Chronic effects of dietary vitamin D deficiency without increased calcium supplementation on the progression of experimental polycystic kidney disease

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Rangan GK, Schwensen KG, Foster SL, Korgaonkar MS, Peduto A, Harris DC. Chronic effects of dietary vitamin D deficiency without increased calcium supplementation on the progression of experimental polycystic kidney disease. Am J Physiol Renal Physiol 305: F574–F582, 2013. First published May 22, 2013; doi:10.1152/ajprenal.00411.2012.—Increasing evidence indicates that vitamin D deficiency exacerbates chronic kidney injury, but its effects on renal enlargement in polycystic kidney disease (PKD) are not known. In this study, male Lewis polycystic kidney disease (LPK) rats received a normal diet (ND; AIN-93G) supplemented with or without cholecalciferol (vitamin D-deficient diet, VDD; both 0.5% calcium), commenced at either postnatal week 3 (until weeks 10–20; study 1) or from week 10 (until week 20; study 2). Levels of 25-hydroxy vitamin D were reduced in groups receiving the VDD (12 ± 1 nmol/l vs. 116 ± 5 in ND; P < 0.001). In study 1, food intake and weight gain increased by ∼25% in LPK rats receiving the VDD ad libitum, and at week 20 this was associated with a mild reduction in the corrected serum calcium (SCa2+, 7.4%) and TKW:BW ratio (8.8%), and exacerbation of proteinuria (87%) and hypertension (19%; all P < 0.05 vs. ND). When LPK rats were pair-fed for weeks 3–10, there was a further reduction in the SCa2+ (25%) and TKW:BW ratio (22%) in the VDD group (P < 0.05 vs. ND). In study 2, the VDD did not alter food intake and body weight, reduced SCa2+ (7.7%), worsened proteinuria (41.9%), interstitial monocyte accumulation (26.4%), renal dysfunction (21.4%), and cardiac enlargement (13.2%, all P < 0.05), but there was a trend for a reduction in the TKW:BW ratio (13%, P = 0.09). These data suggest that chronic vitamin D deficiency has adverse long-term actions on proteinuria, interstitial inflammation, renal function, and cardiovascular disease in PKD, and these negate its mild inhibitory effect on kidney enlargement.

vitamin D; polycystic kidney disease, kidney enlargement, hypertension

POLYCYSTIC KIDNEY DISEASE (PKD) is the most common fatal inherited renal disorder in the world (5). It consists of a family of diseases that arise due to mutations in cilia-related proteins and are characterised by the formation of multiple cysts in the kidney accompanied by renal fibrosis and inflammation as well as hypertension (4, 15, 17). There are several variants of PKD which differ according to the mode of inheritance, underlying genetic mutation and pattern of cystic renal disease. The most common form, autosomal dominant (AD) PKD, has an incidence of approximately one in every 1,000 live births and is estimated to affect 6.5 million people worldwide (5). Autosomal recessive (AR) PKD has an approximate incidence of one in every 20,000 live births, with an estimated 300,000 persons affected globally (45). Nephronophthisis (NPHP) has an autosomal recessive mode of inheritance, and is one of the most frequent genetic disorders causing kidney failure in children and adolescents (41). End-stage kidney failure is the most serious and life-threatening complication of all types of PKD, and the estimated 5-yr survival of PKD patients on dialysis is only 67% (31). Therefore, simple, cost-effective and safe therapies to prevent kidney failure due to PKD would eliminate premature death in these patients and also reduce the overall demand for chronic dialysis throughout the world (39).

Vitamin D is a pro-hormone, whose metabolites are well known for their role in maintaining calcium homeostasis and normal bone health (18). The latter is achieved by mediating calcium reabsorption in the intestine and distal nephron, and through direct effects on osteoblasts (11). In addition to the classic (calcemic) functions, vitamin D has a number of important non-classic (non-calcemic) actions (3), which include the promotion of cellular differentiation and suppression of proliferation (34); anti-inflammatory, immunomodulatory, and antifibrotic effects (38); and inhibition of renin gene transcription, hypertension, and left ventricular hypertrophy (6). Consequently, there is increasing evidence that inadequate levels of vitamin D can lead to or exacerbate cardiovascular disease, type 2 diabetes, cancers of the gastrointestinal and urogenital tracts, and autoimmune disorders (20). In nephropathy, there is already substantial experimental and clinical evidence that both adequate vitamin D intake as well as the exogenous administration of vitamin D analogs are renoprotective (38, 40), such that randomized clinical trials in humans to reduce the progression of chronic kidney disease (CKD) and attenuate cardiovascular mortality are currently being planned or are in progress (10, 23, 38).

The role of vitamin D in renal and cyst growth, tubulointerstitial disease, and hypertension associated with PKD is not known. Vitamin D deficiency is a prevalent condition and estimated to affect up to 50% of the community (20) and is accentuated by CKD, because proximal tubular epithelial cells are an active site of calcitriol synthesis, and serum levels of calcitriol decline as renal injury progresses (21). In humans, low vitamin D levels were directly correlated to increased renal interstitial inflammation in glomerulonephritis (44) and proteinuria in the NHANES III national cohort registry (9) and

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were an independent predictor of disease progression and death in stages 2–5 CKD (32). Collectively, these studies suggest that vitamin D may potentially modulate the progression of PKD. Therefore, the aim of this study was to determine the long-term effects of vitamin D deficiency on cystic renal disease and hypertension in PKD. We hypothesized that vitamin D deficiency would worsen cystic renal disease and hypertension. To test this hypothesis, Lewis PKD (LPK) rats (a hypertensive rodent model of PKD which phenotypically resembles ARPKD but is a genetic ortholog of human NPHP9) (24, 29, 35) received a normal diet (ND; AIN-93G formulation) supplemented with or without cholecalciferol (vitamin D-deficient diet; VDD; both diets contained 0.5% calcium), commencing at either postnatal week 3 (and continuing until weeks 10 and 20; study 1) or from week 10 (until week 20; study 2).

MATERIALS AND METHODS

Animals

Animals were housed at the Research Facility in the Institute of Clinical Pathology and Medical Research (ICPMR; Westmead Hospital) under standard conditions (artificial lighting, light-dark cycle 1800–0600) and were allowed free access to tap water. LPK rats and Lewis/SSN rats were obtained from the Westmead breeding colony (29, 35). The LPK rat colony was derived from a single founder homozygous male and female breeder pairs. All protocols and procedures were approved by the Animal Ethics Committee, Westmead Hospital (Protocol 4100), and animals were handled according to the guidelines of the National Health and Medical Research Council of Australia.

Experimental Design

Three studies were undertaken.

Study 1. The effects of vitamin D deficiency on the progression of early-stage PKD was assessed by feeding male rats either a semipurified AIN-93G diet (ND) supplemented with or without cholecalciferol (1,000 IU/kg; VDD), from postnatal week 3 and continuing until week 20. Study 1 consisted of two parts: 1) study 1a: groups of LPK (n = 9 each) and Lewis rats (n = 4–6 each) were fed the experimental diets ad libitum and euthanized at two time points (postnatal weeks 10 and 20; total rats n = 56); and 2) study 1b: one group of male Lewis rats (n = 3 each) were fed the experimental diets ad libitum from week 3 and killed at week 20 (total n = 6).

Study 2. The effect of vitamin D deficiency on the progression of late-stage PKD was assessed by commencing the experimental diets at week 10 and continuing until week 20 (n = 6–7 each for the LPK groups and n = 3 for Lewis groups; total rats n = 19). The rats received food ad libitum.

Study 3. Because prepubertal LPK and Lewis rats fed the VDD in study 1 had increased food intake compared with those receiving the ND (see RESULTS), groups of LPK (n = 10 each) and Lewis rats (n = 4 each) were pair-fed the diets from week 3 and euthanized at week 10 (total rats n = 28).

Experimental Diets and Monitoring of Food Intake

The vitamin D-sufficient and -deficient diets were based on the AIN-93G formulation and prepared by a commercial vendor (Speciality Feeds, Glen Forrest, Western Australia) using pure raw materials. The calculated nutritional parameters were as follows: 19.4% protein, 7.0% total fat, and 56.9% total carbohydrate. The diets (12-mm-diameter pellets) were not irradiated and stored according to the manufacturer’s recommendations. Three different batches of diets were used in this study. Each batch (consisting of equal amounts of the vitamin D-sufficient and -deficient diets, 10–20 kg each) was manufactured on the same date and used the same raw materials. In addition, for each experimental study, equal numbers of rats in the vitamin D-deficient and -sufficient groups received the same batch of a diet at any one time. Because intracellular calcium signaling is altered in PKD (1, 43) and as there are no previous published data in PKD, both the vitamin D-sufficient and -deficient diets contained the same amount of calcium (0.5%). Food intake was monitored daily at 0900 by weighing the pellets remaining in the food hoppers at the end of a 24-h period. In study 1 and 2, food was provided ad libitum. In study 3, groups receiving the VDD were pair-fed the same amount as the ND groups.

Assessment of Serum 25-Hydroxy Vitamin D, Corrected Serum Calcium and Phosphate, and Renal Function

Serum for analysis of 25-hydroxy (OH) vitamin D, calcium, phosphate, urea, creatinine, and albumin were collected at the time of death, and analyzed by the ICPMR at Westmead Hospital. To assess urine volume, proteinuria, and creatinine clearance, rats were placed in metabolic cages for 16 h. Creatinine clearance was corrected for body surface area.

Assessment of Kidney Enlargement

This was assessed by two methods: 1) total kidney-to-body weight ratio (TKW-BW) was the weight of both kidneys at the time of death, corrected for body weight and expressed as a percentage; 2) serial assessment of total kidney volume by magnetic resonance imaging (MRI) was assessed at weeks 9 and 19 in a subset of LPK animals in study 1a (ND, n = 4; VDD, n = 5). MRI was performed using a clinical 3 Tesla GE SIGNA HDxt human MR scanner running version 15 M4 software in conjunction with A cylindrical Mayo transmit/receive coil (Mayo Clinic Medical Devices) clinically used for human wrist imaging (Department of MRI, Westmead Hospital). Animals were anesthetized with isoflurane inhalation (2–5%) and scanned supine and tail first using a coronal and axial T2-W 3D FIESTA sequence. The scan parameters for the two sequences were coronal: FOV = 10 cm, phase FOV = 1 cm, TR/TE = 12.1 ms/4.1 ms, flip angle = 45°, 352*256 acq. matrix, R/L Freq direction, 32 locations per slab with effective slice thickness 0.8 mm, 2 NEX, acquisition time = 6 min 44 s; axial: FOV = 9 cm, phase FOV = 0.7 cm, TR/TE = 13.7 ms/4.3 ms, flip = 45°, 352*256 acq. matrix, R/L Freq direction, 2 locations per slab with effective slice thickness 0.8 mm, 2 NEX, acquisition time = 5 min 26 s. Both the left and right kidneys were segmented from the coronal volume acquisition, using a semiautomatic fast marching segmentation algorithm implemented in the 3D SLICER toolkit (www.slicer.org). The analyst (K. G. Schwensen) was blinded to the categorization of the animals. Kidney volumes were computed from the segmented images. Due to differences in body weight in study 1a (see RESULTS), the total kidney volume (TKV) was divided by the corresponding body weight (BW) at weeks 9 and 19 and expressed as a percentage.

Assessment of Renal Histology

Midcoronal sections of kidney were immersion-fixed, in either 10% neutral buffered formalin or methyl-carnoy solution for 24 h. For the assessment of percent cyst area and interstitial fibrosis, paraffin sections were used. Cysts were determined using whole-slide scanning (Aperio; Aperio Technologies). To perform quantitative image analysis, sections were digitized with a whole-slide scanner (Scanscope CS2, Aperio, CA). The calculated nutritional parameters were as follows: 19.4% protein, 7.0% total fat, and 56.9% total carbohydrate. The diets (12-mm-diameter pellets) were not irradiated and stored according to the manufacturer’s recommendations. Three different batches of diets were used in this study. Each batch (consisting of equal amounts of the vitamin D-sufficient and -deficient diets, 10–20 kg each) was manufactured on the same date and used the same raw materials. In addition, for each experimental study, equal numbers of rats in the vitamin D-deficient and -sufficient groups received the same batch of a diet at any one time. Because intracellular calcium signaling is altered in PKD (1, 43) and as there are no previous published data in PKD, both the vitamin D-sufficient and -deficient diets contained the same amount of calcium (0.5%). Food intake was monitored daily at 0900 by weighing the pellets remaining in the food hoppers at the end of a 24-h period. In study 1 and 2, food was provided ad libitum. In study 3, groups receiving the VDD were pair-fed the same amount as the ND groups.

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Table 1. Effects of early vitamin D deficiency in rats receiving the diet ad libitum (study 1a)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time Point, wk</th>
<th>Lewis + ND</th>
<th>Lewis + VDD</th>
<th>LPK + ND</th>
<th>LPK + VDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-OH vitamin D, mmol/l</td>
<td>10</td>
<td>114 ± 9</td>
<td>3 ± 3*</td>
<td>109 ± 3</td>
<td>7 ± 3†</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>138 ± 6</td>
<td>9 ± 3*</td>
<td>101 ± 9</td>
<td>17 ± 3†</td>
</tr>
<tr>
<td>Serum calcium, mmol/l</td>
<td>10</td>
<td>2.8 ± 0.1</td>
<td>2.8 ± 0.03</td>
<td>2.8 ± 0.06</td>
<td>2.6 ± 0.04†</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.8 ± 0.03</td>
<td>2.8 ± 0.02</td>
<td>2.7 ± 0.03</td>
<td>2.5 ± 0.08†</td>
</tr>
<tr>
<td>Serum phosphate, mmol/l</td>
<td>10</td>
<td>3.4 ± 0.4</td>
<td>2.6 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.8 ± 0.2</td>
<td>2.4 ± 0.05</td>
<td>2.7 ± 0.3</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>TKW:BW ratio, %</td>
<td>10</td>
<td>1.05 ± 0.04</td>
<td>0.76 ± 0.01*</td>
<td>4.85 ± 0.20</td>
<td>5.07 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.75 ± 0.03</td>
<td>0.64 ± 0.01*</td>
<td>7.93 ± 0.18</td>
<td>7.23 ± 0.26†</td>
</tr>
<tr>
<td>TKV:BW ratio, %</td>
<td>9</td>
<td></td>
<td></td>
<td>5.16 ± 0.61</td>
<td>5.31 ± 1.30</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td></td>
<td></td>
<td>8.75 ± 0.41</td>
<td>8.75 ± 0.18</td>
</tr>
<tr>
<td>24-h Urine volume, ml</td>
<td>10</td>
<td>8 ± 0</td>
<td>12 ± 2</td>
<td>14 ± 1*</td>
<td>20 ± 2†</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>5 ± 1</td>
<td>8 ± 0*</td>
<td>26 ± 2*</td>
<td>36 ± 2†</td>
</tr>
<tr>
<td>Urine protein:creatinine, mg/mmol</td>
<td>10</td>
<td>35 ± 22</td>
<td>56 ± 11</td>
<td>189 ± 24*</td>
<td>238 ± 28</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>15 ± 4</td>
<td>23 ± 3</td>
<td>372 ± 28*</td>
<td>696 ± 82†</td>
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<tr>
<td>Serum urea, mmol/l</td>
<td>10</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
<td>12 ± 1*</td>
<td>12 ± 1</td>
</tr>
<tr>
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<td>20</td>
<td>5 ± 0</td>
<td>6 ± 1</td>
<td>41 ± 3*</td>
<td>51 ± 12</td>
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<tr>
<td>Serum creatinine, μmol/l</td>
<td>10</td>
<td>34 ± 6</td>
<td>22 ± 1</td>
<td>38 ± 2</td>
<td>38 ± 2</td>
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<tr>
<td></td>
<td>20</td>
<td>28 ± 3</td>
<td>28 ± 5</td>
<td>124 ± 9*</td>
<td>152 ± 14</td>
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<tr>
<td>CrCl, μl.min⁻¹.cm⁻²</td>
<td>10</td>
<td>10.9 ± 1.1</td>
<td>13.0 ± 0.7</td>
<td>6.0 ± 0.3*</td>
<td>6.5 ± 0.4</td>
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<tr>
<td></td>
<td>20</td>
<td>9.1 ± 2.3</td>
<td>11.0 ± 0.5</td>
<td>1.8 ± 0.2*</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Cyst area, %</td>
<td>10</td>
<td></td>
<td></td>
<td>49.6 ± 1.9</td>
<td>48.6 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td></td>
<td>53.9 ± 0.6</td>
<td>52.8 ± 1.2</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>10</td>
<td>125 ± 8</td>
<td>125 ± 8</td>
<td>125 ± 7</td>
<td>125 ± 7</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>81 ± 6</td>
<td>85 ± 3</td>
<td>124 ± 7</td>
<td>148 ± 5†</td>
</tr>
<tr>
<td>HW:BW ratio, %</td>
<td>10</td>
<td>0.36 ± 0.02</td>
<td>0.32 ± 0.01</td>
<td>0.40 ± 0.01</td>
<td>0.38 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.28 ± 0.01</td>
<td>0.25 ± 0.01</td>
<td>0.42 ± 0.01</td>
<td>0.45 ± 0.02†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9 each for Lewis polycystic kidney disease (LPK) groups and n = 6 each for Lewis groups. VDD, vitamin D-deficient diet; ND, normal diet; TKW, total kidney weight; TKV, total kidney volume; BW, body weight; HW, heart weight. CrCl, endogenous creatinine clearance; BP, blood pressure. *P < 0.05 compared with the Lewis + ND group. †P < 0.05 compared with the LPK + ND group.

The percent area occupied by tissue or immunohistochemical stain was calculated using the positive pixel count algorithm. The percent cyst area was defined as the total section area minus the tissue area, divided by the total section area multiplied by 100.

Assessment of Cardiovascular Disease

Tail systolic blood pressure was measured noninvasively in conscious rats using a tail sensor (piezoplethysmography, MacLab, AD Instruments) and tail-cuff inflation and defined as the appearance of the tail arterial pulse wave with cuff deflation. Heart weight at the time of death, corrected for body weight, was also assessed.

Statistics

Results are presented as means ± SE. The data were analyzed with the JMP statistical software package (version 4.04, SAS Institute) and Prism (version 5, GraphPad Software). Comparisons between the
RESULTS

**Effects of Early Vitamin D Deficiency in LPK Rats Receiving the Diets Ad Libitum (Study 1)**

Serum levels of 25-OH vitamin D were reduced in groups receiving the VDD (Table 1). The corrected serum calcium did not differ between the diet groups in Lewis rats, whereas there was a mild reduction in LPK rats with vitamin D deficiency (Table 1). There was a trend for a reduction in serum phosphate ($P < 0.05$ and $0.07$ at week 10 and 20, respectively) in Lewis rats fed the VDD compared with the ND.

Lewis and LPK rats receiving the VDD had increased food consumption and body weight gain over time compared with the groups fed the ND (Fig. 1). By gross morphology, at the time of death, there was an increased deposition of abdominal visceral fat in vitamin D-deficient rats. The latter was verified in a separate study of Lewis rats (study 1b) showing that the total visceral abdominal adipose tissue mass was increased by the VDD (Lewis+ND: 17.2 ± 1.0 g; $P < 0.05$) and abdominal girth (Lewis+ND: 29.6 ± 1.0 vs. Lewis+VDD: 32.1 ± 1.0 g; $P < 0.05$) whereas rat length (tip of nose-to-body-to-tail length) (Lewis+ND: 35.9 ± 0.3 vs. Lewis+VDD: 36.4 ± 0.3 cm; $P$ not significant) was not altered. The increased adiposity was associated with a trend for an elevation in blood glucose in vitamin D-deficient LPK rats (week 10: ND: 9.6 ± 0.5 vs. VDD: 11.0 ± 0.5; $P = 0.17$; week 20: ND: 9.4 ± 0.4 vs. VDD: 12.3 ± 1.6 mmol/l; $P = 0.07$).

At weeks 9 and 10, kidney enlargement, as assessed by either the TKW:BW ratio or TKV:BW on MRI, was similar in both LPK groups (Fig. 2 and Table 1). At week 20, there was a 8.8% reduction in the TKW:BW ratio in the LPK rats receiving the VDD compared with the ND ($P < 0.05$) (Table 1), whereas the the TKV:BW ratio did not reach statistical significance in the MRI substudy (Table 1), presumably because of the smaller sample size. The reduction in the TKW:BW ratio with VDD was also present in the Lewis groups (Table 1).

Urine output increased in the week 10 ($P = 0.0055$) and week 20 ($P = 0.0011$) LPK groups receiving the VDD compared with the ND (Table 1). At week 20, proteinuria was exacerbated in the LPK+VDD compared with the LPK+ND group ($P = 0.0018$), whereas renal dysfunction (as assessed by the serum urea, serum creatinine, and the decline in creatinine clearance) was not affected (Table 1). By light microscopy, disease in LPK rats was characterized by diffuse tubular cyst formation affecting the cortex and outer medulla with relative sparing of the inner medulla (Fig. 3). However, there was no significant change between the LPK groups at weeks 10 and 20 in the percent cyst area (Table 1) or in the expression of renal Ki-67, CD68, and α-SMA, and renal interstitial fibrosis (data...
not shown). Last, hypertension was exacerbated in LPK rats receiving the VDD at week 20 (Table 1).

**Effects of Delayed Vitamin D Deficiency in LPK Rats Receiving Diets Ad Libitum (Study 2)**

In contrast to study 1, weight gain and food intake were similar in both groups (Fig. 4). At week 20, there was a trend for a reduction in the TKW:BW ratio and the percent cyst area in the VDD group compared with the ND group ($P = 0.09$ and $0.07$, respectively) (Table 2). However, proteinuria, creatinine clearance, interstitial monocyte accumulation, and the heart weight-to-body weight ratio were exacerbated in the VDD LPK group (Table 2), and there was also trend for an exacerbation of hypertension ($P = 0.09$). Renal cell proliferation and other markers of interstitial disease were not different between the two groups (Table 2).

**Effects of Early Vitamin D Deficiency in Pair-Fed Lewis and LPK Rats (Study 3)**

To exclude the confounding effects of weight gain in study 1, the experiment was repeated in rats that were pair-fed the diets from postnatal weeks 3 to 10. As shown in Fig. 5, body weight gain was identical in the Lewis and LPK rats, receiving either diet. At week 10 in LPK rats, the corrected serum calcium was reduced by 25% in the VDD group (Table 3). In addition, there was a 23% reduction in the TKW:BW ratio and a trend for a decrease in the percent cyst area ($P = 0.10$) (Table 3). Urine volume and proteinuria was also reduced by 41 and 34%, respectively, at week 10 in the LPK+VDD group compared with the LPK+ND group (Table 3).

**DISCUSSION**

The results of this study suggest that vitamin D has differential and stage-specific effects on kidney enlargement, renal function, and hypertension in PKD. The key findings of were as follows: 1) vitamin D deficiency, during either the early or late phase of PKD, caused a mild, but consistent, reduction in kidney enlargement; 2) during the late phase of PKD, vitamin D deficiency exacerbated proteinuria, renal dysfunction, interstitial monocyte accumulation, and markers of cardiovascular disease; and 3) food intake and weight gain were increased in Lewis and LPK rats when the VDD was commenced during the prepubertal period but not when it was started in adult rats.

The LPK rat model is a robust model of cystic renal disease, particularly useful for assessing the long-term cardiorenal effects of treatment interventions in PKD. The model phenotypically resembles ARPKD but is genetically orthologous to human NPHP9 (24) and arises from a point mutation in the NIMA (never in mitosis gene a)-related kinase 8 (Nek8) gene (24), as in the juvenile cystic kidney (jck) murine model of PKD (16, 36) and in human NPHP9 (28). However, because both Nek8 and Pkd1 (the gene mutated in the majority of patients with ADPKD) function in a common pathway to regulate cystogenesis (26, 37), the results of the present study

![Fig. 3. Effect of early vitamin D deficiency (study 1) on renal histology. Shown are representative whole-slide digital images of sections stained with Sirius red from the experimental groups at weeks 10 and 20.](http://ajprenal.physiology.org)
have potential relevance for all types of PKD. Phenotypically, the LPK rat is characterized by diffuse collecting duct ectasia, hypertension, and cardiac enlargement that progresses slowly towards end-stage renal failure from weeks 3 to 20 (29, 35). From postnatal weeks 3 to 10, renal disease is predominantly due to collecting duct dilation which causes a sixfold increase in kidney enlargement, whereas from weeks 10 to 20 the disease is characterized by the development of interstitial fibrosis, renal impairment, and hypertension.

The effects of dietary vitamin D deficiency on kidney enlargement in experimental models of PKD have not previously been reported. In addition, there are little published data in humans with PKD. In a preliminary cross-sectional study, Gitomer et al. (14) reported that the severity of total kidney volume (as assessed by MRI) was negatively correlated with serum 25-OH vitamin D levels in humans with ADPKD. However, the sample size in this study was small, and the causal effects between vitamin D deficiency and kidney enlargement could not be established. In the present study, we unexpectedly observed that a VDD had a mild inhibitory effect on the progression of kidney enlargement in LPK rats. The reduction in the TKW:BW ratio in vitamin D-deficient LPK rats was 8.8% (at week 20 in study 1) and 22.4% (at week 10 in study 3), and in study 2 there was a trend for a reduction at week 20 (P = 0.09). In studies 2 and 3, there was also a trend for a reduction in the percent cyst area (P = 0.07 and 0.10, respectively). In addition, in study 1 the kidney weight-to-body weight ratio was also reduced in vitamin D-deficient Lewis rats (26.2% at week 10 and 14.7% at week 20), but this was not noted in studies 2 and 3.

The mechanisms underlying the reduction in kidney enlargement in vitamin D-deficient rats in the present study require further study.

### Table 2. Effects of delayed vitamin D deficiency in rats receiving the diet ad libitum (study 2)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lewis + ND</th>
<th>Lewis + VDD</th>
<th>LPK + ND</th>
<th>LPK + VDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-OH vitamin D, nmol/l</td>
<td>106 ± 7</td>
<td>0 ± 0</td>
<td>153 ± 14</td>
<td>26 ± 1†</td>
</tr>
<tr>
<td>Serum calcium, mmol/l</td>
<td>2.7 ± 0.006</td>
<td>2.7 ± 0.01</td>
<td>2.6 ± 0.03*</td>
<td>2.4 ± 0.1†</td>
</tr>
<tr>
<td>Serum phosphate, mmol/l</td>
<td>2.5 ± 0.1</td>
<td>2.7 ± 0.2</td>
<td>2.8 ± 0.1*</td>
<td>3.2 ± 0.008†</td>
</tr>
<tr>
<td>TKW:BW ratio, %</td>
<td>0.64 ± 0.03</td>
<td>0.65 ± 0.02</td>
<td>6.78 ± 0.37*</td>
<td>5.87 ± 0.30</td>
</tr>
<tr>
<td>24-h Urine volume, ml</td>
<td>8 ± 0</td>
<td>7 ± 0</td>
<td>30 ± 2*</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>Urine protein:creatinine, mg/mmol</td>
<td>21 ± 3</td>
<td>33 ± 4</td>
<td>332 ± 34*</td>
<td>471 ± 27†</td>
</tr>
<tr>
<td>Serum urea, mmol/l</td>
<td>5 ± 0</td>
<td>5 ± 0</td>
<td>44 ± 2*</td>
<td>50 ± 2</td>
</tr>
<tr>
<td>Serum creatinine, μmol/l</td>
<td>24 ± 2</td>
<td>23 ± 2</td>
<td>142 ± 8*</td>
<td>158 ± 5</td>
</tr>
<tr>
<td>CrCl, μl·min⁻¹·cm⁻²</td>
<td>13.5 ± 1.4</td>
<td>13.1 ± 2.0</td>
<td>1.4 ± 0.1*</td>
<td>1.1 ± 0.1†</td>
</tr>
<tr>
<td>Cyst area, %</td>
<td>58.0 ± 0.9</td>
<td>53.6 ± 2.1</td>
<td>1.57 ± 0.07*</td>
<td>1.58 ± 0.15</td>
</tr>
<tr>
<td>Renal Ki-67, %</td>
<td>0.31 ± 0.02</td>
<td>0.29 ± 0.04</td>
<td>3.90 ± 0.26*</td>
<td>4.93 ± 0.60†</td>
</tr>
<tr>
<td>Renal CD68, %</td>
<td>0.13 ± 0.03</td>
<td>0.21 ± 0.07</td>
<td>10.10 ± 0.10</td>
<td>12.53 ± 0.75</td>
</tr>
<tr>
<td>Renal a-SMA, %</td>
<td>1.2 ± 0.08</td>
<td>1.6 ± 0.3</td>
<td>31.4 ± 1.9*</td>
<td>28.5 ± 1.9</td>
</tr>
<tr>
<td>Renal interstitial fibrosis, %</td>
<td>1.3 ± 0.2</td>
<td>1.6 ± 0.3</td>
<td>31.4 ± 1.9*</td>
<td>28.5 ± 1.9</td>
</tr>
<tr>
<td>HW:BW ratio, %</td>
<td>0.27 ± 0.003</td>
<td>0.27 ± 0.004</td>
<td>0.38 ± 0.02*</td>
<td>0.43 ± 0.01†</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>90 ± 4</td>
<td>87 ± 1</td>
<td>100 ± 10*</td>
<td>122 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6–7 each for LPK groups and n = 3 each for Lewis groups. SMA, smooth muscle actin. *P < 0.05 compared with the Lewis + ND group. †P < 0.05 compared with the LPK + ND group.
Effects of early vitamin D deficiency in rats receiving the diet by pair-feeding (study 3)

Published data on the adverse long-term effects of vitamin D deficiency have demonstrated exacerbation of hypertension (4). These data support previous observations that they could respond to changes in the circulating level of vitamin D. On the other hand, in the current study there was a correlation between the magnitude of reduction in the serum calcium and kidney enlargement, and therefore vitamin D-induced hypocalcemia might also explain these results. Although the latter may appear to be counterintuitive in the context of PKD (5), previous studies show that impaired hepatic regeneration in vitamin D-deficient rats was explained by hypocalcemia (rather than the level of vitamin D) (13). Collectively, these hypotheses require further analysis in future studies using in vitro and in animal models of PKD.

Regardless of the mechanisms of the reduction in kidney enlargement, the net effects of vitamin D deficiency in LPK rats suggest that it is most likely to be detrimental to the course of PKD. This conclusion is primarily because of the data in study 2, which showed that vitamin D deficiency during the established phase of PKD worsened the progression of not only chronic renal injury (proteinuria, interstitial monocyte accumulation, and renal dysfunction) and markers of cardiovascular injury (cardiac enlargement and there was a trend for an exacerbation of hypertension). These data support previous published data on the adverse long-term effects of vitamin D deficiency in CKD (2, 12, 19), hypertension, renin gene transcription (30), and cardiac hypertrophy (8). The results of study 1 also support this hypothesis in PKD. However, due to the weight gain and increased food consumption that occurred in rats receiving the VDD, it is not possible to differentiate whether the exacerbation of cardiorenal injury in study 1 was due to the vitamin D deficiency per se and/or the metabolic effects of weight gain and insulin resistance (33).

An intriguing observation in the present study (and unrelated to the pathogenesis of PKD) was that the commencement of the VDD led to increased food consumption and subsequent weight gain in prepubertal (but not in adult) LPK/Lewis rats. In a separate study of Lewis rats, we verified that the increase in weight gain was associated with greater abdominal visceral adipose tissue deposition. The mechanisms of these effects are not clear, have not been previously reported, and could be strain specific. The manufacturer of the diets used in the present study reported that batch-to-batch variations are minimal and unlikely to explain the effects observed in study 1. Furthermore, as discussed in MATERIALS AND METHODS, studies of the vitamin D-sufficient and -deficient groups were performed in parallel, using the same batch of diet. Certainly, the increased intake in the vitamin D-deficient group in study 1 could reflect a compensatory feeding behavior to overcome the nutritional effects of reduced serum calcium. This hypothesis has been suggested in previous studies of vitamin D-deficient pregnant rats who preferentially self-select a diet that is high in calcium (7). If this is the case, it indicates that there might be specific neuroendocrine effects on feeding-related signals from adipose tissue, the gut, and brain (42) during the early and developmental phase of life (but to a lesser extent in adulthood), in relation to reductions in serum calcium associated with vitamin D deficiency. It would be an interesting hypothesis to test in further experimental studies, in light of the relationship between vitamin D deficiency and metabolic syndrome that has been noted in cross-sectional studies in humans (27).

In conclusion, this study demonstrates that vitamin D deficiency has divergent effects on kidney enlargement, renal function, and hypertension in PKD. Although vitamin D deficiency has inhibitory actions on kidney enlargement in LPK rats, this beneficial effect was mild and negated by adverse long-term effects on proteinuria, renal interstitial inflammation and function, and cardiovascular disease. Further studies should be undertaken to verify the effects of vitamin D deficiency in PKD. This conclusion is primarily because of the data in study 2, which showed that vitamin D deficiency during the established phase of PKD worsened the progression of not only chronic renal injury (proteinuria, interstitial monocyte accumulation, and renal dysfunction) and markers of cardiovascular injury (cardiac enlargement and there was a trend for an exacerbation of hypertension). These data support previous published data on the adverse long-term effects of vitamin D deficiency in CKD (2, 12, 19), hypertension, renin gene transcription (30), and cardiac hypertrophy (8). The results of study 1 also support this hypothesis in PKD. However, due to the weight gain and increased food consumption that occurred in rats receiving the VDD, it is not possible to differentiate whether the exacerbation of cardiorenal injury in study 1 was due to the vitamin D deficiency per se and/or the metabolic effects of weight gain and insulin resistance (33).
ciency in genetically orthologous models of ADPKD as well as the effects of increased calcium supplementation in vitamin D deficiency on kidney enlargement in PKD. In parallel, it would be of interest to evaluate the associations of vitamin D deficiency in a large clinical cohort of PKD. Collectively, these studies should provide further evidence for the effects of vitamin D deficiency on the progression of human PKD.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


