Role of Vitamin A in Determining Nephron Mass and Possible Relationship to Hypertension¹,²

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Abstract

Vitamin A (retinol) and its analogs (retinoids) are important regulators of cell proliferation, differentiation, immune function, and apoptosis. The kidneys are target organs for vitamin A action. Retinoic acid (RA), a vitamin A metabolite, is involved in embryonic kidney patterning through the control of receptor tyrosine kinase expression, which modulates ureteric bud branching morphogenesis. Vitamin A status of the mother profoundly affects kidney organogenesis of the newborn. In rodents, mild vitamin A deficiency results in a 20% reduction of nephron number. In adult humans, nephron number varies between 0.3 and 1.3 million per kidney, which is accepted as normal. However, recent studies indicate that a large amount of vitamin A is also transported protein from mothers to their fetus (1). However, recent studies indicate that humans at the low end of nephron number are predisposed to primary hypertension. Because RA regulates nephron mass, its optimal availability during nephrogenesis is critical. RA levels in the embryo are affected by several factors, such as maternal vitamin A nutrition and disturbances in retinol metabolism. Maternal vitamin A deficiency during pregnancy is widespread in developing countries and segments of these populations may be exposed to low vitamin A during fetal life when nephron number is determined. Infants are likely to be born with suboptimal nephrons and may develop primary hypertension later in life. Although maternal vitamin A deficiency is not common in developed countries, congenital nephron number nevertheless varies widely, indicating low fetal RA levels due to common variants of the enzymes that convert retinol to RA. These infants might require heightened surveillance for hypertension later in life.

Fetal vitamin A metabolism

Fetal vitamin A is acquired from the maternal circulation via the placenta. Vitamin A is transported bound to retinol-binding protein from mothers to their fetus (1). However, recent studies indicate that a large amount of vitamin A is also transported bound to lipoproteins that may be taken up by the fetus (2). Maternal vitamin A levels are influenced by the dietary intake of retinoids. In the diet, vitamin A is present in preform as carotenoids and retinyl esters from plants and animal sources, respectively. Once taken up by fetal tissue, metabolic enzymes convert retinol to the active metabolite retinoic acid (RA), which then regulates vitamin A signaling (3). RA is formed from retinol by a 2-step oxidation process (4). First, retinol is oxidized to retinal by retinol dehydrogenases and then retinal is further converted to RA by retinal dehydrogenases (RALDH). Retinol oxidation is ubiquitous, whereas retinal oxidation is highly tissue specific during development. Although several enzymes (alcohol dehydrogenases [ADH], short-chain dehydrogenases/reductases) that catalyze retinol oxidation to retinal have been described, only ADH3 and retinal dehydrogenase 10 have recently been shown to play a role in embryogenesis (4,5). On the other hand, the enzymes (RALDH) that convert retinal to RA are well characterized and their specific role in RA formation during development has been clearly established (6).

RALDH are cytoplasmic enzymes that include 3 isozymes, now commonly referred to as RALDH1–3, the specificities of which for retinal substrates have been determined (7–9). RALDH1–3 begins to express at embryonic day (E) 9, E7.5, and E8.75, respectively, in mouse embryos (10,11), indicating that the fetus can synthesize its own RA after E7.5 and needs RA from the maternal circulation for early embryonic development. Among the RALDH1–3, RALDH2 is indispensable, generating RA during fetal life, as evidenced by its expression pattern in the embryo and the early embryonic lethality of RALDH2 knockout mice (12). RALDH1 appears to play a role in RA generation, because it is needed for the maintenance of adulthood (6). RALDH3 expression is highly restricted to the ocular and nasal regions and its deletion causes choanal atresia with respiratory death in early postnatal life (13).

The enzyme which catalyzes RA in the fetus, CYP26A1, is a member of the cytochrome P450 system and is expressed from E8 onwards in mice. CYP26A1 inactivation causes spina bifida resembling the teratogenic effects of RA excess and this phenotype is rescued by RALDH1 inactivation (14). RALDH2 and CYP26A1 set the fine-tuning of RA levels in many embryonic tissues. The enzymatic steps involved in the activation of retinol and degradation of RA are summarized in Figure 1.

Kidney development

Human kidneys begin to develop at 4–5 wk of gestation. During nephrogenesis, inductive interactions between the ureteric bud epithelium and metanephric mesenchyme result in the formation of the collecting duct system and optimal nephron number. These 2 developmental pathways are regulated by several
transcriptional factors and protooncogenes, polypeptide growth factors acting as signaling molecules, and their receptors. Gene disruption studies have shown the involvement of at least 11 genes that are crucial for metanephric kidney development (15). They are: WT1 (a transcriptional factor with a zinc finger domain), Pax2 (a transcriptional factor of the paired box family), BF-2 (a transcriptional factor of the winged helix family), c-ret (a receptor tyrosine kinase, a protooncogene), GDNF (glial cell line-derived neurtrophic factor), a member of the transforming growth factor-beta (TGF-\(\beta\)) family and ligand for c-ret, PDGFR\(\beta\) (platelet-derived growth factor, a receptor tyrosine kinase), PDGF B (a PDGF and ligand for PDGFR\(\beta\)), BMP-7 (bone morphogenetic protein, another member of the TGF-\(\beta\) family), Wnt-4 (a secreted glycoprotein), \(\alpha\)8\(\beta\)1, and \(\alpha\)3\(\beta\)1 (integrins). Figure 2 illustrates the essential events that take place during early nephrogenesis and some of the genes involved in this process.

By \(~36\) wk of gestation, new nephron formation is almost complete. Recent studies indicate that an unfavorable prenatal environment, such as vitamin A deficiency or protein-energy malnutrition, profoundly influences the process of nephrogenesis (16).

**Vitamin A and congenital nephron number**

In the early 1950s, Wilson et al. (17) observed that maternal vitamin A deficiency resulted in renal hypoplasia in rats that could be prevented by vitamin A administration to pregnant animals, suggesting the direct involvement of vitamin A in kidney development. Studies on RA receptor (RAR) knockout mice demonstrated the specific role of vitamin A in renal organogenesis (18). Furthermore, renal agenesis was found in mice deficient in the RA-generating enzyme RALDH2, indicating the need for RA for proper kidney development (12). RA restored nephron endowment to normal in the offspring of rats subjected to maternal protein restriction (19). Recent studies have shown that, in kidney development, RA acts on mesenchymal cells expressing RAR\(\alpha\) and \(\beta\), stimulating these cells to release key branching morphogens (20,21). RA has been found to induce kidney tubulogenesis in tissue culture by enhancing the deposition of laminin (22). Retinoids promote branching nephrogenesis in the E14 fetal kidney in vitro (23). Interestingly, in vitro studies have revealed that retinoids modulate nephron number in a dose-dependent manner, suggesting the need for optimal retinoid levels in the fetal kidney for normal nephrogenesis. This is supported by the fact that even modest maternal

**FIGURE 1** Metabolism and action of vitamin A. Retinyl esters and \(\beta\)-carotene from the diet are converted into retinol, which is esterified to retinyl esters in the liver by lecithin retinyl acyl transferase (LRAT). In the liver, retinyl esters are hydrolyzed by retinyl ester hydrolase (REH) to retinol that is successively oxidized to retinol by ADH and to RA by RALDH. RA is also generated from retinol formed by the cleavage of \(\beta\)-carotene. RA enters the nucleus and binds to RAR and retinoic acid x receptor (RXR) that regulate target genes. RA also undergoes oxidation by CYP26A1 to polar metabolites. * indicates the possible dietary and metabolic routes that may affect RA homeostasis, which may result in impaired nephrogenesis, perhaps ultimately leading to the development of hypertension in adulthood.

**FIGURE 2** (from vegetable sources) 

\[\text{Retinyl esters} \quad \xrightarrow{\text{circulated bound to RBP}} \quad \text{Retinol} \quad \xrightarrow{\text{REH}} \quad \text{Retinoic acid} \quad \xrightarrow{\text{CYP26A1}} \quad \text{Polar metabolites} \]

\[\text{Nucleus} \quad \text{RAR} \quad \text{RXR} \quad \text{DNA} \]

Regulation of target genes, such as: lim-1, RAR\(\alpha\)/\(\beta\), c-ret and EGFR expressed during kidney development.
vitamin A deficiency (a 50% decrease in circulating vitamin A concentrations) reduces nephron number by 20% in 21-d-old fetuses (24). In that study, the authors also observed a close correlation between nephron number and circulating vitamin A in term fetuses. Recent experiments compared vitamin A levels in pregnant women from Bangalore (India) and Montreal (Canada) with the kidney sizes of their offspring and determined that circulating retinol concentrations and kidney sizes were lower in the Bangalore group compared with the Montreal group (25).

Several genes expressed during renal organogenesis that are regulated by RA have been identified. Transcriptional factors, such as the Hox family, hepatic nuclear factor 1, lim-1, RARα2, and β2, are potential targets of RA. In addition, c-ret, epidermal growth factor receptor, and transferrin receptor, which are important for nephron formation, are regulated by RA (26). Thus, a growing number of studies point to the strong involvement of vitamin A in nephrogenesis, with adequate vitamin A supply being crucial in determining final nephron numbers.

**Nephron number and primary hypertension**

Recently, several studies provided evidence that low nephron number, as a result of prenatal growth restriction, leads to hypertension later in life (27,28). Restriction of food or protein during pregnancy elicits a lower number of nephrons with the development of hypertension in adult offspring (29). Using the conditional knockout approach, Poladia et al. (30) demonstrated a link between reduced nephron number and hypertension in mice. In a recent investigation, hypertensive patients had fewer glomeruli and larger mean glomerular volume than nonhypertensive people (31). A wide variance in nephron number has been observed in adults (0.3–1.3 million nephrons/kidney), which was once considered normal. However, Brenner et al. (32) have suggested that humans at the low end of the nephron endowment spectrum are susceptible to primary hypertension due to a relatively high glomerular filtration rate in each available nephron. This is supported by animal experiments showing lower nephron number in inbred hypertensive compared with normotensive control rats (33). In another study, a significant decrease in nephron number in mice, because of heterozygous mutations of the GDNF gene, resulted in the development of hypertension in adult offspring (34). An interesting observation was reported by Keller et al. (35), who compared the kidneys of 10 people who had died in accidents and who had hypertension with kidneys from 10 age-matched, normotensive controls. They discovered that the kidneys from hypertensive people had significantly fewer glomeruli (46.6%) per kidney than the matched controls. In addition, glomerular volume was greater (233% of control values) in hypertensive kidneys, than the matched controls. In addition, glomerular volume was greater, indicating that glomeruli overwork in people with hypertension to restore total glomerular volume per kidney to normal. In another recent investigation, Hoy et al. (36) noted racial differences in nephron number in Australian adults. Aboriginals had significantly fewer nephrons compared with Caucasians, with a higher incidence of end-stage renal disease in the former group. These studies strongly support the hypothesis that reduced nephron endowment results in glomerular hypertrophy, which may lead to hypertension later in life.

In conclusion, recent experiments in mice and humans support the hypothesis that perturbations in maternal nutrition have a greater impact on nephrogenesis, which may evoke hypertension in later life. One nutritional factor that affects fetal renal development is insufficient vitamin A. Vitamin A deficiency may occur at the nutrition level or through a defect in metabolism (Fig. 1). Potentially reversible maternal nutritional vitamin A deficiency is widespread in developing countries, so that infants born to these mothers may have suboptimal nephrons and may be at a high risk of developing primary hypertension later in life. If this is the case, the public health implications for developing countries are enormous. In developed countries, maternal vitamin A deficiency is rare. However, congenital nephron number varies widely, suggesting that suboptimal RA levels are caused by the possible presence of common variants of vitamin A-metabolizing enzymes (see Fig. 1). Future research identifying common variant enzymes involved in vitamin A metabolism, such as RALDH2, which is essential for fetal RA synthesis, or CYP26A1, which catabolizes RA, should clarify the role of these vitamin A-metabolizing enzymes in nephrogenesis and hypertension.

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**Literature Cited**


