Cardiovascular Function in Mice During Normal Pregnancy and in the Absence of Endothelial NO Synthase

Shathiyah Kulandavelu, Dawei Qu, S. Lee Adamson

Abstract—In humans, the increased cardiovascular demands of pregnancy are met by increases in cardiac output (CO), stroke volume (SV), plasma volume (PV), and cardiac and aortic inner dimensions and a concurrent decrease in arterial pressure that indicates a fall in total peripheral vascular resistance. The mechanisms responsible for these changes are incompletely understood, but NO synthase (NOS) is believed to play a central role. We assessed whether C57Bl/6J (B6) mice show similar changes and whether these changes are altered in mice lacking the gene for endothelial NOS (eNOS). The CO of B6 mice increased 28% by day 9.5 of gestation because of a 25% increase in SV, and increased 48% by day 17.5 because of a 41% increase in SV. The increase in SV at day 17.5 was associated with a 27% increase in PV, a 15% decrease in arterial pressure, and 10% to 15% increases in aortic and left-ventricular inner dimensions. In the absence of eNOS, CO increased 22% by day 9.5 because of increases in SV (14%) and heart rate (9%), but increased no further by day 17.5. SV near term was lower than B6 mice despite similar 26% increases in PV and 14% decreases in arterial pressure in association with blunted left-ventricular chamber enlargement. All reported changes are $P<0.05$. We conclude that cardiovascular changes during pregnancy are similar in B6 mice and humans. eNOS plays a critical role in increasing stroke volume in late gestation by promoting cardiac remodeling. (***Hypertension*****. 2006;47:1175-1182.)**

Key Words: nitric oxide synthase ■ pregnancy ■ cardiac output ■ arterial pressure ■ blood flow velocity ■ echocardiography ■ remodeling

In the first half of pregnancy, the maternal cardiovascular system preadapts in anticipation of the physiological demands of pregnancy and the growing perfusion and exchange requirements of the conceptus and changes further in the last half of gestation when the most rapid growth of the conceptus occurs. Failure to make or to sustain these changes may result in impaired fetal growth and/or preeclampsia, the 2 most common and serious complications of human pregnancy.[1,2] Although the mechanisms are not fully understood, there is considerable evidence that NO plays an important role in mediating maternal cardiovascular changes during pregnancy in humans, rats, and other species.[3–6] During pregnancy in humans, there is a 30% decrease in the circulating levels of asymmetrical dimethylarginine,[7] an endogenous inhibitor of NO synthase (NOS) activity. Furthermore, a nonselective NOS inhibitor caused a greater decrease in blood flow in the forearm circulation of pregnant versus nonpregnant women,[3] which suggests that an increase in bioactive NO contributes to the decrease in peripheral vascular resistance during pregnancy in humans. NO also appears to be important in rats during pregnancy because plasma and urinary levels of nitrites and nitrates (metabolites of NO) and cGMP (second messenger of NO) are increased in pregnant rats,[5,8] although whether similar changes occur in human pregnancy is less certain.[5,9] Furthermore, treatment of rats in late pregnancy with nonselective NOS inhibitors blunts or indeed reverses the normal decrease in arterial blood pressure,[4,6] abolishes the normal increase in plasma volume,[6] and causes fetal intrauterine growth restriction and preeclamptic-like changes in the mother.[4]

Whereas there is considerable experimental evidence supporting a role for NO in mediating the normal cardiovascular changes during pregnancy, the NOS isoform responsible is less well established. Most studies investigating a role for NO have used L-arginine analogs that are nonselective competitive inhibitors of inducible NOS (iNOS), neuronal NOS (nNOS), and endothelial NOS (eNOS). Of the 3 isoforms, eNOS is likely the most important isoform in that increases in eNOS protein and mRNA levels have been shown in the myocardium,[10] aorta, and the mesenteric artery, whereas iNOS and nNOS levels remain unchanged.[11,12] In addition, eNOS is an important mediator of cardiovascular remodeling. For example, activation of eNOS in endothelial cells exposed to high shear stress promotes arterial vasodilation and eventual structural enlargement.[13–15]

In the current study, we hypothesized that eNOS plays a central role in mediating cardiovascular adaptations to preg-

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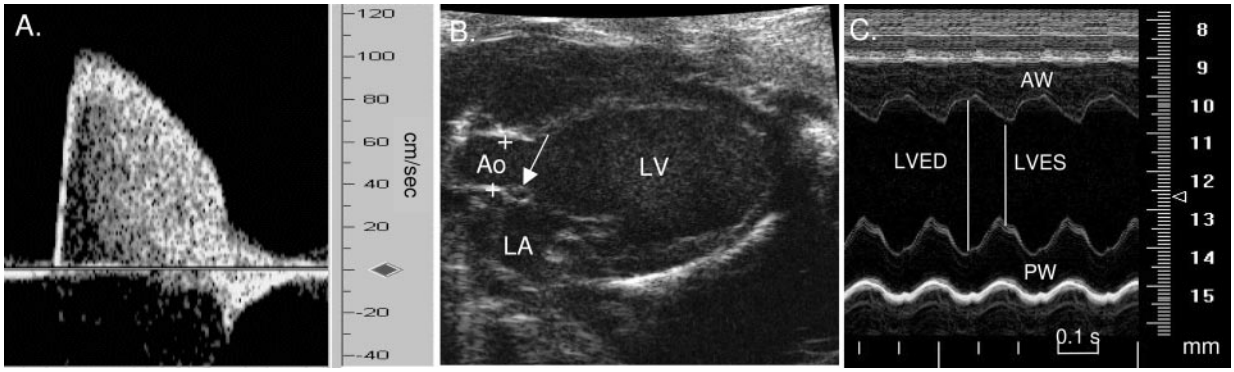

Figure 1. Ultrasound evaluation of cardiac structure and function. A, Doppler blood velocity waveform recorded from the ascending thoracic aorta. B, Long-axis view of the left ventricular outflow tract showing aortic calipers and open valve (arrow). C, M-mode image of the left ventricle. Ao indicates ascending aorta; AW, anterior wall; LA, left atrium; LV, left ventricle; LVED, left ventricular end-diastolic dimension; LVES, left ventricular end-systolic dimension; PW, posterior wall.

nancy. Therefore, we determined the effect of pregnancy on cardiac structure and function using ultrasound in lightly anesthetized mice, and on arterial blood pressure, heart rate, and plasma volume in awake mice in both the eNOS knockout and in the background strain for the knockout mice C57Bl/6J (B6).

Methods

All procedures were approved by the Animal Care Committee of Mount Sinai Hospital and were conducted in accordance with the guidelines of the Canadian Council of Animal Care. An extended Methods section can be found in an online supplement available at http://www.hypertensionaha.org.

Breeding and Genotyping

Virgin female wild-type B6 mice and eNOS knockout ($^{-/-}$) mice were either purchased when 4- to 6-weeks old from Jackson Laboratories (Maine) or raised in-house from the same stock. Between 8 to 12 weeks of age, eNOS knockout females were bred with eNOS knockout males or with B6 males. For the control strain, we used B6 females mated with B6 males. The presence of a sperm plug was defined as day 0.5 of pregnancy. Age-appropriate nonpregnant mice of both strains were studied at equivalent intervals to serve as time controls (n=7 to 8). Experimental time points included before breeding, day 9.5 (mid-gestation, start of umbilico-placental perfusion), day 17.5 (late gestation, 2 days before normal term delivery), and 3 weeks after delivery (at weaning). Mice were genotyped by polymerase chain reaction (PCR) using genomic DNA extracted from the tail.

Hemodynamics and Hematology

eNOS knockout females (n=12) were bred with eNOS knockout males or with B6 males. Male strain caused no significant differences, so the data were pooled. B6 females (n=8) were bred with B6 males.

Mice were lightly anesthetized with 1% to 2% isoflurane in oxygen. This anesthetic minimally affects cardiovascular function in mice.[16] A 20-MHz pulsed Doppler system with a hand-held probe was used to obtain transcutaneous blood velocity waveforms from the ascending thoracic aorta (Figure 1A) and mitral orifice as previously described.[17,18] We then measured ascending aortic diameter during systole from an image of the long-axis of the left ventricular (LV) outflow tract obtained using an ultrasound biomicroscope (UBM; Model VS40; 19-MHz transducer, VisualSonics, Toronto, Canada) (Figure 1B). The mean value of 10 aortic diameter measurements obtained during systole was used to calculate vessel cross-sectional area [π(diameter/2)^2].

The aortic blood velocity waveform was analyzed to obtain heart rate, stroke distance (velocity envelope integrated over ejection time), and mean velocity (velocity envelope averaged over the cardiac cycle). Mean velocity and stroke distance were multiplied by the luminal cross-sectional area to obtain cardiac output and stroke volume, respectively. The mitral flow velocity was analyzed to obtain peak and time durations for flow velocity in early diastole (E wave) and atrial contraction (A wave), and peak E/A ratio and diastolic filling time were calculated.

Arterial blood pressure and heart rate were measured between 9:00 AM and 11:30 AM in awake mice using an automated tail cuff system (BP-2000, Visitech Systems, Apex, NC). Our laboratory previously showed that tail-cuff measurements accurately reflect mean carotid arterial blood pressure measured using a chronic arterial catheter in mice.[17] Pre-pregnancy values obtained on 3 consecutive days were averaged. During pregnancy, measurements were taken every 2 to 3 days and grouped into early (days 2.5, 5.5), mid (days 9.5, 11.5) and late (days 13.5, 17.5) gestation.

Blood (≈15 uL) was collected from the saphenous vein and analyzed in a hematology analyzer (AcT Diff, Beckman Coulter, Toronto, Canada).

LV Geometry

In a separate series of animals, eNOS knockout and B6 females were bred with males of the same strain. A newer model UBM (Model Vevo660, 30-MHz transducer) was used to measure LV geometry in lightly isoflurane-anesthetized, pregnant (B6, n=8; eNOS knockout, n=6) and nonpregnant time controls (B6, n=6; eNOS knockout, n=10) (Figure 1C).

Plasma Volume Determination

In a separate series of animals, eNOS knockout and B6 females were bred with males of the same strain. Plasma volume was determined in awake pregnant (day 17.5 of gestation) and nonpregnant mice (n=6 in each group), using an Evan blue dye dilution method modified from that used previously in rats.[6]

Statistical Analysis

Results are reported as means±SEM, where n is number of animals. Significance was tested using 1-way and 2-way repeated-measures ANOVA followed by Student–Newman–Keuls tests for multiple comparisons. $P<0.05$ was considered significant.

Results

Cardiovascular Changes During Pregnancy in B6 Mice Are Similar to Humans

In B6 mice, body weight increased by 26% by day 9.5 of gestation (Figure 2). Cardiac output increased by 28%, and blood pressure decreased by 15% (Figures 3 and 4). The increase in cardiac output was attributed to a significant 25%

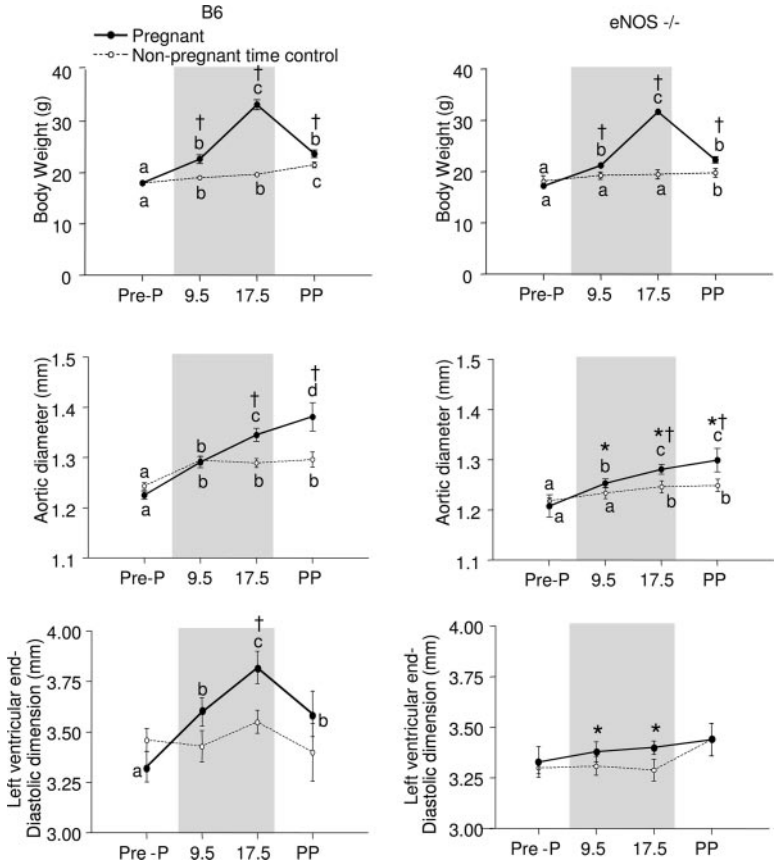

Figure 2. Body weight, aortic diameter, and LVED dimensions under light anesthesia in B6 and eNOS knockout mice. The shaded area highlights the time when the pregnant group was pregnant. Different superscript letters indicate significant changes over time within each strain ($P<0.05$). *$P<0.05$, pregnant eNOS knockout vs pregnant B6 controls. †$P<0.05$, pregnant vs nonpregnant time controls within each strain; Mean±SEM where n=7 to 12 at each point. Pre-P indicates before pregnancy; PP, postpartum.

increase in stroke volume, whereas heart rate in both awake and anesthetized mice did not change significantly (Figures 3 and 4 and Table). The increase in calculated stroke volume was attributed to significant increases in stroke distance (12%) and aortic area (aortic diameter increased 5%) (Figure 2, Table 1). Left-ventricular chamber enlargement (significant 8% increase in left-ventricular end-diastolic [LVED] dimension) (Figure 2) caused the increase in stroke volume, as fractional shortening (FS) did not change significantly (Table I, available online). These findings indicate that pronounced maternal cardiovascular changes occur early in gestation in mice, as in humans.[19,20]

By day 17.5 of gestation, maternal body weight increased by 85% (Figure 2). Cardiac output increased significantly by 48% relative to pre-pregnancy because of a significant 41% increase in stroke volume, whereas heart rate in anesthetized mice remained unchanged (Figure 3, Table). Heart rate in awake mice studied using the tail-cuff system also did not change significantly during pregnancy (Figure 4). The increase in stroke volume was associated with increases in LVED dimension by 15%, aortic diameter by 10%, and plasma volume by 27% and a decrease in hematocrit by 13% (Figures 2 and 5, all changes significant). Arterial pressure in awake mice was slightly but significantly reduced throughout pregnancy with a nadir of 15% in mid-pregnancy (Figure 4). At day 17.5, calculated LV mass was 37% higher, whereas FS remained unchanged when compared with before pregnancy (Table I). Unlike humans, platelet count was 39% higher when compared with before pregnancy ($P<0.05$, Table II, available online).

By 3-weeks postpartum, body weight (+32%), aortic diameter (+13%), stroke volume (+33%), and cardiac output (+27%) remained significantly elevated when compared with before pregnancy and to the time controls (Figures 2 and 3). The magnitude of the cardiovascular changes in pregnancy and the delayed recovery postpartum are similar to that of humans.[19,20]

eNOS Is Required For the Normal Increase In Cardiac Output During Pregnancy

Before pregnancy, eNOS knockout mice were similar to B6 mice in their body weight, cardiac output, aortic diameter, and LV geometry parameters but they had significantly elevated arterial pressures and stroke volumes and lower heart rates (Figures 2, 3, and 4; Table; and Table I).

By day 9.5 of gestation, weight gain in eNOS knockout mice was similar to B6 mice but the increases in aortic diameter and LVED dimension were significantly reduced (Figure 2). Cardiac output increased by 22% in eNOS knockout mice but, compared with B6 mice, this was achieved by a smaller increase in stroke volume (14%) and by an increase in heart rate (9%) measured under light anesthesia (Figure 3 and Table). Heart rate also significantly increased when studied in awake mice (10%) (Figure 4). The increase in calculated stroke volume in eNOS knockout mice was primarily attributed to the small increase in aortic luminal diameter (4%), whereas the smaller increase in stroke distance was not statistically significant (Figure 2 and Table). These results indicate that in early pregnancy, the remodeling

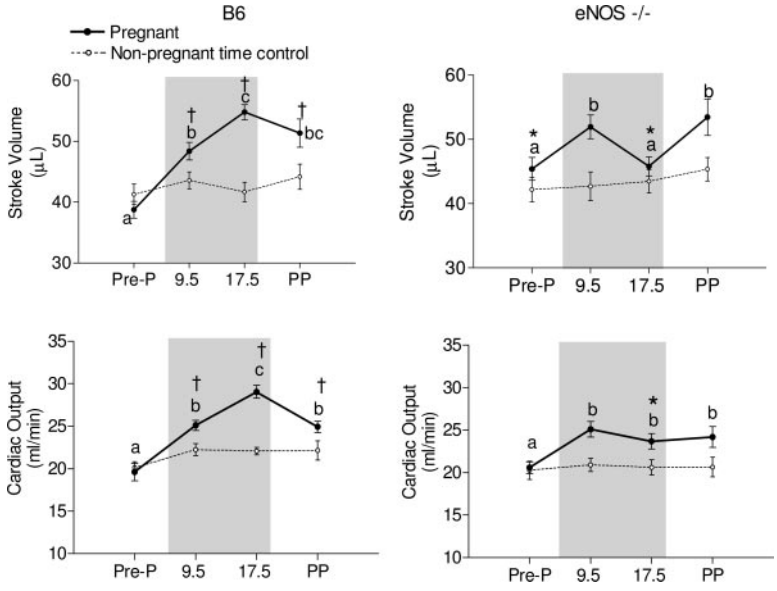

Figure 3. Stroke volume and cardiac output under light anesthesia in B6 and eNOS knockout mice. The shaded area highlights the time when the pregnant group was pregnant. Different superscript letters indicate significant changes over time within each strain ($P<0.05$). *$P<0.05$, pregnant eNOS knockout versus pregnant B6 controls. †$P<0.05$, pregnant versus nonpregnant time controls differ within each strain; Mean±SEM where n=7 to 12 at each point. Pre-P indicates before pregnancy; PP, postpartum.

of the heart is absent, the enlargement of the aorta is blunted, and, unlike controls, an increase in heart rate is an important contributor to the increase in cardiac output in eNOS knockout mice.

By day 17.5 of gestation, the gain in maternal body weight (84%) in the eNOS knockout was almost identical to that of B6 mice (Figure 2). In contrast, the increase in aortic diameter was significantly blunted, and there was still no significant enlargement of LVED dimension in the eNOS knockout mice (Figure 2). Also at late gestation, cardiac output in the eNOS knockout mice was significantly lower than B6 mice because of a significantly lower stroke volume (Figure 3). This occurred even though FS was not significantly different and the peak E/A ratio was significantly improved (because of significantly lower peak A), suggesting that the lower stroke volume was not caused by an impairment in cardiac systolic or diastolic function (Table, and online Tables I and III). The failure of cardiac output to increase in late gestation in the eNOS knockout mice may account for the significant continued decline in arterial pressure in late gestation in these mice, which contrasted with the fairly stable decrement in arterial pressure throughout pregnancy in the B6 mice (Figure 4). Nevertheless, the 26% increase in plasma volume, the 13% decrease in hematocrit, and the 37% increase in platelet count observed at day 17.5 of gestation in eNOS knockout mice did not differ significantly from the values observed in B6 mice at the same stage of gestation (Figure 5, Table II). In contrast to the substantial (37%) gain in LV mass observed in B6 mice, no significant change relative to pre-pregnancy was observed in eNOS knockout mice (Table I). These findings indicate an essential role for eNOS in maintaining an increase in cardiac output in late gestation by promoting LV chamber enlargement.

By 3-weeks postpartum, as in B6 mice, body weight (+30%), aortic diameter (+7%), stroke volume (+18%), and cardiac output (+18%) of eNOS knockout mice remained

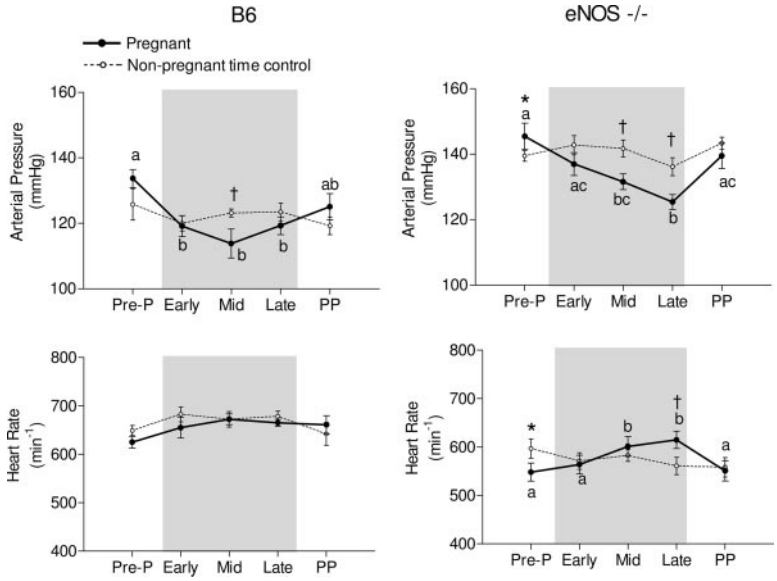

Figure 4. Arterial pressure and heart rate measured using the tail-cuff system in awake B6 and eNOS knockout mice. The shaded area highlights the time when the pregnant group was pregnant. Different superscript letters indicate significant changes over time within each strain ($P<0.05$). *$P<0.05$, pregnant eNOS knockout vs pregnant B6 controls. †$P<0.05$, pregnant vs nonpregnant time controls differ within each strain; Mean±SEM where n=7 to 10 at each point. Pre-P, indicates before pregnancy; early, days 2.5 and 5.5; mid, day 9.5 and 11.5; late, days 13.5 and 17.5; PP, postpartum.

Doppler Parameters in B6 and eNOS Knockout Mice Before, During, and After Pregnancy

Hemodynamic Parameter	Strain	Before Pregnancy	9.5 Days of Gestation	17.5 Days of Gestation	Postpartum
Stroke distance (cm)	B6	3.29±0.10[a]	3.70±0.11[b]†	3.86±0.07[b]†	3.42±0.05[a]
	eNOS$^{-/-}$	4.02±0.15[a]*	4.12±0.17[a]*	3.55±0.11[b]	4.04±0.22[a]*
Mean velocity (cm/s)	B6	27.7±1.22[a]	32.0±1.09[b]†	34.1±0.95[b]†	27.8±0.97[a]
	eNOS$^{-/-}$	30.4±1.12[a]	34.0±1.26[b]	30.6±0.93[a]*	30.4±1.32[a]
Heart rate (anesthetized) (min^{-1})	B6	506±14	524±14	530±9	515±19
	eNOS$^{-/-}$	456±14[a]*	496±18[b]	517±7[b]†	449±9[a]
Peak E/A ratio	B6	1.41±0.07	1.55±0.16	1.33±0.05	1.33±0.07
	eNOS$^{-/-}$	1.58±0.14	1.43±0.07	1.76±0.11*	1.45±0.06

Values are mean±SEM with n=7 to 12 in each group. Along each row, values with different superscript letters indicate significant differences over time within each strain ($P<0.05$).

*$P<0.05$, pregnant eNOS knockout vs pregnant B6 controls; †$P<0.05$, pregnant group vs nonpregnant time control (data not shown) within each strain.

significantly elevated relative to pre-pregnant levels (Figures 2 and 3). The strains differed, however, in that stroke volume increased from late gestation to postpartum, whereas heart rate decreased back to its pre-pregnancy level in eNOS knockout mice only (Figure 3, Table). The increase in stroke volume was sufficient to offset the decrease in heart rate so that cardiac output remained stable postpartum in eNOS knockout mice, in contrast to the postpartum decrement in B6 mice.

Discussion

Our study showed that mice model human cardiovascular changes during pregnancy, including increases in cardiac output, stroke volume, plasma volume, LV and aortic inner dimensions, and decreases in arterial pressure and hematocrit, and many of these changes are present early in pregnancy. The primary novel finding of this study was that eNOS was shown to play an important role in mediating maternal cardiovascular adaptations during pregnancy in the mouse. The normal increase in cardiac output was blunted at late gestation by knockout of the eNOS gene, which was attributed to a reduction in stroke volume that was partially offset by an increase in heart rate. Lower stroke volume in late gestation was associated with inadequate ventricular remodeling.

Effects of Pregnancy on Cardiovascular Function in eNOS Knockout Mice

Arterial Pressure

Arterial blood pressure was elevated in nonpregnant eNOS knockout mice, as in prior reports,[21,22] presumably because of reduced smooth muscle vasorelaxation mediated by a reduction in endothelium-derived NO,[22] which is not completely offset by augmented roles of other vasodilators[22] including endothelium-derived hyperpolarizing factor, prostaglandin, and nNOS. Increased vasoconstrictor stimulus may have contributed because plasma renin levels have been shown to be elevated in eNOS knockout mice,[21,22] which may lead to an increase in circulating levels of the vasoconstrictor, angiotension II. Interestingly, despite being chronically hypertensive, the LV wall was not hypertrophied in nonpregnant eNOS knockout mice as in a prior report.[23]

Arterial pressure in eNOS knockout mice decreased during pregnancy to become similar to that of pregnant B6 controls. Interestingly, arterial pressure also decreases during pregnancy in chronically hypertensive women,[24] thus eNOS knockout mice may provide a useful model for studying this phenomenon. Our study was limited in that arterial pressure and cardiac output were not measured simultaneously and under the same experimental conditions (awake or anesthetized). However, when values obtained in the same animal on

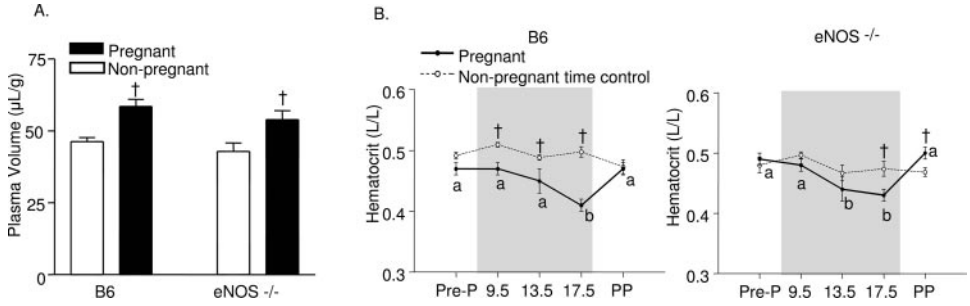

Figure 5. A, Plasma volume in nonpregnant, awake mice (n=6, open bars) and at day 17.5 of gestation (n=6, closed bars). B, Plasma hematocrit levels (n=7 to 12) in awake mice where the shaded area highlights the time when the pregnant group was pregnant. Different superscript letters indicate significant changes over time within each strain ($P<0.05$). †$P<0.05$, pregnant vs nonpregnant time controls within each strain. Pregnant eNOS knockouts did not significantly differ from pregnant B6 controls. Mean±SEM. Pre-P indicates before pregnancy; PP, postpartum.

the same gestational day were used to estimate peripheral vascular resistance, it was found that both strains exhibited similar decreases in peripheral vascular resistance in early pregnancy (B6, –29%; eNOS knockout, –26%), whereas in late gestation, the percent decrease was greater in B6 (−36%) than in eNOS knockout mice (−24%; $P<0.05$ by unpaired t test) (Table III). Thus, results suggest that eNOS-derived NO is less important in mediating maternal peripheral vasodilation in early than in late pregnancy, at which stage it appears to mediate ≈40% of the response. This is in agreement with prior work showing a role for other vasodilators such as endothelium-derived hyperpolarizing factor[25] and prostaglandins[26] in mediating peripheral vasodilation in pregnancy.

Nonspecific NOS inhibitors cause preeclamptic-like symptoms in pregnant rats, including hypertension, thrombocytopenia, and a blunted rise in plasma volume.[4,6] Our results suggest that these changes may be caused by iNOS or nNOS inhibition or the acute effects of eNOS inhibition, because they did not occur in eNOS knockout mice. Our finding that eNOS knockout mice do not exhibit a further significant rise in blood pressure during pregnancy is in agreement with earlier reports.[27,28] On the other hand, eNOS appears to be important in maintaining normal fetal growth because embryo weight at term was significantly reduced in eNOS knockout pregnancies (−17%, data not shown) as reported previously in eNOS knockout mice[29,30] and in pregnant rats treated with NOS inhibitors.[4] Thus, our results suggest that inadequate maternal cardiovascular changes may contribute to intrauterine growth restriction in eNOS knockout pregnancies.

Cardiac Output

Although blood pressure in pregnancy did not differ, the maternal hemodynamic response to pregnancy was abnormal in eNOS knockout mice. Cardiac output in eNOS knockout mice was lower than B6 controls in late pregnancy because of a significantly lower stroke volume, and this occurred despite a preload increase (ie, increased plasma volume), afterload decrease (ie, decreased arterial pressure, increased aortic diameter), and augmented diastolic function (ie, increased peak E/A ratio) relative to the nonpregnant eNOS state. Significantly lower stroke volume in late gestation may be due to the failure of the LV to enlarge. Reduced LV remodeling may be caused by reduced hemodynamic stimuli (ie, reduced cardiac output), reduced response to the hemodynamic stimuli, and/or a reduced response to a hormonal signal.

In the vasculature, shear stress exerted by blood flow on endothelial cells activates PI3K-Akt and eNOS resulting in NO release, thereby contributing to vasodilation in response to increases in blood flow.[13–15] Arterial enlargement in response to a chronic increase in arterial flow also appears to involve NO because NOS inhibition blunts arterial enlargement caused by an arteriovenous shunt in vivo.[13–15] In the heart, a chronic increase in cardiac output can be experimentally induced by creating an arteriovenous anatomosis. Cardiac output progressively increases over several weeks and is associated with structural enlargement of the LV chamber[14,31] and activation of the Akt pathway,[31] a pathway known to be important in regulating myocardial growth.[32] NOS activation appears to be important in this response because NOS inhibition blunts the increase in cardiac output and the ventricular enlargement induced by arteriovenous anastomosis.[14] Similarly, despite the initial increase in cardiac output and mean blood velocity in the aorta in early pregnancy in our study, the LV chamber failed to enlarge and the increase in aortic diameter was blunted in late pregnancy. Thus, our results suggest it is the eNOS isoform that is responsible for the blunting of the remodeling response. This is supported by the observation that uterine artery remodeling is also blunted in eNOS knockout mice.[29] It is likely that other vascular beds also failed to remodel normally during pregnancy because, even in late gestation, uterine blood flow represents only 7% to 16% of cardiac output in human and animal pregnancies,[19] so changes in this one bed would be insufficient to explain the 23% reduction in cardiac output observed in late pregnancy in eNOS knockout mice. Whether blunted remodeling was caused by or caused the failure to sustain an increase in stroke volume and hence a normal increase in cardiac output is unclear. We speculate that blunted cardiovascular remodeling in the knockout mouse blunts the increase in cardiac output, which feeds back to further blunt the remodeling process. Thus, results show that eNOS plays an important role in promoting the progressive increase in cardiac chamber dimensions and output and the enlargement of the aorta during pregnancy.

The vasodilatory hormones, estrogen and relaxin, are increased during pregnancy[19] and interact with the eNOS pathway. Estrogen increases eNOS mRNA expression and activity and increases NO bioavailability by reducing the rate of NO destruction in the endothelium.[11,33] Relaxin activates eNOS via the endothelin B receptor (ET_B) in the endothelium of the renal arteries.[34] Vasodilation initially caused by these hormones may be augmented further by flow-induced activation of the eNOS pathway in endothelial cells.[35] Thus, blunting of the normal decrease in systemic vascular resistance in late gestation in eNOS knockout mice may be attributed to either a blunting of the vasodilation mediated by estrogen and/or relaxin or a blunting of the flow-mediated amplification of the vasodilatory response. This mechanism may have contributed to the blunted rise in cardiac output observed in eNOS knockout mice in the current study.

Heart Rate

In awake, nonpregnant eNOS knockout mice, heart rate was lower than in the control strain as in previous reports,[21,22] whereas there was no significant difference in cardiac output before pregnancy in the 2 strains. The lower heart rate in eNOS knockout hearts is attributed to extrinsic factors because heart rates of isolated hearts in vitro do not differ from controls.[36] Lower heart rates may be due to a baroreflex-mediated augmentation of vagal tone caused by systemic hypertension in eNOS knockout mice. Interestingly, other mouse models with chronic hypertension have normal heart rates,[37] suggesting that eNOS may be required for baroreceptor resetting. The progressive increase in heart rate during pregnancy in both awake and anesthetized eNOS knockout mice may be a baroreceptor-mediated response to the pro-

gressive decrease in arterial pressure. In contrast, heart rate was unchanged during pregnancy and postpartum in B6 mice. If vascular eNOS expression is enhanced during pregnancy in mice as in other species,[10–12] then results suggest this increase may blunt baroreceptor sensitivity during pregnancy in normal, but not in eNOS knockout mice. Heart rate did not increase during pregnancy in B6 mice as in a prior report,[38] whereas we previously observed a significant increase in heart rate during pregnancy in an outbred strain of mice[17] suggesting there are strain-dependent differences in this response.

Limitations

Knockout mouse models provide useful tools for studying the role of specific gene products in mediating physiological responses because elimination of the product is specific and complete. However, in any physiological system, removal of one element can induce compensatory changes in others. Compensatory changes in other NOS isoforms and in other vasodilatory pathways have been described in adult eNOS knockout mice.[22] In addition, single genes may serve multiple functions during development and in the adult. In the case of eNOS knockout mice, ventricular septal defects and bicuspid aortic valves are more common in neonates with this genotype,[30,39] and pulmonary hypovascularity is a common embryonic phenotype that leads to heart failure and death of ≈85% of neonates.[30,39] The eNOS knockout mice used in the current study were the subset that escaped neonatal lethality and therefore were those that more effectively compensated for the role of eNOS in these developmental pathways. How this selection process or the presence of residual developmental effects would impact on adult cardiovascular function is unknown. Another limitation is that ultrasound measurements were determined under light isoflurane anesthesia. Isoflurane has fewer systemic hemodynamic effects in mice than other nonvolatile anesthetics.[16] Cardiac index and cardiac output in anesthetized nonpregnant control mice in the current study (0.90 mL/min per g, 20 mL/min) were slightly higher than previous reports in awake mice (0.75 mL/min per g,[16] 16 mL/min[40]), suggesting that light isoflurane anesthesia had minimal cardiodepressive effects.

Effects of Pregnancy on Cardiovascular Function in B6 Mice

This study also provides novel information on maternal cardiovascular changes during pregnancy in B6 mice, a commonly used inbred strain. Half the total increase in cardiac output during pregnancy in B6 mice occurred by 9.5 days of gestation, at a time when maternal weight gain was modest and embryos were at an early stage of organogenesis. Nevertheless, pronounced peripheral vasodilation had occurred by this stage, because, although not measured simultaneously, arterial pressure had decreased and cardiac output had increased. Therefore, as in humans, pronounced maternal cardiovascular changes occur early in gestation[19,20] at a stage when the conceptus presents minimal perfusion demands. By late gestation, calculated LV mass increased 37% and LVED dimensions by 15% similar to a prior report in B6 mice.[38] We further showed that in late gestation, cardiac output had increased 48%, plasma volume by 27%, and aortic diameter by 10%, and hematocrit had decreased by 13%. Similar changes are observed during pregnancy in human, rats, and other species.[19,20,41] Thus, results suggest that genetically altered mice will provide useful new models for expanding our limited understanding of the mechanisms responsible for cardiovascular changes during pregnancy.

Perspectives

Our results in eNOS knockout mice highlight the inadequacy of using arterial pressure alone to demonstrate the normalcy of hemodynamic changes during pregnancy in mouse models, and indeed, human pregnancy. Most women with chronic hypertension exhibit a decline in arterial pressure during pregnancy, but nevertheless the risk of perinatal death and fetal growth–restriction is twice that of women who are normotensive before pregnancy.[24] Whether increases in cardiac output and stroke volume and decreases in peripheral vascular resistance are blunted during pregnancy in such women, as in our chronically hypertensive eNOS knockout mice, is not known and should be explored. Interestingly, women who have intrauterine growth–restricted fetuses without preeclampsia exhibit significantly reduced increases in cardiac output, stroke volume, and LV mass and diastolic volume.[1] Thus, it is possible that in human pregnancy inadequate maternal hemodynamic changes may contribute to fetal growth restriction, as indeed may be the case in the eNOS knockout mouse. Finally, although a missense polymorphism in the eNOS gene has been associated with preeclampsia in some human populations,[42] our results show that eliminating the function of this gene fails to generate a preeclamptic phenotype in mice, suggesting that other genetic and environmental factors are of primary importance.

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