Periconceptional undernutrition and being a twin each alter kidney development in the sheep fetus during early gestation

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MacLaughlin SM, Walker SK, Kleemann DO, Tosh DN, McMillen IC. Periconceptional undernutrition and being a twin each alter kidney development in the sheep fetus during early gestation. Am J Physiol Regul Integr Comp Physiol 298: R692–R699, 2010. First published January 6, 2010; doi:10.1152/ajpregu.00495.2009.—Adaptive growth responses of the embryo and fetus to nutritional restraint are important in ensuring early survival, but they are implicated in the programming of hypertension. It has been demonstrated that kidney growth and nephrogenesis are each regulated by intrarenal factors, including the insulin-like growth factors, glucocorticoids, and the renin-angiotensin system. Therefore, we have investigated the impact of periconceptional undernutrition (PCUN; from ~6 wk before to 7 days after conception) in singleton (control, n = 18; PCUN, n = 16) and twin pregnancies (control, n = 6; PCUN, n = 5) on the renal mRNA expression of 11β-hydroxysteroid dehydrogenase type 1 and type 2 (11β-HSD-1 and -2), the glucocorticoid (GR), and mineralocorticoid receptors, angiotensinogen, angiotensin receptor type 1 (AT1R) and 2 (AT2R), IGF-1 and IGF-2, and IGF1R and IGF2R at ~55 days gestation. There was no effect of PCUN or fetal number on fetal weight on relative kidney weight at approximately day 55 of gestation. There was an inverse relationship between the relative weight of the fetal kidney at approximately gestation. There was no effect of PCUN or fetal number on fetal weight on relative kidney weight at approximately day 55 of gestation. There was an inverse relationship between the relative weight of the fetal kidney at approximately day 55 and maternal weight loss during the periconceptional period in fetuses exposed to PCUN. Exposure to PCUN resulted in a higher expression of IGF1 in the fetal kidney in singleton and twin pregnancies. Being a twin resulted in higher intrarenal expression of IGF-1 and IGF-2, GR, angiotensinogen, AT1R, and AT2R mRNA at 55 days gestation. Renal 11β-HSD-2 mRNA expression was higher in PCUN singletons, but not PCUN twins, compared with controls. Thus, there may be an adaptive response in the kidney to the early environment of a twin pregnancy, which precedes the fetal growth restriction that occurs later in pregnancy. The kidney of the twin fetus exposed to periconceptional undernutrition may also be less protected from the consequences of glucocorticoid exposure.

A series of clinical and experimental studies have shown that maternal nutrition and placental substrate supply during pregnancy are each important in determining the pattern of fetal kidney growth and the functional capacity of the kidney and cardiovascular system in postnatal life (23, 28). In the human, nephrogenesis is complete by 32–34 wk of gestation and a nephron deficit present at birth will, therefore, persist through life (23, 28). Kidney growth is significantly slower in the intrauterine growth-restricted (IUGR) human fetus, and total nephron number is also lower in the IUGR fetus compared with normally grown fetuses (14, 15, 21). In the sheep, twinning, a naturally occurring form of fetal growth restriction, results in a ~40% lower nephron endowment compared with singleton fetuses (25). Similarly in the rat, maternal protein restriction throughout pregnancy or induction of uteroplacental insufficiency results in a decrease in kidney growth and in the number of nephrons (2, 17, 24, 35, 40, 44). A low-protein diet throughout pregnancy in the rat also programs an upregulation of the renal glucocorticoid receptor (GR) and separately, administration of glucocorticoids at 15–16 days of gestation results in a reduction in glomerular number and hypertension (32, 33). It has been proposed that there is a critical window during which glucocorticoids have their maximum impact on renal development, which is the period when nephrogenesis commences in the permanent metanephric kidney (26). These findings are supported by studies in sheep, in which glucocorticoids have been administered at 26–28 days of gestation (term = 150 ± 3 days of gestation). In the sheep fetus, mesonephric development occurs between 17 and 57 days of gestation, and metanephric development occurs from around 27 to approximately 135 days of gestation (29). At 26–28 days of gestation, the fetal sheep kidney comprises a mass of metanephric mesenchyme, into which the ureteric bud has grown and branched once (26, 29). Offspring from glucocorticoid-exposed mothers have a significantly lower nephron number and are hypertensive in later life (5–7, 27, 29, 42, 43).

In the sheep, exposure to excess glucocorticoids at days 26–28 of pregnancy also results in an increase in the expression of renal mineralocorticoid (MR) and GR, angiotensinogen, and the angiotensin II type 1 (AT1R) and type 2 (AT2R) receptors in the fetal kidney at 130 days gestation (27). The intrarenal renin-angiotensin system (RAS) plays a key role in renal organogenesis, nephrogenesis, and vascularization, and experimental disruption of the RAS has been shown to result in persistent abnormal changes in kidney growth and function (12, 13, 26, 31, 37, 39). It has, therefore, been proposed that glucocorticoid-induced stimulation of the renal RAS results in a premature completion of nephrogenesis and an associated reduction in the final nephron number (26).

The IGFs have also been implicated in the regulation of renal growth and development during late pregnancy (18, 22, 38). Intrafetal infusion of IGF-1 in the fetal sheep stimulates renal growth and function, as well as activation of the fetal RAS (22). The role of IGFs during the phase of renal growth and development in early pregnancy is, however, less clear. Although the impact of maternal and fetal undernutrition imposed throughout pregnancy on renal growth and develop-
ment has been extensively studied, fewer studies have investigated the impact of maternal undernutrition during the periconceptional period on kidney growth and development. Restricting either the protein or energy content of the maternal diet for limited periods before and after conception, when the nutrient demands of the early conceptus are minimal, has long-term consequences for the development of the hypothalamo-pituitary-adrenal (HPA) axis and cardiovascular system in the rat, mouse, and sheep (3, 4, 8–11, 16, 19, 20, 41). In pregnant rats fed a low-protein diet for the first 4.25 days after conception, the offspring had an increased relative kidney weight and raised blood pressure in postnatal life (16). Similarly, exposure of the mouse embryo to a maternal low-protein diet for 3.5 days after conception results in an increase in systolic blood pressure in postnatal life (41). Interestingly, in the sheep, it has also been shown that the effects of periconceptional undernutrition on the development of the HPA and cardiovascular axes are more profound in twin fetuses compared with singletons (8, 9).

In this study, we hypothesized that maternal undernutrition during the periconceptional period (for ~6 wk before to 1 wk after conception) or being a twin fetus would each separately result in activation of the renal GR and RAS system at a stage in pregnancy (55 days of gestation) when nephrogenesis has started but has not completed (29). This is also a stage in gestation when there is no evidence for the impact of placental and fetal growth restriction, which emerges in later gestation in the twin pregnancy (20). We also hypothesized that exposure to periconceptional undernutrition (PCUN) may have a different impact on kidney growth and development in the twin compared with the singleton fetus, given that the twin fetus is more vulnerable to the effects of PCUN on the fetal HPA and on fetal blood pressure than the singleton fetus (8, 9). We have, therefore, investigated the impact of maternal periconceptional undernutrition on fetal kidney weight and on the mRNA expression of the intrarenal growth factors, IGF1 and IGF2, and their receptors in singleton and twin pregnancies. We have also determined the effects of PCUN on the intrarenal expression of 11β-hydroxysteroid dehydrogenase-2 (11β-HSD-2), which is a unidirectional nicotinamide dinucleotide-dependent enzyme that catalyzes the conversion of biologically active cortisol to the inert cortisone and the expression of 11β-HSD-1, which is a reduced nicotinamide adenine dinucleotide phosphate-dependent isoform that acts to convert cortisone to cortisol. Finally, we have determined the effects of PCUN and fetal number on the intrarenal expression of GR, MR, angiotensinogen, AT1R, and AT2R in the singleton and twin fetal sheep at ~55 days gestation.

MATERIALS AND METHODS

Ethical approval. All procedures were approved by The University of Adelaide Animal Ethics Committee and by the Primary Industries and Resources South Australia Animal Ethics Committee.

Animals. Forty-five South Australian Merino ewes were used in this study, and the feeding and breeding protocols were as previously reported (20). All ewes were weighed, and a body condition score was assessed by an experienced assessor employing a 1–5 scale with 0.5 intervals (20). In this scale, a body condition score of 1 represents an extremely emaciated animal and a body condition score of 5 represents a morbidly obese animal. During a 2-wk period, ewes were acclimatized to a pelleted diet containing cereal hay, lucerne hay, barley, oats, almond shells, lupins, oat bran, lime, and molasses (Johnsons & Sons, Kapunda, South Australia, Australia). The pellets provided 9.5 MJ/kg of metabolizable energy and 120 g/kg of crude protein and contained 90.6% dry matter. All ewes received 100% of nutritional requirements (7.6 MJ/day for the maintenance of a 64-kg nonpregnant ewe), as defined by the Agricultural and Food Research Council (1). Ewes were then randomly assigned to one of two feeding regimes, a control regime (C, n = 24), in which ewes received 100% of nutritional requirements or a periconceptional restricted regime (PCUN, n = 21), in which ewes received 70% of the control allowance. All of the dietary components were reduced by an equal amount in the restricted diet. Control ewes were maintained on the control diet for 62 ± 5 days, and the ewes in the PCUN group were maintained on the 70% diet for 55 ± 2 days prior to conception.

Ewes in the PCUN group were maintained on the 70% restricted diet for 7 days after conception. From 7 days after mating, all ewes were fed a control diet (100% of requirements) until autopsy at days 53–56 of pregnancy. Pregnancy was diagnosed, and fetal number was estimated by ultrasound at day 45 of pregnancy, generating four treatment groups: control singleton pregnancies (n = 18), PCUN singleton pregnancies (n = 16), control twin pregnancies (n = 6), and PCUN twin pregnancies (n = 5).

Collection of tissues. Ewes were killed with an overdose of pentobarbital sodium (Virbac, Peakhurst, NSW, Australia) between day 53 and day 56 of pregnancy, and the utero-placental unit was delivered by hysterotomy. Fetal organs were dissected and weighed.

Maternal blood samples. Maternal blood samples (10 ml) were collected by venepuncture at autopsy into chilled heparinized tubes. All samples were centrifuged at 1500 g for 10 min, and plasma was separated into aliquots and stored at −20°C.

Cortisol radioimmunoassay. Maternal plasma samples were extracted and assayed as previously described (19). Briefly, cortisol was extracted from the plasma using dichloromethane (recovery >85%). Samples were reconstituted in buffer (Tris hydrochloride: bovine serum albumin; sodium azide). Standards were serially diluted in buffer, from a stock (1000 ng/ml) solution (range 0.78–100 ng/ml). Anticortisol (100 µl; 1:15 dilution; Orion Diagnostica, Turku, Finland) was added followed by 125I-labeled cortisol (100 µl; Amersham Pharmacia Biotech, UK). Tubes were vortexed and incubated at 37°C for 1 h before addition of goat anti-rabbit serum (initial dilution 1:30; 100 µl) and polyethylene glycol (1 ml; 20%; BDH Laboratory Supplies, Poole, Dorset, UK). Tubes were vortexed before centrifugation at 3700 g and 4°C for 30 min. The supernatant was aspirated, and the precipitate was counted on a gamma counter (Packard, Downers Grove, IL). The sensitivity of the assay was 0.2 nmol/l. The intra-assay and interassay coefficients of variation were <15%.

RNA isolation and cDNA synthesis. RNA was isolated from frozen kidney tissue samples using TRIzol reagent (Invitrogen, Leek, The Netherlands) and purified using the RNeasy Mini Kit (Qiagen, Basel, Switzerland) (19). Genomic DNA contamination was minimized by treating each sample with DNase 1 (Ambion, Austin, TX), and RNA was quantified by spectrophotometric measurements at 260 nm and 280 nm. cDNA was synthesized from 5 µg RNA using Superscript III (Invitrogen) reverse transcription. Controls containing no RNA transcript or no superscript were used to test for DNA contamination.

Quantitative real-time RT-PCR. The relative abundance of IGF-1, IGF-2, IGF-1R, IGF-2R, GR, MR, AT1R, and AT2R, angiotensinogen, 11β-HSD-1, and 11β-HSD-2 mRNA transcripts in fetal kidney tissue were measured by quantitative real-time RT-PCR (qRT-PCR) using the SYBR Green system in an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) (6, 19). In brief, each qRT-PCR well contained 5 µl SYBR Green Master Mix (Applied Biosystems), 1 µl each of forward and reverse primer (GeneWorks, South Australia, Australia) for the appropriate gene (6, 19), water (2 µl), and 50 ng/µl cDNA (1 µl) to give a total volume of 10 µl. Controls for each primer set containing no cDNA were included on each plate. Three replicates of cDNA from each kidney
were performed for each gene on each plate, and each plate was repeated three times to ensure a consistent result. Amplification efficiencies were determined, from the slope of a plot of Ct (defined as the threshold cycle with the lowest significant increase in fluorescence) against the log of the cDNA template concentration (ranging from 1 to 100 ng/μl). The abundance of each transcript relative to the abundance of the reference gene, ribosomal protein P0 (RpP0), was calculated using Q-Gene analysis software (30).

**RESULTS**

**Maternal weight change, cortisol, and fetal kidney growth.**

Ewes in the PCUN group lost significantly more weight (singleton PCUN, −5.3 ± 0.78 kg; twin PCUN, −2.20 ± 2.38 kg) than control ewes (singleton control, 0.44 ± 0.76 kg; twin control, 0.83 ± 1.44 kg, P < 0.0001) between the start of the feeding regime and by day 10 after conception. Similarly, there was a greater decrease in the body condition score of ewes in the PCUN group (singleton PCUN, 0.46 ± 0.10; twin PCUN, 0.61 ± 0.23, P < 0.05). There was no difference in maternal weight loss or in body condition score during the periconceptional period between ewes carrying singleton or twin pregnancies. There was also no difference in maternal plasma cortisol concentrations in ewes carrying singleton or twin pregnancies or in ewes exposed or not exposed to PCUN (singleton control, 13.3 ± 2.4 nmol/l; singleton PCUN, 13.5 ± 2.1 nmol/l; twin control, 14.2 ± 2.8 nmol/l; twin PCUN, 14.0 ± 2.6 nmol/l).

**Table 1. Effect of PCUN and fetal number on fetal and kidney weights**

<table>
<thead>
<tr>
<th>Organ weight</th>
<th></th>
<th>Singles</th>
<th></th>
<th>Twins</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal weight, g</td>
<td>26.26 ± 1.24</td>
<td>28.11 ± 0.78</td>
<td>28.45 ± 0.89</td>
<td>25.89 ± 1.87</td>
<td></td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>0.329 ± 0.018</td>
<td>0.362 ± 0.031</td>
<td>0.344 ± 0.022</td>
<td>0.302 ± 0.029</td>
<td></td>
</tr>
<tr>
<td>Relative kidney weight, g/g</td>
<td>0.0126 ± 0.0005</td>
<td>0.0128 ± 0.0009</td>
<td>0.0121 ± 0.0008</td>
<td>0.0115 ± 0.0009</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. PCUN, periconceptional undernutrition.

**Fig. 1.** There was a significant inverse relationship between relative kidney weight at approximately day 55 of pregnancy (y-axis) and the change in maternal weight during the periconceptional period (x-axis) in control twins (y = −0.0008x + 0.0312, r = 0.90, P < 0.001; ○), but not control singleton pregnancies (●). There was, however, a significant inverse relationship between relative kidney weight and the change in maternal weight during the periconceptional period in PCUN singleton (y = −0.0008x + 0.0088, r = 0.63, P < 0.009, ▲) and PCUN twin pregnancies (y = −0.0004x + 0.0109, r = 0.68; P < 0.05; △).
PCUN, 14.9 ± 3.4 nmol/l; twin control, 18.8 ± 4.2 nmol/l; twin PCUN 10.6 ± 2.4 nmol/l).

There was no effect of PCUN or fetal number on fetal weight or on absolute or relative kidney weight at approximately day 55 gestation (Table 1). In singleton pregnancies, in the PCUN, but not the control group, there was an inverse relationship between the relative weight of the fetal kidney at approximately day 55 (y-axis) and maternal weight loss during the periconceptional period (x-axis) (Fig. 1). In twin pregnancies, there was an inverse relationship between relative kidney weight at approximately day 55 (y-axis) and maternal weight loss during the periconceptional period (x-axis) in both the control and PCUN groups (Fig. 1).

**PCUN and renal IGF-1, IGF-1R, IGF-2, and IGF-2R mRNA expression.** Renal IGF-1 mRNA expression was higher in twin fetuses compared with singleton fetuses (P < 0.002, Fig. 2A and B) and was also higher in the PCUN group compared with controls, independently of fetal number (P < 0.008, Fig. 2A and B) at approximately day 55 of pregnancy. Conversely IGF-1R mRNA expression was lower in twin fetuses compared with singleton fetuses (P < 0.0001, Table 2) and was also lower in the PCUN compared with the control groups (P < 0.04, Table 2).

There was a significant relationship between relative kidney weight (y-axis) and renal IGF-1 (x-axis) mRNA expression (y = 0.33x + 0.0084, r = 0.52, n = 16, P < 0.05) in control singletons but not in control twins or in either the PCUN singleton or twin groups (Fig. 3).

Renal IGF-2 mRNA expression was higher (P < 0.0001, Fig. 2D), and IGF-2R expression was lower (P < 0.008, Table 2) in twins compared with singletons (Fig. 2C and Table 2). There was no effect of PCUN, however, on renal expression of IGF-2 or IGF-2R mRNA in either singleton or twin fetuses.

**PCUN and renal GR, MR, 11β-HSD-1, 11β-HSD-2, angiotensinogen, AT1R, and AT2R mRNA expression.** There was no effect of PCUN on renal GR, 11β-HSD-1, angiotensinogen, AT1R, or AT2R mRNA expression in either singleton or twin fetuses (Table 2 and Fig. 4).

Renal expression of GR, angiotensinogen, AT1R, and AT2R mRNA levels were each higher in twin fetuses compared with singleton fetuses, and this was independent of the level of maternal nutrition during the periconceptional period (Table 2, Fig. 4).

In singleton, but not twin fetuses, renal 11β-HSD-2 mRNA expression was higher in the PCUN compared with the control

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**Table 2. Effect of fetal number and PCUN on renal expression of IGF-1R, IGF-2R, MR, and GR at approximately day 55 gestation**

<table>
<thead>
<tr>
<th>Normalized mRNA Expression</th>
<th></th>
<th>Singles</th>
<th></th>
<th>Twins</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>PCUN</td>
<td></td>
<td>Control</td>
<td>PCUN</td>
</tr>
<tr>
<td>IGF-1R: RpP0</td>
<td>0.184 ± 0.006</td>
<td>0.174 ± 0.007*</td>
<td></td>
<td>0.118 ± 0.002†</td>
<td>0.078 ± 0.005†</td>
</tr>
<tr>
<td>IGF-2R: RpP0</td>
<td>0.471 ± 0.015</td>
<td>0.448 ± 0.018</td>
<td></td>
<td>0.394 ± 0.031†</td>
<td>0.399 ± 0.014†</td>
</tr>
<tr>
<td>MR: RpP0</td>
<td>0.018 ± 0.001</td>
<td>0.018 ± 0.001</td>
<td></td>
<td>0.020 ± 0.002</td>
<td>0.018 ± 0.002</td>
</tr>
<tr>
<td>GR: RpP0</td>
<td>0.141 ± 0.007</td>
<td>0.142 ± 0.009</td>
<td></td>
<td>0.184 ± 0.016†</td>
<td>0.165 ± 0.013†</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. *Significant difference between control and PCUN pregnancies (P < 0.04). †Significant difference between singleton and twin pregnancies (P < 0.001).
Kidney growth and renal IGF expression. In this study, we found no effect of PCUN or fetal number on the relative weight of the fetal sheep kidney at 55 days of gestation. A novel finding, however, was that a greater maternal weight loss during the periconceptional period was associated with an increase in the relative weight of the fetal kidney in control twin, but not singleton pregnancies and in both PCUN singleton and twin pregnancies. This occurred in the absence of any difference in maternal weight change between ewes carrying singleton and twin pregnancies in either the control or PCUN groups. This suggests that there are mechanisms that protect the growth of the fetal kidney in the face of a maternal weight loss experienced before or around conception in twin pregnancies, independently of the level of maternal nutrition, and that are recruited in the singleton pregnancy on exposure to PCUN. These effects may be mediated, in part, through placental growth responses, as in sheep, the normal positive relationship between maternal weight gain and placental growth, which is present in singleton pregnancies, is disrupted after maternal
PCUN (20). Furthermore, in twin pregnancies, an inverse relationship between placental growth and maternal weight gain emerges after exposure to PCUN, such that placental growth increases as maternal weight decreases (20).

In the present study, there was a significant and positive relationship between relative kidney weight and renal IGF1 expression in the control singleton fetus at 55 days of gestation. It has been shown that IGF1 plays an important role in metanephric morphogenesis, as blocking endogenous IGF1 expression or the presence of low circulating IGF1 levels are each associated with impaired renal growth and a nephron deficit (37). Furthermore, it has been shown that a 10-day infusion of IGF1 between 120 and 130 days gestation in the fetal sheep results in an increase in kidney growth and in sustained activation of the fetal RAS characterized by an increase in the synthesis and release of renin by the fetal kidney (18, 22). It appears from the present study that there is a relationship between renal growth and intrarenal IGF1 expression from as early as 55 days gestation. Interestingly, exposure to maternal PCUN or being a twin each separately resulted in an increase in renal IGF1-1 expression and a concomitant decrease in renal IGF-1R mRNA expression. One possibility why this occurs is that the increase in renal IGF1 expression in these groups is mediated, in part, through the placental growth responses, which occur after PCUN. As discussed above, in twin pregnancies, an inverse relationship between placental growth and maternal weight change emerges, such that placental growth increases as maternal weight decreases after exposure to PCUN (20). In the PCUN groups and control twins, however, the direct relationship between relative kidney weight and renal IGF1 expression was not present, and there was no increase in relative kidney weight, despite the increase in IGF1 expression. It is likely that the reciprocal decrease in renal IGF1R expression, which occurs in response to the increase in IGF1 expression present after PCUN and in a twin pregnancy, may limit the full impact of the increased IGF1 expression on the growth and development of the fetal kidney. Renal IGF-2 mRNA expression was also higher, and IGF-2R mRNA expression was correspondingly lower in kidneys from twin fetuses compared with singleton fetuses at approximately day 55 gestation. IGF2 is expressed in the metanephric mesenchymal cells and prevents these cells from undergoing apoptosis (37). As IGF2R acts as a clearance receptor, the decrease in IGF2R may result in an increased action of IGF2 within the kidney of the twin fetus. In the sheep, while fetal growth is restricted in twins, it has been shown that kidney growth is maintained or tends to be increased in twins compared with singletons in late gestation (10, 25). Despite the maintenance of kidney growth in the twin, nephron endowment is ~40% lower in the twin compared with the singleton fetus at 140 days of gestation (25). Ewes with a low body condition score around the time of conception also have lambs with a lower number of nephrons present at 6 mo of age compared with those born to ewes with a normal body condition score (11). The data from the present study highlight that there are early changes in the expression of intrarenal IGF1 and IGF2 in the fetal kidney in a twin pregnancy and in intrarenal IGF1 after exposure to PCUN, and these changes may precede the emergence of the kidney growth phenotype in later gestation.

**PCUN, intrarenal glucocorticoids, and the RAS.** Glucocorticoid receptors are present in the fetal sheep kidney from as early as 30 days gestation (34), and it has been shown that exposure to excess maternal glucocorticoids during early pregnancy results in a lower nephron number in the kidney in postnatal life (42, 43). In the present study, there was a higher expression of renal 11β-HSD-2 mRNA expression in PCUN singleton fetuses compared with control singletons. Therefore, in contrast to our initial hypothesis, the kidney in the PCUN singleton fetus may be protected from exposure to excess glucocorticoids. There was, however, no upregulation of 11β-HSD-2 expression in the PCUN twin group, and renal GR expression was also higher in the twins than the singletons.

A novel finding was that renal angiotensinogen, AT1R, and AT2R expression were each increased in the twin compared with the singleton fetus, independent of the level of periconceptional nutrition. In the sheep, exposure to excess glucocor-
ticipoids at days 26–28 of pregnancy results in an upregulation in the expression of renal MR and GR, angiotensinogen, AT1R, and AT2R in the fetal kidney at 130 days gestation (26, 27). Disruption of the RAS during critical windows of perinatal development has been shown to result in persistent abnormal changes in kidney growth and function (12, 13, 26, 31, 36, 39). It has, therefore, been proposed that exposure to excess glucocorticoids during the early stages of nephrogenesis upregulates the intrarenal RAS, which results in activation and premature completion of nephrogenesis and a reduction in the final nephron number (26). Upregulation of the GR and components of the RAS in the kidney of the twin fetus in early pregnancy may, therefore, be one contributor to the lower nephron endowment present in the kidney of the twin fetus in late gestation (25).

**Perspectives and Significance**

Exposure of the mouse, rat, or sheep embryo to a maternal nutrient restriction in the periconceptional period results in adaptive growth responses of the embryo, placenta, and fetus (16, 20, 41). While such growth responses may have a survival value when poor fetal nutrition continues beyond the periconceptional period, they result in a subsequent vulnerability to later adult disease when the level of nutrition is restored to normal either during fetal or postnatal life (4, 16, 23, 41).

The present study highlights that in an animal model in which nephrogenesis is complete before birth, as in the human, IGF1 expression is upregulated in the fetal kidney during the early period of nephrogenesis in response to maternal undernutrition during the periconceptional period. Interestingly, 11β-HSD-2 expression was also upregulated in the kidney of the singleton fetus exposed to maternal PCUN, and an increase in this enzyme may act to protect the fetal kidney from exposure to excess glucocorticoids during early or later gestation, as PCUN is associated with an increased activation of the fetal HPA axis (3, 8). In contrast, however, intrarenal 11β-HSD-2 expression was not increased in the twin fetuses in the PCUN group, and thus, the kidney in this instance may be more vulnerable to excess glucocorticoid exposure in early or late gestation. In this context, it may be relevant that we have also demonstrated that expression of GR and components of the RAS system are increased in the kidney of the twin fetus in early pregnancy. We speculate that the upregulation of intrarenal GR and RAS expression in the twin may anticipate a requirement for an earlier structural differentiation of the kidney in the twin fetus, which is at risk of intrarenal growth restriction in later gestation. One consequence of such an earlier differentiation may be a lower nephron endowment, as has been shown to be present in the kidney of the twin and IUGR fetus in late gestation (14, 15, 21, 25).

It will be important to understand the relative importance of each of the hormonal, metabolic, and/or epigenetic mechanisms that contribute to the structural and functional adaptations of the kidney to poor maternal nutrition in the periconceptional period and to the early environment of a twin/IUGR pregnancy. It appears from the current study that intervention in early pregnancy may be required to limit the adverse impact of such adaptations on adult cardiorenal health.

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**DISCLOSURES**

No conflicts of interest are declared by the authors.

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