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Decreased blood pressure with acute administration of quercetin in L-NAME-induced hypertensive rats

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Abstract

Quercetin is known to reduce blood pressure (BP); however, its acute effects are unclear. We investigated the acute effects of quercetin on BP, aortic mechanical properties and vascular reactivity in female Sprague–Dawley (SD) rats. Hypertension was induced using L-NAME (40 mg/kg/day). Quercetin (4.5 mg/kg) was administered intravenously. Mechanical properties of the aortae were measured by echo-tracking in normotensive and hypertensive rats. L-NAME and quercetin quantities in the aorta were determined using AP-MALDI-MSI. Vascular reactivity was performed in mesenteric and renal arteries. L-NAME increased BP and PWVβ while decreasing strain. Quercetin decreased BP and ameliorated PWVβ in L-NAME-induced hypertensive rats. Ex vivo, the acetylcholine (ACh)-induced increase in tension at 100 μM was reduced in renal arteries when exposed to quercetin while phenylephrine (Phe)-induced contractile response was augmented. In quiescent rings of renal arteries incubated with L-NAME (10 μM) and TRAM-34 (1 μM), the ACh-induced vasoconstrictions were inhibited by quercetin. Quercetin resulted in concentration-dependent vasodilation in mesenteric arteries and increased its sensitivity to ACh-induced relaxations. Quercetin lowered BP in L-NAME-induced hypertensive rats, likely due to changes in aortic mechanical properties and relaxation of resistance arteries. Further research is warranted to clarify the acute effects of quercetin on renal arteries in this hypertensive model.

KEYWORDS
AP-MALDI-MSI, blood pressure, echo-tracking, quercetin, vascular reactivity

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Plain English Summary

In this study, we explored how quercetin, a natural compound found in many fruits and vegetables, can quickly lower blood pressure in a rat model of hypertension. We induced high blood pressure in female rats and then administered quercetin to see its immediate effects. Our results showed that quercetin effectively reduced blood pressure and improved the flexibility of the aorta, a major blood vessel. It also enhanced the function of mesenteric arteries, making them relax more easily. This suggests that quercetin might be a useful, fast-acting treatment for managing high blood pressure.

1 | INTRODUCTION

Quercetin, a polyphenolic flavonoid, is naturally present in various fruits, vegetables, leaves, seeds and grains.[1] Common dietary sources that contain appreciable quantities of quercetin include capers, red onions and kale. Quercetin is frequently used as an ingredient in dietary supplements, beverages and various food products.[2] In traditional and complementary medicine, quercetin has been widely used to treat metabolic syndrome.[3] In preclinical and clinical studies, quercetin has demonstrated considerable therapeutic potential against cardiovascular disease, possibly through a protective effect against hypertension.[1,2,4–14] In this regard, numerous human and animal studies have demonstrated that chronic treatment with quercetin decreases systolic and diastolic blood pressure (BP).[2,4,9,15–18] In the setting of hypertension, quercetin may protect the vasculature against several pathological mechanisms including oxidative stress and inflammation, thereby preventing endothelial dysfunction.[2,4,9,19,20] Vasodilation has also been proposed as an important mechanism responsible for the BP-lowering effect of quercetin.[9] Indeed, quercetin has been shown to induce vasodilation in isolated umbilical arteries of healthy women.[21] Moreover, several studies have shown that quercetin induces vasodilation in isolated blood vessels of hypertensive and normotensive male rodents.[22–30]

Despite these promising results reported with chronic quercetin treatment, only a few studies have investigated the acute effects of quercetin on vasodilation and BP in vivo, without definitive findings.[15,21,31,32] In this regard, quercetin did not result in vasodilation or BP-lowering effect in healthy normotensive men and women.[33] Another study, however, showed that quercetin induced vasodilation which did not translate into a BP-lowering effect in healthy men and women.[21] Similarly, despite improvements in flow-mediated dilation, quercetin did not translate into a BP-lowering effect in men and women at risk for cardiovascular disease.[32] In contrast, quercetin decreased BP without inducing vasodilation in hypertensive men.[15] Finally, no change in BP or haemodynamic parameters has been shown with quercetin in normotensive and spontaneously hypertensive male and female rats.[31] These inconclusive findings on the acute BP-lowering effect and in vivo vasodilation induced by quercetin underscores the need for further investigation.

Except for one study in males,[15] studies on the acute effects of quercetin on BP have been conducted in a combined sample of healthy or diseased males and females.[21,31,32] Yet, none of these studies have demonstrated a sex-specific difference in the acute effects of quercetin on BP, most probably because of the small sample size.[31–33] Compared to men of similar age, women have a lower risk of hypertension and cardiovascular disease, likely due to the cardioprotective effects of oestrogen.[34] Because quercetin exhibits phytoestrogenic properties, it may modulate these oestrogen-mediated effects.[9] Hence, investigating the effects of quercetin in females may be particularly pertinent in the setting of hypertension.

Therefore, the present study aimed to determine the acute effects of quercetin on BP and the mechanical properties of the abdominal aortae in vivo using echo-tracking in female normotensive and L-NAME-induced hypertensive Sprague Dawley (SD) rats. In addition, L-NAME and quercetin's presence and abundance in the abdominal aorta were determined using atmospheric pressure matrix-assisted laser desorption ionisation mass spectrometry imaging (AP-MALDI-MSI). Finally, the effects of quercetin on vascular reactivity were assessed ex vivo in renal and mesenteric arteries.

2 | METHODS

2.1 | Animals and housing

This study was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (Approval references: 2017/02/03/C, 2019/11/67/A and 2020/02/03C) and was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and are reported in accordance with the ARRIVE guidelines. Additionally, the study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies.[35] The experiments were conducted on 3-month-old female SD rats ($n = 35$) that were housed

under conditions of controlled ambient temperature with a light–dark cycle of 12 hours, at the Wits Animal Research Facility (WRAF). Twenty[20] rats were assigned to the in vivo procedures and 15 to the ex vivo procedures. Rats were given ad libitum access to drinking water and standard laboratory animal chow (Labchef, Johannesburg, South Africa).

2.2 | Induction of hypertension and non-invasive blood pressure measurement

Twenty ($n = 20$) 2-month-old, female SD rats were randomly assigned to either a normotensive control group or hypertensive group ($n = 10$ each). Control rats received tap water for 4 weeks. The hypertension group received N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME, Sigma Aldricht,), at a concentration of 40 mg/kg/day in drinking water for a period of 4 weeks. Conscious, non-anaesthetised rats were subjected to weekly BP measurement using a tail-cuff technique which uses non-invasive BP amplifiers (Biopac Systems, Santa Barbara, CA, USA), as previously reported.[36] Rats were habituated to the procedure during a 2-week acclimation period. Measurements were taken at midday to avoid diurnal variation in BP. Systolic and diastolic BPs were determined by the average of 5 readings.

2.3 | Echo-tracking of the abdominal aortae and intravenous administration of quercetin

After 4 weeks of the intervention, the L-NAME ($n = 10$) and control ($n = 10$) rats were anaesthetised using isoflurane (5% induction followed by 2% for maintenance) via 100% O_2 mask inhalation to determine the acute effects of quercetin in vivo. Non-invasive BP and abdominal aorta ultrasound were performed on anaesthetised rats to assess their mechanical properties using echo-tracking before and after the injection of quercetin. The parameters derived from echo-tracking require an assessment of BP, ideally measured within the abdominal aorta. However, achieving this would necessitate catheter insertion into the lower abdominal aorta via the femoral artery, a procedure incompatible with echo-tracking. Therefore, we measured BP in anaesthetised rats using the tail-cuff method as an alternative. This approach allowed us to balance the technical constraints while maintaining the integrity of our data collection. After basal measurements, an intravenous injection of quercetin at a concentration of 4.5 mg/kg dissolved in a maximum 0.3 ml of 0.1% methylnitrocellulose[31,37] was administered in the left saphenous vein ($n = 7$ per group), as previously reported. The remaining three ($n = 3$) rats per group received the vehicle (0.3 ml of 0.1% methylnitrocellulose). Non-invasive BP and abdominal aorta mechanical properties were re-assessed 10 minutes after injection of quercetin.[31,37] As it has been previously reported in Vrolijk et al[31] and Sánchez et al,[37] intravenous administration is preferred in order to address the low oral bioavailability of quercetin and ensure effective loading of the drug into the system.

For echo-tracking, transabdominal grayscale ultrasonography was performed on anaesthetised rats using an ultrasound (Affiniti CVx, Philips Healthcare, Andover, Massachusetts) by a single experienced observer that was blinded to the experimental design. A longitudinal segment of the abdominal aorta was imaged 2 to 3 cm distal to the iliac artery. The probe was placed vertically to the artery wall to obtain the inner diameter (mm) of the vessel during systole and diastole. In this way, the vessel inner diameter, including systolic diameter (Ds) and diastolic diameter (Dd), were obtained on 5 stable waveforms within 12 cardiac cycles. Thereafter, the mechanical properties of the abdominal aortae were computed using standard formulae (supplemental information file 1). Echo-tracking was performed by a trained ultrasonographer who was blinded to group allocation.

2.4 | Atmospheric pressure matrix-assisted laser desorption ionisation mass spectrometry imaging (AP-MALDI-MSI) of blood vessels

Aortic tissue samples preserved in 10% buffered formalin were processed using standard procedures for paraffin embedding. Sections (5 μm) were prepared from these embedded tissues with a microtome and then deparaffinised. In brief, the formalin-fixed paraffin-embedded (FFPE) tissue sections were deparaffinised in 100% xylene with two changes of 2 and 1 minute each.[38,39] Following deparaffinisation, the sections were air-dried at room temperature. Next, the tissues were coated with a 2,5-dihydroxybenzoic acid (DHB, 50 mg/ml) matrix, prepared in 70% acetonitrile, 30% H_2O and 0.1% trifluoracetic acid (TFA), using a SunCollect Sprayer (Sunchrom Technologies, Germany). Sprayer settings were as follows: Z-distance (25 mm), flow rate (0.1 ml/min), velocity (1200 mm/min), track spacing (2 mm), 8 passes and pressure (2.5 bar).

Following matrix application, the tissue samples were analysed using AP-MALDI-MSI to confirm the presence of L-NAME and quercetin in the aortic tissue. Mass spectrometry imaging was carried out using AP-MALDI

(MassTech, US) coupled to a Thermo Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, US). The mass spectrometer was operated in positive ion mode; scan range, 150–500 m/z, with a mass resolution of 70 000, a scan rate of 3.7 scan/sec. The AP-MALDI source was operated using Target Next software (MassTech, USA) to define regions of interest (ROIs) for imaging experiments. All experiments were run at a spatial resolution of 20 μm. L-NAME (234.12 *m/z*) and quercetin (303.05 *m/z*) were visualised, and relative intensities extracted using the Mozaic software (SpectroSwiss, Switzerland).

2.5 | Vascular reactivity in isolated renal and mesenteric arteries

Fifteen ($n = 15$) 3-month-old female SD rats that did not receive L-NAME were anaesthetised with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (15 mg/kg), and a thoracotomy was performed. The kidneys and intestines were removed and placed in bicarbonate control Krebs–Henseleit solution containing: NaCl (118 mmol/l); KCl (4.7 mmol/l); $CaCl_2$ (2.5 mmol/l), $MgSO_4$ (1.2 mmol/l); KH_2PO_4 (1.2 mmol/l); $NaHCO_3$ (25 mmol/l); and glucose (11.1 mmol/l). The renal arteries and the second-order branches of the mesenteric arteries were isolated from the kidneys and the intestines, respectively. Both renal and mesenteric arteries were cleaned of fat and connective tissue; dissected into small rings (1.89 ± 0.09 mm in length) and mounted onto a wire myograph (Model 610 M, Danish Myo Technology, Aahrus, Denmark). Contraction data is presented as wall tension (mN/mm). In preparations used to assess the role of the endothelium, the vessels were denuded of the endothelium by mechanical friction using a hair. The rings were suspended between two stainless steel wires with a diameter of 40 μm in an organ chamber filled with a control solution kept at 37°C and aerated with 95% O_2 and 5% CO_2. One of the wires was connected to a movable holder supporting a tension transducer (Grass Instrument Co., Quincy, MA, USA) to collect the isometric force measurements by a data acquisition system (PowerLab 4SP, ADInstruments, United States). The rings were placed under an optimal resting tension according to a previously described method.[40] After a 60-minute stabilisation period, the arteries were exposed to potassium chloride (KCl) (80mM) which produced a maximal contraction response to potassium (K^+) and served as the reference contraction. The degree of contraction caused by phenylephrine was standardised relative to the contraction induced by the bicarbonate control Krebs–Henseleit solution. The preconstriction levels were as follows: 5.24 ± 0.61 mN/mm in mesenteric arteries, and 5.03 ± 1.46 mN/mm in renal arteries. This was important for ensuring that any observed vasorelaxation induced by quercetin was not influenced by variability in initial preconstriction levels. The detailed protocol has been provided in supplemental information file 2.

3 | STATISTICAL ANALYSIS

Data analysis was performed using GraphPad Prism version 10.3.1 for Windows (GraphPad Software, Boston, Massachusetts USA, www.graphpad.com). All continuous variables were examined for normality using a Shapiro–Wilk test. Normally distributed data is expressed as mean ± standard error of the mean (SEM), and non-normally distributed data as median [IQR]. For vascular reactivity, changes in isometric tension under baseline tension were recorded as wall tension (mN/mm). The relaxation evoked by quercetin, acetylcholine and sodium nitroprusside is expressed as a percentage of the tension during contractions induced by phenylephrine (30 μM). The concentrations that produced 50% of the maximal contractile response (EC_{50}) and the maximal responses (E_{max}) were determined from regression analysis logistic sigmoid function curves and compared by Student's *t*-test for unpaired observations for EC_{50} and E_{max}. To assess changes in SBP and DBP over time in control and L-NAME rats, a repeated measures analysis of variance (ANOVA) test was used. An unpaired *t*-test was used to determine differences between control and L-NAME rats for haemodynamic parameters, organ weights and mechanical properties of the abdominal aorta. For the in vivo protocol, an a priori sample size calculation using a two-tailed *t*-test, with an alpha of 0.05 and 80% power of detection, determined that a total sample size of $n = 20$ rats ($n = 10$ rats per group) was required (G*Power, version 3.1.9.2). Two-way repeated measures ANOVA were conducted in control and L-NAME rats before and after administration of vehicle or quercetin. The effects of vehicle/quercetin administration on SBP and DBP are presented using mean difference as the point estimate, with a corresponding 95% CI. For the ANOVA tests with hypertension/control and quercetin/vehicle as the main effects, a Tukey post hoc test was performed. $P < 0.05$ was considered as statistically significant.

3.1 | Pharmacological agents for vascular reactivity

Acetylcholine chloride, indomethacin, N^{ω}-nitro-L-arginine methyl ester hydrochloride, methylnitrocellulose, quercetin, phenylephrine hydrochloride, sodium nitroprusside

and TRAM-34 were purchased from Sigma-Aldrich (St Louis, MO, USA). All drugs were dissolved in deionised water except indomethacin (70% ethanol), quercetin (0.1% methylnitrocellulose), TRAM-34 (100% dimethyl sulphoxide). Further dilutions were performed in deionised water. Concentrations are expressed as final molar concentrations in the bath solution.

4 | RESULTS

4.1 | Characterisation of experimental model

4.1.1 | Systolic and diastolic blood pressure in response to L-NAME administration

Figure 1 shows the changes in systolic (Figure 1A) and diastolic (Figure 1B) blood pressure of female SD control and L-NAME rats over a 4-week intervention period. After habituation (week 0), the systolic and diastolic blood pressures were similar between the control and L-NAME rats ($P = 0.93$ and $P = 0.98$, respectively). From weeks 1 to 4, the systolic and diastolic blood pressures were significantly higher in the L-NAME compared to the control rats ($P < 0.05$ at all time points).

At the termination of the experimental protocol, Table 1 shows that under anaesthesia, the systolic and diastolic blood pressure of the L-NAME rats were significantly higher than those of the control rats ($P = 0.0002$ and $P = 0.0003$, respectively). Body and organ masses were similar between the groups (all $P > 0.05$).

4.1.2 | Mechanical properties of the abdominal aorta at termination of experimental protocol

The systolic (Figure 2A) and diastolic (Figure 2B) diameters of the abdominal aortae of the control and L-NAME rats were similar ($P = 0.40$ and $P = 0.65$, respectively). The percentage change in lumen diameter from systole to diastole (strain, Figure 2C) was significantly lower in L-NAME compared to control rats ($P < 0.0001$). The abdominal aortic compliance (Figure 2G) was similar between L-NAME and the control rats ($P = 0.052$). The Peterson's elastic modulus (Figure 2H) and pulse wave velocity β (Figure 2I) were significantly greater in L-NAME compared to controls rats ($P = 0.009$ and $P = 0.035$, respectively). The abdominal aortic flow velocity (Figure 2D), distensibility coefficient (Figure 2E) and stiffness parameter β (Figure 2F) were similar between the groups (all $P > 0.05$).

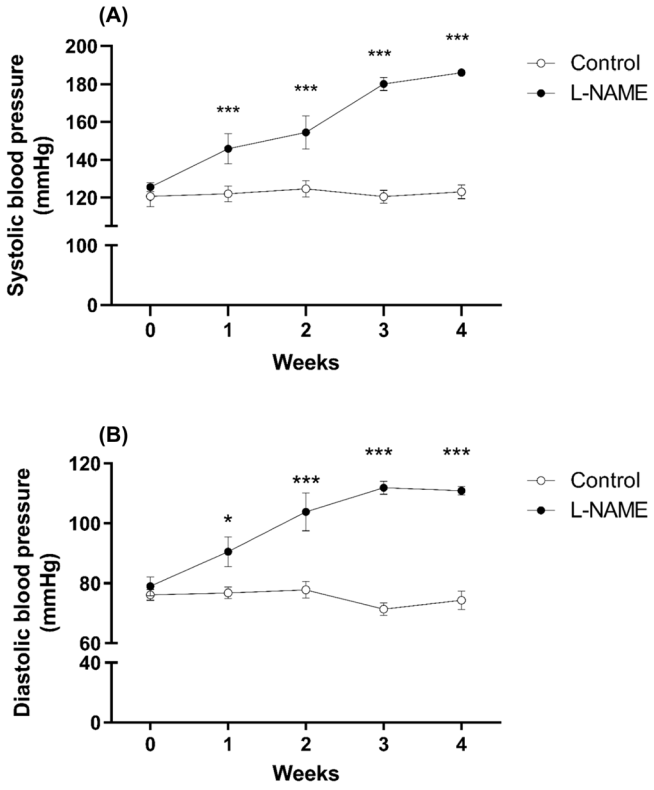


FIGURE 1 Systolic (A) and diastolic (B) blood pressure of female Sprague–Dawley rats that received L-NAME over a 4-week intervention period (L-NAME, $n = 10$) or not (control, $n = 10$). Data expressed as mean ± SEM. *$P < 0.05$; ***$P < 0.0001$; and ****$P < 0.00001$ vs control (2-way repeated measures ANOVA).

4.1.3 | Effects of vehicle and quercetin administration on blood pressure and mechanical properties of the abdominal aortae

First, to confirm that 0.3 ml of 0.1% methylnitrocellulose serves as an appropriate vehicle for the administration of quercetin, and to demonstrate that the vehicle itself did not influence the haemodynamic and echo-tracking parameters, a small sample of $n = 3$ rats was administered the vehicle. Here, we show that intravenous injection of the vehicle had no effects on the L-NAME-induced increases in systolic and diastolic blood pressure, and no effects on L-NAME-induced changes in echo-tracking parameters in these rats (all $P > 0.05$) (Table S1).

Second, in a separate sample of $n = 7$ rats, we tested the effects of quercetin administration on the L-NAME-induced changes in haemodynamic and echo-tracking parameters. We show that rats that received L-NAME exhibited greater systolic and diastolic blood pressure compared to control rats ($P = 0.0014$ and $P = 0.0003$, respectively, Table 2). The systolic ($P = 0.0007$), but not the diastolic blood pressure ($P = 0.08$), in control and L-NAME female SD rats was significantly lower after

TABLE 1 Haemodynamic parameters, and organ masses of control and L-NAME induced hypertensive female Sprague–Dawley rats.

	Control $n = 10$	L-NAME $n = 10$	P value
Anaesthetised BP			
SBP (mmHg)	123 ± 2	161 ± 4	**0.0002**
DBP (mmHg)	78 ± 4	112 ± 4	**0.0003**
Organ mass			
Body mass (g)	278 ± 7	266 ± 5	0.2119
Heart mass (g)	0.99 ± 0.03	1.01 ± 0.03	0.7052
Left ventricular mass (g)	0.74 ± 0.03	0.72 ± 0.03	0.5202
Right kidney mass (g)	1.01 ± 0.03	0.93 ± 0.03	0.0584
Liver mass (g)	9.67 ± 0.32	8.97 ± 0.35	0.0963

Note: Data expressed as mean ± SEM. SBP, systolic blood pressure; DBP, diastolic blood pressure. (Unpaired *t*-test).

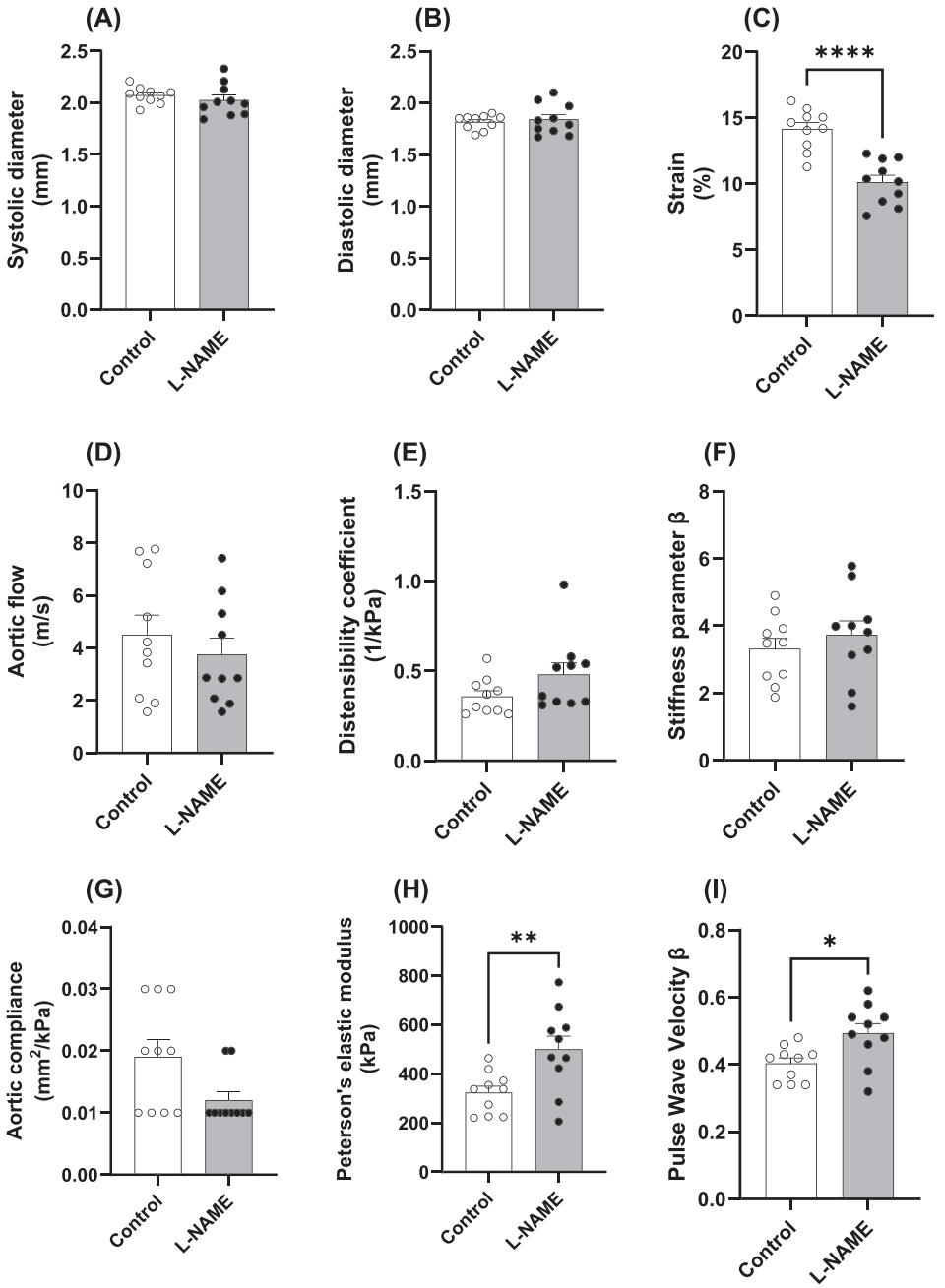


FIGURE 2 Aortic mechanical properties in control and L-NAME induced hypertensive rats. Systolic diameter (A), diastolic diameter (B), strain (C), aortic flow (D), distensibility coefficient (E), stiffness parameter β (F), aortic compliance (G), Peterson's elastic modulus (H) and pulse wave velocity β (I). Data expressed as mean ± SEM. *$P < 0.05$; **$P < 0.001$; and ****$P < 0.00001$ (unpaired *t*-test or Mann–Whitney U test).

TABLE 2 Effects of quercetin administration on blood pressure and mechanical properties of the abdominal aortae of L-NAME and control female Sprague–Dawley rats.

	Control $n = 7$		L-NAME $n = 7$		*P* value		
	Before	**After**	**Before**	**After**	**L-NAME**	**Quercetin**	**Interaction**
Blood pressure (under anaesthesia)							
SBP (mmHg)	125 ± 2	120 ± 2	163 ± 6	142 ± 9*	**0.0014**	**0.0007**	**0.0132**
DBP (mmHg)	81 ± 5	80 ± 4	113 ± 4	98 ± 5	**0.0003**	0.0786	0.1184
Abdominal aorta parameters							
Systolic diameter (mm)	2.06 ± 0.03	2.14 ± 0.02	2.06 ± 0.07	2.12 ± 0.06	0.8063	**0.0436**	0.6728
Diastolic diameter (mm)	1.81 ± 0.03	1.88 ± 0.02	1.88 ± 0.06	1.91 ± 0.05	0.3824	0.0999	0.3443
Strain (%)	13.9 ± 0.7	13.9 ± 0.6	9.3 ± 0.5	11.0 ± 0.6	**<0.0001**	0.2937	0.2624
Distensibility coefficient (1/kPa)	0.37 ± 0.04	0.41 ± 0.04	0.52 ± 0.09	0.53 ± 0.13	0.2602	0.6103	0.7377
Peterson's elastic modulus (kPa)	321 ± 33	299 ± 36	546 ± 68	405 ± 47	**0.0223**	**0.0288**	0.0961
Stiffness parameter β	3.24 ± 0.41	3.07 ± 0.40	4.02 ± 0.53	3.39 ± 0.44	0.3104	0.2887	0.5356
Aortic compliance (mm^2/kPa)	0.018 ± 0.002	0.022 ± 0.002	0.012 ± 0.002	0.017 ± 0.002	0.1138	**0.0306**	0.4301
Aortic flow (m/s)	3.7 ± 0.8	3.9 ± 0.7	3.3 ± 0.7	2.9 ± 0.7	0.3539	0.9288	0.6564
PWVβ	0.40 ± 0.02	0.39 ± 0.02	0.51 ± 0.04	0.44 ± 0.03	**0.0399**	**0.0352**	0.1427

Note: Data expressed as mean ± SEM. SBP, systolic blood pressure; DBP, diastolic blood pressure; PWV, pulse wave velocity.
*$P < 0.05$ vs before. (2-way RM ANOVA).

compared to before the injection of quercetin. There was a significant interaction for the effect of quercetin on systolic blood pressure ($P = 0.013$); wherein, quercetin decreased systolic blood pressure in L-NAME rats (Mean difference [95% CI]; 20.6 [10.5 to 30.6], $P = 0.0004$) but not in control rats (4.4 [−5.6 to 14.5], $P = 0.48$). L-NAME rats had significantly lower strain compared to control rats ($P < 0.0001$), and significantly greater Peterson's elastic modulus and pulse wave velocity β compared to control rats ($P = 0.02$ and $P = 0.04$, respectively). The systolic diameter in control and L-NAME rats was significantly greater after compared to before the injection of quercetin ($P = 0.04$). The Peterson's elastic modulus and pulse wave velocity β were significantly lower in control and L-NAME rats after compared to before injection of quercetin ($P = 0.03$ and $P = 0.035$, respectively). The compliance of the abdominal aortae in control and L-NAME rats was significantly greater after compared to before injection of quercetin ($P = 0.03$). All other parameters measured were similar between the experimental groups (all $P > 0.05$).

4.1.4 | Tissue distribution of L-NAME and quercetin in the aortae

In rats administered with L-NAME daily via drinking water, MSI analysis confirmed the presence of L-NAME in the blood vessels of experimental animals, median [IQR]: 620 [121–1649] (Figure 3A). Similarly, in rats that received quercetin intravenously, quercetin was detected in aortic tissue, median [IQR]: 2058 [197–3298] (Figure 3B). MSI analysis of the control animals showed only the presence of the matrix (DHB, 273.04 *m/z*) in the aortic walls (C), but neither L-NAME nor quercetin (C).

4.2 | Ex vivo effects of quercetin

4.2.1 | Quercetin-induced relaxations

Quercetin did not induce relaxations in rings of renal arteries of female SD rats (data not shown). Figure 4 shows that quercetin induced significant concentration-dependent decreases in phenylephrine-induced contraction compared to the vehicle.

4.2.2 | Effects of quercetin on vasoconstriction

The EC_{50} of phenylephrine-induced contractions in rings of renal arteries (Figure 5A) was significantly shifted to the left when incubated in the presence of 10 μM quercetin (0.74 ± 0.15 μM vs 2.16 ± 0.35 μM, $P = 0.002$). Yet,

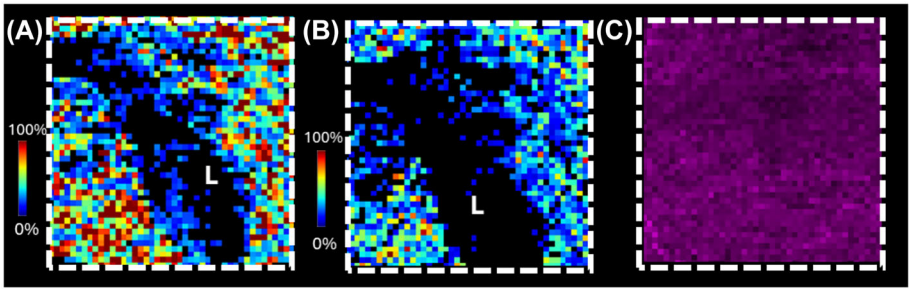


FIGURE 3 The tissue distribution of L-NAME and quercetin in rat abdominal aortae was evaluated using AP-MALDI MSI. Panel **A** illustrates the distribution of L-NAME (234.05 *m/z*) in aortic tissue from L-NAME-induced hypertensive rats. Panel **B** displays the distribution of quercetin (303.05 *m/z*) in aortic tissue following quercetin administration. Panel C shows the distribution of the matrix (DHB, 273.04 *m/z*) in the background, but the absence of L-NAME and quercetin in control rats. **Key:** L- lumen of blood vessel.

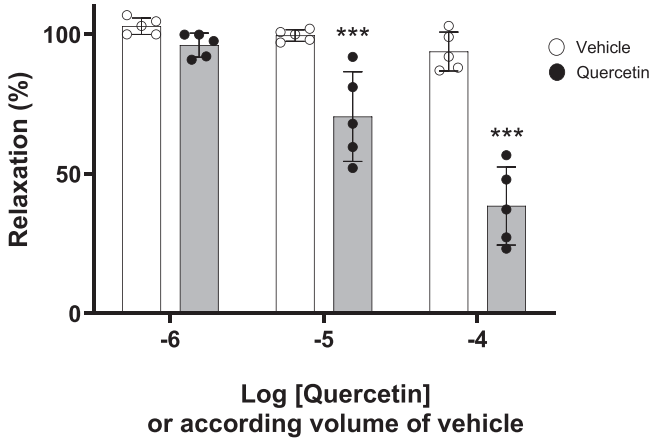


FIGURE 4 Quercetin-induced vasodilation in mesenteric arteries of female Sprague–Dawley rats ($n = 5$). Incremental concentrations of quercetin (1, 10 and 100 μM) or the according volume of the vehicle (0.1% methylnitrocellulose) were added on phenylephrine (30 μM)-induced contracted rings of mesenteric arteries in organ chambers. Data expressed as mean ± SEM. *** $P < 0.0001$ vs vehicle (ANOVA two-way).

the KCl-induced contraction in rings of renal arteries was similar in the presence or absence of 10 μM quercetin (Figure 5C). In rings of mesenteric arteries (Figure 5B and D), the KCl and phenylephrine-induced contractions were similar in the presence or absence of 10 μM quercetin ($P > 0.05$).

4.2.3 | Effect of quercetin on vasodilation

In rings of mesenteric arteries (Figure 6A), the ACh-induced relaxations were significantly shifted to the left in the presence compared to the absence of 10 μM quercetin, suggesting that quercetin ameliorated vasodilation. The EC_{50} of acetylcholine-induced relaxations were significantly decreased in rings of mesenteric arteries in the presence of 10 μM quercetin (0.08 ± 0.02 μM vs 0.36 ± 0.10 μM, $P = 0.03$). In the presence or absence of 10 μM quercetin, the SNP-induced relaxations (Figure 6C) were similar in rings of mesenteric arteries ($P > 0.05$). In rings of renal arteries, the Ach (Figure 6B) or SNP-induced relaxations (Figure 6D) were similar in the presence or absence of 10 μM quercetin ($P > 0.05$). Further, the ACh-induced increase in tension at 100 μM was significantly decreased in the presence compared to the absence of 10 μM quercetin ($P < 0.0001$).

4.2.4 | Effect of quercetin on ACh-induced contractions

In quiescent rings incubated with L-NAME and TRAM-34, ACh did not induce contractions in mesenteric arteries (data not shown). In quiescent rings incubated with L-NAME and TRAM-34, Ach (Figure 7) induced significant concentration-dependent vasoconstriction in renal arteries ($P < 0.0001$). The ACh-induced vasoconstriction was significantly inhibited by indomethacin, the removal of the endothelium or 10 μM quercetin (all $P < 0.0001$).

5 | DISCUSSION

In the present study, an acute intravenous administration of quercetin lowered systolic BP in female L-NAME-induced hypertensive rats, but not in the normotensive controls. For the first time, AP-MALDI-MSI analysis confirmed in rats administered L-NAME daily via drinking water the presence of the inhibitor in the aorta. Similarly, quercetin was detected in the aortic tissue of rats administered quercetin intravenously. In vivo, quercetin ameliorated the L-NAME-induced increased aortic stiffness, indexed by Peterson's elastic modulus and pulse wave velocity β, as well as L-NAME-induced impaired elastic recoil indexed by strain and aortic compliance. Ex vivo,

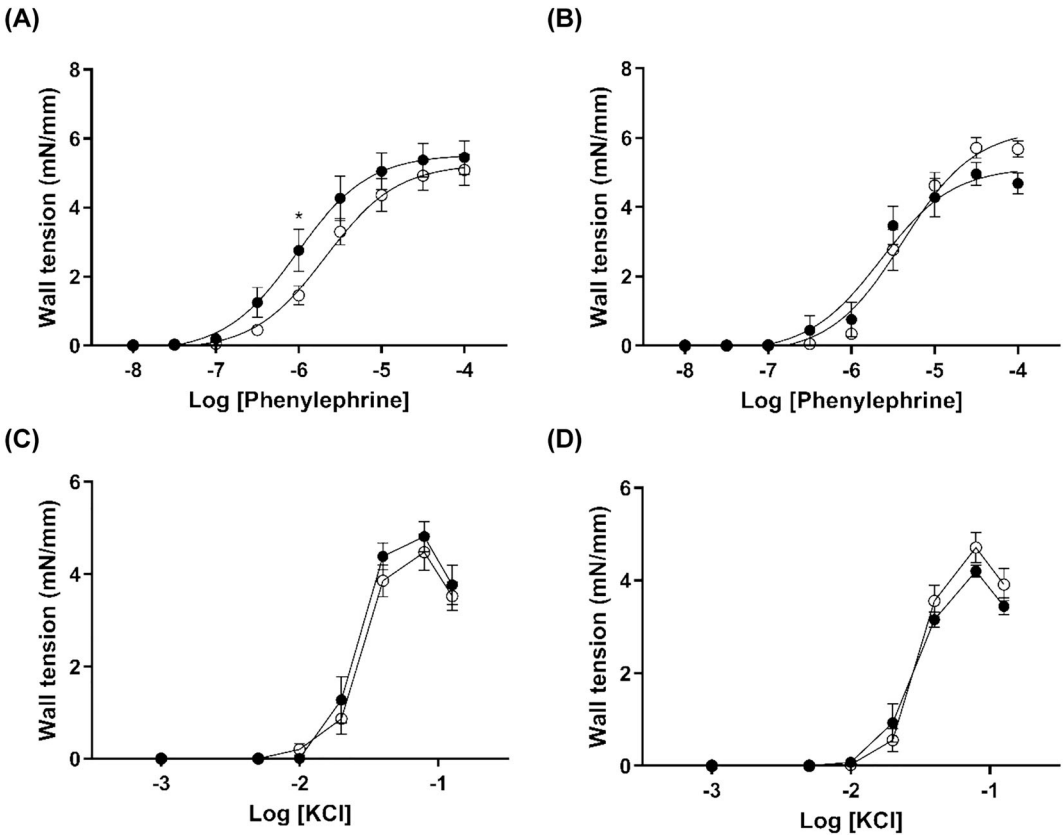


FIGURE 5 Phenylephrine ($n = 9$ per group) and potassium chloride ($n = 7$ per group)-induced contractions in the presence (10 μM) or absence of quercetin (closed and open circles, respectively) in renal arteries (A and C) and mesenteric arteries (B and D) of female Sprague–Dawley rats. Data expressed as mean ± SEM. * $P < 0.05$ vs vehicle (ANOVA two-way).

quercetin induced relaxation in mesenteric arteries but not in renal arteries of female SD rats. Relaxation in response to ACh was improved in the presence of quercetin in mesenteric arteries, but not in renal arteries. In renal arteries, quercetin impaired the ACh-induced increases in tension and the endothelium-dependent contractions. Taken together, these results suggest that quercetin may have an acute effect on endothelial and vascular function, and may confirm its beneficial effect against the development of hypertension.

Hypertension induced by L-NAME has been well-studied in male rodents.[41,42] The administration of L-arginine analogues, such as L-NAME, inhibits nitric oxide synthase (NOS) and decreases NO biosynthesis.[43] The NO deficiency results in endothelial dysfunction, increased total peripheral resistance and increased blood pressures.[44] Using AP-MALDI-MSI analysis, the present study confirms that L-NAME penetrated and localised into blood vessels. Consequently, a 4-week intervention with L-NAME induced hypertension in female Sprague–Dawley rats. Although less studied in females, our results align with previous findings showing that L-NAME induces hypertension in female rodents as well.[45,46]

The potential changes in mechanical and dynamic aortic properties that may explain the increased BP have been investigated in response to L-NAME administration. In this regard, an increased aortic stiffness measured by pulse wave velocity as well as an increased characteristic impedance and pulse wave reflection have been shown in L-NAME-induced hypertensive male rats.[47] Our in vivo assessment of the mechanical properties of the abdominal aortae showed a lower percentage change in lumen diameter from systole to diastole (strain) in L-NAME-induced hypertensive female rats, suggesting an impaired elastic recoil. In addition, the pressure change required for a theoretical 100% increase in diameter (Peterson's elastic modulus) and pulse wave velocity β were increased suggesting greater aortic stiffness following the L-NAME intervention. The L-NAME-induced increases in the production of reactive oxygen species as well as inflammation in male rodents have been shown to cause vascular remodelling.[43,48,49] The disruption of elastic fibres may reduce aortic elasticity and affect the overall aortic mechanical properties.[50] The present study suggests that, similar to male rats, a chronic intervention with L-NAME may induce adverse vascular remodelling

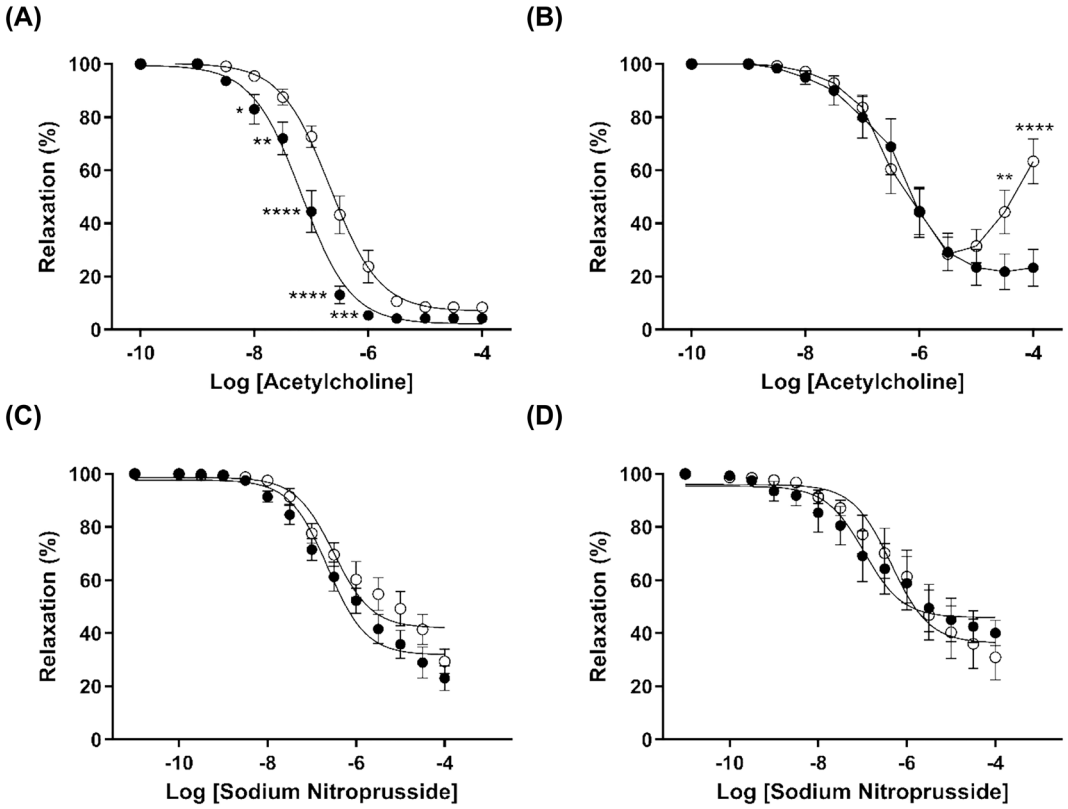


FIGURE 6 Acetylcholine ($n = 9$ per group) and sodium nitroprusside ($n = 7$ per group)-induced relaxations in the presence (10 μM) or absence of quercetin (closed and open circles, respectively) in mesenteric arteries (A and C) and renal arteries (B and D) of female Sprague–Dawley rats. The vessels were exposed to quercetin for 30 min in the bath. Data expressed as mean ± SEM. *$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$, ****$P < 0.00001$ vs vehicle (ANOVA two-way).

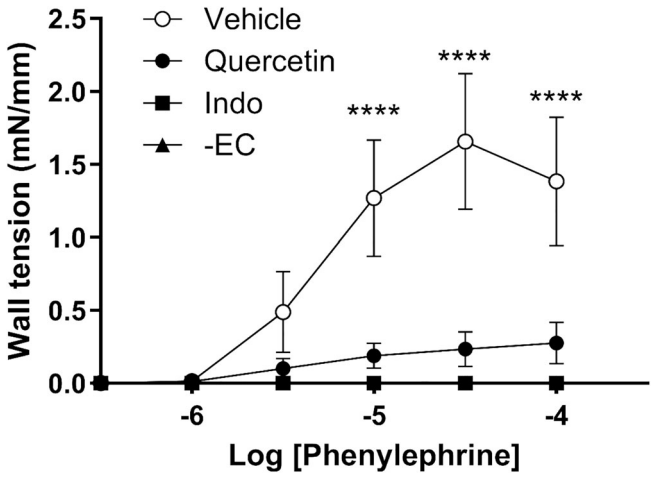


FIGURE 7 Acetylcholine-induced contractions in renal arteries of female Sprague–Dawley rats incubated with 10 μM N^{ω}-nitro-L-arginine methyl ester hydrochloride and 1 μM TRAM-34 and in the presence of vehicle (open circles, $n = 5$), 10 μM quercetin (closed circles, $n = 5$), 10 μM indomethacin (closed squares, $n = 5$) or without endothelium (closed triangles, $n = 5$). Data expressed as mean ± SEM. ****$P < 0.00001$ vs vehicle (ANOVA two-way).

and aortic stiffening, which may result in increased blood pressure in female rats.

Using the L-NAME-induced hypertensive female rats, the present study showed that an acute intravenous administration of quercetin lowered systolic blood pressure. The presence of quercetin in the blood vessels confirmed by AP-MALDI-MSI analysis suggests that quercetin may be responsible for the decrease in blood pressure in hypertensive animals. Similar to previous findings, we did not show an effect of quercetin on systolic blood pressure in the normotensive controls despite confirmation of its presence. This suggests that the effect of quercetin on blood pressure is less potent in the absence of endothelial dysfunction than in hypertension characterised by disruptions to the NO-ergic system. In contrast, the acute administration of quercetin did not lower arterial BP in both male and female spontaneously hypertensive rats (SHRs).[31] The complex aetiology of hypertension in SHRs, which involves the activation of various pathophysiological mechanisms, may explain the difference in results with the present study where induction of hypertension is solely attributable to NO-withdrawal, nitrosative stress and its consequences.

To the best of our knowledge, the present study is the first to determine the acute effects of quercetin on aortic mechanical properties in vivo. Quercetin ameliorated dynamic properties, aortic stiffness and compliance, that had been impaired with L-NAME-induced hypertension. The echo-tracking parameters are computed as a function of BP. Because quercetin elicited a pressure-lowering effect, it is possible that the observed acute improvements in echo-tracking parameters may reflect the BP-lowering effect in response to quercetin. Indeed, the determination of Peterson's elastic modulus, pulse wave velocity β and aortic compliance are load-dependent. Hence, the reduction in pressure may also be due to a reduction in total peripheral resistance resulting from the relaxation of resistance arteries in response to quercetin administration. In this regard, it has previously been shown that an aqueous extract of the *moringa oleifera* leaf, which contains large amounts of quercetin, decreases total peripheral resistance and BP by attenuating hyperreactivity to adrenergic-mediated vasoconstriction and decreasing impairment of endothelium-dependent vasorelaxation in the mesenteric vascular bed of L-NAME-induced hypertensive rats.[51] In the present study, quercetin induced vasorelaxation and improved the ACh-induced relaxations in mesenteric arteries of SD rats.

The reversal of the mechanical parameters measured may also result from improved vasodilation or reduced vasoconstriction in the aortae and conductance arteries. Indeed, acute administration of quercetin causes in vivo vasodilation of the brachial artery in healthy participants[21] or those at risk for cardiovascular disease.[32] In addition, numerous studies have shown that quercetin induces ex vivo relaxation of the aorta of male hypertensive rats including the L-NAME-induced hypertensive rats.[21,22,25,28] The pharmacokinetic profile of quercetin administered in vivo at a dose of 4.5 mg/kg translated effectively into micromolar concentrations ex vivo, where it exerted significant modulatory effects on vascular reactivity through both endothelial and smooth muscle pathways. This demonstrates a robust pharmacokinetic-pharmacodynamic (PK-PD) relationship, with the in vivo BP-lowering effects of quercetin being closely mirrored by its ability to reduce vasoconstriction and enhance vascular relaxation in isolated vessels.

Finally, quercetin may have reduced the production of or counteracted the action of endothelium-derived contracting factors (EDCF). L-NAME-induced hypertension has been associated with augmented EDCF response in the aorta.[52] Quercetin has been shown to inhibit ACh-induced increases in tension during potassium chloride or phenylephrine-induced contractions in aortic rings of hypertensive rat models.[53,54] The inhibition of EDCF by quercetin has, however, not been confirmed in male SHRs,[55,56] which may also explain the absence of haemodynamic effects following administration of quercetin in SHRs.[31] In the present study, the reduced increase in tension at high concentrations of ACh in renal arteries with quercetin suggests that quercetin inhibits an endothelium-dependent response. In line with this finding, quercetin abolished the endothelium- and cyclooxygenase-dependent ACh-induced contractions induced in the presence of a NO synthase inhibitor and a selective inhibitor of intermediate-conductance Ca^{2+}-activated potassium (IKCa) channels in renal arteries, suggesting that quercetin inhibits or counteracts the EDCF release in female SD rats. On the other hand, the leftward shift in the EC_{50} of phenylephrine-induced contractions suggests that quercetin enhances contractile responses in renal arteries. This duality suggests that quercetin acts as a modulator of vascular tone, potentially shifting the balance toward contractility while simultaneously promoting vasodilatory responses mediated by the endothelium.

There are some limitations in the present study. Firstly, the experiments were only conducted in female SD rats, and the oestrous cycle was not assessed. Future studies using both sexes may shed light on sex-specific differences in the model of L-NAME-induced hypertension. Secondly, functional changes in the aorta were only studied by echo-tracking in vivo, and not by vascular reactivity in the ex vivo protocol. Further, incubation with L-NAME was only performed in quiescent rings of renal arteries and the responses of quercetin may have been stronger when the rings were incubated with L-NAME. Thirdly, despite a demonstrated BP depressor effect of quercetin, the tail-cuff method is less accurate than radiotelemetry.[31] Telemetry may also enable testing of the effects of oral administration of quercetin in conscious animals. Finally, the abdominal aortae mechanical properties assessed in vivo by echo-tracking were calculated as a function of BP and the cause-and-effect relationship cannot be determined. In this respect, molecular and histological techniques may have improved the interpretation of the results.

Taken together, the present study demonstrated that acute intravenous injection of quercetin decreased BP in L-NAME-induced hypertensive female rats and ameliorated aortic stiffness and elastic recoil. The acute BP-lowering effect of quercetin may be attributable to the improvement of endothelium-dependent relaxation of resistance arteries, inhibition of ECDF release by conductance arteries, as well as to changes in the mechanical properties of the aorta. Further research is needed to clarify the mechanisms by which acute quercetin administration exerts its effects in the setting of L-NAME-induce hypertension.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicting interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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