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Effects of Estrogen in Gender-dependent Fetal Programming of Adult Cardiovascular Dysfunction

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Abstract

Background: Epidemiological studies and experimental studies have demonstrated that intrauterine adverse environment increases the risk of cardiovascular disease (CVD) in adulthood. However, whether an individual develops a cardiovascular dysfunctional phenotype may depend on genetic background, age, and sex.

Methods: In this review, we summarize some of the recent experimental animal studies in the developmental programming of adult CVD with an emphasis on sex differences and the potential role of estrogen in mediating sexual dimorphism.

Results: Few epidemiological studies report the effect of sex on the developmental programming of CVD. However, numerous experimental animal studies have shown a sex difference in fetal programming of adult cardiovascular dysfunction. Most of the animal studies indicate that male offspring develop cardiovascular dysfunction and CVD in adulthood, whereas adult females appear to be protected. Estrogen is one of the key factors that contributes to the sex difference of adult CVD. Estrogen/its receptor (ER) may interact with the RAS system by changes of DNA methylation patterns at the target gene promoter, serve as an antioxidant to counteract the prenatal insults-induced heightened ROS, and function as an eNOS activator to increase vasodilation, resulting in the protection of female offspring from the development of hypertension and other CVDs.

Conclusion: These studies suggest that estrogen/ER may contribute to sex differences in cardiovascular response to an adverse intrauterine environment and play a significant role in modulating the cardiovascular response in adulthood.

Graphic abstract diagram.

Role of estrogen in the sex difference of development of CVD phenotype in adult offspring.

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Keywords

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1. INTRODUCTION

Cardiovascular disease (CVD) is the leading global cause of death. The most important risk factors of CVD are unhealthy diet, physical inactivity, tobacco addiction, alcohol addiction and other stresses. A growing body of evidence has shown that intrauterine exposure to these risk factors during a critical period of fetal development might result in fetal adaptive changes that have led to an increased risk of CVD in postnatal life [1, 2]. However, the impact of this adverse intrauterine environment on the fetal programming of adult CVD is complex. The individual genetic background, postnatal environments and sex difference could modify the fetal origins of CVD in adulthood. It has been reported that intrauterine adverse environments enhance blood pressure in spontaneously hypertensive rats but not in normotensive rats [3], which suggest that genetic background might modify the fetal development of CVD in the adulthood. In addition, the sex differences of fetal programming of cardiovascular dysfunction in offspring have also been reported in many different animal models [4, 5]. Previous studies have indicated that male offspring were more likely to develop hypertension and CVD later in life, whereas female offspring appeared to be protected. These findings suggest that sex hormones such as estrogen may contribute to sex differences in cardiovascular response to an adverse intrauterine environment in adulthood.

Therefore, in this review we discuss the sex differences of cardiovascular dysfunction in different programmed animal models. Then we discuss the role of estrogen in the sex-dependent fetal programming of an adult cardiovascular dysfunction. Finally, we summarize the potential molecular mechanisms underlying the estrogen-mediated sex differences of the programmed cardiovascular dysfunctional phenotype later in life.

2. THE SEX DIFFERENCES OF PROGRAMMED CARDIOVASCULAR DYSFUNCTION

Epidemiologic studies show that CVD is the leading cause of mortality in both men and women. However, there is a gender difference in the development of CVD [6]. Among adults, men are at greater risk for developing CVD and hypertension than age-matched premenopausal women [7]. After menopause, the risk of developing CVD in women is significantly increased at a later age [6]. The increased risk of CVD in postmenopausal women has been linked to the decrease in plasma estrogen levels, which suggests a gender-dependent pathophysiology of CVD and implies that estrogen may play a key role in differentiating the incidence of CVD between men and women [8]. Similarly, sex differences are widely reported in developmental programming of CVD in human [9, 10] and experimental animal studies [4, 5, 11–17]. It has been shown in epidemiological studies that intrauterine growth restriction (IUGR) could cause a sexdifferent increase in the risk of CVD later in life [9, 10]. Law et al. in a longitudinal study showed that a young men with a low birth weight had a higher blood pressure than the women with a low birth weight [9]. Barker et al. also reported in a large cohort studies that there were no significant difference of blood pressure between the low birth weight boys and girls at 10 years of age [10]. However, this study further reported that the systolic blood pressure was higher in men than in women at 36 years of age [10]. These epidemiological studies suggest a sex difference in development of CVD risk in an IUGR individual. Furthermore, the transition from childhood through puberty and before the time onset of menopause may also contribute to sex difference in programmed CVD risk that occurs with aging. Yet, experimental animal studies provide the direct cause-effect molecular mechanisms that contribute to the increased CVD risk and the mechanisms underlying the sexual dimorphic programming of CVD. Previous studies [13] in pregnant rat model of fetal programming induced by placental insufficiency have demonstrated significant increases in blood pressure in both prepubertal male and female offspring without sexual difference. However, after puberty, only male animals show persistent hypertension whereas females normalize their blood pressure. Furthermore, recent studies show that male fetuses may be more susceptible to intrauterine nutrient deprivation, while female fetuses seem to have increased susceptibility to maternal overnutrition in programming of cardiovascular dysfunction [15–17]. Our previous studies on the nicotine-infused pregnant rat model have demonstrated that prenatal nicotine exposure increased both vascular contractility and blood pressure response in adult males but not in female offspring [5]. Furthermore, in the maternal hypoxia rat model we found that gestational hypoxia could result in a gender-dependent increase in blood pressure and vascular reactivity in the adult male, but significantly decrease in blood pressure and vascular reactivity in the adult female offspring [18]. These studies have suggested complex gender-dependent differential

responses to different intrauterine adverse stimuli in the developmental programming of CVD in adulthood.

3. THE ROLE OF ESTROGEN IN SEX DIFFERENCES OF THE PROGRAMMED CARDIOVASCULAR DYSFUNCTION

There are a variety of mechanisms that contribute to the sex differences in the programmed CVD. These differences in the development of CVD may be due to intrinsic sex differences in cardiovascular function, a sex dichotomy in the genes expressed in male and female placentas, or sex differences of fetuses in adaptation to developmental stressors. Of importance, sex steroids play a significant influence on the programming of CVD in adulthood [19, 20]. Elegant experimental studies in a pregnant rat model have demonstrated placental insufficiency during gestation results in significant increases in blood pressure in both prepubertal male and female offspring. After puberty, only male rather than female offspring remained with high blood pressure [13]. Furthermore, ovariectomy (OVX) induces hypertension in adult female offspring, which is normalized by estrogen replacement [18]. In a male rat model, treatment with estradiol significantly improves the ischemia/reperfusion (I/R) injury and cardiac function [21]. Our previous studies in the pregnant rat model have also demonstrated that prenatal nicotine exposure causes a gender-dependent alteration of vascular function, resulting in increased risk of hypertension in adult male but not female offspring [5]. In our further studies [22] of female offspring, we have demonstrated that OVX uncovers nicotine-induced programming of heightened vascular reactivity and hypertensive response in the female offspring. Treatment with estrogen in the OVX offspring eliminates the differences of contractions and blood pressure responses between nicotine-treated and saline control ovariectomy rats. The findings from those of other reports [13, 21] and ours [5, 22] suggest that estrogen may contribute to sex differences in the blood pressure response to an adverse intrauterine environment and play an important role in modulating cardiovascular response in adulthood. However, the effect of estrogen in programmed cardiovascular dysfunction is complex. In our recent study, we found that the plasma estrogen levels were not significant difference between saline control and maternal nicotine-treated adult offspring [22]. Consistent with our finding, Ojeda et al. also reported that in utero adverse environmental exposure did not affect the circulating estrogen levels in adult female offspring [13]. These observations suggest that the role of estrogen in the regulation of cardiovascular function may be not only dependent on estrogen levels per se, but also on its effect on downstream signaling or other regulatory systems. Our previous studies in rats have shown that gestational hypoxia exposure could result in a gender-dependent increase for cardiac vulnerability to I/R injury in the adult male, but not female offspring [25, 26]. In the same animal model, we have further demonstrated that estrogen may not be directly responsible for sex dimorphism in the fetal programming of heart ischemic vulnerability in female offspring exposed to antenatal hypoxia, but rather protect against cardiac I/R injury in both the normoxic control and antenatal hypoxic female offspring [25]. Interestingly, the decreased blood pressure that estrogen caused in the OVX rats only appeared in the control group instead of the hypoxic group [18]. From these results, we have proposed the potential mechanisms of underlying estrogen with antenatal hypoxia-induced hypotensive responses in the female offspring. We speculate that estrogen could decrease the arterial blood

pressure, however other ovarian-released factors such as progesterone could counteract the inhibitory effect of estrogen on blood pressure. Given the fact that maternal hypoxia has no effect on plasma estrogen levels in female adult offspring [18, 25], maternal hypoxia may predominately alter ovarian progesterone or other ovarian-released factors. The overall re-programming of the balance between estrogen and progesterone/other factors by maternal hypoxia results in the development of hypotensive phenotype in female offspring. The complex mechanisms underlying perinatal stress-induced re-programming of the ovarian steroid hormones (such as estrogen/progesterone) function and cardiovascular reactivity are needed to further investigate in our future studies.

4. THE POTENTIAL MECHANISMS OF ESTROGEN IN REGULATION OF PROGRAMMED CARDIOVASCULAR DYSFUNCTION

Most studies in programmed animal models have indicated that male offspring develop cardiovascular dysfunction, whereas female offspring appeared to be protected [11–13, 26]. These studies have suggested a significant role of estrogen in modulating cardiovascular function in adulthood [27]. Herewith, we discuss some of the key molecular mechanisms underlying the estrogen-induced protective effects on cardiovascular dysfunction in response to intrauterine insults in the programmed animal models from our and other laboratories.

4.1. Modulation of Renin-Angiotensin System (RAS).

Estrogen is a steroid hormone which is secreted mainly from the ovaries. Estrogens exert their biological effects through estrogen receptors (ER), ER α and ER β , which are widely expressed not only in reproductive organs but also in many tissues including the heart and vasculature. The role of estrogen in the regulation of cardiovascular function may be dependent not only with its own direct effect on the cardiovascular system, but also its indirect effects on other systems. RAS is one of the potential systems targeted by estrogen. Growing evidence has shown a key role of RAS in the pathogenesis of cardiovascular dysfunction in the model of fetal programming [13, 28–30]. Estrogen may activate the RAS by augmenting levels of renin and angiotensinogen or their downstream signaling pathways, which then can shift the vasoconstrictor-vasodilator balance of the RAS, resulting in protection against hypertension [31].

Recent studies [13] have suggested that modulation of the Angiotensin H receptor (ATR) by estrogen is one of the key mechanisms that contributes to the sex difference in fetal programming of adult cardiovascular dysfunction. Angiotensin II (Ang II) regulates vascular resistance through receptors (AT1R and AT2R). Activation of the AT1R by Ang II induces vasoconstriction, whereas activation of AT2R opposes the classic actions of the AT1R, resulting in vasodilation. Previous studies have indicated that renal AT1R expression was relatively greater in the male rats than in the female rats [32]. In the models of placental insufficiency, renal AT1R expression was increased in the hypertensive male offspring, but no changes happened in the normotensive female offspring [33]. In the pregnant rat models of those exposed to nicotine, we have demonstrated that prenatal nicotine exposure gender-dependently enhances the vascular AT1R protein levels, resulting in a significantly increased vascular reactivity and elevated blood pressure response to Ang II in adult male offspring [5,

22]. In the female offspring, ovariectomy increases the Ang II -induced vascular contraction and blood pressure response in the nicotine-exposed group as compared with the saline control group [22], which is associated with an increased vascular AT1R expression. However, estrogen replacement can reverse the effect of ovariectomy on the nicotine-induced response. These studies have suggested that the estrogen-induced counteraction of nicotine-induced exaggerated AT1R gene expression may be one of the key mechanisms for the sex difference in the development of a hypertensive phenotype.

The molecular and cellular mechanisms underlying the protective effect of estrogen on the prenatal nicotine-mediated over-expression of AT1R are unclear. DNA methylation is one of the most key epigenetic mechanisms in the regulation of gene expression. It occurs at the cytosine in the CpG dinucleotide sequence. Previous studies have indicated that CpG methylation in the sequence-specific transcription factor binding sites have significantly caused epigenetic alteration of the gene expression patterns in developing fetuses in different programmed models [34, 35]. Recently, we have found that several CpG units are located in the transcription factor binding sites in the rat AT1R promoter region [35]. Of these CpG sites, nicotine selectively decreased the methylation levels at -449 CpG locus, suggesting that hypo-methylation of the selective CpG locus may be one of the key epigenetic mechanisms in up- regulation of AT1R gene expression of vasculatures in response to perinatal nicotine exposure. Because the CpG is at the site of -449, located at the ER α , β the transcription factor binding site of the AT1aR promoter region, we speculate that in the adult female offspring, a higher estrogen/ ER α , β level binding at the AT1aR promoter may alter the specific CpG methylation pattern at ER α , β binding site. This will lead to normalizing the enhanced AT1aR promoter activity and gene expression.

In addition to regulation of target gene expression via interacting with CpG methylation, estrogen/ER also can epigenetically modulate gene transcription via non- CpG methylation (CpH) mechanism. Methylation at CpH dinucleotide (H means A, C, and T) has emerged as one of the important epigenetic marks in mammalian embryonic cells [36]. Recent studies reported that estrogen (17 β -estradiol) treatment increased the expression of DNA methyltransferases, Dnmt3a and Dnmt3b, and selectively increased CpH methylation in MyoD gene, leading to reduction of MyoD gene expression and muscle growth [37]. Furthermore, estrogen/ER signaling also can epigenetically regulate gene expression via histone modification at the ER target gene promoter [38]. ER α is a ligand-activated transcription factor. Upon estrogen stimulation, ER α recruits a number of co-regulators to the estrogen response elements (ERE), modulating gene activation or repression. Most coregulatory complexes contain histone-modifying enzymes to control ER α target gene expression in an epigenetic manner [38]. Estrogen/ER's ability to modulate epigenetic changes by regulating DNA methylation and histone modification may provide a novel molecular mechanism of a sex difference in programmed CAD.

4.2. Estrogen Serves as an Antioxidant.

Previous studies with estrogen suggested that many of the cardiovascular effects of sex hormones were related to their role in the modulation of oxidative stress. Furthermore, increasing bodies of evidence have shown a significant role of oxidative stress in the fetal

programming of CVD [39–41]. Studies in different programmed animal models have indicated that estrogen may serve as an antioxidant to protect female offspring against prenatal insults such as induced oxidative stress which contributes to the sex difference in the development of the cardiovascular dysfunctional phenotype in male but not female offspring [22, 39, 41]. Placental insufficiency in the pregnant rat results in a significant increase in renal reactive oxygen species (ROS) production in hypertensive male offspring but not in the normotensive female offspring [39]. Treatment with a superoxide dismutase (SOD) mimetic abolishes hypertension in the male offspring, which indicates that increased oxidative stress contributes to the gender-dependent development of the hypertensive phenotype in adult male offspring [39]. Our previous studies have demonstrated that prenatal nicotine exposure-induced hypertensive reactivity in adult male offspring is associated with an increase in vascular NADPH oxidase (Nox)-mediated ROS production [41]. Furthermore, treatment with an inhibitor of Nox (apocynin) significantly abolishes prenatal nicotine-induced exaggerated vascular contractions in the adult male offspring [41], indicating that nicotine-mediated oxidative stress may play a key role in the programming of hypertensive phenotype in adult male offspring. In another study with female offspring [22] vascular ROS levels had no significant difference between the saline control and prenatal nicotine exposed female offspring, but were significantly increased in the nicotine-treated offspring as compared with the saline control offspring in the ovariectomy rats, which was also abolished by estrogen replacement. These findings have suggested that nicotine-mediated vascular ROS production may be modulated by estrogen. Similarly, previous studies have shown that estrogen directly inhibits the NADPH oxidase activity via the estrogen receptor, leading to attenuate ischemic oxidative damage [42]. Therefore, these studies have suggested that estrogen may serve as an antioxidant to inhibit prenatal insults-mediated ROS production in adult female offspring, which may contribute to the sex differences of the programmed cardiovascular dysfunction in adult offspring.

4.3. Modulation of Nitric Oxide-Mediated Signaling by Estrogen.

In addition to the modulation of oxidative stress, estrogen may also modulate nitric oxide (NO)-dependent signaling. There are two major molecular mechanisms of endothelial nitric oxide synthase (eNOS) activation by estrogen: one is up-regulation of eNOS expression and another one is alteration of eNOS sensitivity by increased eNOS phosphorylation and eNOS-caveolin-1 interaction. In human endothelial cells, estrogen treatment significantly increases NO release by estrogen receptor (ER)-dependent activation of eNOS [43]. Activation of ERs could result in vasodilation of the small artery via elevation of endothelial NO release [44]. On the other hand, a sex difference of NO-dependent relaxation has also been reported in rats, where the relaxation is more pronounced in female than in male rats. This is most likely due to the increased eNOS expression by endogenous estrogen [45]. Indirectly, estrogens could also regulate the expression of the vascular endothelial growth factor receptor 2 and soluble fms-like tyrosine kinase-1 receptor, and then acts on NO signaling [46]. However, the role of estrogen-mediated NO dependent signaling in the gender difference of programmed cardiovascular dysfunction is somehow very complex. Our previous studies have demonstrated that prenatal nicotine exposure attenuates NO-dependent relaxations in male offspring but enhances them in females without the alteration of vascular eNOS gene expression [8]. These results suggest that prenatal nicotine selectively decrease

eNOS activity, which can be reversed and further enhanced by estrogen, resulting in altered NO-mediated vasodilation in a gender- dependent manner.

5. CONCLUSION

Sex differences are well demonstrated in several animal models of fetal programming of adult hypertension and cardiovascular dysfunction. Estrogen is one of the key factors that significantly contributes to the sex difference of fetal programming of adult CVD. Findings from previous studies in different animal models suggest that estrogen may interact with the RAS system, serve as an antioxidant to counteract the prenatal insults-induced heightened ROS, and function as an eNOS activator to increase vasodilation, resulting in the protection of female offspring from the development of hypertension and other CVDs (Graphic abstract diagram). As shown in the graphic abstract diagram, prenatal insults (such as nicotine) exposure during pregnancy can cause fetal programming of adult cardiovascular disease (CVD) via epigenetic molecular mechanisms. Typically, in utero stimuli impair target gene promoter activity via either alteration of specific CpG (or CpH)-DNA methylation patterns at a specific transcription factor binding site (e.g. ER α / β) of target gene (such as AT1aR) promoter region or histone modification, which lead to an epigenetic regulation of the target gene expression in offspring. The change of target gene expression (such as AT1R overexpression) may lead to increased ROS production, resulting in an increase in vascular contraction and blood pressure response. In addition, utero adverse stimuli may also impair the vascular endothelium function and eNOS activity, resulting in an increase in ROS production and a decrease in vasodilation. However, in adult female offspring, the ovaries could release estrogen and other ovarian hormones. The released estrogen may directly bind to the transcription factor binding site (ER α / β) at the target gene (AT1aR) promoter region, which leads to modify the CpG (or CpH) methylation pattern, counteracting prenatal insults-induced changes in the target gene (AT1R) expression and the gene-mediated effects in female offspring [33]. Although it is generally accepted that ovary-released estrogen may play an important role in the counteraction of prenatal insults-mediated in the development of hypertension and cardiovascular dysfunction, the interaction between estrogen and cardiovascular function is far more complex and controversial. In conclusion, recent experimental animal studies provide strong proof of principle that prenatal insult can cause a fetal programming of a sex difference in CVD in adulthood. Animal studies also demonstrate a role of sex steroids (estrogen) in regulation of the RAS, ROS and NO release, which may control or normalize the development of CVD following a prenatal insult. Although some epidemiological studies support the link between prenatal insult during early life and later cardiovascular disease, further studies are needed to clarify regulatory mechanisms of estrogen and other sex hormones involved in the fetal programming of adult cardiovascular dysfunction in the clinical implications. Understanding these mechanisms will provide a molecular basis for development of sex-specific strategies for prevention and treatment of fetal originated CVD.

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ABBREVIATIONS

Ang II	Angiotensin II ATR II Angiotensin II receptor CVD = Cardiovascular disease E2 = Estradiol
eNOS	Endothelial nitric oxide synthase
ER	Estrogen receptors
I/R	Ischemia/reperfusion
iNOS	Inducible nitric oxide synthase
NO	Nitric oxide
Nox	NADPH oxidase
OVX	Ovariectomy
RAS	Renin-Angiotensin System
ROS	Reactive oxygen species
SOD	Superoxide dismutase

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