Angiotensin-Converting Enzyme 2 Deficiency Is Associated With Impaired Gestational Weight Gain and Fetal Growth Restriction

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Abstract—Angiotensin-converting enzyme 2 (ACE2) is a key enzyme of the renin-angiotensin system that influences the relative expression of angiotensin II (Ang II) and Ang-(1-7). Although ACE2 expression increases in normal pregnancy, the impact of ACE2 deficiency in pregnancy has not been elucidated. We determined the influence of ACE2 deficiency on circulating and tissue renin-angiotensin system components, fetal and maternal growth characteristics, and maternal hemodynamics (mean blood pressure and cardiac output output) at day 18 of gestation. Gestational body weight gain was lower in the ACE2 knockout (KO) versus C57BL/6 (wild-type) mice (30.3±4.7 versus 38.2±1.0 g; P<0.001). Fetal weight (0.94±0.1 versus 1.24±0.1 g; P<0.01) and length (19.6±0.2 versus 22.2±0.2 mm; P<0.001) were less in KO. Mean blood pressure was significantly reduced in C57BL/6 with pregnancy; it was elevated (P<0.05) in the KO virgin and pregnant mice, and this was associated with an increased cardiac output in both C57BL/6 and KO pregnant mice (P<0.05). Plasma Ang-(1-7) was reduced in pregnant KO mice (P<0.05). Placenta Ang II levels were higher in KO mice (52.9±6.0 versus 22.0±3.3 fmol/mg of protein; P<0.001). Renal Ang II levels were greater in KO virgin mice (30.0±1.7 versus 23.7±1.1 fmol/mg of protein; P<0.001). There was no change in the Ang-(1-7) levels in the KO placenta and virgin kidney. These results suggest that ACE2 deficiency and associated elevated placenta Ang II levels impact pregnancy by impairing gestational weight gain and restricting fetal growth. (Hypertension. 2011;58:00-00.)

Key Words: Ang-(1-7) ■ ACE2 ■ renin-angiotensin system ■ pregnancy ■ fetus

Maternal weight is an important determinant of optimal fetal development, pregnancy outcome, and lifelong health as an adult. Fetal growth restriction remains a leading cause of perinatal morbidity and mortality in humans. Although there are many potential causes of fetal growth restriction, the most common identifiable cause is suboptimal uteroplacental perfusion. Vasoactive mediators play an important role in the regulation of the vasculature of the uteroplacental bed. Among these are the peptide components of the renin-angiotensin system (RAS), primarily angiotensin II (Ang II) and Ang-(1-7). Angiotensin-converting enzyme (ACE) 2 is a pleiotropic monocarboxypeptidase shown to efficiently metabolize Ang II to Ang-(1-7). Ang II has well-characterized vasoconstrictive, proliferative, and angiogenic actions. In contrast, Ang-(1-7) has vasodilatory, anti-proliferative, and antiangiogenic actions. In Ang II–induced cardiac hypertrophy and remodeling and pressure-overload heart failure, simultaneous administration of Ang-(1-7) counterbalances the detrimental effects of Ang II. ACE2-deficient mice have impaired cardiovascular function, enhanced oxidative stress, and inflammatory cytokine expression. These studies illustrate a critical role for the ACE2-dependent balance of Ang II and Ang-(1-7).

The circulating RAS is elevated in normal pregnancy, with hemodynamics characterized by a reduction in systemic vascular resistance and no increase or a reduction in systemic blood pressure. Pregnancy is associated with increased renal and uterine ACE2 mRNA expression and activity as compared with virgin rats. In the current study, we hypothesized that increases in ACE2 expression in the kidney and reproductive tissues during pregnancy have a protective role in renal, cardiovascular, and reproductive function. Therefore, we determined the effect of pregnancy on circulating and tissue RAS components, fetal and maternal growth characteristics, and maternal hemodynamics (blood pressure and cardiac output [CO]) in both the ACE2 knockout (KO) and C57BL/6 (wild type [WT]) mice.

Materials/Methods
For a description of materials and methods please see the online Data Supplement at http://hyper.ahajournals.org.
Results

Figure S1, available in the online Data Supplement, shows the ACE2 protein in placental tissue from ACE2 KO mice, as well as WT females, by Western blot. As expected, the glycosylated 125-kDa form of ACE2 was detected in the WT placenta, whereas it was not seen in the placenta from KO mice (Figure S1A). On preincubation of the ACE2 antibody with the antigenic peptide, the 125-kDa immunoreactive band was absent in the WT, further confirming the specificity of our antibody for mouse ACE2 (Figure S1B).

Maternal Characteristics

Figure 1 shows the maternal characteristics of the WT and KO virgin and pregnant mice. There was no difference in body weight between virgin KO and WT females (16.1±0.3 versus 16.6±0.4 g, respectively). Gestational body weight gain was significantly lower in the KO mice compared with WT mice (30.3±4.7 versus 38.2±1.0 g, respectively; P<0.001; KO versus WT). This difference (23±1.3 versus 28±0.5 g; P<0.05) persisted even when the total pup weight/pregnancy was subtracted. Mean blood pressure was significantly higher in KO virgin females as compared with WT virgins (86±2 versus 76±1 mm Hg; P<0.01). Mean blood pressure was reduced in WT pregnant animals (76±1 versus 69±2 mm Hg; P<0.05). In contrast to the pregnant WT, the higher pressure was maintained in the pregnant KO (84±2.5 versus 86±2 mm Hg; P value not significant, pregnant versus virgin KO). There was a significant effect of pregnancy on CO (20.8±1.6 versus 25.3±2.7 mL/min [WT, n=10, 4] and 20.2±1.3 versus 25.3±2.8 mL/min [KO, n=8, 9], virgin versus pregnant P<0.05).

Fetal Characteristics

Figure 2 provides the fetal characteristics for KO and WT mice. Average pup weight (0.94±0.1 versus 1.24±0.01 g; 30.3±4.7 versus 38.2±1.0 g, respectively; P<0.001; KO versus WT). This difference (23±1.3 versus 28±0.5 g; P<0.05) persisted even when the total pup weight/pregnancy was subtracted. Mean blood pressure was significantly higher in KO virgin females as compared with WT virgins (86±2 versus 76±1 mm Hg; P<0.01). Mean blood pressure was reduced in WT pregnant animals (76±1 versus 69±2 mm Hg; P<0.05). In contrast to the pregnant WT, the higher pressure was maintained in the pregnant KO (84±2.5 versus 86±2 mm Hg; P value not significant, pregnant versus virgin KO). There was a significant effect of pregnancy on CO (20.8±1.6 versus 25.3±2.7 mL/min [WT, n=10, 4] and 20.2±1.3 versus 25.3±2.8 mL/min [KO, n=8, 9], virgin versus pregnant P<0.05).

Figure 1. Maternal characteristics of virgin and pregnant C57BL/6 and angiotensin-converting enzyme 2 knockout (ACE2KO) females. A, Pregnant ACE2KO body weight was significantly reduced compared with C57BL/6 pregnant females (n=7). B, ACE2KO maternal weight —total pup weight per pregnancy was significantly reduced vs C57BL/6 females (n=7). C, Mean blood pressure was significantly higher for virgin and pregnant ACE2KO (n=5, 4) females vs the respective C57BL/6 females (n=7, 5). D, Correlation of maternal weight to total pup weight. Values are mean±SEM; * P<0.05 vs virgin; #P<0.01; ##P<0.001 vs C57BL/6.

Figure 2. Eighteen-day gestation fetal characteristics in C57BL/6 and angiotensin-converting enzyme 2 knockout (ACE2KO). Pup weight (A) (n=64, 48), pup length (B), and total pup weight/pregnancy (C) (n=8, 7) were significantly reduced in ACE2KO. The number of resorption sites (D) was significantly higher in ACE2KO mice (n=8, 7). Values are mean±SEM; #P<0.05; ##P<0.01 vs C57BL/6.
Pregnant

for WT and KO mice (185.17 ± 17.16 fmol/mL per minute; P < 0.005). In WT mice, ACE activity was >30-fold higher compared with ACE2 activity, indicating that ACE is considerably more abundant in the circulation than ACE2.

**RAS Components in the Kidney of KO and WT Mice**

Figure 4A shows the peptide profile in the KO and WT virgin and pregnant female kidney tissue. Ang II levels were greater in virgin KO versus WT mice (30.0 ± 1.7 versus 23.7 ± 1.1 fmol/mg of protein; P < 0.001) but not in the kidneys from pregnant KO versus WT mice. Ang I and Ang-(1-7) levels were higher in WT pregnant animals compared with virgins. Figure 5B shows ACE and nephrilysin (NEP) activity levels in the kidney. The KO animals had higher NEP activity compared with WT mice. There was no difference in kidney tissue ACE activity in KO versus WT virgin and pregnant mice.

**RAS Components in the Placenta of KO and WT Mice**

Figure 5A shows the peptide profile in the placenta of KO and WT mice. Ang II levels were 2.5-fold greater in KO versus WT mice (52.9 ± 6.0 versus 22.0 ± 3.3 fmol/mg of protein; P < 0.01); however, Ang-(1-7) and Ang I levels were unchanged. Placental ACE activity was reduced in KO mice compared with WT (13.8 ± 1.7 versus 28.0 ± 2.6 nmol/h per milligram of protein; P < 0.005), and there was no difference in the NEP activity (Figure 5B).
RAS Peptide Levels in the Heart, Uterus, and Fetal Membranes of KO and WT Mice

Tables S1 through S3 provide the Ang I, Ang II, and Ang-(1-7) levels in the heart, uterus, and fetal membranes from WT and KO virgin and pregnant mice. There were no differences in the levels of peptides in the heart, uterus, or fetal membranes. Ang II and Ang-(1-7) content in the fetal membranes was markedly higher than all of the other tissues studied.

Discussion

This is the first report to demonstrate the impact of ACE2 deficiency on maternal, as well as fetal, growth. ACE2, a carboxypeptidase, is a homologue of ACE that cleaves Ang II into Ang-(1-7) with high efficiency. ACE2 acts in a counter-regulatory manner to ACE to shift the balance between Ang II and Ang-(1-7). There are limited data on the specific functions of ACE2 in vivo, and no data on ACE2 function in pregnancy. Therefore, the present study was undertaken to

Figure 4. A, Angiotensin peptide levels in the kidney of virgin and pregnant C57BL/6 and angiotensin-converting enzyme 2 knockout (ACE2KO) mice (n=20, 12, 8, 7). Angiotensin II (Ang II) levels were significantly increased in virgin ACE2KO females. Angiotensin I (Ang I) and angiotensin-(1-7) (Ang-(1-7)) were significantly increased in pregnant C57BL/6 mice. B, ACE and neprilysin (NEP) activity in the kidney (n=5, 5, 4, 5). NEP was significantly increased in virgin and pregnant ACE2KO mice. Values are mean±SEM; **P<0.01; ***P<0.001 vs virgin of the same background; #P<0.05; ##P<0.01; ###P<0.001 vs C57BL/6.

Figure 5. A, Angiotensin peptide levels in the placenta of pregnant C57BL/6 and angiotensin-converting enzyme 2 knockout (ACE2KO) mice. Angiotensin II (Ang II) levels were increased in ACE2KO females vs C57BL/6 (n=6, 7). B, ACE and neprilysin (NEP) activity in the placenta (n=6, 4). ACE was decreased in ACE2KO, whereas NEP was unchanged as compared with C57BL/6. Values are mean±SEM; ###P<0.01 vs C57BL/6.
study the impact of ACE2 deficiency on maternal and fetal growth in mice during pregnancy. We showed that gestational body weight gain and average and total pup weight were lower in the KO mice compared with the WT mice. We also evaluated the impact of the ACE2 deficiency on the major RAS peptides and maternal hemodynamics. Ang II was increased in the placenta of KO animals and in the kidney of virgin KO animals. The increase in Ang II in virgin kidney was absent in KO pregnant animals. Plasma Ang-(1-7) was decreased in the KO pregnant mice, a finding consistent with the circulating profile of preeclamptic women.10

Maternal gestational body weight gain was decreased in pregnant KO females compared with pregnant WT females. Body weight increase during gestation is associated with increased food and fluid intake, expansion of plasma volume, and enhanced CO.14,15 These changes are usually associated with a normal or decreased blood pressure, reflecting the reduction in total peripheral resistance attributed to the prominent vasodilation.15 With pregnancy, WT mice showed a significant reduction in mean arterial pressure (MAP) as compared with virgins. In virgin KO females, MAP was higher than that in WT virgin females. The increase in MAP is in agreement with the original study by Gurley et al.,16 where blood pressure was increased in the male KO. These observations were made in KO mice on C57BL/6 background. In the KO mice on a 129/SvEv background, however, they did not see any difference in blood pressure compared with WT mice, emphasizing the different phenotypic outcomes for KO mice of different genetic backgrounds. In our study, the increase in MAP in KO virgin mice may reflect higher kidney Ang II content without a change in Ang-(1-7), indicating a shift in the balance of local RAS tissues toward Ang II. An increase in renal Ang II could contribute to increased vasoconstriction and fluid and water retention. In pregnant KO mice, MAP remained elevated compared with pregnant WT females. In these animals that exhibit no increase in renal Ang II, the basis for the increase in blood pressure may reflect greater placental Ang II, resulting in an increased placental bed resistance.

In spite of the increase in blood pressure, Gurley et al.16 did not report any differences in cardiac dimensions in the KO mice on the 2 backgrounds, which is different from the study of Crackower et al.,4 reporting that KO mice on a mixed background develop left ventricular dilation. Pregnancy is well known to have substantial hemodynamic changes and CO increases by 35% to 40%.15 As expected with normal pregnancy, CO increased in WT pregnant mice; in the pregnant KO mice CO increased to a similar level. Because CO was similarly increased in both WT and KO pregnant animals, our data would suggest that the impaired gestational weight gain and fetal growth restriction in ACE2 KO pregnancy cannot be attributed to inadequate maternal systemic CO changes.

Previous studies using the antagonist [D-Ala7]-Ang-(1-7) to block the endogenous actions of Ang-(1-7) in pregnant rats revealed reduced water and food intake, as well as a lower amount of urine.14 It is possible that, in pregnancy, ACE2 deficiency and the associated reduced plasma Ang-(1-7) adversely affected the thirst and hunger centers of the brain, resulting in decreased water and food intake, thereby leading to reduced body weight. Because the reduced water and food intake accompanied the antidiuresis, the preponderance of effects in KO pregnant animals appears to be central actions of the reduced Ang-(1-7) controlling food and water intake. The central effects would be balanced by the reduced diuresis that is also observed in pregnant animals, which may contribute to the increase in CO. The basis for the maternal weight reduction, together with the increased MAP and CO in the KO, requires further exploration.

ACE2 deficiency resulted in significant inhibition of fetal growth, in addition to impaired gestational weight gain. Fetal weight and length were lower in the KO mice compared with WT mice, and there was ~1 fetal resorption per pregnancy. These findings point to an important survival role of ACE2 in normal pregnancy. The mechanism for the resorptions is unknown. Crackower et al.4 reported in the first study describing the KO mice on a mixed genetic background that mice appeared healthy, without any gross detectable alterations in all of the organs analyzed and at the expected mendelian frequency. However, the assessment of fetal characteristics was not the primary goal of their study, and only qualitative observations were presented. Maternal body size has an influence on fetal growth.17-19; smaller mothers produce smaller offspring than larger mothers, and this may be one of the causes of poor fetal growth in the KO females. Our data confirm this observation by the strong positive correlation between maternal growth and fetal growth. In addition, the uterine environment is a key determinant of fetal growth.20 The increased Ang II in the placenta of the pregnant KO mice could lead to increased placental ischemia. Increased Ang II was observed in the chorionic villi of the placenta of preeclamptic women21 and in both the maternal and fetal components of the placenta of the preeclampsia rat transgenic model arising from the cross of the female human angiotensinogen with the male human renin.22 In both preeclamptic women and the human transgenic rat model of preeclampsia, fetal growth restriction accompanied the activated placental RAS. Finally, because of the observation of prepregnancy elevated blood pressure and elevated kidney Ang II levels in the virgin KO animals, the question of systemic maternal factors contributing to later detrimental outcomes during pregnancy cannot be eliminated.

No differences in plasma Ang II were detected among KO and WT pregnant and virgin mice. Gurley et al.16 also reported no differences between WT and KO mice in circulating Ang II levels. These results differ from the report by Crackower et al.,4 who showed that plasma Ang II was increased in the KO model. The disparity in these findings may be attributed to the differing backgrounds, the sex and age of the animals, and the accompanying cardiovascular disease in the ACE2 KO mice from the study by Crackower et al.4 at the time of measurement. On the other hand, plasma Ang-(1-7) in our study was significantly decreased in KO pregnant mice. Previously we reported that preeclamptic women at late gestation showed lower plasma Ang-(1-7).10 In the present study, the profile in circulating peptides was accompanied by a lower serum ACE activity in the pregnant as compared with the virgin females in both WT and KO animals. The decrease of ACE in
pregnancy observed in this study is consistent with previous studies conducted during pregnancy and with estrogen treatment in rats and mice. Another aspect of these studies is that we used the endogenous substrates Ang I and Ang II assayed under identical conditions to provide an accurate assessment of the relative activities of ACE and ACE2 in the serum. We demonstrated that serum ACE activity was 30-fold higher compared with serum ACE2 activity. This differential expression of the 2 enzymes in the circulation with ACE2 being present at substantially lower levels may explain why there was no change in serum peptides with ACE2 deletion. Serum ACE2 activity was increased in pregnant compared with virgin WT mice. This pattern of opposite changes in ACE2 and ACE activities is consistent with the counterregulatory mechanism of their actions on peptide formation.

In the kidney, Ang II levels were increased in virgin KO mice compared with virgin WT mice. Gurley et al showed a 6-fold increase in renal Ang II in KO males after Ang II infusion but did not report baseline Ang II values. Crackower et al reported increased Ang II content in the kidney of KO mice. Thus, the increase in Ang II in the kidney is consistent with the lack of ACE2 enzyme in the KO virgin mice. In the present study, this increase was absent in pregnant mice.

To further investigate the basis for the angiotensin peptide profile in pregnant KO mice, we determined NEP and ACE activity levels in the kidney. NEP activity was higher in virgin and pregnant KO mice compared with WT, suggesting a negative feedback regulation between ACE2 and NEP in virgin and pregnant KO mice compared with WT, suggesting a non-NEP enzyme that may be compensating for the reduction in ACE2. In the kidney, Ang II levels were increased in virgin KO mice compared with virgin WT mice. Gurley et al showed a 6-fold increase in renal Ang II in KO males after Ang II infusion but did not report baseline Ang II values. Crackower et al reported increased Ang II content in the kidney of KO mice. Thus, the increase in Ang II in the kidney is consistent with the lack of ACE2 enzyme in the KO virgin mice. In the present study, this increase was absent in pregnant mice.

In ACE2 KO mice, a decrease in Ang-(1-7) was expected in the placenta, which they propose is the site of increased clearance of the peptide. Placental Ang II was higher in ACE2-deficient mice compared with WT mice. This finding suggests that Ang II is a primary substrate for ACE2 in the placenta, because its degradation is prevented in the absence of ACE2. In contrast to the expected decrease in Ang-(1-7) in the placenta of the KO, Ang-(1-7) levels were not changed as compared with the WT mice. This finding suggests that redundant enzymes may contribute to the formation of Ang-(1-7) in the placenta. Our study demonstrated no difference in the placental NEP but a decrease in ACE activity in the KO. The absence of ACE2 with a reduction in ACE in the presence of elevated Ang II suggests that ACE2 is the predominate enzyme that is responsible for the breakdown of Ang II with a lesser contribution of ACE to the generation of Ang II. Because ACE can degrade Ang-(1-7) and convert it to Ang-(1-5), a decrease in ACE activity in the placenta is consistent with a decrease in degradation of Ang-(1-7) by ACE, which could contribute to the maintenance of Ang-(1-7) in the placenta. In contrast to the kidney and placenta, the lack of change of angiotensin peptides in the uterus, heart, and fetal membranes indicates that there is tissue-specific regulation of peptide expression and that different tissues show a distinct profile of peptide changes.

This study surprisingly showed very little change in the Ang-(1-7) levels in tissue. The RAS system is complicated, and part of the complexity is the redundancy of enzymes to act on multiple substrates. Thus, the endopeptidases nephrilysin, prolyl oligopeptidase, and thimet oligopeptidase, as well as prolyl carboxypeptidase, all can generate Ang-(1-7). In this model, any of these enzymes can contribute to maintain Ang-(1-7) in the face of increased Ang II. Because ACE2 is an Ang-(1-7) synthesizing enzyme, a reduction in Ang-(1-7) levels in the tissue was anticipated in the KO animals, especially in the placenta and virgin kidney, where Ang II was increased. In the placenta, the reduction in ACE activity is consistent with its contribution to the maintenance of Ang-(1-7), but the lack of change in NEP points to a non-NEP enzyme that may be compensating for the reduction in ACE2. In the kidney, the increase in NEP could contribute to the maintenance of Ang-(1-7).

Finally, ACE2 acts on substrates unrelated to the RAS. Apelin is a multifunctional peptide that is catabolized by ACE2 and has major actions related to cardiovascular function, body fluid homeostasis, and energy metabolism. Van Mieghem et al showed that apelin levels drop by 50% in the last week of gestation and proposed that the drop in apelin was because of the increased expression of ACE2 in the placenta, which they propose is the site of increased clearance of the peptide.

**Perspectives**

In the present study we demonstrated that ACE2 deficiency is associated with impaired gestational weight gain and restricted fetal growth. ACE2 deficiency affected the angiotensin profiles in the kidney of virgin animals and the placenta with an increase in Ang II. Only in the circulation was the shift in the Ang II/Ang-(1-7) balance accompanied by a decrease in Ang-(1-7). Our findings have uncovered a unique, protective role for ACE2 in normal pregnancy. Correcting the ACE2 deficiency during abnormal pregnancy may provide the potential to prevent impaired gestational weight gain and fetal growth restriction. The study has also raised questions about the complexity and redundancy of enzymes of the RAS, which resulted in the unexpected maintenance of tissue Ang-(1-7) levels.

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**Disclosures**

None.

**References**


