 5)
Liver Q	0.09 ± 0.03	0.12 ± 0.04
Liver conjugated Q	0.17 ± 0.04	0.19 ± 0.03
Liver 3-*O*-methyl Q	0.19 ± 0.07	0.14 ± 0.05
Liver conjugated 3-*O*-methyl Q	1.05 ± 0.10	1.30 ± 0.10
Plasma Q*	3.26 ± 0.05	3.96 ± 0.40

*All isoforms of Q combined.

Myocardial Function

Heart rate (bpm) was similar among SHAM (389 ± 10), AAC (390 ± 10), SHAMQ (392 ± 10), and AACQ (413 ± 10) rats. Echocardiographic estimates of LV mass indicated that AAC-evoked increases in LV mass (g; 1.55 ± 0.19) were abolished in AACQ (1.19 ± 0.07) animals (Fig. 1E). SHAM (1.08 ± 0.08) and SHAMQ (1.17 ± 0.10) had similar LV mass. There were no differences in cardiac output, ejection fraction, and fractional shortening among groups, indicating that cardiac function was normal in both AAC and AACQ compared with sham groups.

Vascular Reactivity

Vessel characteristics are shown in Table 2. ACh-evoked vasorelaxation was less in thoracic aortas from AAC versus SHAM animals regardless of Q treatment (Fig. 2A), whereas responses from the coronary and mesenteric arteries were similar among groups (Fig. 2B, C). For all vessel types (aorta, coronary, mesentery), SNP-evoked dose–response curves (10^{-9}–10^{-4} mmol/L) were not different among the 4 groups, demonstrating that a functional smooth muscle layer existed (Table 3). Precontraction tension between the ACh and SNP dose-response curves was similar for all groups and all vessel types. Basal nitric oxide synthase activity, estimated as tension development in response to L-NMMA, was similar among groups for all vessel types (Table 3). Likewise, receptor-mediated (ie, NE 10^{-8}–10^{-4} mmol/L, aorta and mesentery) and nonreceptor mediated (KCl, 10–100 mmol/L, all vessels) contractile responses were not different among groups (Table 3).

Signaling Proteins

PKCβII translocation was greatest in LV tissue from AAC versus AACQ, SHAM, SHAMQ rats, whereas PKCα, PKCβI, PKCδ, or PKCε were similar among all groups (Fig. 3A, B). Increased membrane levels of PKCβII in AAC rats coupled with no change in cytosolic levels suggested increased PKCβII protein expression, which was verified by greater protein levels of PKCβII from whole heart homogenates in AAC versus all other groups (Fig. 3C). Although serine phosphorylation of endogenous PKC substrates normalized to α-tubulin levels was greater in AAC, AACQ versus SHAM, SHAMQ (Fig. 3D), no differences existed between AAC and AACQ rats. None of the measured PKC isoforms were altered in SHAMQ rats (Fig. 3A, B). In contrast, both AAC and AACQ rats had increased ERK1/2 activation (p-ERK: total ERK) compared to SHAM and SHAMQ (Fig. 4). Activation of Akt (p-Akt: total Akt) was similar among groups (data not shown).

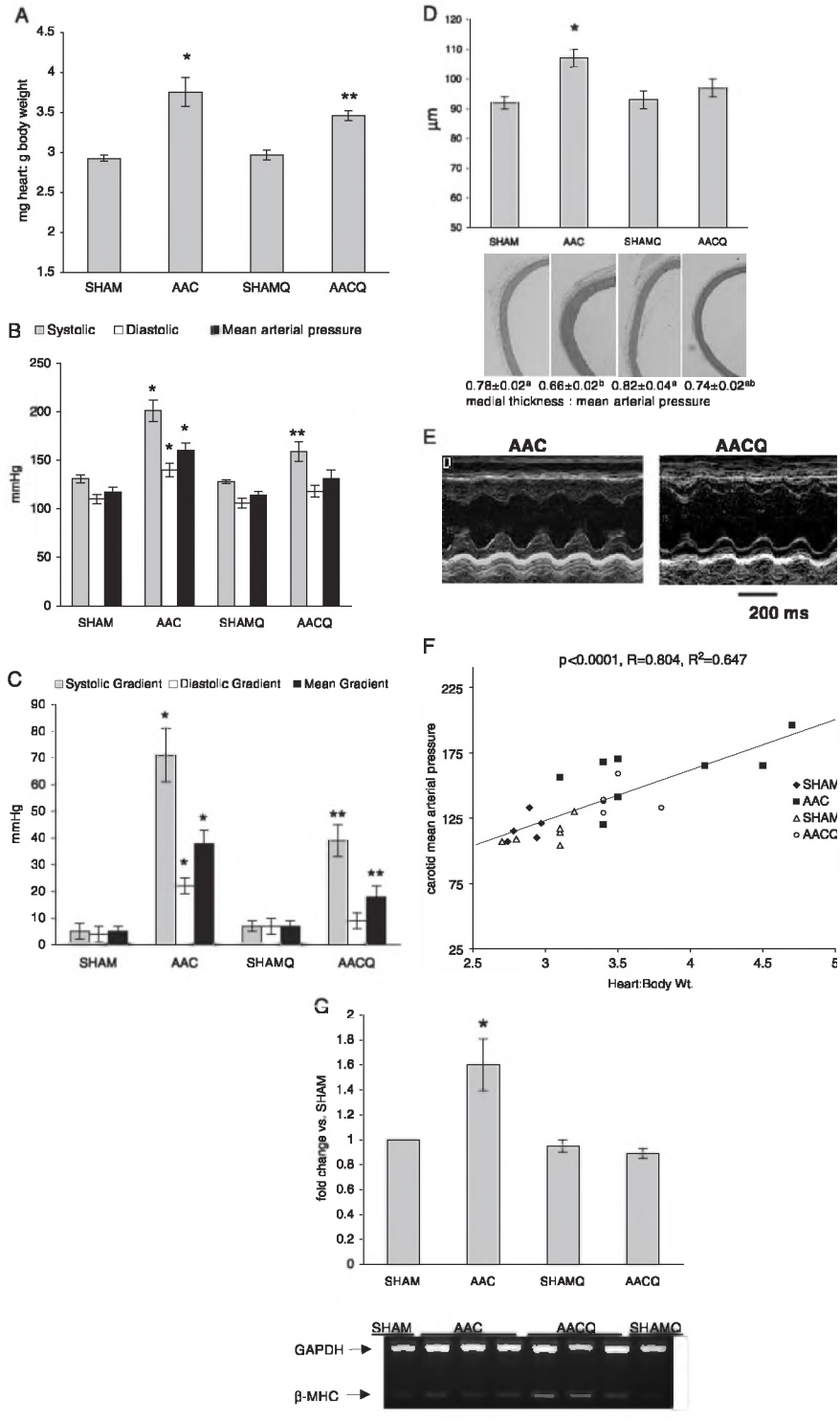

FIGURE 1. A, AACQ rats have less cardiac hypertrophy than AAC. *$P<0.05$ versus SHAM, SHAMQ, AACQ; **$P<0.05$ versus SHAM, SHAMQ, AAC. B, AACQ rats have lower systolic carotid pressure and normal diastolic and mean arterial pressure compared to AAC rats. *$P<0.05$ versus SHAM, SHAMQ, AACQ; **$P<0.05$ versus SHAM, SHAMQ, AAC. C, The pressure gradient across the site of aortic constriction is lower in AACQ rats versus AAC. *$P<0.05$ versus SHAM, SHAMQ, AACQ; **$P<0.05$ versus SHAM, SHAMQ, AAC. D, Aortic hypertrophy is prevented in AACQ animals as determined by medial thickness (µm; n = 5/group, 3–5 sections/animal). Magnification level used for analysis was 4×. *$P<0.05$ versus SHAM, SHAMQ, AACQ. E, M-mode images demonstrate that AAC rats had greater wall thickening than AACQ (n = 5/group). F, Regression analysis of heart:body weight versus carotid arterial pressure. A strong correlation exists between blood pressure and cardiac hypertrophy. G, β-MHC mRNA expression normalized to GAPDH mRNA is increased 60% in AAC versus SHAM, but normalized in AACQ. *$P<0.05$ versus SHAM, SHAMQ, and AACQ. Results are average of 3 independent trials. SHAM, sham operated and fed control chow; SHAMQ, sham operated and fed Q-enriched chow; AAC, abdominal aortic constriction and fed control chow; AACQ abdominal aortic constriction and fed Q-enriched chow.

Estimates of Oxidant Load

Thiobarbituric acid reactive substances (nmol malondialdehyde/mg protein) were lower in liver from SHAMQ and AACQ rats compared to SHAM and AAC animals (Fig. 5). There was a trend toward lower ($P = 0.058$) liver protein carbonyls (nmol/mg protein) in Q-treated versus untreated rats (SHAMQ 1.42 ± 0.11; AACQ 1.34 ± 0.13; SHAM 1.62 ± 0.18; AAC 1.70 ± 0.10).

DISCUSSION

The major finding of this study was that AAC rats consuming the flavonoid Q had less cardiac hypertrophy compared with untreated AAC rats. The most likely

TABLE 2. Vessel Characteristics

	SHAM	AAC	SHAMQ	AACQ
Aorta				
Baseline internal diameter, μm	1686 ± 46	1680 ± 32	1629 ± 41	1658 ± 29
Internal diameter at 2*g* tension, μm	2694 ± 35	2780 ± 28	2774 ± 35	2767 ± 37
Vessel length, μm	3314 ± 70	3443 ± 48	3348 ± 61	3346 ± 66
Mesentery				
Baseline internal diameter, μm	188 ± 13	186 ± 22	175 ± 11	195 ± 9
Internal diameter at L_{max}, μm	463 ± 10	455 ± 19	485 ± 26	445 ± 26
Vessel length, μm	3859 ± 134	4216 ± 150	4094 ± 122	4148 ± 224
Coronary				
Baseline internal diameter, μm	125 ± 8	126 ± 3	122 ± 7	128 ± 8
Internal diameter at 2*g* tension, μm	347 ± 16	376 ± 18	381 ± 20	389 ± 16
Vessel length, μm	1223 ± 47	1300 ± 35	1339 ± 43	1293 ± 39

mechanism for this effect is a decreased arterial blood pressure and a secondary reduction in the activation of hypertrophic signaling pathways in the heart. These data support our original hypothesis and agree with previous studies that oral administration of Q attenuates blood pressure in spontaneously hypertensive rats[2] and rats with hypertension evoked by chronic nitric oxide synthase inhibition.[31] Furthermore, the structural and hemodynamic benefits we observed in AACQ animals were not accompanied by deleterious changes to myocardial or vascular function. Our results contribute importantly to a growing body of literature concerning the potential for polyphenolic compounds to reduce indices of cardiovascular risk.[32–36]

Originally we hypothesized that reduced cardiac hypertrophy in AACQ would be accompanied by reduced activation of PKC and/or ERK1/2. Although this hypothesis was supported by the observation that Q consumption normalized PKCβII translocation in AACQ, cardiac ERK1/2 activation persisted in these animals. The cause for this finding is unclear, but it may be related to the mild degree of hypertension that was present in AACQ rats compared with the severe hypertension experienced by AAC animals. However, despite normal translocation levels of PKC isoforms in AACQ hearts, levels of serine phosphorylated PKC substrates increased similarly between AAC and AACQ hearts. Although the cause of this cannot be defined precisely, PKC isoforms not examined in the present study may have remained activated in AACQ and contributed to serine phosphorylation.

Previous studies have reported that antihypertensive drugs can reduce cardiac hypertrophy in pressure-overloaded rats;[37,38] however, little is known about the effect on cardiac PKC activation following blood pressure reduction. Fedorova et al reported that hypertensive Dahl salt-sensitive rats treated with cicletanine (antihypertensive) had reduced blood pressure, cardiac PKCβII

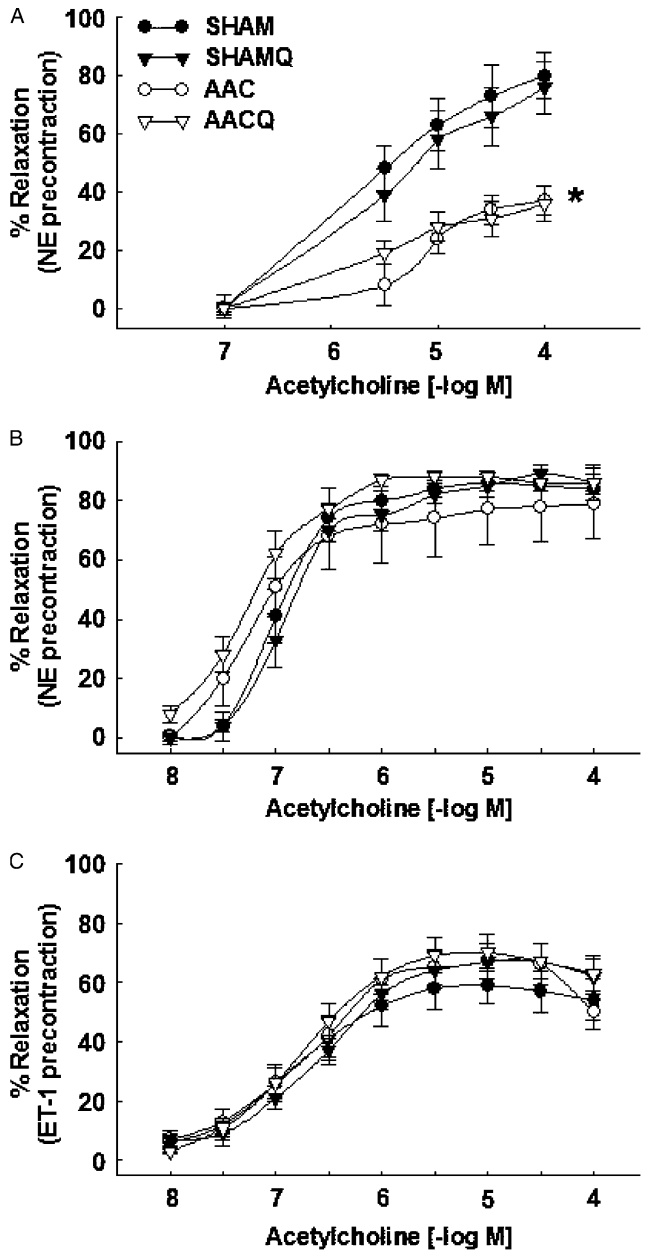

FIGURE 2. (A) ACh-evoked vasorelaxation in aortas; (B) mesenteric arteries; and (C) coronary arteries. SHAM, sham operated and fed control chow; SHAMQ, sham operated and fed Q-enriched chow; AAC, abdominal aortic constriction and fed control chow; AACQ, abdominal aortic constriction and fed Q-enriched chow. Data are mean ± SE. **P*<0.05 versus SHAM, SHAM Q.

translocation, and cardiac hypertrophy.[39] The authors of that study suggested that because cicletanine is also a PKC inhibitor in vitro, cardiac PKCβII inhibition may have occurred at least in part because of the direct effects of cicletanine on PKC itself. Along these lines, previous investigations have also reported that Q can also inhibit PKC in vitro via competitive inhibition when adenosine triphosphate (ATP) and Q concentrations are similar.[40]

TABLE 3. Vessel Reactivity Data

	SHAM	AAC	SHAMQ	AACQ
Aorta				
Maximal % relaxation, 10^{-3} mmol/L SNP	88 ± 4	92 ± 6	85 ± 4	85 ± 5
% tension development, 10^{-3} mmol/L L-NMMA	19 ± 2	15 ± 2	19 ± 2	20 ± 2
mg tension development, 10^{-4} mmol/L NE	2336 ± 38	1767 ± 183*	2197 ± 154	1706 ± 114*
mg tension development, 100 mmol/L KCl	1937 ± 69	1804 ± 128	1846 ± 67	1912 ± 70
Mesentery				
Maximal % relaxation, 10^{-3} mmol/L SNP	96 ± 4	92 ± 6	106 ± 4	94 ± 4
% tension development, 10^{-3} mmol/L L-NMMA	8 ± 3	15 ± 2	19 ± 2	20 ± 2
mg tension development, 10^{-4} mmol/L NE	1962 ± 165	1920 ± 65	2032 ± 132	1992 ± 100
mg tension development, 100 mmol/L KCl	957 ± 32	841 ± 43	907 ± 62	875 ± 149
Coronary				
Maximal % relaxation, 10^{-3} mmol/L SNP	96 ± 4	92 ± 6	106 ± 4	94 ± 4
% tension development,10^{-3} M L-NMMA	19 ± 2	15 ± 2	19 ± 2	20 ± 2
mg tension development, 100 mmol/L KCl	476 ± 28	498 ± 88	534 ± 120	585 ± 143

Responses in aortas and mesentery to SNP and L-NMMA were performed on vessels precontracted with NE. Responses in coronary arteries to SNP and L-NMMA were performed on vessels precontracted with endothelin-1. KCl, potassium chloride.

*$P < 0.05$ vs respective control group.

However, we do not believe our findings observed in vivo can be attributed to a direct effect of Q on PKCβII. In this regard, ATP concentrations are ≈5 μmol/g wet heart weight in the rat,[41] whereas plasma and liver Q concentrations in our animals were much lower (Table 1). Because of the competitive relationship between ATP and Q and the differences in their respective concentrations, it is unlikely that Q directly inhibited PKCβII in AACQ rats. Furthermore, given the mechanism of Q inhibition of PKC, only the catalytic activity of PKC would be affected, not the translocation of the enzyme as measured in our study.

The reduction of myocardial PKCβII translocation we observed in AACQ versus AAC rats is likely secondary to the blood pressure-lowering effects of Q (ie, the stimulus to activate hypertrophic signaling kinases was reduced). At least 1 other study provides evidence to support this statement. When chronically hypoxic rats with pulmonary hypertension were treated with nifedipine, right ventricular hypertrophy was lower and PKC expression normalized.[42] Further evidence supporting our contention that lower blood pressure was responsible for reduced cardiac hypertrophy is provided by the strong correlation between blood pressure and cardiac mass ($r = 0.804$, $P < 0.001$). Although previous investigations report that antihypertensive drugs reduce cardiac hypertrophy in pressure-overloaded rats,[37,38] our study is the first to show that the polyphenolic compound Q reduces arterial pressure, cardiac hypertrophy, PKCβII translocation, and β-MHC expression. This is an important finding because transgenic mice with cardiac-specific PKCβII overexpression have myocardial hypertrophy that is characterized by fibrosis and poor cardiac function,[43] and humans with heart failure demonstrate PKCβ isoform activation.[44]

The precise mechanism or mechanisms responsible for the ability of Q to reduce blood pressure are unclear, particularly in the setting of a fixed, mechanical obstruction. One possibility is that Q preferentially dilated resistance vessels in the upper body proximal to the aortic constriction. This is plausible because Q has been shown to evoke arterial vasodilation that is inversely proportional to vessel diameter.[45] This is relevant physiologically because arterial pressure and blood flow are regulated to a greater degree by resistance-sized arteries (eg, skeletal muscle arteries) versus conductance-sized arteries (eg, aorta).[46] Although we did not find differences in endothelial function from arteries proximal to the site of aortic constriction (aorta and coronary arteries), this does not exclude Q-induced in vivo effects on the smaller peripheral arteries and arterioles in the skeletal muscle. A second possible explanation for our results concerning blood pressure involves the renal response to AAC. Acute AAC causes renal hypoperfusion that leads to increased plasma renin activity.[47,48] Greater plasma renin activity increases circulating levels of angiotensin I,[47] and ultimately of angiotensin II, a known stimulator of hypertension and cardiac hypertrophy.[49] Importantly, Q has been reported to be an inhibitor of angiotensin 1-converting enzyme in vitro and in vivo.[50–53] Therefore, it is possible that inhibition of angiotensin 1-converting enzyme by Q may have limited AAC-evoked hypertension.

Though our results were associated with a general reduction in oxidative stress (decreased levels of liver malondialdehyde [$P = 0.05$] and trend to lower protein carbonyls [$P = 0.06$] in SHAMQ and AACQ), the specific consequence of this reduction in oxidative stress is unclear. For example, previous studies have reported that antioxidants can reduce hypertension by improving vascular dilation,[54–56] but lower blood pressure in our study was not accompanied by improved endothelial-dependent relaxation in the vessels we examined. Furthermore, inhibition of ERK1/2 and PKC in vitro via an antioxidant mechanism has also been reported.[57] Although we found AAC-evoked increases in PKCβII translocation were prevented by Q, we did not find any decrease in cardiac ERK1/2 phosphorylation. Therefore,

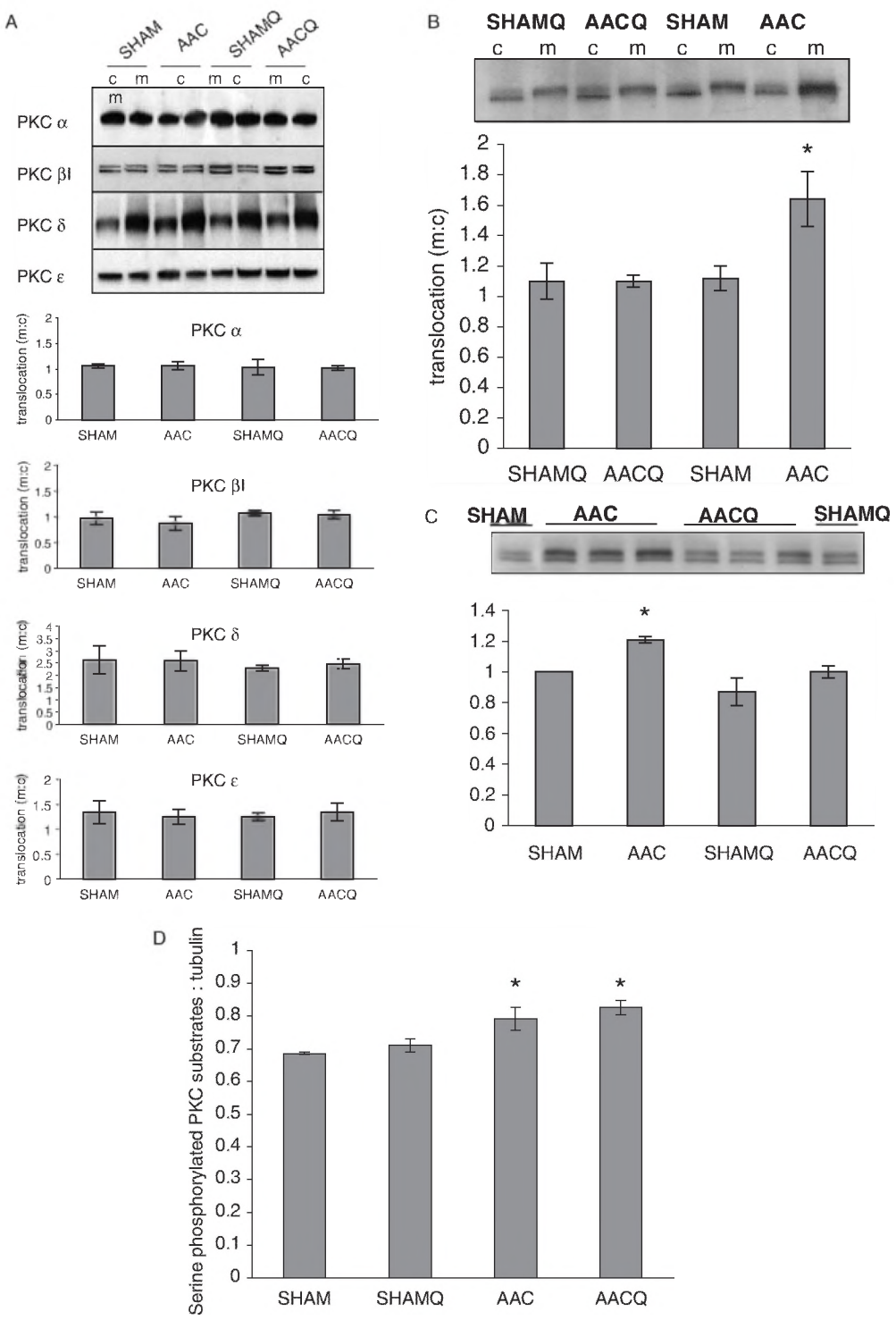

FIGURE 3. A, Representative immunoblots of PKC isoforms measured from LVs of rats. PKCα, βI, δ, and ε were unchanged among groups. B, PKCβII membrane density was increased and translocation was greater in AAC versus SHAM. AACQ rats had similar PKC βII translocation compared to SHAMQ. *$P<0.05$ versus SHAM, AACQ, SHAMQ. C, Total protein levels of PKCβII were greater in AAC, but normalized in AACQ. *$P<0.05$ versus SHAM, AACQ, and SHAMQ. D, Levels of serine phosphorylated PKC substrates taken from whole left ventricular homogenates were greater in AAC and AACQ versus SHAM and SHAMQ. However, there was no difference in degree of serine phosphorylation between AAC and AACQ controls. α-Tubulin was used as loading control and lane density of serine phosphorylated PKC substrates was normalized to levels of tubulin. $P<0.05$ versus SHAM, SHAMQ. c, cytosolic fraction; m, membrane fraction; SHAM, sham operated and fed control chow; SHAMQ, sham operated and fed Q-enriched chow; AAC, abdominal aortic constriction and fed control chow; AACQ, abdominal aortic constriction and fed Q-enriched chow; n = 5 for each group.

our present data do not provide strong evidence for the reduction of oxidative stress as a mechanism to explain the effect of Q.

At present, the efficacy of antioxidants such as vitamins or flavonoids to limit/prevent progression of cardiovascular disease is controversial. Vitamin C has been shown to decrease vascular dysfunction and hypertension in rats[58] and guinea pigs,[4] whereas vitamin E can decrease hypertension in humans.[59] However, such reports are tempered by a recent science advisory statement by the American Heart Association reporting that more null than beneficial effects are observed in clinical trials using vitamins C and E.[60] In contrast, there are a growing number of studies that demonstrate the

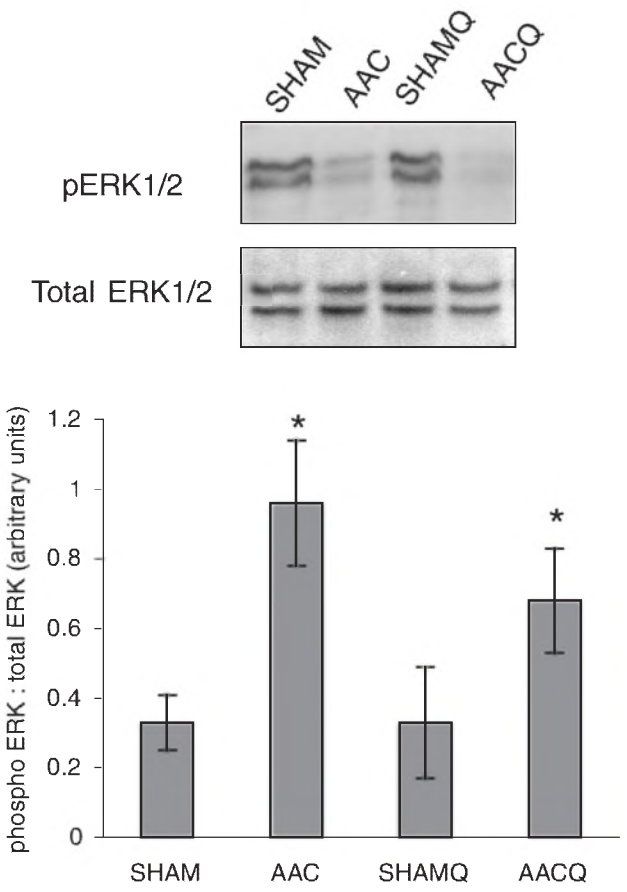

FIGURE 4. Activation of ERK1/2 (p-ERK1/2:total ERK1/2) was greater in AAC and AACQ rats compared with SHAM and SHAMQ, however, there was no difference in ERK1/2 activation between AAC and AACQ. *$P<0.05$ versus SHAM, SHAMQ. SHAM, sham operated and fed control chow; SHAMQ, sham operated and fed Q-enriched chow; AAC, abdominal aortic constriction and fed control chow; AACQ abdominal aortic constriction and fed Q-enriched chow; n = 5 for each group.

benefits of flavonoids to prevent cardiovascular disease. For example, isorhapontigenin, an analog of the flavonoid resveratrol, reduces cardiac hypertrophy and blood pressure in pressure overloaded rats.[57] Furthermore, Q attenuates blood pressure in spontaneously hypertensive rats,[2,61] rats with surgically induced renovascular hypertension,[56] rats with chronic nitric oxide synthase inhibition,[31] deoxycorticosterone acetate-salt hypertensive rats,[62] and rats with abdominal aortic constriction (present study). Taken together, results from the previous studies suggest that polyphenolic compounds such as Q supplemented in the diets of experimental animals are efficacious in reducing blood pressure. The implications of these data with regard to reduction of cardiovascular disease risk in humans with chronic consumption of lower amounts of dietary Q are not clear. Although the amounts of Q consumed by rats in this study are comparatively higher than those reported in humans, it is noteworthy that dietary Q consumption in humans has been reported to be inversely associated with cardiovascular disease risk.[5,6]

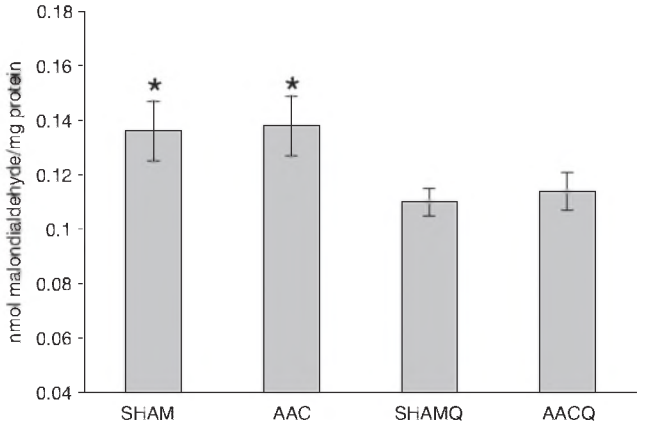

FIGURE 5. Animals consuming Q-supplemented diets had less oxidative stress as determined by liver thiobarbituric acid reactive substances. *$P<0.05$ versus SHAMQ, AACQ. SHAM, sham operated and fed control chow; SHAMQ, sham operated and fed Q-enriched chow; AAC, abdominal aortic constriction and fed control chow; AACQ, abdominal aortic constriction and fed Q-enriched chow; n = 5 for each group.

CONCLUSIONS

In summary, our findings demonstrate that rats with aortic constriction fed Q-supplemented diets had attenuated cardiac hypertrophy, lower arterial blood pressure, decreased aortic medial thickening, and normalized cardiac PKCβII translocation. These beneficial effects were not accompanied by deleterious changes to cardiac or vascular function.

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