The developmental programming effects of vitamin D on renal nephron endowment and gene expression of the renin angiotensin system in weanling male rats

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Fetal vitamin D levels are determined by maternal status, and even in Australia, maternal vitamin D deficiency is common (Bower *et al*. 2009). There is limited and conflicting evidence about the effects of vitamin D deficiency on fetal nephrogenesis, and the consequences of an excess of vitamin D on nephrogenesis have not been explored. Therefore in this study we investigated the effects of maternal vitamin D deficiency and excess on nephron endowment in weanling male rats. As well, since vitamin D is known to suppress the renin angiotensin system (Li *et al*., 2002) and the intrarenal renin angiotensin system is essential for normal kidney development (Guron & Friberg, 2000), we also examined the expression of the vitamin D receptor (VDR) gene and genes involved in the renin angiotensin system.

One month old virgin female Sprague Dawley rats were maintained in UVB-free housing and separated into three groups: control (CON; n=10), vitamin D deficient (DEF; received no Vit D in the diet; n=10) and vitamin D supplemented (HIGH; received daily injections of 1,25 $(OH)_2D_3$ s.c.; n=10). Dams were mated with vitamin D replete males from 10 weeks of age and the pups for the present study were removed for post-mortem analysis at postnatal day 20. Pups were deeply anaesthetized (80 mg/kg ketamine and 10 ng/kg xylazine i.p.) before they were killed by decapitation and tissues removed, weighed and stored. The right kidney was collected and fixed in 10% buffered formalin solution while the left kidney was snap frozen in liquid nitrogen and stored at −80°C. The right kidney was processed in paraffin and exhaustively sectioned into 5µm thick sections, which were stained with H&E and scanned into Aperio ImageScope Image Analysis software. Glomerular number was determined using the physical dissector/fractionator combination (Cullen-McEwen *et al.*, 2012). Total RNA was extracted from homogenized whole left kidney and reverse transcribed to cDNA. Real-time qPCR was carried out using TaqMan assays with 18S as the endogenous housekeeper and the comparative cycle threshold technique.

There was no significant difference in bodyweights of 20 day old male offspring between the 3 treatment groups, but the kidney was a smaller percentage of body weight in both DEF and HIGH groups than in CON (P<0.05). Nephron number (mean ± S.E.M.) was increased in DEF (23384 ± 337, n=7) compared to HIGH (20489 ± 400, n=6, P=.001). Furthermore, DEF rats had more glomeruli per mg kidney (78.09 ± 2.63) than control (55.42 ± 2.83, n=6 ; P<0.001) and HIGH (58.26 ± 3.69; P=0.001) animals. Expression of the genes for the VDR, angiotensinogen, (pro)renin and angiotensin converting enzyme 1 (ACE1) were downregulated in DEF offspring (P<0.05). However, there were no differences in renal mRNA levels of the (pro)renin receptor, ACE2 or any of the angiotensin receptors (ATR1a, ATR1b or ATR2) between treatment groups.

We conclude that maternal vitamin D deficiency promotes nephrogenesis in rats, but maternal excess vitamin D does not alter nephrogenesis. Although maternal vitamin D deficiency increases nephron number in male offspring, we have found that in adulthood these offspring have reduced creatinine clearance (Boyce *et al.* 2013), so these nephrons may be functionally impaired. While we demonstrated that several genes of the renin angiotensin system were down regulated in these weanling rats, at E20, a time when nephrogenesis was still occurring, renin gene expression was upregulated (Boyce *et al.* 2013). Thus, it is possible that vitamin D deficiency stimulates nephrogenesis *via* its effects on the renin angiotensin system.

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