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Oral Estrogen Reverses Ovariectomy-Induced Morning Surge Hypertension in Growth Restricted Mice

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Abstract

Perinatal growth restriction is associated with heightened sympathetic tone and hypertension. We have previously shown that naturally occurring neonatal growth restriction (GR) programs hypertension in male but not female mice. We therefore hypothesized that intact ovarian function or post-ovariectomy estrogen administration protects GR female mice from hypertension. Utilizing a non-interventional model that categorizes mice with weanling weights below the 10th percentile as GR, control and GR adult mice were studied at three distinct time points: baseline, post-ovariectomy (OVX), and post-OVX with oral estrogen replacement. OVX elicited hypertension in GR mice that was significantly exacerbated by psychomotor arousal (systolic blood pressure at light to dark transition: control 122 $\pm$ 2; GR 119 $\pm$ 2; control-OVX 116 $\pm$ 3; GR-OVX 126 $\pm$ 3 mmHg). Estrogen partially normalized the rising blood pressure surge seen in GR-OVX mice (23 $\pm$ 7% reduction). GR mice had left ventricular hypertrophy, and GR-OVX mice in particular had exaggerated bradycardic responses to sympathetic blockade. For GR mice, a baseline increase in baroreceptor reflex sensitivity and high frequency spectral power support a vagal compensatory mechanism, and that compensation was lost following OVX. For GR mice, the OVX-induced parasympathetic withdrawal was partially restored by estrogen (40 $\pm$ 25% increase in high frequency spectral power, $P < 0.05$). In conclusion, GR alters cardiac morphology and cardiovascular regulation. The hemodynamic consequences of GR are attenuated in ovarian-sufficient or estrogen replete females. Further investigations are needed to define the role of hormone replacement therapy targeted towards young women with estrogen deficiency and additional cardiovascular risk factors, including perinatal growth restriction, cardiac hypertrophy and morning surge hypertension.

Keywords

autonomic; developmental origins; echocardiography; heart rate variability

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Competing Interests

None

Author Contributions

Conception and design of the experiments: SEH, VP, BER, CZ, RDR

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INTRODUCTION

Perinatal growth restricted infants face a constellation of challenges and must compensate to minimize the risk of life-long morbidity (1). In addition to the reprogramming of metabolism to optimize the incorporation of limited nutritional resources, the circulatory system must adapt to altered caloric demands and body dimensions. Whether a primary consequence of growth restriction (GR) or a compensatory response, former GR infants have increases in sympathetic tone and are at a greater risk of hypertension than the general population (2, 3).

Premature infants face many of the same challenges encountered by intrauterine growth restricted term infants. Both have global impairment in growth and development and marked reductions in nutritional stores. By the time premature infants reach 36 weeks postmenstrual age, nearly 90% have developed ex-utero growth restriction and up to 70% have elevated arterial pressures throughout infancy (4, 5). As adults, former premature infants have restricted cardiac dimensions and increased arterial pressures, with the greatest alterations seen in men (6, 7, 8).

Investigations in rats have shown late gestation uterine artery ligation and maternal malnutrition elicit intrauterine growth restriction with long-term phenotypic changes in adult offspring, including increased sympathetic tone and hypertension (9, 10, 11). Female offspring in these studies are partially protected from the damaging effects of intrauterine GR, but this protection is lost with ovariectomy or age-related ovarian failure (9, 12). Considering the inter-species differences in developmental trajectory, the critical third trimester of human neurodevelopment and cardiac growth may be modelled in neonatal mice. Utilizing a non-interventional model, we have previously shown neonatal GR programs hypertension in male but not female mice (13).

In the context of our neonatal GR model, we sought to define the estrogen-dependent hemodynamic adaptations that occur in young adult female mice that partially protect them from programmed hypertension. We hypothesized that neonatal GR increases sympathetic activation, but ovarian function or estrogen replacement therapy facilitates physiologic compensation through enhanced baroreceptor reflex control to suppress the development of adult hypertension.

METHODS

Ethical approval

All procedures were approved by the University of Iowa Animal Care and Use Committee. The investigation conforms with the *Guide for the Care and Use of Laboratory Animals: Eighth Edition*, published by the National Research Council: National Academies Press, Washington, DC.

Animal model

Adult C57Bl/6J mice (Jackson Laboratory, Bar Harbor, ME) delivered naturally and were maintained on standard rodent chow (7013; Harlan Teklad, Madison, WI) throughout

pregnancy and lactation. Neonatal growth restriction was identified by a day 21 weight either less than 80% of the mean weight of the littermate controls or less than the tenth percentile for the colony (13, 14). Beginning at three months, female mice were utilized for baseline radiotelemetry or echocardiography. To assess the potential protective effect of ovarian function, bilateral ovariectomy (OVX) was completed at 4 months (control 17.7+/-2.2 wk; GR 17.6+/-2.3 wk). Post-ovariectomy data were collected an additional 4 to 6 weeks after the ovariectomies were performed (15). Analgesia during OVX included flunixin meglumine (2.5 mg/kg, subcutaneously once or twice daily for 48h with the first dose given at the time of isoflurane induction) and 0.5% bupivacaine applied to the wound margin. To test the protective effect of estrogen replacement on GR mice, 6 GR and 4 control mice underwent OVX followed 4 to 6 weeks later by post-OVX recordings, then repeat recordings 3 weeks after beginning daily oral estrogen at a dose shown to provide physiologic replacement (1.12 µg 17β-estradiol in 60 mg hazelnut cream) (16).

Radiotelemetry

Carotid radiotelemetry catheters (PA-C10; Data Sciences International) were implanted during isoflurane-induced general anesthesia (17). Flunixin meglumine (2.5 mg/kg) was administered subcutaneously at the time of anesthetic induction and 0.5% bupivacaine was applied to the wound margin. After a 7d recovery period, arterial pressures, heart rate and relative locomotor activity were recorded for 10 sec every 5 min for 60h (encompassing 3 dark cycles and 2 light cycles). Immediately thereafter, arterial waveforms were recorded at 2000 Hz to calculate spontaneous baroreflex activity by the sequence technique and spectral analysis by fast Fourier transformation. This sampling occurred at the end of the light cycle, during a 10 minute phase of inactivity. Baroreflex events were defined as reciprocal changes in blood pressure and heart rate occurring over the course of 4 cardiac cycles, and murine heart rate-specific values were utilized to calculate the absolute power of the low frequency (0.1–1.5 Hz) and high frequency (1.5–4.0 Hz) spectral components, both of which are influenced predominately by parasympathetic alteration (17, 18). After these observational recordings, mice received a single intraperitoneal injection of the α1-adrenergic receptor antagonist prazosin (1 mg/kg), followed in 24h by the nicotinic receptor antagonist chlorisondamine (2.5 mg/kg). Hemodynamic responses were assessed 30–60 minutes after the injections, and the transmitters were then magnetically deactivated and OVX was performed. After 4–6 weeks of recovery, the transmitters were reactivated and the telemetry protocol was repeated in its entirety. For the final cohort of mice, post-OVX recordings were followed by daily estrogen therapy and a final set of recordings 3 weeks thereafter. No mouse underwent all 3 sets of recordings. At the end of the protocol, the mice were euthanized by bilateral thoracotomy while under isoflurane anesthesia.

Echocardiography

Echocardiograms of the left ventricle were obtained using the VisualSonics Vevo 2100 High Resolution Imaging System (VisualSonics Inc., Toronto, ON, Canada) by an investigator blinded to group assignment. Isoflurane was titrated to maintain heart rate between 450 and 600 beats per minute. M-mode recordings were obtained of the left ventricles in the parasternal short axis view at the level of the left ventricular papillary muscles. Measurements were made of the left ventricular internal dimension in diastole and systole

(LVIDd, LVIDs), and left ventricular posterior wall thickness in diastole and systole (LVPWd, LVPWs). These measurements were then used to calculate the left ventricular ejection fraction (EF) and fraction shortening (FS), and left ventricular volumes, as previously described (19).

Estradiol levels

Eight adult mice underwent bilateral ovariectomy. Fourteen days later, plasma was collected for estradiol determination. The oral estradiol replacement regimen was then initiated, and both 3 and 5 weeks into estrogen replacement, 4 hours after the daily dose of estradiol was administered, plasma was again collected. To further define the upper range of physiologic estradiol levels, plasma was collected from 3 dams in the third trimester of pregnancy (2 were at E19 and delivered within 24 hours and the third was at E14 and delivered 5 days later). All samples were collected at the end of the light cycle (to match the timing of the heart rate variability assessments), and all samples were immediately frozen at -80°C until thawed a single time for determination of estradiol concentration by ELISA (Calbiotech, ES180S-100, standard range: 3–300 pg/ml).

Data analysis

All values are presented as mean $\pm$ SE. GR versus control body weights were analyzed by Student's t-test, and GR versus control litter size was analyzed by the Mann-Whitney Rank Sum Test. Dark cycle and light cycle tabulated data were compared by 3-way ANOVA factoring for lighting cycle, GR and OVX status. Hourly blood pressure data were compared within the three cohorts (baseline, OVX and OVX-estrogen) by 2-way repeated measures ANOVA factoring for time of day and GR. All other comparisons were by 2-way ANOVA, factoring for GR and OVX/estrogen status. Post hoc analysis (Holm-Sidak method) was performed if statistically significant differences were detected ($P < 0.05$). All analyses were performed using SigmaStat 3.0 (SPSS Inc., Chicago, IL).

RESULTS

Animal Model

The initial 76 litters spontaneously ranged in size from 2 to 12 pups, and all 486 pups (including 255 females) were fostered by their birth dam. There was an inverse correlation between litter size and weanling weight (Figure 1A, $R = -0.36$, $P < 0.001$). To minimize the number of unused pups, we applied these data to our breeding strategy and increased the likelihood of identifying a balanced ratio of control versus GR mice by cross-fostering < 1 day-old mice into litters of 6 versus 11 or 12 pups. For the 324 female mice subsequently fostered by this complementary approach, weanling weight again inversely correlated with litter size (Figure 1B, $R = -0.66$, $P < 0.001$). Overall, GR, defined by a weanling weight less than the historically determined 10th percentile threshold of 6.8 g (20), developed in 11% of mice from litters containing up to 10 pups and 32% of mice from litters containing 11 or 12 pups. Ultimately, the control group consisted of 30 mice from 19 litters and the GR group consisted of 29 mice from 17 litters. While GR mice tended to come from larger litters than controls (median litter size: 11 versus 6), this did not reach statistical significance ($P = 0.054$), and 9 litters provided both GR and control mice. Birth weights of the mice that went on to

develop neonatal GR did not significantly differ from control values (control: 1.71 ± 0.06 g, GR: 1.85 ± 0.13 g). By definition, neonatal GR mice weighed less than controls at weaning (6.3 ± 0.4 g versus 9.0 ± 0.2 g, $P < 0.001$), but partially caught-up as adults (23.7 ± 0.9 g versus 25.7 ± 0.9 g, $P = 0.11$).

Radiotelemetry

For both GR and control mice, time of day (dark cycle versus light cycle) significantly influenced arterial pressures, heart rate and locomotor activity (all $P < 0.01$) (Table 1). Independent of GR or estrogen therapy, OVX mice had lower heart rates than ovarian sufficient mice (571 ± 4 versus 601 ± 5 beats per minute, $P < 0.001$). Baseline blood pressure was not significantly altered by GR, but following OVX, increased systolic blood pressures were seen in GR versus control mice (Table 1; GR-OVX 124 ± 1 , control-OVX 121 ± 1 mmHg, $P < 0.05$). To better define the blood pressure regulatory pattern of GR and control mice, hourly data were analyzed. This ambulatory blood pressure assessment confirmed normal arterial pressures in pre-OVX GR mice (Figure 2A). In contrast, GR-OVX mice had significantly higher systolic pressures immediately prior to and following the removal of ambient lighting (Figure 2B: light cycle to dark cycle transition occurs at 1800). As shown in Figure 2C, this increase in GR-OVX versus control-OVX arterial pressure at the time of awakening correlated with an increase in GR-OVX versus GR-baseline blood pressure suggesting OVX unmasked a predisposition of GR mice towards “rising surge hypertension”. Applying the conventional equation for surge hypertension (rising blood pressure minus blood pressure nadir at rest), GR-OVX mice had significantly higher surges (GR-OVX: 14 ± 4 ; control-OVX: 7 ± 2 mmHg, $P < 0.05$) and were more likely than control-OVX mice to have a rising surge in excess of 15 mmHg (46% versus 0%, $P < 0.05$ by Fisher exact test). Estrogen replacement effectively normalized the arterial blood pressure of GR mice (Table 1 and Figure 2D) and reduced the rising blood pressure surge of GR-OVX mice to $77 \pm 7\%$ of pre-estrogen replacement values ($P = 0.07$). Taken together, the hemodynamic data suggest the OVX-induced hypertension seen in GR mice may be influenced by estrogen-modulated alterations in arousal-evoked sympathetic activation.

To further assess the potential for autonomic dysregulation, hemodynamics were recorded during pharmacologic challenge. Intraperitoneal injection of prazosin lowered arterial pressures and eliminated the increase in blood pressure seen in GR-OVX mice (Figure 3). Despite the reduction in arterial pressure, prazosin also significantly lowered the heart rate of GR-OVX mice (Figure 3), again suggesting an increase in cardiac sympathetic tone. To further assess autonomic tone, the ganglionic blocker chlorisondamine was administered. Unlike prazosin, chlorisondamine minimally attenuated the systolic blood pressure elevation seen in GR-OVX versus control-OVX mice (92 ± 5 versus 82 ± 5 mmHg; $P = 0.08$; Figure 4). Furthermore, independent of OVX or estrogen therapy, post-chlorisondamine heart rates were consistently higher in GR versus control mice (481 ± 14 versus 434 ± 18 bpm; $P < 0.05$; Figure 4). Taken together the pharmacologic data suggest GR-OVX mice have heightened cardiac sympathetic tone independent of post-ganglionic neurotransmission.

To more specifically analyze resting cardiac autonomic tone, we next interrogated spontaneous baroreceptor reflex activity while mice rested in the midst of the diurnal light

cycle. Consistent with the OVX-induced reduction in baseline heart rate (Table 1), both GR and control mice had fewer baroreceptor events following OVX, with or without estrogen therapy (Figure 5A). Independent of OVX or estrogen, GR mice had decreased baroreceptor event frequency (GR overall: 5.7 ± 0.9 ; Control overall: 10.3 ± 1.1 events per 1000 beats; Figure 5A, $P < 0.01$). For individual events, baroreceptor reflex sensitivity was increased in GR mice at baseline, but this was lost following OVX, and then significantly restored by estradiol therapy (Figure 5B). Subsequent spectral analysis revealed a significant increase in baseline low frequency and high frequency spectral power in GR versus control mice (Figure 6). The increase in GR spectral power was lost following OVX regardless of estrogen therapy, providing converging evidence that the hypertension seen in GR-OVX mice is partially mediated by a loss of compensatory baroreceptor or vagal tone. To screen for the possibility of GR-induced cardiac structural or functional impairment as a contributing factor in the observed cardiovascular dysregulation, echocardiography was performed.

Echocardiography

Independent of ovarian or estrogen status, GR mice had significantly decreased left ventricular volumes in diastole (GR overall: $59 \pm 2 \mu\text{l}$; Control overall: $67 \pm 2 \mu\text{l}$; $P < 0.05$) (Table 2). As estimated by left ventricular fractional shortening, intrinsic cardiac function was not altered by either GR or OVX. Related to the decrease in diastolic volumes with preserved fractional shortening, GR mice had independent reductions in left ventricular stroke volumes (GR overall: $34 \pm 1 \mu\text{l}$; Control overall: $38 \pm 2 \mu\text{l}$; $P < 0.05$). When indexed to current body weight, there were no longer significant differences in left ventricular volumes, but adult left ventricular posterior wall thickness was significantly increased by neonatal GR, regardless of ovarian or estrogen status (GR overall: 36 ± 1 ; Control overall: 31 ± 2 mm/kg; $P < 0.05$).

Estradiol levels

To determine whether the oral estradiol treatment regimen matched the physiologic levels previously reported (16), plasma estradiol concentrations were determined (Figure 7A). Prior to treatment, OVX mice had a mean plasma estradiol level of 5.6 ± 0.8 pg/ml with a range of 2.8 to 8.2 pg/ml. The two levels below the lowest 3.0 pg/ml standard (2.8 and 2.9 pg/ml) were excluded from further analysis. During oral estradiol therapy, the same OVX mice had stable plasma estradiol levels of 38 ± 19 pg/ml after 3 weeks of treatment and 40 ± 15 pg/ml after 5 weeks of treatment, with a final range of 6.5 to 102 pg/ml ($P = 0.07$ versus OVX). In every case, plasma estradiol levels were increased by oral estradiol administration (Figure 7B). In comparison, third trimester maternal plasma estradiol levels were 220 ± 8.9 pg/ml with a range of 205–236 pg/ml, values that were significantly higher than those seen in OVX mice, even after estradiol therapy (Figure 7C).

DISCUSSION

Research into the developmental origins of cardiovascular disease has identified a strong sexual dimorphism with heightened susceptibility towards hypertension seen in males. Utilizing our translational neonatal growth restriction model, we identified key phenotypic

changes in GR-OVX mice that are analogous to those reported in low birth weight or premature infants (2, 5–8), including 1) hypertension, 2) autonomic dysregulation, and 3) abnormalities in cardiac morphology. We further demonstrated the therapeutic utility of daily oral estrogen replacement therapy to restore the cardiac vagal tone of GR-OVX mice, and dampen the emergence of morning surge hypertension in a target population at increased risk of cardiovascular disease.

The light-dark transition surge hypertension we uncovered in GR-OVX mice is a novel finding. Recent studies have demonstrated postmenopausal status strongly predicts nondipping nocturnal and morning surge hypertension (21, 22). Of greatest concern, surge hypertension is strongly associated with cardiovascular mortality (22–24), perhaps through an association with increased sympathetic tone and reduced baroreceptor sensitivity (25–27), and women are exquisitely sensitive to nondipping-associated end organ damage (23).

The data we obtained during ambulatory blood pressure monitoring and pharmacologic autonomic blockade highlight the complexity of cardiovascular regulation and the ways that ovarian status influences the predisposition of GR mice to cardiac dysregulation. At baseline, ovarian sufficient GR mice appeared to have a well-balanced increase in cardiac parasympathetic and sympathetic tone. Following OVX, the significant reduction in cardiac vagal tone, as detected by baroreceptor reflex and spectral analysis, appeared to unmask an underlying increase in sympathetic activation, and this was most dramatically manifest as morning surge hypertension. The presence of a GR-induced increase in sympathetic tone was further supported by the results of autonomic blockade. During prazosin administration, systemic vascular resistance and blood pressure consistently decrease with variable effects on heart rate (28). Typically, prazosin-induced hypotension leads to baroreceptor reflex activation and beta-adrenergic receptor mediated tachycardia, but that reflex tachycardia is partially opposed by the bradycardia that occurs as a direct result of prazosin-induced cardiac alpha-adrenergic receptor blockade (28). For ovarian sufficient GR mice, the heightened baroreceptor reflex sensitivity may have increased reflex tachycardia, but that exaggerated response may have been attenuated to control levels by an increase in prazosin-induced bradycardia. For GR mice, OVX reduced baroreceptor reflex sensitivity to control levels, but might not have attenuated persistent hypersensitivity to direct alpha-adrenergic receptor antagonist-mediated bradycardia, potentially contributing to the observed reduction in post-prazosin tachycardia seen in GR-OVX mice. This indirect evidence of heightened cardiac sympathetic tone is consistent with the increase in post-ganglionic blockade heart rate and the decrease in baroreceptor event frequency demonstrated in GR mice, independent of ovarian or estrogen status. Overall, the relative cardioprotection of GR female versus male mice appears to emanate from an ovarian-dependent enhancement in cardiac vagal tone as a critical buffer for the heightened sympathetic responses that may otherwise be observed (29). This interpretation of our data is consistent with clinical data showing ovariectomy interferes with vagally-mediated heart rate regulation in young women, in a manner this is normalized by estrogen replacement (30, 31).

Among the potential proximal causes of autonomic imbalance, there is experimental support for GR-associated over-activation of the renin-angiotensin system as well as dysregulated central leptin signaling (9, 12). To this list, we now add an association between reduced left

heart volumes and downstream sympathetic activation. We have identified a strikingly similar small left heart syndrome in adult mice exposed to the SSRI sertraline as neonates (19). In these mice, a restriction in neonatal cardiac development led to programmed increases in adult heart rate, metabolic rate and urinary catecholamine excretion. Our investigations have consistently focused on the neonatal window of susceptibility because that is the critical final phase of murine cardiomyocyte proliferation, a developmental stage analogous to the third trimester of human gestation (32, 33). Of concern, former premature or low birth weight infants have now been shown to have a long-term reduction in left ventricular volumes (6, 34).

We previously reported maternal carbenoxolone administration leads to neonatal growth restriction, stress hypertension, heightened superoxide production and impaired baroreceptor sensitivity in male mice, likely a consequence of increased transplacental glucocorticoid exposure (17). While the physiologic effects of ovariectomy extend beyond estrogen deficiency, estradiol is known to attenuate oxidative stress, possibly by reducing angiotensin receptor signaling (35, 36). Notably, estrogen replacement, renin-angiotensin system blockade and renal denervation are each capable of normalizing the blood pressure of ovariectomized rats (9, 10). Clinical studies have shown combined progestin plus estrogen replacement exerts greater effects than placebo or estrogen alone in reducing the morning blood pressure surge seen in postmenopausal women despite unfavorable effects on intraoffice blood pressure readings (37–39). Further investigations are needed to determine the relative importance of perinatal glucocorticoid exposure, oxidative stress and enhanced adrenergic signaling in the development of GR-associated cardiovascular dysregulation and left ventricular hypertrophy. Clinical investigations have already identified morning surge hypertension as an independent predictor of left ventricular hypertrophy in elderly populations (40, 41).

Therapeutically, the estradiol concentrations we measured are consistent with prior studies in mice (16). The observed variability in these investigations has likewise been seen during clinical therapy in both young women with primary ovarian failure and older women with surgically-induced or age-related menopause (42, 43). The pharmacokinetic variability emphasizes the importance of clinical dosing to achieve efficacy, followed by concentration monitoring to minimize the risk of adverse effects. For our investigations, after we documented cardiovascular improvement during estradiol treatment, we obtained levels to confirm treatment was not eliciting concentrations above the physiologic range. While our studies were designed to be translational, extensive monitoring for adverse effects was beyond the scope of these investigations. Meta-analysis in heterogeneous populations has shown cardioprotection when hormone therapy is initiated within 10 years of menopause, but concerns remain regarding thromboembolic risk (44). Assessment of the relative risk versus benefit of estrogen therapy for high-risk subpopulations within existing clinical studies would inform the therapeutic potential of individualized hormone replacement, perhaps targeted towards young adults with a personal history of neonatal growth restriction and subsequent early-onset menopause (45).

In conclusion, neonatal GR leads to reduced adult left ventricular volumes with relative cardiac hypertrophy and markers of increased sympathetic tone. In the presence of intact

ovarian function, arterial pressures are not elevated. Following ovariectomy, there is vagal withdrawal, the bradycardic response to hypertension is attenuated, and a surge hypertension phenotype is observed. The results provide insight into the role of estrogen in programmed hypertension and help to identify a patient population that may benefit from preservation of ovarian function. Further investigations into the links between the early environmental perturbations and later physiologic alterations are needed to identify interventions to prevent or better palliate an important subset of cardiovascular disease.

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Abbreviations

GR	growth restriction
OVX	ovariectomy

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Summary statement

Our translational investigations identify morning hypertension in ovariectomized young adult mice with a history of early life growth restriction. We subsequently correlate that novel finding with converging evidence of autonomic dysfunction and ultimately demonstrate the protective effects of estrogen replacement therapy.

Clinical Perspectives

- Sexual dimorphism has been observed in the developmental origins of cardiovascular disease, with young adult women relatively protected from perinatal growth restriction-induced hypertension.
- Utilizing a translational model, we identified heightened baroreceptor sensitivity and heart rate variability in young adult mice with a history of neonatal growth restriction. Following ovariectomy, those markers of cardiac vagal tone decreased, and growth restricted mice developed morning surge hypertension in the absence of oral estrogen replacement.
- Our findings provide insight into the cardioprotective mechanisms empowered in young women and identify a patient population, those with ovarian insufficiency and growth restriction-related hypertension, which may uniquely benefit from estrogen replacement therapy.

Figure 1. Impact of Litter Size on Neonatal Growth

Female C57BL/6 mice were alternatively kept with their birth dam (A, N=255) or cross-fostered into litters of 6 versus 11 or 12 mice (B, N=324). In both cases, weanling weight was inversely proportional to litter size ($R = -0.36$ and -0.66 , respectively; both $P < 0.001$).

Neonatal growth restriction, defined by a weanling weight below 6.8g, was more likely to occur when mice were fostered in litters of 11 or 12 pups, but it also occurred in 11% of the pups fostered in smaller litters.

Figure 2. Ambulatory Blood Pressures

Systolic blood pressure was measured by radiotelemetry in adult female control mice (solid symbols) and neonatal growth restricted mice (GR, open symbols) at baseline (A, triangles), after bilateral ovariectomy (B and C, circles), and 3 weeks after post-OVX estradiol therapy (D, squares). Compared to control-OVX mice (B) and GR-baseline mice (C), GR-OVX mice had increased arterial pressures in the absence of estrogen replacement. * $P < 0.05$ versus control-OVX and † $P < 0.05$ versus GR-baseline

Figure 3. Hemodynamic Effects of Sympathetic Blockade

Control (gray bars) and GR mice (white bars) were injected with the α_1 -adrenergic receptor antagonist prazosin (1 mg/kg ip). Systolic blood pressure (left column) and heart rate (right column) were recorded over the subsequent 60 minutes. * $P < 0.05$ versus corresponding control and # $P < 0.05$ versus GR-baseline

Figure 4. Hemodynamic Effects of Pharmacologic Denervation

Control (gray bars) and GR mice (white bars) were injected with the nicotinic receptor antagonist chlorisondamine (2.5 mg/kg ip) to induce ganglionic blockade (GB). Systolic blood pressure (left column) and heart rate (right column) were recorded over the subsequent 60 minutes. * $P < 0.05$ versus corresponding control

Figure 5. Effect of Neonatal Growth Restriction on Baroreceptor Reflex Activity and Sensitivity During the light cycle that followed the radiotelemetry recordings, spontaneous baroreceptor reflex events (A) and sensitivity (B) were recorded in adult female control (solid bars) and GR mice (open bars) both before and after bilateral ovariectomy (OVX) +/- estrogen therapy (E2). * $P < 0.05$ versus corresponding control and # $P < 0.05$ versus baseline

Figure 6. Impact of Ovariectomy of Heart Rate Variability

Spectral power was calculated from the same high fidelity radiotelemetry recordings utilized for baroreceptor reflex analysis. Heart rate variability was analyzed at both low frequency (LF HRV, A) and high frequency (HF HRV, B) before and after bilateral ovariectomy (OVX) +/- estrogen therapy (E2). * $P < 0.05$ versus control, # $P < 0.05$ versus GR-baseline and † $P < 0.05$ versus GR-OVX

Figure 7. Plasma Estradiol Levels

Estradiol levels were determined by ELISA with linear values obtained utilizing standards ranging from 3 to 300 pg/ml (A, $R^2=0.97$). Plasma was obtained two weeks after bilateral ovariectomy, 3 weeks after starting estradiol therapy, and again 5 weeks after starting estradiol therapy (B and C; N=6). Comparative plasma was obtained from third trimester pregnant dams that were not receiving exogenous estrogen (C, N=3). * $P<0.05$ versus OVX and OVX-E2.

TABLE 1

Arterial blood pressure (BP), heart rate, and relative locomotor activity were recorded every 5 minutes for 60 hours, allowing for comparison of dark cycle and light cycle values. The effects of neonatal growth restriction (GR), bilateral ovariectomy (OVX) and OVX plus oral estradiol administration (OVX-E2) were analyzed by 3-way ANOVA factoring for time, growth and ovarian status.

	Baseline Control	GR	OVX Control	GR	OVX-E2 Control	GR
Dark Cycle						
Systolic BP (mmHg)	128+/-1	127+/-1	125+/-3	128+/-2 [*]	130+/-2	126+/-2
Mean BP (mmHg)	113+/-1	114+/-2	110+/-2	112+/-2	113+/-3	110+/-2
Diastolic BP (mmHg)	97+/-1	99+/-2	95+/-2	97+/-3	96+/-4	93+/-2
Heart Rate (bpm)	618+/-11	613+/-7	591+/-7 [#]	588+/-7 [#]	569+/-18 [#]	570+/-24 [#]
Activity (AU)	14+/-3	12+/-2	10+/-2	9+/-1	13+/-3	13+/-3
Light Cycle						
Systolic BP (mmHg)	117+/-1	116+/-2	114+/-2	119+/-2 [*]	118+/-1	116+/-2
Mean BP (mmHg)	103+/-1	103+/-2	100+/-1	104+/-2	102+/-2	100+/-1
Diastolic BP (mmHg)	87+/-1	89+/-2	86+/-1	90+/-3	87+/-3	84+/-2
Heart Rate (bpm)	588+/-10	586+/-9	560+/-10 [#]	570+/-9 [#]	552+/-22 [#]	545+/-8 [#]
Activity (AU)	5+/-1	5+/-1	4+/-1	4+/-1	4+/-1	5+/-1

^{*} $P < 0.05$ versus control;

[#] $P < 0.05$ versus baseline;

$n = 11$ control baseline, 7 GR baseline, 9 control OVX, 11 GR OVX, 3 control OVX-E2, 6 GR OVX-E2; AU: arbitrary units

TABLE 2

Left ventricular echocardiography was performed on control and growth restricted mice (GR), including those that underwent bilateral ovariectomy (OVX) alone or OVX with oral estrogen replacement (OVX-E2).

	Baseline Control	GR	OVX Control	GR	OVX-E2 Control	GR
LVPW, d	(mm)	0.80+/-0.04	0.77+/-0.03	0.82+/-0.06	0.80+/-0.06	0.67+/-0.05
	(mm/kg)	30+/-1	33+/-2 *	34+/-3	38+/-3 *	30+/-3
LV Volume, d	(μl)	66+/-2	61+/-3 *	64+/-4	60+/-4 *	70+/-3
	(μl/g)	2.5+/-0.1	2.6+/-0.1	2.7+/-0.2	2.8+/-0.2	3.1+/-0.2
Stroke Volume	(μl)	39+/-2	34+/-2 *	38+/-3	35+/-2 *	37+/-2
	(μl/g)	1.5+/-0.1	1.4+/-0.1	1.6+/-0.1	1.6+/-0.1	1.7+/-0.1
FS	(%)	32+/-2	29+/-2	33+/-3	32+/-3	27+/-1

LVPW: left ventricular posterior wall thickness; FS: fractional shortening

* $P < 0.05$ for GR versus control by 2-way ANOVA;

$n = 15$ control baseline, 16 GR baseline, 11 control OVX, 13 GR OVX, 4 control OVX-E2, 6 GR OVX-E2; Left ventricular volumes were excluded for 3 mice as statistical outliers; those values in μ l were 21 (control mouse at baseline), 107 (GR mouse at baseline), and 19 (GR mouse after OVX-E2).