

Perinatal Resveratrol Supplementation to Spontaneously Hypertensive Rat Dams Mitigates the Development of Hypertension in Adult Offspring

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Abstract—This study was undertaken to determine whether perinatal maternal resveratrol (Resv)—a phytoalexin known to confer cardiovascular protection—could prevent the development of hypertension and improve vascular function in adult spontaneously hypertensive rat offspring. Dams were fed either a control or Resv-supplemented diet (4 g/kg diet) from gestational day 0.5 until postnatal day 21. Indwelling catheters were used to assess blood pressure and vascular function in vivo; wire myography was used to assess vascular reactivity ex vivo. Perinatal Resv supplementation in dams had no effect on fetal body weights, albeit continued maternal treatment postnatally resulted in growth restriction in offspring by postnatal day 21; growth restriction was no longer evident after 5 weeks of age. Maternal perinatal Resv supplementation prevented the onset of hypertension in adult offspring (−18 mmHg; $P=0.007$), and nitric oxide synthase inhibition (with L-NG-nitroarginine methyl ester) normalized these blood pressure differences, suggesting improved nitric oxide bioavailability underlies the hemodynamic alterations in the Resv-treated offspring. In vivo and ex vivo, vascular responses to methylcholine were not different between treatment groups, but prior treatment with L-NG-nitroarginine methyl ester attenuated the vasodilation in untreated, but not Resv-treated adult offspring, suggesting a shift toward nitric oxide-independent vascular control mechanisms in the treated group. Finally, bioconversion of the inactive precursor big endothelin-1 to active endothelin-1 in isolated mesenteric arteries was reduced in Resv-treated offspring (−28%; $P<0.05$), and this difference could be normalized by L-NG-nitroarginine methyl ester treatment. In conclusion, perinatal maternal Resv supplementation mitigated the development of hypertension and causes persistent alterations in vascular responsiveness in spontaneously hypertensive rats. (*Hypertension*. 2016;67:1038-1044. DOI: 10.1161/HYPERTENSIONAHA.115.06793.) • [Online Data Supplement](#)

Key Words: developmental programming ■ hypertension ■ nitric oxide ■ prevention ■ resveratrol
■ spontaneously hypertensive rat ■ vascular function

Hypertension is an important and modifiable risk factor for cardiovascular disease, affecting ≈ 1 in 4 adults worldwide.¹ In the United States, hypertension is estimated to account for >\$46 billion annually in healthcare services, medication, and lost productivity,² and these costs are expected to increase substantially over the coming decades. A universal consensus is that prevention, rather than treatment, is a more strategic and cost-effective approach to reducing the burden of cardiovascular disease in coming decades.

Essential hypertension, in which known primary causes (eg, renovascular disease, pheochromocytoma, monogenic causes, etc) are not present, makes up 95% of hypertension

worldwide.³ Although the pathogenesis of essential hypertension is multifactorial and complex, subclinical changes in vascular function often precede the development of hypertension and circulatory decline.⁴ The spontaneously hypertensive rat (SHR) is a model of essential hypertension in which blood pressure (BP) begins to rise after 6 weeks of age, ultimately reaching stable pressures of ≈ 180 to 200 mmHg. Recently, Komolova et al reported evidence of altered vascular resistance profiles and renal hemodynamics as early as 3 weeks of age in the SHR,⁵ implicating these early functional changes as a cause, rather than a consequence, of hypertension in this model. As such, the developmental period before weaning (ie,

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before 3 weeks) may constitute a critical time for therapeutic intervention.

Accumulating evidence from human and animal studies suggests that an important determinant of chronic disease risk is dictated by the quality of the intrauterine environment.⁶ Because of its phenotypic plasticity, the fetus and neonate are highly susceptible to several insults, including hypoxia, nutritional disturbances, and hormonal influences.⁶ By extension, this period of increased vulnerability also reflects a time in which offspring may be most amenable to therapeutics,⁷ such that targeted interventions may provide lasting benefits, even after treatment cessation.

Resveratrol (Resv) is a natural polyphenol found in relatively high concentrations in grapes and other plants. Studies in rodent models have shown that Resv confers protection against cardiovascular diseases and other chronic health conditions.⁸ Resv treatment has been shown to lower BP and improve nitric oxide (NO) bioavailability (and hence improved vascular function) in adult SHR,^{9,10} albeit these beneficial cardiovascular effects were lost when treatment was discontinued.¹⁰ Here we investigated whether maternal Resv supplementation instituted during gestation and the immediate postnatal phase would have lasting benefits on BP regulation and vascular function in SHR offspring.

Methods and Materials

Methods are available in the online-only Data Supplement.

Animals and Treatments

The experimental protocols described herein were approved by the University of Alberta Animal Care and Use Committee, in accordance with the Canadian Council on Animal Care guidelines. SHR were purchased from Charles River (St Constant, QC) at 12 weeks of age and were mated with male SHRs. After confirmation of pregnancy, dams were randomly assigned to receive either a control diet (AIN-93G, Research Diets Inc, New Brunswick, NJ) or an identical diet supplemented with Resv (Lalilab, Durham, NC; 4 g/kg diet). Dams were maintained on their respective diet until postnatal day 21. After giving birth, rats were left undisturbed to minimize maternal stress. Offspring were weaned onto a standard chow-based diet at postnatal day 21.

Statistical Analyses

Initially, all offspring data from male and female offspring were analyzed separately. However, no sex differences were observed throughout, and therefore, results from both sexes from each litter were pooled. Experimental number represents fetuses or offspring from different dams. Data were analyzed by Student's *t* test or 2-way analysis of variance with Bonferroni post hoc test, as appropriate. For isolated vascular function data, pEC50 values from concentration response curves were calculated by fitting to the Hill equation; comparisons between untreated vessels and those preincubated with antagonists were compared by analysis of variance, with Bonferroni correction. Summary data for big endothelin-1 (bET-1) concentration–response curves were calculated as area under the curve for each curve and compared by Student's *t* test or 2-way analysis of variance. Data are presented as mean±SEM. *P*<0.05 was considered significant.

Results

Pregnancy Outcomes

Resv supplementation had no effect on maternal food intake (Figure S1A in the online-only Data Supplement) or body weight gain (Figure S1B) throughout pregnancy. No differences were observed in uterine artery resistance index (SHR: 0.61±0.02,

n=8; SHR+Resv: 0.59±0.04, *n*=5; *P*=0.61) and pulsatility index (SHR: 0.62±0.02, *n*=5; SHR+Resv: 0.59±0.04, *n*=5; *P*=0.51) or umbilical artery pulsatility index (SHR: 0.97±0.01, *n*=5; SHR+Resv: 0.96±0.01, *n*=5; *P*=0.69) between SHR and their Resv-treated counterparts, suggesting that prenatal Resv treatment had no effect on blood flow patterns in the maternal uterine arteries (which supply blood to the conceptus) or in the umbilical arteries (as an indicator of fetal blood supply). Length of gestation was not different between treatment groups (with 4 and 7 litters in the SHR group giving birth on gestational day [GD] 21 and GD22, respectively, and 2 and 9 litters in the SHR+Resv group giving birth on GD21 and GD22, respectively; *P*=0.63 by Fisher exact test), nor were litter sizes (SHR: 8.1±1.6, *n*=7; SHR+Resv: 7.6±1.6, *n*=5; *P*=0.81). Maternal Resv treatment throughout pregnancy had no effects on fetal body weights or placental weights (shown as litter averages in Table S1); analysis of individual pup body weights (SHR: 4.03±0.06 g, *n*=36; SHR+Resv: 4.04±0.07 g, *n*=38; *P*=0.95) and placental weights (SHR: 0.37±0.01 g, *n*=36; SHR+Resv: 0.39±0.01 g, *n*=38; *P*=0.10), although not different, were calculated to ensure that averaging of litters did not conceal intralitter variability. Organ weights (absolute and normalized to body weight) were not different between groups at GD21, with the exception that male SHR+Resv fetuses had increased heart weights (*P*=0.034), but this difference was no longer significant when normalized to body weight (*P*=0.074; Table S1).

Postnatal Growth Patterns in SHR Offspring

Continued supplementation of maternal diet with Resv postpartum resulted in growth restriction by postnatal day 21, corresponding to 17% reduction in offspring body weights (*P*<0.01; Figure 1) compared with untreated SHR; male and female offspring exhibited nearly identical growth patterns (Figure S2). After offspring were weaned, serum prolactin levels and glucose handling in dams were assessed to gain insights into the effects of Resv supplementation on maternal health. Serum prolactin levels in dams were not different between groups (SHR: 7.3±1.1 ng/mL, *n*=4; SHR+Resv: 11.6±2.3 ng/mL, *n*=4; *P*=0.15). Further, glucose tolerance test testing in dams within 2 days of weaning offspring revealed a trend toward glucose intolerance in Resv-fed dams (glucose tolerance test area under the curve: SHR: 59.84±9.83, *n*=5; SHR+Resv: 90.2±9.7, *n*=5; *P*=0.06).

No differences in body weight were evident at 5 weeks of age (Figure 1). At the time of euthanasia (≈20 weeks), body weight, length, and organ weights were not different between treatment groups (Table S2). To determine whether

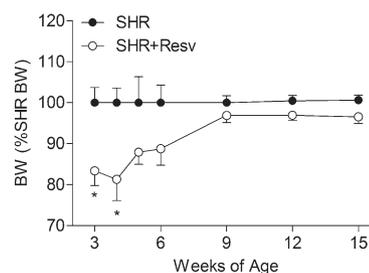


Figure 1. Combined male and female offspring growth patterns expressed as percent of respective untreated spontaneously hypertensive rat (SHR) body weights. **P*<0.001 versus SHR offspring. BW indicates body weight; and Resv, resveratrol.

SHR+Resv–altered growth patterns were associated with changes in body composition or altered metabolic profile in adulthood, we assessed body composition and performed glucose tolerance tests before euthanasia; no differences in body composition were evident between treatment groups (Table S2), and there was no evidence of altered glucose handling in SHR or SHR+Resv adult offspring (Figure S3).

Cardiovascular Outcomes

No sex differences in hemodynamics were observed, and therefore, male and female offspring data from each litter were pooled. Adult SHR+Resv offspring had lower baseline hemodynamics than their respective SHR counterparts (Figure 2), including mean (-17 mmHg; $P=0.007$), diastolic (-18 mmHg; $P=0.007$), and systolic BPs (-15 mmHg; $P=0.02$). Neither pulse pressures (SHR: 53 ± 4 , $n=10$; SHR+Resv: 56 ± 3 , $n=7$; $P=0.60$) nor heart rates (SHR: 366 ± 7 , $n=10$; SHR+Resv: 351 ± 5 , $n=7$; $P=0.15$) were different between treatment groups. Data separated according to sex are shown in Figure S4.

Administration of 0.1, 1, and 10 $\mu\text{g}/\text{kg}$ methylcholine (MCh) caused dose-dependent reductions in mean, diastolic, and systolic BP (Figure S5A). However, the dose of 10 $\mu\text{g}/\text{kg}$ MCh, but not 0.1 or 1 $\mu\text{g}/\text{kg}$, caused a marked, albeit transient, reduction in heart rate ($P<0.001$; Figure S5B), suggesting that this dose impacts cardiac autonomic function. To avoid the confounding effects of reduced cardiac output on hemodynamics, we focused instead on MCh doses of 0.1 and 1 $\mu\text{g}/\text{kg}$. Although there was a dose-dependent lowering effect by MCh ($P<0.001$), there was no difference between SHR and SHR+Resv offspring (Figure S6A–S6C).

Adult offspring were then treated with the nitric oxide synthase inhibitor L-NG-nitroarginine methyl ester (L-NAME) to study the role of NO. L-NAME administration caused a hypertensive response, and this rise in diastolic and mean BP were greater in SHR+Resv than in SHR offspring (Figure 3A and 3B); the rise in systolic BP was not different between groups (Figure 3C). Subsequent administration of MCh in the presence of L-NAME caused a dose-dependent drop in all hemodynamic parameters in both groups, but diastolic and mean BP drops were greater in SHR+Resv compared with SHR offspring (Figure S6D–S6F). As MCh-induced hypotension was not different between perinatal treatment groups (Figure S6A–S6C), the calculated shift in the MCh-induced BP lowering with and without L-NAME was greater in SHR+Resv than in SHR offspring (Figure 4). Finally, to determine whether endothelin-1 (ET-1) production was altered by changes in NO

signaling, a subgroup of rats were treated with the endothelin-converting enzyme inhibitor CGS 35066 (CGS) before L-NAME administration. Pretreatment with CGS partially attenuated the L-NAME-induced rise in diastolic BP ($P=0.05$), albeit by similar amounts in SHR and SHR+Resv offspring (Figure S7). In contrast, CGS had no apparent effects on mean or systolic BPs, suggesting that bET-1 conversion to active ET-1 is not altered in vivo.

To further study the altered vascular signaling pathways in adult offspring, we assessed vascular function in isolated mesenteric arteries. Cumulative concentration–response curves to the α -adrenoceptor agonist phenylephrine (Figure S8A) and ET-1 (Figure S8B) were superimposable between SHR and SHR+Resv offspring. Pretreatment with L-NAME potentiated these vasoconstrictor effects, albeit to a similar extent in SHR and SHR+Resv offspring (Figure S8). In the absence of inhibitors, there were no differences in cumulative concentration–response curves to MCh between SHR and SHR+Resv adult offspring (Figure 5A). However, pretreatment with L-NAME caused a shift in SHR offspring MCh curves ($P<0.01$) that was absent in SHR+Resv offspring (Figure 5A), suggesting loss of vascular NO signaling in this latter group. We next investigated whether vascular responses to the inactive precursor bET-1, which must be cleaved to yield a functional vasoconstrictor peptide,¹¹ were altered because of maternal perinatal Resv treatment. SHR+Resv offspring had reduced bET-1 conversion to ET-1 (area under the curve -28% ; $P<0.05$) compared with SHR offspring (Figure 5B). Pretreatment with L-NAME potentiated bET-1-induced vasoconstriction ($P<0.001$; Figure 5B), and this effect was more pronounced in SHR+Resv offspring compared with untreated offspring (SHR: $+29\pm 12\%$, SHR+Resv: $+73\pm 8\%$; $P=0.02$) such that bET-1-induced vasoconstriction was no longer different between groups ($P=0.63$).

Discussion

In this study, we found that maternal Resv treatment throughout pregnancy caused (1) no effects on fetal growth patterns. Furthermore, continued Resv supplementation in the postnatal (preweaning) period caused (2) growth restriction in the offspring by 3 weeks of age; (3) prevented the development of hypertension in adult offspring; and finally, (4) caused persistent alterations in NO signaling in vivo and ex vivo. Taken together, these results suggest that hypertension in the SHR is amenable to early intervention, and Resv constitutes a promising candidate for early intervention to reduce future

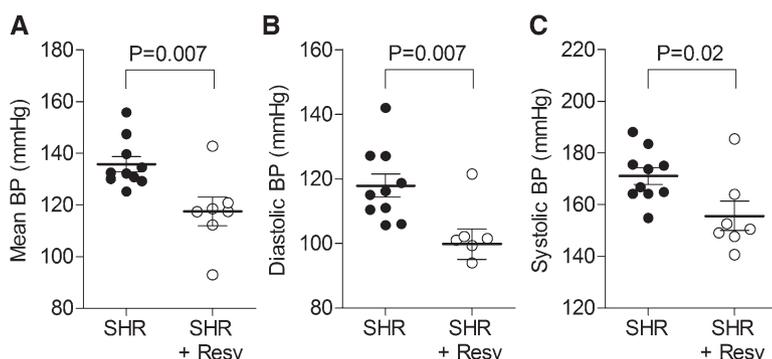


Figure 2. Baseline mean blood pressure (BP; **A**), diastolic BP (**B**), and systolic BP (**C**) values of adult spontaneously hypertensive rat (SHR) and SHR+Resv offspring at ≈ 20 weeks of age. Resv indicates resveratrol.

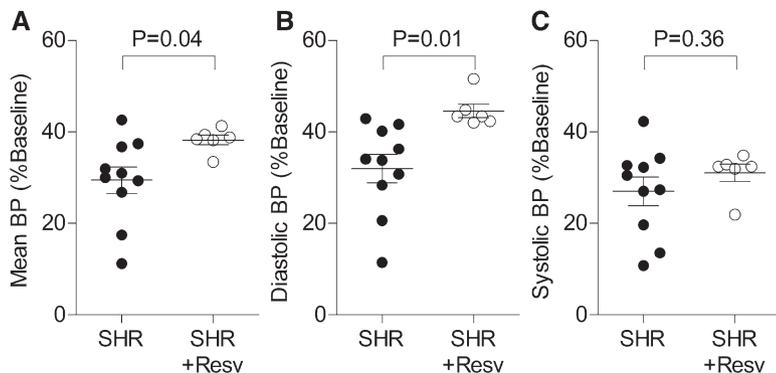


Figure 3. L-NG-nitroarginine methyl ester (L-NAME)-induced rise in mean blood pressure (BP; **A**), diastolic BP (**B**), and systolic BP (**C**). Rise in each hemodynamic parameter was taken as the highest value recorded (continuous average of 30 seconds) within the 15 minutes after L-NAME (30 mg/kg IV initial dose, followed after 10 minutes by 15 mg/kg IV maintenance dose) administration. Mean BP (**A**), diastolic BP (**B**), and systolic BP (**C**), assessed as percentage of baseline BP. Resv indicates resveratrol.

cardiovascular disease risks. However, as discussed later, care should be taken with the use of Resv during development because it may adversely affect neonatal growth as well as long-term cardiovascular outcomes.

The timing and duration of Resv treatment was chosen to maximize offspring exposure during the key periods of growth and development; rats are highly altricial, and thus, organ development continues until the third postnatal week. Moreover, recent work by Komolova et al showed vascular changes are evident as early as 3 weeks of age,⁵ implicating the period before weaning as an important determinant in the development of hypertension in this model.

Maternal Resv supplementation during pregnancy at 4 g/kg diet—which improves pregnancy outcomes in a model of severe hypoxia¹²—did not affect fetal growth trajectories in this study. These findings are consistent with our observations that Resv had little impact on uterine artery and fetal umbilical blood flow patterns. However, whether Resv supplementation improved maternal BP during pregnancy, as it does in adult SHR,^{9,10} is not known and is a limitation of the present study given that maternal hypertension can influence offspring BP with minimal impact on growth patterns.¹³ Interestingly, Roberts et al recently reported that Resv supplementation during pregnancy at a dose similar to that used in the present study affected pancreatic growth in nonhuman primates.¹⁴ Although pancreas weights were not assessed herein, there was no evidence of metabolic dysfunction or altered glucose homeostasis in adult SHR+Resv offspring, suggesting no obvious pancreatic dysfunction. More detailed studies pertaining to the effects of Resv on fetal growth and development are nevertheless warranted.

Continued maternal Resv treatment caused growth restriction in offspring in the postnatal phase, suggesting either a direct effect on offspring growth that is time-dependent or an indirect effect via the dam (eg, Resv may impact the quantity or quality of milk production via altered prolactin synthesis^{15,16}). Although we did not detect any changes in maternal serum prolactin levels in the first day after offspring were weaned, it is possible that a more thorough investigation will reveal time-dependent changes. Resv has also been shown to impact metabolic function in rats,¹⁷ which in turn can influence milk quantity and composition. Our data in a small subset of dams suggest that metabolic function may be altered in dams during lactation. Irrespective of the cause of altered growth in the offspring, SHR+Resv offspring exhibited no apparent changes in body composition or glucose handling in adulthood, suggesting that these altered growth trajectories in early life do not appreciably impact metabolic function in adulthood. Whether underlying metabolic differences ultimately manifest with increasing age (or other metabolic stressors) remains to be determined.

The key finding that maternal Resv supplementation during development caused lower BP in adult offspring is among a few that demonstrate early interventions can impact the development of hypertension in the SHR. Previous studies have shown that Resv treatment instituted postweaning (eg, starting at 3–4 weeks) or later (eg, 10 week of age) successfully lowered BP in SHR,^{9,10,18} although there was reversion to a hypertensive state on cessation of treatment.¹⁰ These findings suggest that targeting the developmental period with Resv is critical to its long-term effects.

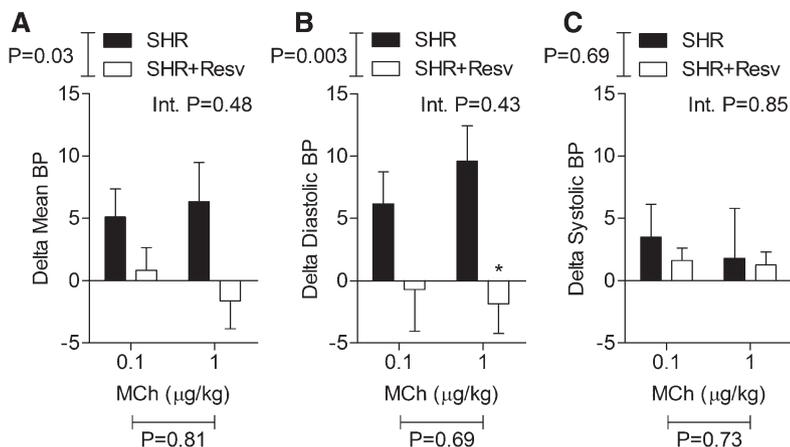


Figure 4. Change in mean blood pressure (BP; **A**), diastolic BP (**B**), and systolic BP (**C**) in response to methylcholine (MCh) between L-NG-nitroarginine methyl ester (L-NAME) and vehicle conditions (data shown in Figure S6) to show NO contribution to vasodilation. **P*<0.05 compared with spontaneously hypertensive rat (SHR) offspring at the same dose of MCh. Mean BP (**A**), diastolic BP (**B**), and systolic BP (**C**), assessed as percentage of baseline BP. Resv indicates resveratrol.

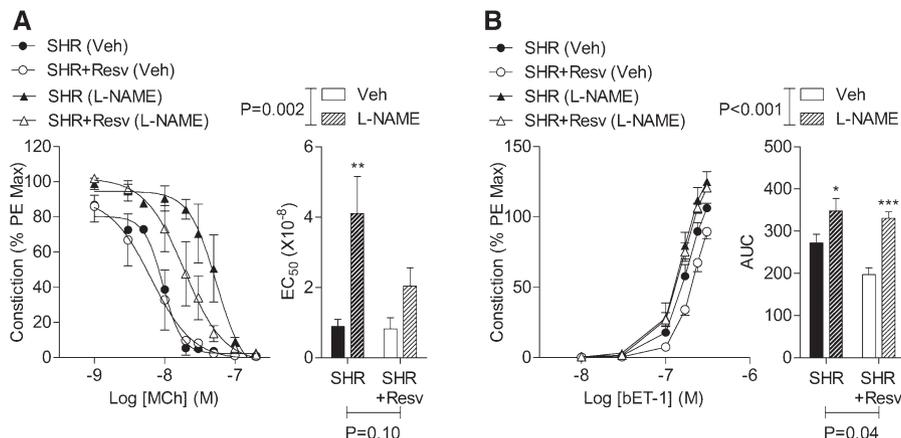


Figure 5. Ex vivo cumulative concentrations–response curves to methylcholine (MCh; **A**) and the inactive precursor big-ET-1 (bET-1; **B**) in isolated mesenteric arteries. **Left.** Cumulative concentration–response curves. **Right.** Summarized data; summarized data are shown as pEC₅₀ (–logEC₅₀) for sigmoidal curves for MCh and area under the curve (AUC) for the nonsigmoidal bET-1 curve. **P*<0.05, ***P*<0.01, ****P*<0.001 compared to vehicle in same perinatal treatment group. L-NAME indicates L-NG-nitroarginine methyl ester; Resv, resveratrol; and Veh, vehicle.

Improved NO bioavailability in the vasculature has been proposed to be an important mechanism by which continued Resv supplementation improves cardiovascular function^{9,10,18} and prevents pathological vascular remodeling.¹⁹ The finding that L-NAME caused a greater rise in BP, effectively normalizing BPs between SHR and SHR+Resv, suggests that a key mechanism by which Resv causes long-term BP normalization is restoration of NO-mediated signaling. To gain insights into the effects of maternal Resv treatment on adult offspring vascular function, we assessed hemodynamic responses to the endothelial-dependent vasodilator MCh in the presence and absence of L-NAME. Interestingly, we found that although MCh-induced vasodilation was not changed between SHR and SHR+Resv offspring, the involvement of NO-dependent pathways in this response is reduced in the SHR+Resv group. Total peripheral resistance is dictated, in large part, by arterioles that exhibit dependence on both NO-dependent and -independent mechanisms, and these findings suggest Resv treatment during development causes a shift toward non-NO dependent mechanisms. The relative contribution of NO-dependent and -independent mechanisms of vascular control seems to be plastic and, thus, permanently altered by influences during pregnancy. For example, prenatal hypoxia has been shown to reduce NO dependence in vascular tone and promote endothelial-derived hyperpolarization–mediated vasodilation.²⁰ Whether this shift in vascular control mechanisms by Resv supplementation during development is mediated by hypoxia-related signaling is an intriguing hypothesis, particularly because Resv is known to interfere with hypoxia-inducible factor expression and adaptations to hypoxia.²¹

In addition to contributing to resting vascular tone, NO has myriad functions, including physiological antagonism of vasoactive mediators, such as ET-1.¹¹ We have previously shown that NO modulates cleavage of bET-1 to active ET-1,²² and this pathway is perturbed in offspring that exhibit a reduced NO-mediated vasodilation.^{20,23} We therefore investigated the role of bET-1 conversion to active ET-1 in baseline and L-NAME-induced hypertension in adult offspring. The endothelin-converting enzyme inhibitor CGS attenuated the

L-NAME–induced rise in BP, without affecting baseline BP, thus confirming our previous reports that NO tonically inhibits the actions of ET-1, in part by inhibiting the conversion of bET-1 to active ET-1.²² However, because CGS did not differentially affect the L-NAME-induced hypertension between groups, it is likely that baseline levels of bET-1 conversion do not appreciably contribute to the persistent lowering of BP.

We sought to gain further insights into the mechanisms underlying this altered vasodilatory pathway by investigating vascular signaling pathways in isolated mesenteric arteries. Consistent with our findings in vivo, MCh-induced vasodilation was unchanged between SHR and SHR+Resv offspring. However, the contribution of NO to this vasodilation was reduced in the SHR+Resv group, based on the lack of shift in EC₅₀ by L-NAME in this group. These findings further support the notion of a greater dependence on NO-independent vascular control mechanisms in the treated offspring. In contrast, L-NAME potentiated vasoconstrictor responses to phenylephrine and ET-1 by comparable amounts in both treatment groups, suggesting residual amounts of NO signaling in blood vessels of SHR+Resv offspring. In fact, we found that bET-1 conversion to active ET-1 is attenuated in SHR+Resv offspring, and this difference between groups could be normalized with L-NAME pretreatment. Because we observed no changes in vasoconstrictor activity to phenylephrine nor ET-1—suggesting unaltered intrinsic vasoconstrictor mechanism and ET-1 receptor signaling in SHR+Resv offspring—these findings suggests improved NO signaling in the context of bET-1 bioconversion in the vasculature of treated offspring. Although this may seem at odds with the in vivo and ex vivo vascular function data showing a shift away from NO-dependent mechanisms, these findings may indicate that different subcellular compartments are more or less vulnerable to loss of NO signaling. Indeed, we have previously shown that levels of NO required to inhibit ET-1 signaling are lower than those needed to induce vasodilation,²² suggesting an intimate coupling between NOS enzymes and the ET-1 synthetic machinery. Future studies are needed to provide more definitive insights into this area of vascular biology.

It is particularly noteworthy that male and female offspring were similarly affected by the perinatal maternal Resv supplementation, considering how developmental stressors can induce a range of sexually dimorphic effects on cardiovascular function in the offspring.²⁴ In many instances, gender-disparities do not manifest until advanced age,²⁴ and thus, sex-differences in long-term cardiovascular function may become evident with time. Alternatively, it may be that Resv treatment prevents early events in the eventual progression to hypertension in the SHR that are common to both sexes. In either case, these results emphasize the need for additional studies focused on early mechanisms by which Resv impacts development, as well as long-term studies investigating the progression of the cardiovascular phenotype with advanced age.

Perspectives

This study offers new insights into therapeutic potential for Resv to prevent the development of hypertension in a model of essential hypertension. Although the mechanisms underlying this improvement in hemodynamics require further investigation, the degree of BP lowering was notable and likely impact long-term cardiovascular health in these offspring. Indeed, the average lifespan of the SHRs is \approx 14 months, which ultimately ends prematurely because of cardiovascular complications associated with unmitigated hypertension. Although rats in the present study were euthanized for cardiovascular assessments, it would be of interest to determine whether perinatal maternal Resv treatment extends lifespan because even reductions of 2 to 3 mmHg have been shown to reduce morbidity and mortality in humans.²⁵ However, it is noteworthy that Racasan et al showed that although prenatal losartan treatment attenuated the development of hypertension in young SHR, these offspring ultimately developed malignant hypertension and died prematurely.²⁶ In the case of SHR+Resv offspring, it is possible that despite maintaining vasodilatory function, the loss of vascular NO signaling may affect vascular health and contribute to accelerated age-related decline in circulatory function. As such, future studies examining the long-term cardiovascular health of prenatal or perinatal Resv treatment are crucial.

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Disclosures

None.

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Novelty and Significance

What Is New?

- Perinatal maternal resveratrol supplementation prevented the development of hypertension in adult spontaneously hypertensive rat offspring.
- Alterations in nitric oxide signaling contribute to this resveratrol-mediated persistent lowering of blood pressure in adult spontaneously hypertensive rat offspring.
- Postnatal (preweaning) resveratrol supplementation caused growth restriction in young spontaneously hypertensive rat offspring.
- Male and female offspring are similarly affected by perinatal resveratrol treatment.

What Is Relevant?

- Hypertension affects ≈25% of adults globally and is an important risk factor for cardiovascular disease. A universal consensus is that prevention of hypertension is a more strategic and cost-effective approach than treatment. The results of the present study demonstrate that resveratrol is a strong candidate for prenatal use as a prevention strategy for hypertension.

Summary

We demonstrate that perinatal maternal resveratrol supplementation prevents the rise in blood pressure and has persistent effects on vascular function in adult offspring.

Perinatal Resveratrol Supplementation to Spontaneously Hypertensive Rat Dams Mitigates the Development of Hypertension in Adult Offspring

Alison S. Care, Miranda M. Sung, Sareh Panahi, Ferrante S. Gragasin, Jason R.B. Dyck, Sandra T. Davidge and Stephane L. Bourque

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PERINATAL RESVERATROL SUPPLEMENTATION TO SPONTANEOUSLY HYPERTENSIVE RAT DAMS MITIGATES THE DEVELOPMENT OF HYPERTENSION IN ADULT OFFSPRING

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METHODS

Animals and Treatments

The experimental protocols described herein were approved by the University of Alberta Animal Care and Use Committee, in accordance with the Canadian Council on Animal Care guidelines. SHR were purchased from Charles River (St Constant, QC) at 12 wk of age and housed in the Animal Care Facility at the University of Alberta, which maintained in a 12:12h light:dark cycle, and an ambient temperature of $22\pm 1^\circ\text{C}$. Throughout the experiments, rats were given *ad libitum* access to food and water. Dams were mated with male SHRs and vaginal smears were checked daily for the presence of sperm; the presence of sperm was considered gestational day (GD)0.5. After confirmation of pregnancy, dams were randomly assigned to receive either a control diet (AIN-93G, Research Diets Inc. New Brunswick, NJ) or an identical diet supplemented with resveratrol (Lalilab, Durham, NC; 4g/kg diet); dams were maintained on their respective diet after giving birth until postnatal day (PD)21. After giving birth, rats were left undisturbed to minimize maternal stress. Offspring were weaned onto a standard chow-based diet at PD21. To ensure weaned offspring were representative of their litters with respect to body weights, pups were chosen pseudo-randomly, taking care to avoid the largest or smallest offspring of each sex within the litter. All adult offspring studied were obtained from either the first or second successful pregnancy; dams were given a 3 week “washout period” between pregnancies, and allocated to the same treatment group in both instances.

Ultrasound Biomicroscopy and Assessment of Pregnancy Outcomes

In a subset of rats, ultrasound biomicroscopy was performed on pregnant dams at GD20 to assess uterine artery as well as fetal umbilical artery and vein hemodynamic parameters, as previously described.¹ Under isoflurane anesthesia (5% induction, 2.5% maintenance, in air) dams were imaged transcutaneously using an ultrasound biomicroscope (model Vevo 2100, VisualSonics, Toronto, ON, Canada) with a 16-21 MHz MicroScan array transducer probe. A 0.2 to 0.5 mm pulsed Doppler gate was used, and the angle between the Doppler beam and the vessel was $<45^\circ$. Doppler waveforms were obtained from uterine arteries as well as from the umbilical artery and vein from three fetuses per dam. Peak systolic velocity and end diastolic velocity averages were obtained from a minimum of three consecutive cardiac cycles. Both resistance index ($\text{RI} = [\text{PSV} - \text{EDV}] / \text{PSV}$) and pulsatility index ($\text{PI} = [\text{PSV} - \text{EDV}] / \text{time averaged velocity [TAV]}$), were calculated.

The following day (GD21; term=GD22), pregnant dams were anesthetized with isoflurane (5% in 100% O_2) and euthanized by exsanguination via a cardiac puncture. Fetuses and their placentae were quickly removed and weighed. Fetuses were then decapitated and organs were collected, weighed and frozen in liquid nitrogen.

Metabolic Assessments in Adult Offspring

Glucose tolerance tests were performed after a minimum 6 h fasting period. Rats were injected with glucose (2g/kg body weight, i.p.), and blood glucose levels were assessed serially using a glucometer (Accu-Chek Nano, Roche Diagnostics, Laval, QC, Canada) from saphenous vein punctures. Data are expressed as absolute change in blood glucose concentrations from baseline (0 min; taken before glucose injection). Body composition was assessed in conscious rats using

quantitative nuclear magnetic resonance technology (Echo Medical Systems, Houston, TX, USA).

Hemodynamic Assessments in Adult Offspring

For direct hemodynamic assessments, adult offspring (~20 wks of age) were kept spontaneously breathing under anesthetized with isoflurane (3-5% in 100% oxygen) and maintained on a warming pad. Once surgical anesthesia was achieved, rats were implanted with a femoral arterial catheter (PE50, 0.58 mm i.d., 0.97 mm O.D., Becton Dickson, Sparks, MD, USA) connected to a pressure transducer (ADInstruments, Colorado Springs, CO) for hemodynamic measurements, and a femoral venous catheter (Silastic®, Cole-Parmer, Montreal QC. 0.51 mm i.d., 0.94 mm o.d.) for drug delivery. Once catheter implantation was completed, inspired isoflurane was set at 1.5% for the remainder of the experiment. To ensure stable hemodynamic parameters, blood pressure was allowed to equilibrate for a minimum of 25 minutes after cannulation, after which time baseline values were collected for 10 minutes.

After baseline recordings, hemodynamic responses to pharmacological agents were assessed in the following sequence: (i) three increasing bolus doses of MCh (0.1, 1, 10µg/kg, IV), with 2 min in between each dose; (ii) a washout period of 15 minutes, followed by administration of vehicle (saline) or the endothelin converting enzyme inhibitor CGS35066 (CGS; 1mg/kg/hr IV); (iii) a 15 minute waiting period, followed by administration of the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) given as an initial dose (30mg/kg IV) followed by a maintenance dose (15mg/kg) after 10 minutes; ^{2, 3} (iv) a 5 minute wait period, followed by three increasing bolus doses of MCh (0.1, 1, 10µg/kg, IV).

All agents were dissolved in sterile saline except CGS, which was dissolved in 97:3 0.25mM NaHCO₃ in PBS:1M NaOH.

Wire Myography

Vascular function was assessed in mesenteric resistance arteries of adult offspring according to established procedures;^{4, 5} rats tested for vascular function *ex vivo* had not been subjected to hemodynamic assessments described above. Briefly, adult offspring were anesthetized with isoflurane (5% in 100% O₂) and killed by exsanguination. Mesentery was rapidly excised and placed in ice-cold HEPES-buffered physiological saline solution (PSS; NaCl-[142mmol/L], KCl [4.7mmol/L], MgSO₄ [1.17mmol/L], CaCl₂ [4.7mmol/L], K₂PO₄ [1.18mmol/L], HEPES [10mmol/L], and glucose [5.5 mmol/L], pH 7.4). Mesenteric arteries were carefully isolated from the surrounding adipose tissue using a binocular microscope, and arteries with internal diameters ranging 150-250 µm were mounted in an isometric myograph system (DMT, Copenhagen, Denmark) using 40 µm tungsten wire. After equilibration, vessels were normalized through a series of stepwise increases in diameter. Following tension optimization, vessels were rinsed with PSS, and given 15 minutes to equilibrate prior to viability testing with PE and MCh to assess contractile and endothelial-dependent function, respectively. Vessels were then treated for a minimum of 30 minutes with vehicle, L-NAME (100µmol/L; Sigma), or a combination of L-NAME+Indomethacin (5µmol/L). After pre-incubation with inhibitors, cumulative concentration response curves were generated with either PE (10µmol/L; Sigma); ET-1 (Calbiochem; 1-100 nmol/L), or bET-1 (Anaspec, Freemont CA; 10-310 nmol/L). For MCh (Sigma, 1nmol/L to 5µmol/L) and SNP (Sigma, 0.1nmol/L to 5µmol/L) cumulative

concentration responses, vessels were pre-constricted to 80% of maximal constriction with PE, and given a minimum of 5 minutes before administration of vasodilators.

Statistical Analyses

Initially, all offspring data (including normalized growth rates and body composition, metabolic function, and hemodynamic data) from male and female offspring were analyzed separately. However, no sex-differences were observed throughout, and therefore results from both sexes from each litter were pooled. Data obtained from the same litter were averaged and treated as a single value; thus, n values reflect the number of litters (i.e. treated dams) and represent data from 1-8 offspring for prenatal and neonatal offspring data, and 1-4 offspring for adult offspring data. Data were analyzed by student's *t* test, or 2-way ANOVA with Bonferonni post hoc test, as appropriate. For isolated vascular function data, pEC50 values from concentration response curves were calculated by fitting to the Hill equation; comparisons between untreated vessels and those pre-incubated with antagonists were compared by ANOVA, with Bonferoni correction. Because bigET-1 concentration-response curves did not exhibit a sigmoidal shape, data was calculated as area under the curve (AUC) for each curve, and compared by Student's *t* test or 2-way ANOVA. Data are presented as Mean±SEM. P<0.05 was considered significant.

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Table S1. Fetal body and organ weights assessed at gestational day 21.

Parameter	Males		Females	
	SHR	SHR+Resv	SHR	SHR+Resv
Body wt (g)	4.2±0.1 (5)	4.2±0.2 (5)	3.8±0.1 (5)	4.0±0.1 (4)
Placenta wt (g)	0.35±0.02 (5)	0.39±0.05 (5)	0.35±0.02 (5)	0.38±0.03 (4)
Placenta wt/Body wt ratio	0.085±0.005 (5)	0.086±0.010 (5)	0.091±0.006 (5)	0.093±0.006 (4)
Absolute Heart wt (mg)	22.1±1.1 (5)	26.0±1.0 (5)*	23.9±1.0 (5)	25.8±1.4 (4)
Relative Heart wt (mg/g)	5.3±0.2 (5)	6.3±0.4 (5)	6.2±0.2 (5)	6.40±0.3 (4)
Absolute Kidney wt (mg)	20.8±1.6 (5)	21.4±0.6 (5)	21.3±0.7 (5)	21.9±1.0 (4)
Kidney wt (mg/g)	5.0±0.4 (5)	5.1±0.2 (5)	5.5±0.2 (5)	5.4±0.2 (4)
Absolute Liver wt (mg)	252.6±19.3 (5)	257.4±21.6 (5)	257.4±8.3 (5)	267.3±25.8 (4)
Liver wt (mg/g)	60.6±5.2 (5)	61.3±3.6 (5)	67.5±3.9 (5)	66.0±4.6 (4)
Absolute Brain wt (mg)	190.4±1.4 (5)	196.7±3.1 (5)	192.6±3.9 (5)	192.8±3.2 (4)
Brain wt (mg/g)	45.5±1.1 (5)	47.3±1.7 (5)	50.3±1.2 (5)	48.0±1.2 (4)

Data are mean±SEM (n). Data obtained from the same litter were pooled and treated as a single value. *P=0.034 compared to male SHR.

Table S2. Adult offspring characteristics.

Parameter	Males			Females		
	SHR	SHR+Resv	P Value	SHR	SHR+Resv	P Value
Body Weight (g)	313±7 (13)	302±9 (11)	0.36	197±2 (13)	196±5 (12)	0.74
% Fat Mass	8.7±0.3 (12)	8.8±0.4 (11)	0.83	8.1±0.3 (14)	8.3±0.4 (11)	0.71
% Lean Body Mass	78.8±1.6 (12)	78.6±1.2 (11)	0.93	78.6±1.3 (14)	77.6±1.1 (11)	0.60
Abdominal girth (cm)	17.6±0.3 (11)	17.4±0.3 (9)	0.67	14.6±0.2 (8)	14.6±0.5 (6)	0.95
Length (cm)	16.4±0.2 (12)	15.8±0.2 (8)	0.09	14.4±0.2 (5)	14.4±0.2 (6)	0.97
Liver Weight (g/kg)	35.4±0.7 (13)	35.7±0.1 (11)	0.31	38.2±0.8 (11)	39.4±1.1 (7)	0.39
Kidney Weight (g/kg)	3.8±0.1 (13)	3.6±0.1 (11)	0.29	3.7±0.1 (11)	3.7±0.0 (7)	0.67
Ventricle Weight (g/kg)	3.4±0.0 (13)	3.3±0.1 (11)	0.69	3.7±0.0 (11)	3.6±0.1 (7)	0.31
Spleen Weight (g/kg)	1.9±0.1 (13)	2.1 ±0.1 (11)	0.39	2.8±0.1 (11)	2.6±0.2 (7)	0.40
Brain Weight (g/kg)	4.3±0.1 (7)	4.3±0.1 (7)	0.91	6.6±0.1 (10)	6.6±0.2 (6)	0.77

Data are mean±SEM (n). Data obtained from the same litter were pooled and treated as a single value. Data were collected at the time of euthanasia (~20wks).

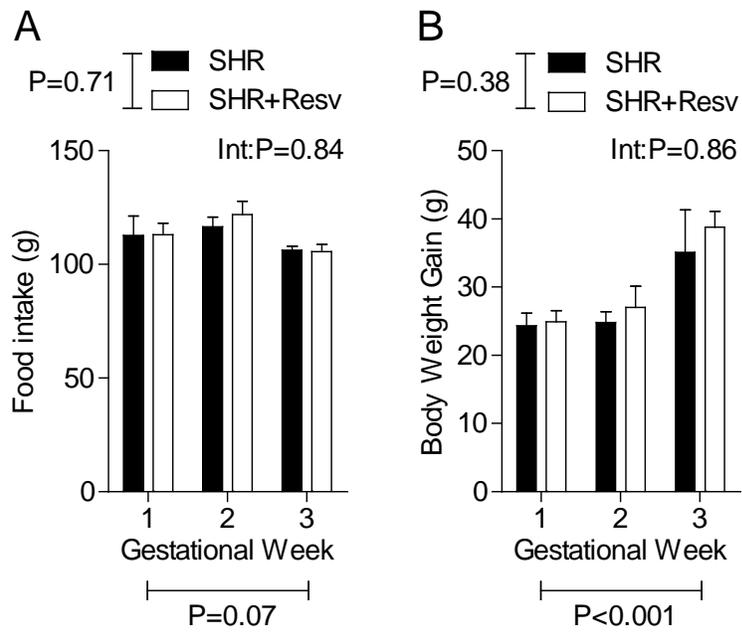
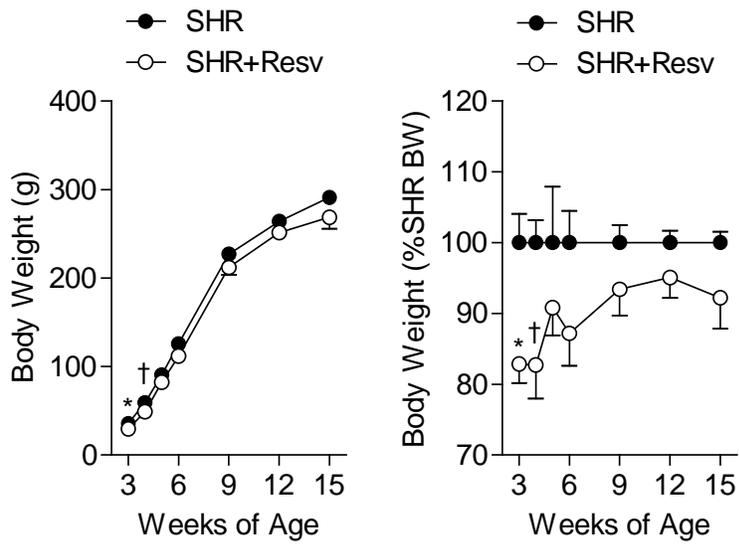


Figure S1. Resveratrol (Resv) supplementation does not impact dam (A) food consumption or (B) weight gain throughout pregnancy.

A. Males



B. Females

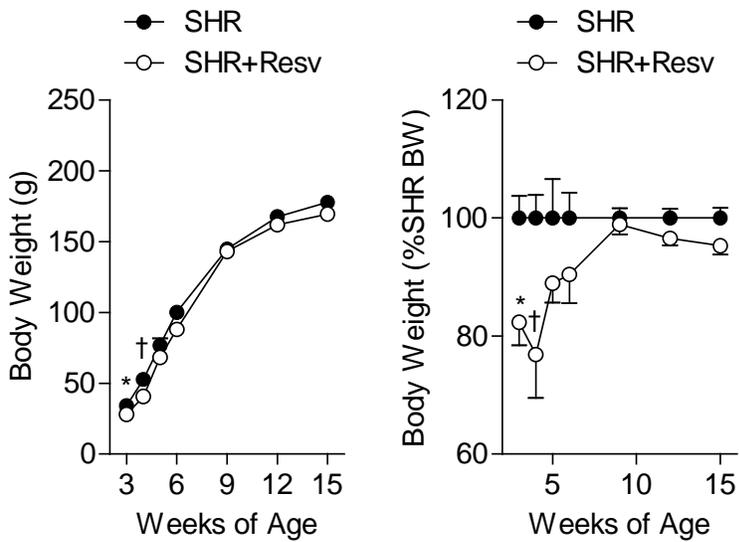
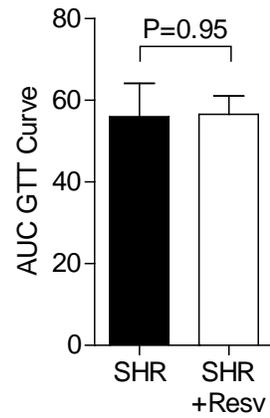
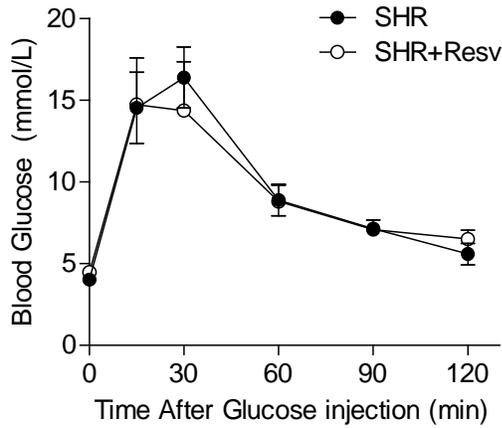


Figure S2. (A) Male and (B) female offspring growth patterns expressed as absolute body weights (left panels) and expressed as percent of untreated SHR body weights (right panels). * $P < 0.001$, † $P < 0.05$ versus SHR offspring. Resv indicates resveratrol.

A. Males



B. Females

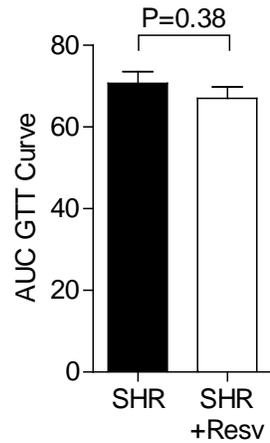
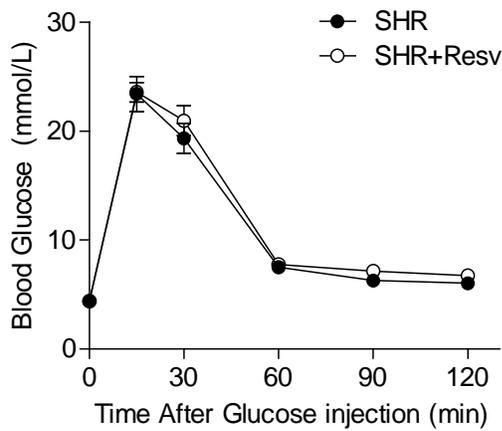
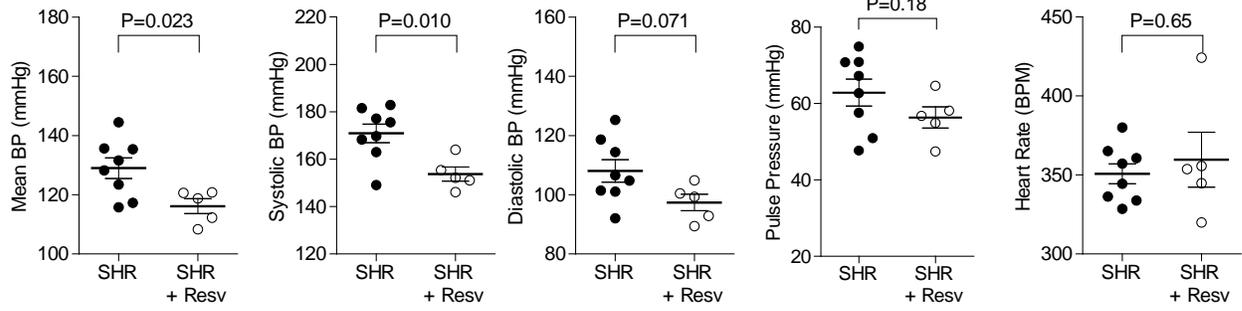


Figure S3. Metabolic function is not different between SHR+Resv adult (A) male or (B) female offspring compared to respective SHR controls. Left panels show glucose tolerance tests, and right panel show summary of the area under the curve (AUC). Resv indicates resveratrol.

A. Males



B. Females

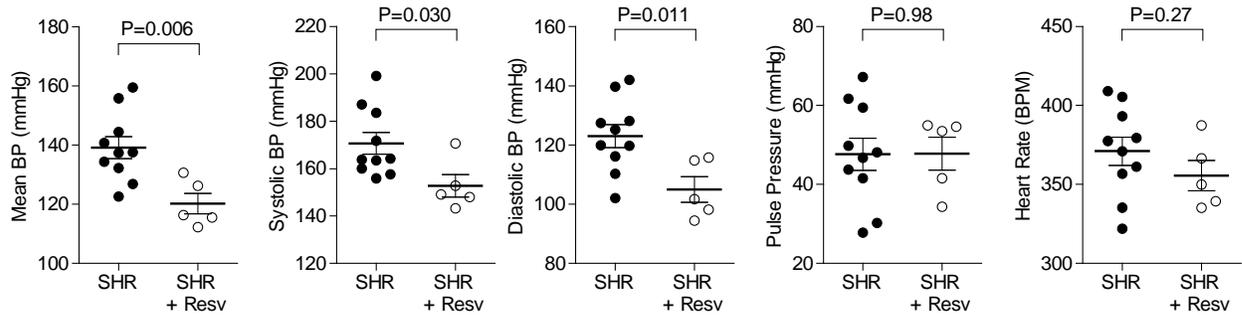


Figure S4. Baseline hemodynamics of adult SHR and SHR+Resv offspring at ~20 wk of age. Each data point reflects 1 litter (with 1-3 offspring sampled per litter). Resv indicates resveratrol.

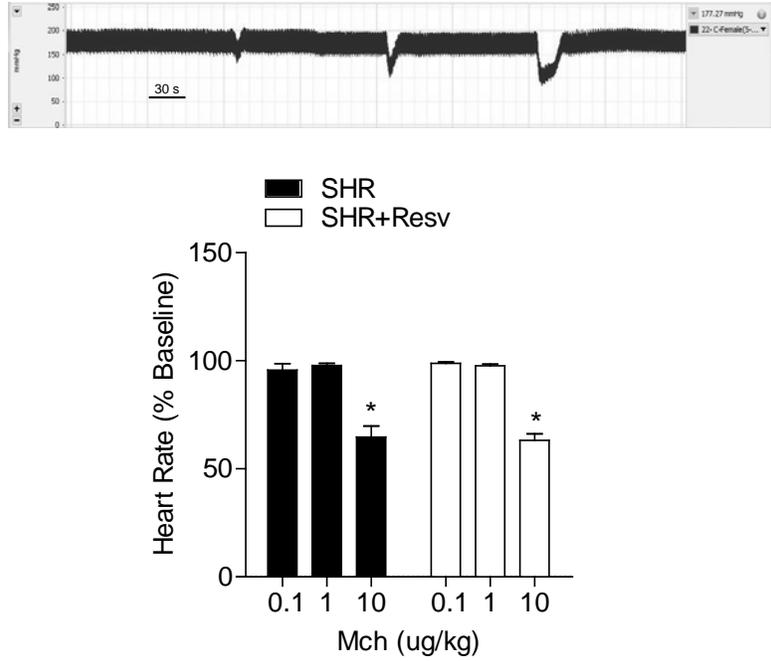


Figure S5. Hemodynamic effects of methylcholine (MCh) administration. (A) Representative trace showing blood pressure responses to MCh doses; (B) summarized effect of MCh on heart rate (expressed as a percentage of baseline). *P<0.001 compared to 0.1 and 1µg/kg in the same group. Resv indicates resveratrol.

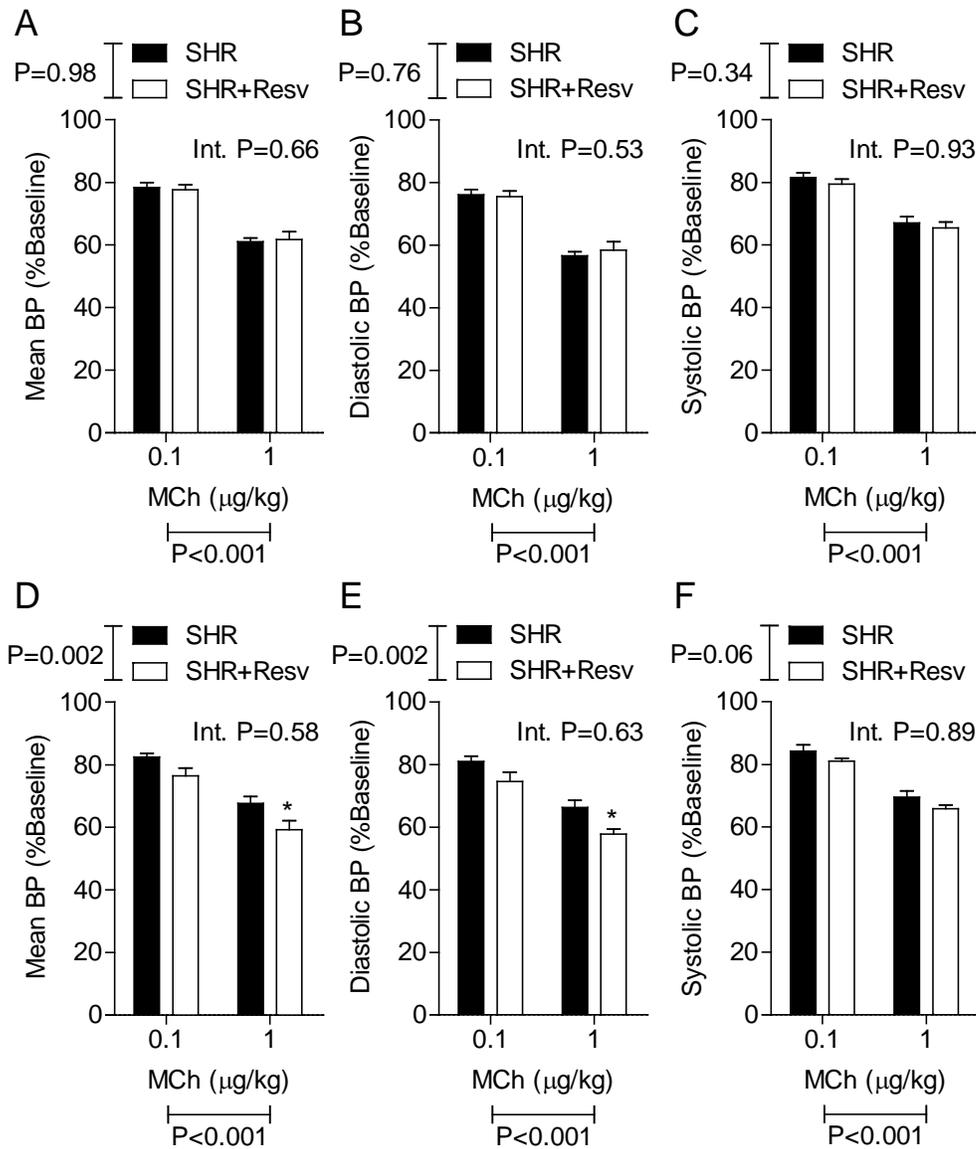


Figure S6. Hemodynamic responses to methylcholine (MCh; 0.1 and 1 µg/kg) in the presence of saline (A-C) or L-NAME (30mg/kg IV initial dose, followed after 10 minutes by 15mg/kg IV maintenance dose) (D-F). Data reflect percent of baseline established after vehicle or L-NAME treatment. *P<0.05 compared to SHR offspring at the same dose of MCh. Resv indicates resveratrol.

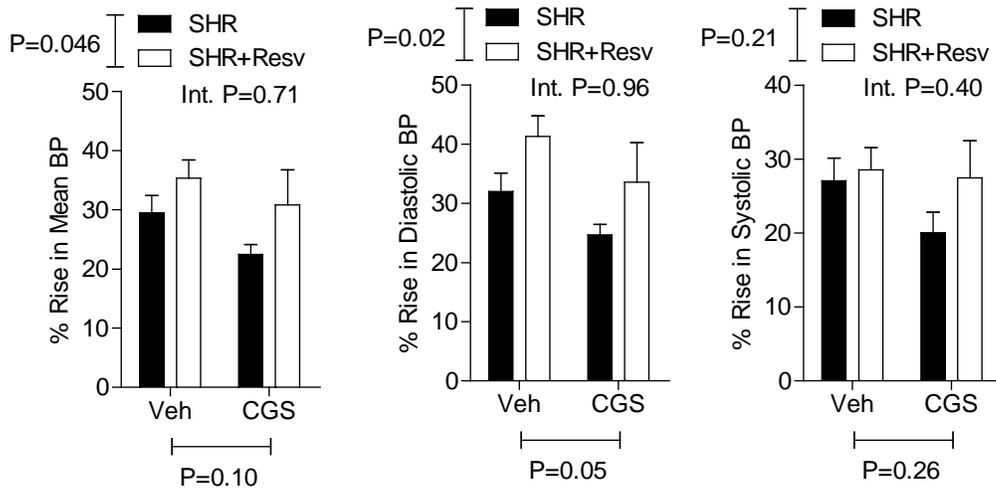


Figure S7. The endothelin converting enzyme inhibitor CGS 35066 (CGS) has modest effects on L-NAME-induced rise in blood pressure compared to vehicle. For clarity, all data show offspring treated with the NOS inhibitor L-NAME (30mg/kg IV initial dose, followed after 10 minutes by 15mg/kg IV maintenance dose) with or without co-treatment of CGS (1mg/kg/hr IV). Resv indicates resveratrol.

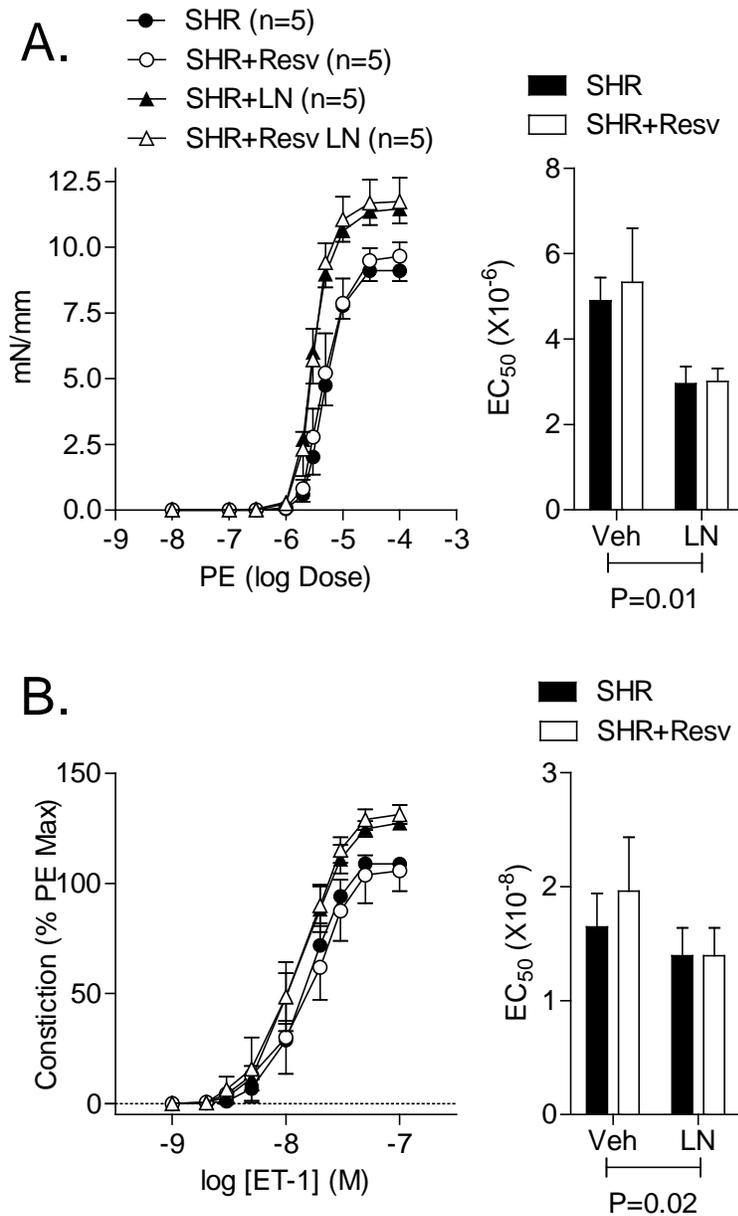


Figure S8. Mesenteric vascular responses, alone or in the presence of L-NAME (100 μ mol/L) to (A) the alpha-adrenoceptor agonist phenylephrine (PE), and (B) endothelin-1 (ET-1). Left panels depict cumulative concentration-response curves to agonists, and right panels depict summarized data expressed as EC₅₀. Resv indicates resveratrol.

Reprogramming Essential Hypertension The Role of Resveratrol

Suttira Intapad

See related article, pp 1038–1044

In this issue of *Hypertension*, Care et al¹ show promising data supporting resveratrol supplementation during gestational and post gestational periods for the treatment of essential hypertension in the offspring. Administration of resveratrol to spontaneously hypertensive rat (SHR) dams prevented high blood pressure (BP) in both male and female SHR progeny. Their results have clinical relevance for they demonstrate a subjugation of genetically inherited high BP (Figure).

Hypertension is a leading health problem worldwide. It has a high prevalence and is associated with increased morbidity and mortality for cardiovascular and renal diseases. An estimated one third of the adult US population is hypertensive and an equivalent percentage experience prehypertension. Individuals with prehypertension are at a particular risk of developing overt hypertension. Furthermore, the costs for treatments and hospitalization are exorbitant.^{2,3} Primary or essential hypertension is the most common form of hypertension. It is typically a genetic, progressive disease with susceptibility increasing based on individual confounding factors/environment. In contrast to secondary hypertension, treatment of essential hypertension cannot be addressed by removing the known cause. Although essential hypertension is treatable, it is not curable. Treatment goals focus on the prevention of the key consequences of elevated BP. Several effective antihypertensive drugs are available nowadays, such as diuretics, angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers, β -blockers, and calcium channel blockers. However, they require constant administration or recurrence may take place. For those reasons, a significant effort should be devoted to the discovery of a strategy for early prevention.

The SHR is a well-known animal model of human essential hypertension. It was developed in the early 1960s by Okamoto and Aoki⁴ by breeding Wistar–Kyoto rats with high BP. Numerous studies have used SHRs to research treatment options. Currently available antihypertensive agents are

effective in controlling hypertension in SHRs. However, as their hypertension is genetically inherited, treatment discontinuation results in the reappearance of high BP.

Resveratrol, or 3,5,4'-trihydroxy-*trans*-stilbene, is a natural polyphenolic molecule naturally found in high concentrations in red grapes as well as in berries and peanuts. The cardioprotective effect of resveratrol was first observed in 1992. Since then, resveratrol supplementation has been considered as a potential therapeutic strategy for the treatment of cardiovascular diseases and other chronic health conditions. The cardioprotective effects of resveratrol are perhaps because of its potent antioxidant properties as well as it being a nitric oxide signaling pathway modulator. These characteristics make it an attractive target for the prevention and treatment of hypertension. Resveratrol has been studied in various animal models of hypertension and in limited human clinical trials.⁵

In 2011, Bhatt et al⁶ administered resveratrol to SHR pups starting at weaning (3–4 weeks of age) for 10 weeks. BP was measured at the end of the experiment and resveratrol supplementation resulted in lower BP (≈ 20 mmHg). In another study,⁷ resveratrol administration to 10-week-old SHRs for 5 weeks lowered systemic arterial pressure starting at 2 weeks after supplementation. However, treatment withdrawal resulted in an immediate restoration of hypertension. The ability of resveratrol to produce sustained, long-term reductions in arterial pressure remains controversial. Similarly to other antihypertensive drugs, resveratrol is considered to have an acute effect and requires continued administration to exhibit uninterrupted effects.

It is well established that BP in later life can be programmed not only by genetic inheritance but also by the pre- and postnatal milieu. Studies using insults during pregnancy (ie, pre-eclampsia, or nutritional restriction) in animal models result in persisting cardiovascular dysfunctions in later part of life of the offspring, despite their genetic background being normal.⁸ Similarly, postnatal exposure to stress by separating the offspring from the dams for a few hours a day also translated into enhanced susceptibility to high BP in response to angiotensin II.⁹ These results suggest that the environment plays a crucial role in the stemming phenotypes. Moreover, despite the genotype being programmed to become hypertensive, cross-fostering of SHR offspring to normotensive Wistar–Kyoto¹⁰ dams results in an intermediate phenotype because they exhibit lower BP compared with non-cross-fostered SHRs. Thus, the progression of high BP in these animals is not strictly predetermined by genetic factors. Rather, a genetic predisposition to hypertension interacts with the preweaning environment to determine an animal's cardiovascular phenotype in adulthood.

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

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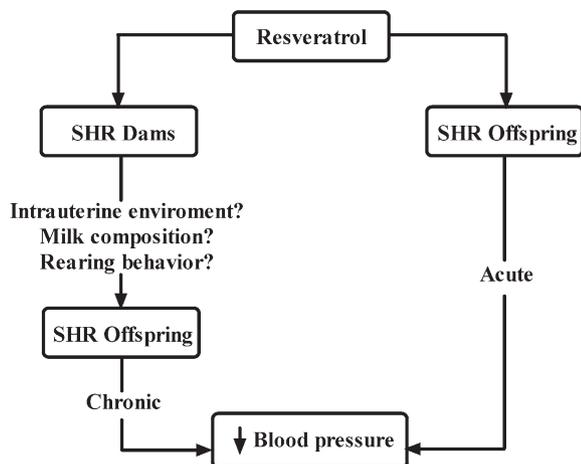


Figure. Diagram for the proposed effects of resveratrol on the blood pressure of the spontaneously hypertensive rat (SHR).

Care et al¹ successfully show that resveratrol administration to the dams, not the pups, during pregnancy and nursing prevents the phenotypic expression of high BP in the SHR offspring. The peculiarity of this study lies in the lowering of BP in the SHR model past adulthood, overcoming genetic programming. In contrast to the aforementioned studies^{6,7} in which resveratrol was given directly to SHR animals to lower their BP, it seems gestational+postnatal intervention is much more effective because the discontinuation of treatment did not reverse the phenotype. The mechanism is likely because of a reset of the vascular responsiveness and nitric oxide bioavailability of the offspring,¹ but how? Neither blood flow patterns from the mother to fetus nor prolactin concentration in the milk is involved.¹ Resveratrol may acutely lower the BP of the dams leading to a less adverse fetal environment to the fetus and modulating the differential turning on and off of genes. Further studies are required to identify how maternal physiology, milk composition, and rearing behavior during resveratrol supplementation lead to the resetting of the offspring phenotype.

In summary, this study by Care et al¹ provides extensive evidence that timing of resveratrol treatment is crucial in preventing hypertension. Nevertheless, there are still many unknowns; for example, safety, dosage, and mechanism.

Studies focused not only on the pups but also the dams could advance our knowledge on the effects of pre- and postnatal resveratrol supplementation in the overcoming of genetic programming in populations at risk of developing essential hypertension.

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Disclosures

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