1. Introduction

The World Health Organization estimates that hypertension affects approximately 25% of adults worldwide [1]. Despite continued improvement in the treatment and management of hypertension, 20–30% of hypertensive patients are resistant to blood pressure (BP) reduction with the maximum tolerated dose of at least three anti-hypertensive drugs [2]. In addition, growing numbers of individuals with the metabolically most effective medications involved. The spontaneously hypertensive rat (SHR) and the angiotensin (Ang)-II infused mouse were used as hypertensive models. Compared to a standard control diet, consumption of diets containing RESV by SHRs and Ang-II hypertensive mice, markedly prevented rises in systolic BP. In addition, flow-mediated vasodilation was significantly improved by RESV in SHRs. RESV also reduced serum and cardiac levels of the lipid peroxidation by-product, 4-hydroxy-2-nonenal in the hypertensive rodents and inhibited the production of superoxide in human-derived endothelial cells. Analysis of mesenteric arteries from SHRs and Ang-II infused mice demonstrated that RESV increased endothelial NO synthase (eNOS) phosphorylation by enhancing the LKB1/adenosine monophosphate (AMP)-activated protein kinase (AMPK) signal transduction pathway. Moreover, RESV reduced hypertrophic growth of the myocardium through reduced hemodynamic load and inhibition of the p70 S6 kinase pro-hypertrophic signaling cascade. Overall, we show that high dose RESV reduces oxidative stress, improves vascular function, attenuates high BP and prevents cardiac hypertrophy through the preservation of the LKB1–AMPK–eNOS signaling axis.

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Abbreviations: AMPK, AMP-activated protein kinase; Ang-II, angiotensin-II; BP, blood pressure; eNOS, endothelial nitric oxide synthase; HNE, 4-hydroxy-2-nonenal; HUVEC, human umbilical vein endothelial cell; HW/TL, heart weight/tibia length; IVRT, intraventricular relaxation time; IVS, intraventricular septum; LVPW, LV posterior wall; NO, nitric oxide; RESV, resveratrol; ROS, reactive oxygen species; SHR, spontaneously hypertensive rat
elevated HNE levels in hypertensive rodents also inhibit the LKB1–AMPK–eNOS signal transduction cascade in endothelial cells or vascular tissues of hypertensive rodents.

We recently showed that calorie restriction (CR) prevents the significant rise of BP in the spontaneously hypertensive rat (SHR) [14] and that the subsequent positive vascular adaptations involved increased NO bioavailability through stimulation of AMPK–eNOS signaling [14]. However, lifestyle modifications, such as CR, require significant long-term patient compliance, which may be difficult to achieve. The utilization of small molecules that activate similar molecular and signal transduction pathways as CR could provide novel therapeutic approaches for hypertension. Interestingly, the natural polyphenolic molecule, resveratrol (RESV), mimics many of the molecular and biological effects of CR [15]. Moreover, we showed that RESV prevented HNE-mediated inhibition of the LKB1–AMPK signaling axis in cardiomyocytes [11]. However, the ability of RESV to regulate BP is unclear. Some studies showed that RESV had no effect on BP [11,16–19], while other reports indicate that RESV lowered BP [20–23]. Due to these divergent findings, the purpose of this study is to clarify how RESV administration influences vascular function and BP in the SHR model of genetically programmed hypertension and the angiotensin (Ang)-II infused hypertensive mouse to investigate the effects of RESV on BP regulation in two distinct non-obese models of hypertension. In addition, we investigated whether the effects of RESV on BP corresponded with lower levels of HNE and the activation of the LKB1–AMPK–eNOS signal transduction cascade that could rescue NO bioavailability.

2. Material and methods

2.1. Materials

Most antibodies were purchased from either Cell Signaling Technology or Santa Cruz Biotechnology. The anti-nitrosocysteine antibody was purchased from Abcam (Cambridge, MA). Most other cell culture reagents and chemicals were purchased from Sigma. Fetal bovine serum was obtained from Invitrogen (Carlsbad, CA). Resveratrol was purchased from Lalilab (Durham, NC).

2.2. Animal care and diets

The University of Alberta Animal Policy and Welfare Committee adheres to the principles of biomedical research involving animals developed by the Council for International Organizations of Medical Sciences (CIOMS). SHRs and C57BL/6 mice were obtained from Charles River Laboratories (St. Foy, QC). Rodents had ad libitum access to water and were fed the AIN93G standard chow (control) diet or an AIN93G standard chow diet that contained 4 g RESV/kg diet (Dyets Inc., Bethlehem, PA), a dosage that was consistent with previous studies [24,25]. For SHRs, the dosage of RESV was equivalent to −146 mg RESV/kg/day and for mice, the dosage was equivalent to −320 mg RESV/kg/day. Alzet Osmotic Micro-pumps (Cupertino, CA) were implanted into C57BL/6 mice for the delivery of saline or Ang-II (1.4 mg/kg/day) by a small incision that was made dorsal interscapularis, to form a small subcutaneous pocket. SHRs consumed the diets for 5 weeks and mice consumed the diets for two weeks following implantation of the osmotic mini-pumps.

2.3. Analysis of tissues

Blood was collected from rodents by cardiac puncture and the tissues were freeze-clamped in liquid nitrogen as described previously [11,24]. Tissues were homogenized and the protein concentration was assayed using Bradford Protein Reagent. 15–20 μg of protein was used for SDS–PAGE and immunoblot analysis. HNE-protein adducts were quantified using the OxiSelect HNE-His Adduct ELISA Kit (Cell Biolabs, San Diego, CA) from 1 μg of protein lysates from heart tissue or serum samples according to the manufacturer’s instructions.

2.4. In vivo assessment of cardiac and vascular function

Transthoracic echocardiography was performed according to our previously reported procedures [14] on mildly anesthetized (sedated with 3% and maintained at 1–1.5% isoflurane) SHRs at 8 and 15 weeks of age using a Vevo 770 High-Resolution Imaging System equipped with a 17.5-MHz transducer (Visual Sonics, Toronto, ON). For mice, transthoracic echocardiography was performed on 24 and 26-week old mice using a 30-MHz transducer. Flow-mediated vasodilation of the femoral artery was performed according to procedures we described previously [14]. BP was recorded using a telemetric BP recording device, TA11PA-C10 (Data Science International, St. Paul, MN), and heart rate (HR), and mean arterial (MAP), systolic (SP), and diastolic (DP) pressures were measured weekly using PowerLab 4/30 Acquisition and LabChart 7 software (ADInstruments, Colorado Springs, CO). See online supplement for detailed methods.

2.5. Human umbilical vein endothelial cell (HUVEC) culture

Cells were isolated from human umbilical cords obtained from the Royal Alexandra Hospital in Edmonton, AB. The protocol was approved by the University of Alberta Ethics Committee and conformed to the principles outlined in the Declaration of Helsinki and also Title 45, US Code of Federal Regulations, Part 46, Protection of Human Subjects. All subjects provided written informed consent. HUVECs were grown at 37 °C with 5% CO2/95% air in M199 medium supplemented by 20% fetal bovine serum, L-Glutamine, Penicillin–Streptomycin and 1% endothelial cell growth supplement. Superoxide detection and S-nitrosocysteine measurements were performed as described previously [26]. See online supplement for detailed methods.

2.6. Statistical analysis

Data are expressed as mean ± standard error of the mean (S.E.M.). Comparisons between treatment groups were performed using the unpaired Student’s two-tailed t-test or analysis of variance (ANOVA) with a Bonferroni post-hoc test of pairwise comparisons between groups, where appropriate. The repeated measures test was used wherever applicable. A probability value of <0.05 was considered significant.

3. Results

3.1. Resveratrol improves vascular function and prevents the rise of BP in hypertensive rodents

A RESV containing diet was administered to SHRs at 10 weeks of age and SHRs were maintained on this diet until they were 15 weeks of age. These ages were selected since SHRs advance from mild (10 weeks of age) to pronounced hypertension (15 weeks of age) during this period and display compensatory increases in concentric LVH [11]. Consistent with our previous findings [11], RESV had no effect on body weight in the SHRs at 15 weeks of age (Table S1). Since recent work has established that RESV improves vascular function using isolated vessels ex vivo [17,23,27,28], we investigated whether RESV could also improve vascular function in the SHR in vivo. To do this, we initiated RESV when the SHRs were mildly hypertensive, but prior to developing increased LV wall thickness (Table S1) and then monitored femoral artery vasodilation and flow velocity using ultrasound at 15 weeks of age. Hyperemic vasodilation was greater in the RESV-SHRs compared to the control-SHRs, following temporary ischemia (Fig. 1A). Furthermore, femoral artery blood flow velocity following temporary ischemia was significantly higher in RESV-SHRs compared with control-SHRs (Fig. 1B) and the effective arterial elastance (Fig. 1C) was significantly reduced.
the RESV-SHRs, suggesting improved arterial distensibility and reduced peripheral resistance. As expected from improved vascular function, RESV treatment prevented the significant rise in MAP, as well as SP and DP in the SHRs (Fig. 1D–F). In agreement with RESV being responsible for the BP effects, removal of RESV from SHRs resulted in a rapid (2 weeks) and striking rise in BP in previously treated SHRs to levels observed in the control-SHRs (Fig. 1D–F).

To confirm that the RESV-mediated effects on BP were not confined to the SHR model or to rats in general, we evaluated RESV in a mouse model of Ang-II induced hypertension. Elevated systemic levels of Ang-II significantly increased SP in the mice compared to saline-infused mice (Fig. 2A), though not DP (Fig. 2B). Consistent with the effects of RESV in the SHRs, the addition of RESV to the diets of Ang-II infused mice prevented the significant rise of SP (Fig. 2A), though RESV did not affect DP (Fig. 2B). RESV also improved in vivo vascular function in Ang-II infused mice, as determined by femoral artery flow velocity using Doppler flow ultrasound. Mice infused with Ang-II had significantly reduced femoral artery blood flow velocity following temporary ischemia, compared with saline controls (Fig. 2C). In the Ang-II infused mice, RESV significantly increased the femoral artery blood flow velocity following temporary ischemia (Fig. 2C) and RESV attenuated the Ang-II induced rise in effective arterial elastance (Fig. 2D). These observations demonstrate that RESV administration improved vascular function and prevented the rise in BP in two distinct rodent models of hypertension.

3.2. RESV increases arterial eNOS and AMPK activities in hypertensive rodents

Based on the effects of RESV on the vascular function of the hypertensive rodents, we hypothesized that endogenous NO production was higher in RESV-treated rodents. To investigate this, we measured phosphorylated serine-1177 eNOS (P-eNOS) as a surrogate marker of eNOS activity. Increased P-eNOS was observed in mesenteric arteries isolated from RESV-SHRs compared to control-SHRs (Fig. 3A). In addition, Ang-II infusion was associated with lower P-eNOS levels in mesenteric arteries and this decrease was prevented by RESV (Fig. 3B). These observations likely reflect a RESV-induced increase of NO bioavailability in the two distinct hypertensive models.

Since AMPK has been shown to be activated by RESV [11,29] and AMPK activates eNOS by phosphorylating the serine-1177 residue [13], we measured the levels of phosphorylated AMPK at its activation site, threonine-172 (P-AMPK). P-AMPK levels were 3-fold higher in the arteries of RESV-SHR compared with controls (Fig. 3C). Correspondingly, RESV increased P-AMPK in the arteries of saline and Ang-II infused mice (Fig. 3D). As phosphorylation and stimulation of AMPK occurs via the action of its upstream activating kinase, LKB1, we also investigated the activity of LKB1 using phosphorylation of LKB1 at serine-428 (P-LKB1) as a surrogate marker of LKB1 activity [30]. RESV increased P-LKB1 3-fold in SHRs compared with controls (Fig. 3E). In addition, RESV increased P-LKB1 in the saline and Ang-II infused mice (Fig. 3F).
Next, we investigated the association between vascular dysfunction, oxidative stress and LKB1–AMPK–eNOS signaling. Previous work demonstrated that the lipid peroxidation by-product, HNE, is elevated in SHRs [9] and inhibits LKB1 activity [11]. Therefore we measured plasma HNE levels in both of our models. In the present study, we observed that the circulating HNE levels were significantly lower in RESV-SHRs compared to control-SHRs (Fig. 3C). Moreover, serum HNE levels were significantly elevated in the mice infused with Ang-II compared to saline-infused mice (Fig. 3H). More importantly, this Ang-II induced increase in serum HNE levels was attenuated by RESV (Fig. 3H), which corresponds with elevated LKB1–AMPK–eNOS signaling in the arteries of hypertensive rodents treated with RESV (Fig. 3A–F).

### 3.3. RESV prevents the deleterious effects of HNE on endothelial NO generation

In order to determine whether HNE regulates endothelium superoxide and NO levels via the LKB1–AMPK–eNOS signal transduction pathway in the absence of pre-existing hypertension and/or elevated Ang-II (i.e. was HNE sufficient to recapitulate the alterations in signaling that we observed in mesenteric arteries from hypertensive rodents), we utilized a human-derived HUVEC cell culture system. As expected, HNE potentiated the production of superoxide in HUVECs (Fig. 4A), whereas RESV did not alter baseline S-nitrosothiols in HUVECs, RESV prevented the decrease in S-nitrosylation levels (Fig. 4B). While HNE treatment did not alter baseline S-nitrosylation levels by HUVECs, RESV prevented the HNE-induced decrease in S-nitrosylation levels (Fig. 4B). Thus, it appears that RESV stimulates the LKB1–AMPK signal transduction pathway and not Akt, to prevent the HNE-induced inhibition of P-eNOS.

To understand the mechanisms regulating eNOS, we examined the expression of regulators of eNOS phosphorylation in HUVECs. Consistent with our analysis of arteries from hypertensive rodents (Fig. 3), RESV increased P-AMPK 2-fold in control cells, while HNE markedly inhibited P-AMPK in HUVECs (Fig. 4D). However, the co-treatment of endothelial cells with HNE and RESV completely abrogated the inhibitory effects of HNE on P-AMPK. Since our previous work demonstrated that HNE inhibited LKB1 activity [11], we also investigated whether HNE altered levels of P-LKB1 in HUVECs. While HNE reduced P-LKB1 levels, RESV completely prevented this decrease, which resulted in a 2-fold increase in P-LKB1 levels (Fig. 4E). Since eNOS is also phosphorylated at serine-1177 by Akt [31], we measured levels of Akt phosphorylation in response to HNE. Unexpectedly, HNE treatment of endothelial cells increased Akt phosphorylation, while the treatment of cells with RESV and HNE attenuated HNE-induced P-Akt (Fig. 4F). These data suggest that in HNE-treated HUVECs, Akt activation alone is not sufficient to induce eNOS phosphorylation. In order to further investigate this pathway, AMPK was inhibited with compound C and HUVECs were treated with HNE in combination with RESV, as before. Interestingly, compound C prevented the increase in P-eNOS in the presence of RESV (Fig. 4G). Thus, it appears that RESV stimulates the LKB1–AMPK signal transduction pathway and not Akt, to prevent the HNE-induced inhibition of P-eNOS.

In order to determine whether eNOS activation was required for AMPK activation by RESV, HUVECs were pre-treated with L-NAME, followed by RESV. As expected, L-NAME did not prevent the stimulation of P-AMPK by RESV (Fig. 4H). On the other hand, L-NAME pre-treatment of HUVECs blocked the ability of RESV to prevent the HNE-induced decrease in S-nitrosylation levels (Fig. 4I). Overall these findings suggest that elevated HNE levels modified the LKB1–AMPK–eNOS signal transduction cascade and reduced protein nitrosylation, even in the absence of hypertension.

### 3.4. Resveratrol prevents LVH in hypertensive rodents

Since RESV improved vascular function and prevented elevated BP, we investigated whether RESV also prevented the development of LVH in hypertensive rodents. Consistent with the reduced hemodynamic load, RESV treatment resulted in a substantial reduction in both the heart weight/tibia length (HW/TL) ratio and the LV mass index in SHRs (Fig. 5A, Table S1) and Ang-II infused mice (Fig. 5B, Table S2).
compared to their respective controls. RESV also prevented the increase in LV posterior wall (LVPW) thickness in SHRs (Fig. 5C) and Ang-II infused mice (Fig. 5D). Echocardiographic assessment revealed that RESV did not significantly influence HR or parameters of systolic (ejection fraction) and diastolic (intraventricular relaxation time; IVRT) function in either the SHRs (Table S1) or the Ang-II infused mice (Table S2), compared with their respective controls. Nevertheless, stroke work and LV wall stress of RESV-SHRs was significantly lower than control-SHRs (Fig. 5E, G). Infusion of mice with Ang-II increased stroke work and LV wall stress, while RESV attenuated the effects of Ang-II on the LV (Fig. 5F, H). Together, these results indicate that RESV prevents hypertension-induced LVH, likely secondary to preventing elevated BP.

Fig. 3. Arterial signaling pathways in hypertensive rodents. Immunoblot analysis was performed on mesenteric artery homogenates isolated from Control-spontaneously hypertensive rat (SHR) and resveratrol (RESV)-SHRs as well as saline or angiotensin (Ang)-II infused (1.4 mg/kg/day) mice that consumed control or RESV diets. Phosphorylated serine-1177 eNOS (P-eNOS) quantified by densitometry and normalized against total eNOS (A, B). Phosphorylated threonine-172 AMPK (P-AMPK) normalized against total AMPK (C, D). Phosphorylated serine-428 LKB1 (P-LKB1) normalized against total LKB1 (E, F). Serum levels of 4-hydroxy-2-nonenal (HNE)-protein adducts (G, H). Values are mean ± S.E.M. (n = 6). **Significant difference (p < 0.05) between Control-SHR and RESV-SHRs using Student’s t-test. §Significant difference (p < 0.05) between Control and RESV mice and a *significant difference (p < 0.05) between the AngII-Control group and other groups of mice using a two-way ANOVA with Bonferroni post-test.
3.5. RESV increases cardiac LKB1/AMPK phosphorylation in accordance with the reduction of cardiac HNE and LVH

The p70S6 kinase (p70S6K) pathway regulates protein synthesis and promotes hypertrophic growth of the myocardium [29]. To investigate whether RESV attenuated LVH through direct effects on molecular signaling cascades in the heart, we measured phosphorylated levels of p70S6K (P-p70S6K) in SHRs since this pathway regulates protein synthesis and promotes hypertrophic growth of the myocardium [29]. P-p70S6K was reduced in the hearts of RESV-SHRs compared to controls.

Fig. 4. Regulation of nitric oxide production in response to resveratrol (RESV) and 4-hydroxy-2-nonenal (HNE) in human umbilical vein endothelial cells (HUVECs). HUVECs were treated with 10 μM HNE, in combination with 10 μM RESV or vehicle-treated control (CON). Representative micrographs from three independent experiments are shown for intracellular superoxide production determined by dihydroethidium staining in cells treated for 1 h (A) and immunostaining for nitrosocysteine in cells treated for 24 h (B). Phosphorylated serine-1177 eNOS (P-eNOS) was quantified by densitometry and normalized against Actin (C). Phosphorylated threonine-172 AMPK (P-AMPK) was normalized against total AMPK (D). Phosphorylated serine-428 LKB1 (P-LKB1) was normalized against total LKB1 (E). Phosphorylated serine-473 Akt (P-Akt) was normalized against total Akt (F). P-eNOS in HUVECs pre-treated with compound C (1 μM) for 30 min (G). P-AMPK in HUVECs pre-treated with N-omega-nitro-l-arginine methyl ester (L-N; 200 μM) for 30 min followed by RESV treatment for 1 h (H). Nitrosocysteine levels in HUVECs pre-treated with L-N for 30 min (I). Representative immunoblot images from 3 to 5 independent experiments are shown. *Significant difference (p < 0.05) compared with CON. §§Significant difference (p < 0.05) compared with HNE treated cells.
control-SHRs (Fig. 6A). In hearts from Ang-II infused hypertensive mice, P-p70S6K was higher than saline-controls, while RESV significantly reduced P-p70S6K (Fig. 6B). These results demonstrate an anti-hypertrophic effect of RESV [11,29], which we previously demonstrated to occur via the activation of AMPK [29]. In agreement with this, RESV increased P-AMPK in SHR hearts (Fig. 6C). Moreover, Ang-II induced hypertension reduced cardiac P-AMPK levels while RESV stimulated P-AMPK in both saline and Ang-II infused mice (Fig. 6D). Consistent with increases in P-AMPK levels, RESV increased P-LKB1 levels in hearts from SHRs (Fig. 6E). Ang-II induced hypertension markedly inhibited cardiac P-LKB1 levels while RESV prevented the inhibition of P-LKB1 (Fig. 6F). Since increased cardiac HNE levels were associated with reduced LKB1-AMPK signaling [11], we examined the effect of RESV on HNE levels in the hearts of hypertensive rodents. RESV reduced cardiac HNE concentrations in SHRs (Fig. 6G). Ang-II infusion elevated HNE levels in the heart and RESV markedly attenuated this increase (Fig. 6H).

4. Discussion

Herein, we provide evidence that RESV can prevent the rise in BP in two distinct hypertensive rodent models, the SHR and Ang-II infused mice. In both models, RESV induced several positive vascular adaptations including reduced oxidative damage and improved hyperemic vasodilation that correlated with activation of eNOS by the LKB1-AMPK pathway. In addition, RESV contributed to reduced oxidative damage to the heart, reduced cardiac LVH and inhibition of pro-hypertrophic signaling pathways.

NO is the primary vasodilatory molecule in the circulation and reduced NO bioavailability contributes to the pathogenesis of hypertension [7]. A major factor that contributes to low NO bioavailability is oxidative stress-mediated NO scavenging [7]. In fact, elevated vascular ROS detrimentally affects endothelial function [5], which precedes the development of hypertension. The generation of ROS is a feature of hypertension in SHRs and Ang-II infused mice [9,32]. In particular, the lipid peroxidation by-product, HNE, has been reported to mediate damage due to ROS in endothelial cells [33]. Consistent with this, we observed that Ang-II elevated HNE levels in the serum and hearts of mice. Furthermore, we showed that in HUVECs, HNE inhibited nitrosocysteine formation and enhanced the production of superoxide by the cells. While it was previously reported that HNE caused a dose-dependent inhibition of nitrite levels (a surrogate marker of NO bioavailability) in endothelial cells, the mechanism underlying this observation was unknown [33]. Our data show that HNE inhibited NO mediated nitrosocysteine formation possibly via reduced phosphorylation of eNOS on serine-1177. While eNOS is phosphorylated and activated by both AMPK and Akt [13,31], our data show that HNE inhibits AMPK activation and stimulates Akt, suggesting that the inhibition of
eNOS by HNE is a consequence of AMPK inhibition and not Akt. In endothelial cells, LKB1 activity has been shown to correlate with its phosphorylation at serine-428 [30]. Consistent with the reduced P-AMPK, P-LKB1 was also reduced by HNE, suggesting that HNE can directly inhibit LKB1 in the endothelium, similar to its effects in HEK293T cells [12] and cardiac myocytes [11]. However, one major limitation to our study is the fact that we were not successful in immunoprecipitating LKB1 from mesenteric arteries or HUVECs. As such, we cannot provide unequivocal evidence that HNE adducts are formed on LKB1 in hypertensive rodents or whether RESV prevents this from occurring. That said, we have previously shown that HNE adducts are formed on LKB1 in the hearts of SHRs and that RESV can prevent these HNE adducts on LKB1 and subsequently rescue LKB1-AMPK signaling [11]. These previous findings are consistent with the data provided herein showing that RESV can lower HNE levels in the circulation of SHRs and Ang-II hypertensive mice. However, we must acknowledge the fact that at present, these findings are only correlative.

To investigate whether RESV increased NO bioavailability, we treated cultured human endothelial cells with HNE in combination with RESV. Notably, RESV attenuated the effects of HNE on superoxide...
production and prevented reduced S-nitrosocysteine levels. RESV also reduced HNE levels in the circulation of SHRs and Ang-II hypertensive mice, which is consistent with the notion that the reduction of HNE levels improves vascular function in hypertensive rodents. AMPK also appears to be involved in this process since RESV activates AMPK and AMPK directly phosphorylates eNOS, which increases NO production [13]. However, it has also been shown that NO can activate AMPK, placing eNOS upstream of AMPK [34]. In contrast to the latter finding, our study showed that the pre-treatment of HUVECs with L-NAME did not block activation of AMPK by RESV. Moreover, inhibition of AMPK with compound C prevented the phosphorylation of eNOS in response to RESV treatment. In addition, other studies have shown that inhibition of AMPK with compound C abolishes the ability of RESV to improve endothelium dependent vasodilation in aortic rings [35]. Furthermore, L-NAME did not inhibit AMPK activation by RESV in the carotid arteries of rats [36]. Together, our findings extend these previous observations and identify a strong correlation between the stimulation of the LKB1-AMPK-eNOS pathway and improved vasodilatory properties both in cultured human endothelial cells and arteries isolated from both SHRs and Ang-II induced hypertensive mice.

Isolated artery preparations were used to demonstrate that RESV stimulated endothelium dependent relaxation [17,23,27,28]. Our results confirm these findings and extend them further to show that in two different hypertensive rodent models, RESV improves flow-mediated vasodilation in vivo. Vascular dysfunction is associated with high systemic BP, suggesting that the ability of RESV to improve vascular function could contribute to lowering BP. Indeed, chronic RESV administration reduced BP in DOCA-salt sensitive rats [20], partially nephrectomized rats [22], and in two-kidney one-clip hypertensive rats [21]. However, other studies showed that RESV did not affect the BP of the SHR [11,17,18], stroke-prone SHR [16] or Dahl-salt sensitive rat [19]. The differences between the in vivo effects of RESV in hypertensive rodents are likely a consequence of different mechanisms involved in the development of high BP in the various animal models, the dose of RESV administered or the length of treatment. Our findings demonstrate that chronic and high doses of RESV can prevent elevated BP in SHRs, as well as in mice infused with Ang-II (Figs. 1, 2). Although multiple molecular mechanisms could be involved in the effects of RESV on the vessels of hypertensive rodents [15], our results are consistent with several reports that RESV stimulated eNOS in endothelial cells [21,37,38]. Furthermore, we recently reported that RESV prevented neointimal growth following vascular injury in wild-type mice, but that this effect was lost in eNOS knock-out mice [36]. Together, these data provide solid evidence that the beneficial vascular effects of RESV require activation of eNOS [36]. While the effects of RESV may extend beyond NO bioavailability, we hypothesize that the rapid increase in BP following the removal of RESV indicates NO-mediated effects as opposed to RESV-mediated suppression of remodeling of the artery wall or changes in transcriptional levels of relevant genes.

As expected, the ability of RESV to prevent elevated BP also protected the hearts of SHRs and Ang-II infused mice from LVH and significantly reduced the strain on the LV (Fig. 5). Previous studies
showed that RESV prevented LVH in hypertensive rodents due to lower SP [20–22]. However, RESV may have direct effects on the growth of the cardiac myocyte since RESV also prevented LVH in the absence of reduced BP in hypertensive rodents [11,16–19]. In this study, we did not ascertain whether the effect of RESV on cardiac growth was mediated via the reduction of BP or through direct effects on the growth of the cardiac myocyte [29]. Nevertheless, our data supports the previous report that RESV reduced cardiac HNE levels and prevented the inhibition of LKB1-AMPK signaling [11]. Although we show that RESV was able to prevent the development of hypertension and the resulting cardiac hypertrophy, RESV treatment of hypertensive SHR at 16 weeks of age did not result in a regression of the phenotype (data not shown). While we do not know the reasons for this, it is likely that once negative vascular remodeling occurs, RESV is unable to reverse it (at least in the short-term; i.e. 5 weeks). That said, although we did not test this in the current study, it is possible that longer-term treatment with RESV may improve the phenotype. Taken together, we suggest that chronic administration of high dose RESV may reduce cardiac mass through the reduction of BP as well as the inhibition of pro-hypertrophic signals (p70S6K) and stimulate anti-hypertrophic signaling pathways (LKB1/AMPK) in the cardiac myocyte.

5. Conclusions

Our data demonstrate that RESV improves vasodilatory function and attenuates the development of hypertension in SHRs and Ang-II infused mice. RESV induced improvements of vasodilatory functions were associated with improved NO bioavailability. RESV also reduced HNE levels in the hearts and circulation of hypertensive SHRs and Ang-II infused mice, which was associated with an enhanced LKB1-AMPK-eNOS signaling pathway in isolated rodent arteries and hearts (Fig. 7). These positive adaptations induced by RESV likely contributed to the prevention of LVH. Recently, a clinical trial showed that 30 days of RESV treatment reduced BP in overweight middle-aged men [39]. Based on these findings and our results, we propose that RESV may be used as an additional approach for the treatment of hypertension and associated LVH even in the absence of obesity.

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Appendix A. Supplementary data

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