



## NOTE

Anatomy

# Novel intrauterine growth retardation model: effects of maternal subtotal nephrectomy on neonates

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**ABSTRACT.** Changes in body weight (BW), systolic blood pressure (SBP), and localization of renin in the kidneys of neonates born to normal mothers (C neonates) or to five-sixths (5/6) nephrectomized (2/3 left kidney and right kidney) mothers (Nx neonates) were studied. Maternal 5/6 nephrectomy caused weight loss in neonates but no differences in SBP or renin localization. Culling Nx neonates to a litter of 3 at 1 day after birth resulted in growth catching up with C neonates from 3 weeks old and increases in both SBP and renin-positive cells in neonatal kidney. These findings revealed that maternal 5/6 nephrectomy results in low-birth-weight neonates and that these neonates are at increased risk of metabolic syndrome by catch-up growth.

**KEYWORDS:** intrauterine growth retardation, maternal subtotal nephrectomy, neonatal rat kidney

When a pregnant mother is exposed to under nutrition, lack of placental blood flow, or renal disease, fetal growth retardation can result [15]. This can lead to intrauterine growth retardation (IUGR). In addition to low birth weight, IUGR neonates reportedly show increased risks of metabolic syndrome [1] and delayed mental development [3] after maturation. Reports have described IUGR models resulting from gestational maternal undernutrition [2, 12] or impaired placental blood flow [13], but little information is available on IUGR models attributed to maternal renal disease. We have reported that in pregnant rats, a slight but significant increment in blood urea nitrogen (BUN) concentration was induced after removing 2/3 of the left kidney, and an intense increase in BUN was induced after removing the right kidney compared with sham-operated rats [14]. Further, maternal renal dysfunction resulting from subtotal nephrectomy is associated with low fetal body weight [8]. Han *et al.* [5] recently reported elevated systolic blood pressure and progressively increasing blood creatinine in five-sixths (5/6) nephrectomized pregnant rats compared with sham-operated rats, and the remaining kidney in the 5/6 nephrectomized rats showed interstitial inflammation and mild fibrosis. In the present study, neonates from 5/6 nephrectomized mothers were examined to determine their potential utility as an IUGR model from maternal renal dysfunction.

Wistar-Imamichi rats were purchased from CLEA Japan (Osaka, Japan), reared under ordinary conditions (temperature,  $24 \pm 1^\circ\text{C}$ ; relative humidity,  $55 \pm 5\%$ ; 12 hr light, 12 hr dark) and were provided with commercial diet (CE-2; CLEA Japan) and water *ad libitum*. The present study was conducted in accordance with the Guidelines for Animal Experimentation of Osaka Prefecture University, Japan and was approved by the Animal Experiment Committee at Osaka Prefecture University (approval no. 30-74). The day following overnight mating, with sperm observed in vaginal smears, was determined as day 1 of pregnancy. To induce maternal renal dysfunction, 5/6 nephrectomy was performed according to a modification of the method from our previous study [11]. On day 5 of pregnancy, the left kidney was pulled out through a left dorsolateral incision to expose the renal vessels and ureter under isoflurane anesthesia. The renal vessels were clipped together by a clamp and the cranial and caudal parts of the organ were cut to make the kidney one-third of its original size. The cut surfaces were then coated with adhesive gum (Aron alpha A Sankyo; Daiichi Sankyo, Tokyo, Japan) and the clamp was released. The treated left kidney was returned to the abdominal cavity and the incision was then sutured. One week after the first operation, the right kidney was pulled out to expose the renal vessels and ureter through a right dorsolateral incision under isoflurane anesthesia. The renal vessels and ureter were ligated with cotton thread and cut between the hilus and ligated portion to remove the kidney. After removal of the kidney, the incision was sutured. After delivery, neonates were weighed on the first day of life and male neonates that met the definition of IUGR [6] as “weight of control group  $-2 \times$  standard deviations” from mothers with 5/6 nephrectomy were designated as Nx neonates. Male neonates from mothers without the operation were designated as C neonates. Since no difference in body weight was seen between neonates born to sham-operated mothers and neonates born to untreated mothers (unpublished data), neonates from untreated mothers (but not neonates from sham-operated mothers) were used as controls.

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The number of nursing pups was litter-adjusted to 8 on the first day of life. According to the methods of Martin-Gronert *et al.* [10] to increase the amount of milk given per newborn, in some Nx group litters, the number of nursing pups was litter-adjusted to 3 instead of 8 on the first day of life; neonates from these groups were designated as L3 neonates. Neonates were weighed for 7 consecutive days immediately following birth and were weighed once a week from 2–4 weeks after birth. At 4 weeks after birth, systolic blood pressure of rats was measured using a BP98A non-invasive continuous blood pressure measuring device for animals (Softron, Tokyo, Japan). Five consecutive measurements were taken, and after excluding the maximum and minimum values, the mean of the remaining three measurements was used as the systolic blood pressure for each individual. The blood of neonates was then collected via the femoral artery under triple-drug anesthesia using a mixture of medetomidine (0.3 mg/kg), midazolam (4 mg/kg) and butorphanol (5 mg/kg). The left kidneys were quickly removed by mid-abdominal incision. Collected blood was used for estimation of BUN, creatinine (Cre), high-density lipoprotein cholesterol (HDLC), total cholesterol (T-Cho), and triglycerides (TG). The kidney was fixed for 15 min in methanol-Carnoy solution (a 6:3:1 mixture of methanol, chloroform, acetic acid), then dehydrated through a graded series of alcohol, embedded in Tissue Prep (Fisher Scientific, Fair Lawn, NJ, USA) and cut into 6- $\mu$ m sections. These sections were used to examine the localization of renin by immunohistochemical methods. Renin immunostaining was performed as follows. After deparaffinization with xylene, sections were transferred to distilled water through a series of degraded ethanol and phosphate buffered saline. Sections were incubated with 3% hydrogen peroxide for 15 min to quench endogenous peroxidase. After treatment with 5% skim milk for 20 min, the aforementioned sections were incubated with rabbit anti-rat renin antibody (Proteintech Japan, Tokyo, Japan) at 1:1,000 dilution overnight at 4°C. Sections were then incubated with HISTOFINE simple stain Rat Max-PO (MULTI) (Nichirei Bioscience, Tokyo, Japan) for 60 min at room temperature. Following incubation with HISTOFINE, sections were incubated with diaminobenzidine for 5 min. The sections were counterstained with methyl green. Negative controls to determine background staining were generated by incubating with non-immunized normal immunoglobulin G instead of the primary antibody (renin) during the immunohistochemical protocol. Analysis of data was performed using the Tukey-Kramer test. Values of  $P < 0.05$  were considered statistically significant.

The gestation period of the rats used was 22 days, with both Nx and C neonates born at full term. From 1 day through 2 weeks after birth, body weights of Nx and L3 neonates were significantly lower than those of C neonates. The body weights of Nx neonates were significantly lower than those of C and L3 neonates at 3 and 4 weeks after birth (Table 1).

No significant differences in BUN, Cre, HDLC, or T-CHO were observed among C, Nx, and L3 neonates. TG in 4-week-old L3 neonates was significantly higher than in age-matched C and Nx neonates (Table 2).

Renin-positive cells were localized to the vascular pole of the renal corpuscle in the kidneys of 4-week-old C, Nx, and L3 neonates. Renin-positive cells were more frequent in the kidneys of L3 neonates than in age-matched C and Nx neonates (Fig. 1).

Systolic blood pressure was significantly higher in 4-week-old L3 neonates than in age-matched C and Nx neonates (Table 3).

The present study reveals that neonates from mothers with renal dysfunction were smaller in weight, and that these neonates are at increased risk of metabolic syndrome by catch-up growth.

In the present study, the weight of L3 neonates was significantly smaller than that of C neonates at birth. However, L3 neonates gained weight rapidly and no longer showed significant differences in weight from C neonates at 3 weeks old. This finding indicates that low birth weight neonates from mothers with renal dysfunction show catch-up growth due to excessive nutrition by the litter-adjustment immediately after birth. Further, this finding is supported by the report from Martin-Gronert *et al.* [10] that in an IUGR rat model created by maternal nutrient retardation, adjusting the number of nursing neonates to four induced rapid growth in neonates, which reached the same weight as control newborns at 3 weeks of age.

Unlike L3 neonates, the weight of Nx neonates remained significantly below that of C neonates from immediately after birth until

**Table 1.** Changes in body weight of neonates at 4 weeks after birth, from normal mothers (C neonates), from 5/6 nephrectomized mothers (Nx neonates), and the number of nursing pups from 5/6 nephrectomized mothers was litter-adjusted to 3 (L3 neonates)

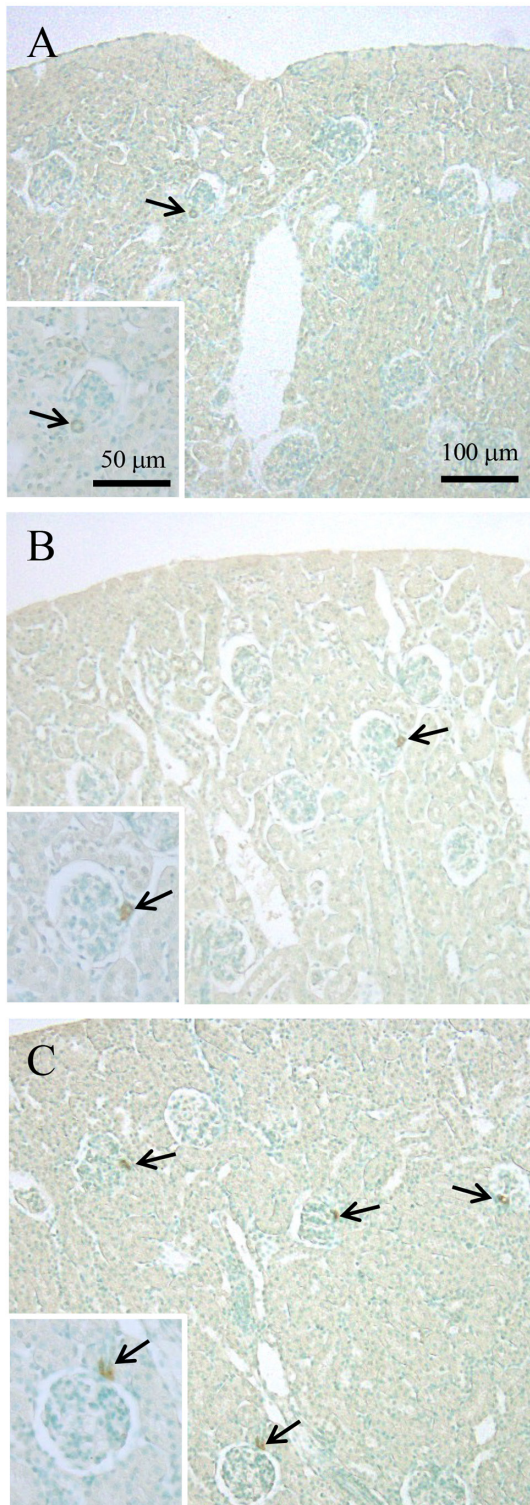
Age	C neonates 44 (8)*	Nx neonates 39 (8)*	L3 neonates 6 (2)*
1 day	6.15 ± 0.29	5.27 ± 0.28 <sup>a</sup>	5.05 ± 0.34 <sup>a</sup>
2 days	6.81 ± 0.43	5.78 ± 0.45 <sup>a</sup>	5.78 ± 0.50 <sup>a</sup>
3 days	7.87 ± 0.72	6.65 ± 0.66 <sup>a</sup>	6.70 ± 0.54 <sup>a</sup>
4 days	9.13 ± 0.87	7.74 ± 0.85 <sup>a</sup>	7.90 ± 0.48 <sup>a</sup>
5 days	10.39 ± 1.16	9.07 ± 0.91 <sup>a</sup>	8.90 ± 0.48 <sup>a</sup>
6 days	12.23 ± 1.06	10.69 ± 1.13 <sup>a</sup>	10.01 ± 0.53 <sup>a</sup>
7 days (1 week)	14.09 ± 1.24	12.06 ± 1.10 <sup>a</sup>	11.76 ± 0.46 <sup>a</sup>
2 weeks	26.91 ± 1.91	22.80 ± 2.28 <sup>a</sup>	23.92 ± 1.63 <sup>a</sup>
3 weeks	41.64 ± 2.59	32.83 ± 3.91 <sup>a</sup>	38.20 ± 1.63 <sup>b</sup>
4 weeks	72.35 ± 4.27	60.60 ± 6.99 <sup>a</sup>	69.96 ± 3.44 <sup>b</sup>

All values (g) are expressed as mean ± SD. \*: Number of litters (number of dams). a, b: Significantly different from C and Nx neonates, respectively ( $P < 0.05$ ).

**Table 2.** Serum blood urea nitrogen (BUN), creatinine (Cre), high density lipoprotein cholesterol (HDLC), total cholesterol (T-Cho), and triglyceride (TG) levels of neonates at 4 weeks after birth, from normal mothers (C neonates), from 5/6 nephrectomized mothers (Nx neonates), and the number of nursing pups from 5/6 nephrectomized mothers was litter-adjusted to 3 (L3 neonates)

	C neonates 10 (3)*	Nx neonates 9 (5)*	L3 neonates 6 (2)*
BUN	13.9 ± 4.1	18.5 ± 4.5	14.4 ± 2.9
Cre	0.23 ± 0.05	0.22 ± 0.04	0.18 ± 0.08
HDLC	60.7 ± 8.5	66.3 ± 6.7	58.2 ± 5.3
T-Cho	107.8 ± 11.1	115.4 ± 9.6	114.0 ± 40.0
TG	77.2 ± 20.9	76.2 ± 13.9	112.2 ± 22.0 <sup>a,b</sup>

All values (mg/dL) are expressed as mean ± SD. \*: Number of litters (number of dams). a, b: Significantly different from C and Nx neonates, respectively ( $P < 0.05$ ).



**Fig. 1.** Kidney sections from C (A), Nx (B), and L3 (C) neonates at 4 weeks after birth, stained with anti-rat renin antibody. Renin-positive cells (arrows) are seen in L3 neonates (C), while appear rare in C neonates (A) and Nx neonates (B). In the inset, renin-positive cells (arrows) are seen in the juxtaglomerular apparatus of the three neonates in A–C.

**Table 3.** Systolic blood pressure of neonates at 4 weeks after birth from normal mothers (C neonates), from 5/6 nephrectomized mothers (Nx neonates), and the number of nursing pups, from 5/6 nephrectomized mothers was litter-adjusted to 3 (L3 neonates)

	No. of animals*	Systolic blood pressure
C neonates	15 (5)	86.9 ± 8.9
Nx neonates	14 (7)	81.3 ± 9.9
L3 neonates	11 (4)	96.3 ± 7.1 <sup>a,b</sup>

All values (mmHg) are expressed as mean ± SD. \*: Number of litters (number of dams). a, b: Significantly different from C and Nx neonates, respectively ( $P < 0.05$ ).

4 weeks after birth. This finding indicates that neonates from mothers with renal insufficiency do not show catch-up growth. No significant difference in any of the blood chemistry tests (including TG) was seen between Nx neonates and C neonates at 4 weeks old in this study. In contrast, TG in L3 neonates was significantly higher than that in Nx or C neonates. This suggests that IUGR neonates with catch-up growth show elevated TG, while IUGR neonates without catch-up growth do not. This in turn suggests that TG is elevated in IUGR neonates with catch-up growth, but not in IUGR neonates without catch-up growth, consistent with both a report by Malo *et al.* [9] that no increase in serum TG was seen in an IUGR model without catch-up growth and a study by Desai *et al.* [4] that plasma TG is increased in an IUGR model with catch-up growth at 3 weeks of age, suggesting that catch-up growth affects lipid metabolism.

In the present study, a significant increase in systolic blood pressure was evident among L3 neonates compared to C and Nx neonates at 4 weeks old. This is consistent with the report by Huxley *et al.* [6] that infants born with IUGR show hypertension when catch-up-growth occurs.

The renin-angiotensin system (RAS) plays a central role in regulating blood pressure and fluid volume [7, 16]. Renin is secreted by juxtaglomerular cells in the kidney and converts angiotensinogen to angiotensin I. In the present study systolic blood pressure was significantly higher in L3 neonates than in Nx or C neonates. Since the positive region of renin was more abundant in L3 neonates than in Nx or C neonates, increased renin expression appears to be involved in the elevated blood pressure seen in this IUGR model.

The present study reveals that neonates from mothers with renal dysfunction are at increased risk of metabolic syndrome due to catch-up growth by culling to 3 neonates and can serve as a model for IUGR.

**CONFLICT OF INTEREST.** The authors declare that there are no conflicts of interest.

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