Renal function and structure in a rat model of arterial calcification and increased pulse pressure

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Gaillard V, Jover B, Casellas D, Cordaillat M, Atkinson J, Lartaud I. Renal function and structure in a rat model of arterial calcification and increased pulse pressure. Am J Physiol Renal Physiol 295: F1222–F1229, 2008. First published August 20, 2008; doi:10.1152/ajprenal.00081.2008.—Clinical studies suggest a strong link between tissue calcification and pressure hyperpulsatility in end stage renal disease patients. Using a Wistar rat model of arterial calcification and hyperpulsatility [vitamin D and nicotine (VDN) treatment], we evaluated the relative importance of tissue calcification and hyperpulsatility in the etiology of renal failure. VDN rats showed significant increases in aortic wall calcium content (50 times; 992 ± 0.32 μmol/g dry wt) and pulse pressure (1.5 times; 61 ± 4 vs. control 40 ± 2 mmHg). Significant renal calcification (16 times; 124 ± 27 vs. control 8.1 ± 0.7 μmol/g dry wt) occurred mainly within the media of the preglomerular vasculature and in the areas of interstitial fibrosis in VDN. Extensive renal damages (5 times; 26 ± 5% of collapsed-atrophic or sclerotic glomeruli, or glomerular cysts vs. control 5.2 ± 0.3%; 28 times; 61 ± 12% areas of focal, cortical areas exhibiting interstitial fibrosis per section vs. control 2.2 ± 0.6%) were observed histologically. The glomerular filtration rate significantly decreased (880 ± 40 vs. control 1,058 ± 44 μl·min⁻¹·g kidney wt⁻¹). Albuminuria increased six times (1.6 ± 0.4 vs. control 0.27 ± 0.04 mg/h). There were significant linear relationships between albuminuria and pulse pressure (r² = 0.408; n = 24) or renal calcium content (r² = 0.328; n = 24; P < 0.05) and between glomerular filtration rate and pulse pressure (r² = 0.168; n = 27). To our knowledge, this study provides the first evidence of links between both 1) hyperpulsatility and renal dysfunction, and 2) renal calcification and renal dysfunction. Given the increasing frequency of end-stage renal disease, this model could prove useful for preclinical evaluation of drugs that prevent or attenuate hyperpulsatility and/or tissue calcification.

pulse pressure; renal failure; albuminuria; renal histology

In patients suffering from end stage renal disease (ESRD), there are strong links between chronic renal failure and tissue (renal) calcification (4, 6, 37, 38). The mechanisms responsible are complex, and work on this problem has concentrated so far on altered phosphocalcium metabolism. Calcification also occurs in other tissues such as the aorta in these patients (17, 27–29), and this leads to increased arterial pulse pressure (hyperpulsatility; Refs. 17, 27–29). O’Rourke and Safar (35), after revisiting the work of Byrom, suggested that organs with low vascular resistance such as the kidney are susceptible to the damaging effect of increased central pulse pressure. An increase in pulse pressure is one the most frequent cardiovascular risk factors in ESRD (18, 27–29).

We approached the problem therefore of whether hyperpulsatility in addition to renal calcification is involved in the etiology of human renal failure. We used a preclinical, animal model, where hypervitaminosis D [plus nicotine (VDN)] produces, 3 mo later, aortic wall calcification with increased pulse pressure (26, 34, 40).

In the light of the above, we evaluated in the VDN rat model the relative importance of renal calcification and increased central pulse pressure in the etiology of renal failure. Only one high dose of vitamin D₃ (+2 of nicotine) is required to induce, 2–6 days later, aortic wall calcification followed by aortic wall stiffening then hyperpulsatility (19, 20, 25, 42). The latter remains elevated for >1 yr (2). Given the long half-life of vitamin D₃ (16 h in rats; Ref. 23) and the fact that high doses of vitamin D₃ initially decrease renal blood flow by constraining the renal vasculature (36), we cannot exclude an initial phase of renal failure in VDN rats as a result of vitamin D toxicity. To avoid this initial phase of vitamin D toxicity, experiments on renal function have been performed in the present study 3 mo after VDN treatment, when hyperpulsatility is established (2, 15, 25, 26, 34, 40). We evaluated increased central arterial pulse pressure and aortic wall calcification and stiffness. The degree of renal calcification was measured by atomic absorption spectrophotometry (total calcium content) and localization of calcium deposits by staining. Renal function was evaluated by measuring urinary albumin excretion, plasma creatinine and urea concentrations, glomerular filtration rate, and renal plasma flow. Renal structural alterations were quantified by two histological indexes (5): an index of glomerular structural alteration (collapsed-atrophic glomeruli, sclerotic glomeruli, and glomerular cysts) and total number of focal, cortical areas of interstitial fibrosis per section.

METHODS

Animals. Seven-week-old, male, normotensive, outbred Wistar rats (Ico/Wi, IOPS AF/Han, Charles River Laboratories, L’Arbresle, France) were kept under standard conditions (temperature: 21 ± 1°C; lights on 6 AM to 6 PM) and given a rat diet (A04, Safe, Villemoisson-sur-Orge, France; calcium: 150 mmol/kg) and water (Aqua-clear, Culligan, Northbrook, IL; calcium: 28 μmol/l) ad libitum for 1 wk before experiments. Experiments were performed in accordance with the guidelines of the French Ministry of Agriculture (permit numbers 54-5 and 54-70).

On day 0, 38 rats (VDN rats; starting body wt = 237 ± 2 g) were injected with vitamin D₃ (300,000 IU/kg im) and nicotine (2 × 25 mg/kg, 5 ml/kg po) to induce elastocalcinosis as previously described.
HYPERPULSATILITY, CALCIFICATION, AND RENAL FUNCTION

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Three VDN rats (8%) died between days 2 and 6. We (19, 20, 42) previously reported a similar percentage mortality after VDN treatment. Control rats (n = 24; starting body wt = 238 ± 3 g) received 0.15 M NaCl im and two gavages of distilled water. None of the control rats died. Renal and vascular parameters were evaluated 90 days later.

Measurement of aortic wall mechanics. On day 90, rats were anesthetized (pentobarbital sodium: 60 mg/kg ip) and polyethylene cannulas, connected to low-volume pressure transducers, were introduced into the descending thoracic and abdominal aorta for measurement of baseline central and peripheral blood pressures for 30 min. An algorithm detected systolic and diastolic pressures and calculated mean pressure (waveform area), pulse pressure (systolic-diastolic), heart rate, and thoraco-abdominal pulse wave transit time (15, 26, 34). Hyperpulsatility was evaluated from increases in pulse pressure. Pulse wave velocity was calculated as the distance between the two cannula tips (measured in situ after death) divided by the pulse wave transit time.

Evaluation of renal function. A femoral venous cannula was implanted for infusion of the radiolabeled tracers. Glomerular filtration rate and renal plasma flow were estimated from the clearance of [99mTc]-diethylenetriaminepentaacetic acid and [131I]-orthoiodohippurate using the continuous infusion technique as previously reported (10) and expressed per gram of kidney wet weight.

Urine samples were collected from freely moving rats, stabilized for 2 days before measurement of glomerular filtration rate and renal plasma flow. Urinary excretion of albumin (mg/24 h) was expressed as urinary albumin concentration (immunonephelometric method; Refs. 5, 9) × 24-h urine volume (mean of 2 days).

Before the animals were killed, 1 ml of arterial blood was collected. Plasma and urinary concentrations of Na+, K+, Cl−, Ca2+, creatinine, and urea were measured (CX3, Synchron Clinical System, Beckman). Urinary excretion was expressed as urinary concentration × 24-h urine volume.

Measurement of total tissue calcium content. After death, a 1-cm sample (15 mg) of the descending thoracic aorta (just above the diaphragm) and segments (350 mg) of the right kidney (pole) were excised. Tissues were dehydrated at 110°C, and total calcium content was determined by atomic absorption spectrophotometry after mineralization and nitric acid digestion (19).

Evaluation of renal structure. For histological studies, the vascular pedicle of the right kidney was tied, and the left kidney was perfused then fixed in situ (Krebs-Ringer bicarbonate buffer + 1% bovine albumin followed by 10% buffered formalin) via the abdominal aorta (5). Animals were then killed with a pentobarbital sodium overdose (100 mg/kg iv).

The right and left kidneys were removed and weighed. The left kidney was kept in fixative overnight and then embedded in paraffin and cut into mid sagittal sections (3–5 μm thick). Collagen deposition was assessed by staining with picrosirius red and counterstaining with Weigert’s hematoxilin (5). Renal structural alterations were quantified as described previously (5). Two histological indexes of renal damage were used. An index of glomerular structural alteration was assessed as the percentage of total glomeruli with signs of anatomical alteration (collapsed-atrophic glomeruli, sclerotic glomeruli, and glomerular cysts; see Fig. 4A). Countings were performed in a blind fashion on three noncontiguous sections stained with picrosirius red and hema-toxilin, representing 600–800 glomeruli per rat. Results obtained in the same rat were averaged. The second index was defined as the total number of focal, cortical areas of interstitial fibrosis per section. The evaluation was done on the three nonconsecutive sections previously used to assess glomerular alteration. Results obtained in the same rat were averaged.

The presence of crystallized calcium apatite as revealed by black deposits using the von Kossa technique (44). Some sections were counterstained in red with safranin-o; the procedure was validated in serial sections by the absence of black deposits after 1-h exposure of the sections to 3% citric acid (14).

Statistics. Values are given as means ± SE. Differences between groups were evaluated using a Student t-test (P < 0.05).

Drugs. Vitamin D3 (Duphafral D3 1000) was purchased from Fort Dodge Santé Animale (37204 Tours, France), pentobarbital sodium was from Ceva Santé Animale (33501 Libourne, France), and all other chemicals were from Sigma Chemical (St. Louis, MO).

RESULTS

Body weight fell in VDN from days 2 through 6 and then stabilized (results not shown). After this initial period, VDN recovered normal growth and their body weight was similar to controls on day 90 (Table 1). VDN had no effect on heart rate.

Central aortic pulse pressure increased in VDN (1.5 times vs. control; P < 0.05; Fig. 1). Central aortic mean blood pressure was similar in both groups, central diastolic pressure fell slightly but not significantly, and systolic pressure increased in VDN rats (P < 0.05; Table 1).

VDN rats showed kidney calcification (calcium content 16 vs. control; P < 0.05) and aortic wall calcification (50 times; calcium content vs. control; P < 0.05; Table 1) and stiffening (1.4 times; pulse wave velocity vs. control; P < 0.05; Fig. 1).

The plasma calcium concentration (VDN 2.42 ± 0.05 vs. control 2.39 ± 0.04 mmol/l) and urinary excretion of calcium (VDN 14 ± 3 vs. control 8 ± 2 μmol/24 h) were similar in both groups, as were plasma urea concentration (VDN 6.1 ± 0.5 vs. control 6.2 ± 0.6 mmol/l) and plasma creatinine concentration (VDN 37 ± 3 vs. control 26 ± 2 μmol/l; P > 0.05). Plasma concentrations and urinary excretions of Na+, K+, and Cl− were similar in both groups (results not shown).

Albuminuria was higher in VDN (6 times vs. control; Fig. 1). The glomerular filtration rate decreased (P < 0.05), but renal plasma flow remained within the normal range (P > 0.05; Fig. 1), as did filtration fraction (33 ± 1%; P > 0.05 vs. control 38 ± 2).

There were significant positive relationships in VDN rats between albuminuria or glomerular filtration rate (dependent variable) and central aortic pulse pressure (independent variable), and between albuminuria and kidney calcium content (Fig. 2), but not between glomerular filtration rate and renal calcium content (r² 0.168; n = 27; P > 0.05).

Table 1. Central blood pressures, body weight, left ventricle/body weight, and tissue calcium content in control and VDN rats 90 days after VDN treatment

<table>
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<tr>
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<th>Control</th>
<th>VDN</th>
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<tr>
<td>Central aortic blood pressure, mmHg</td>
<td>Mean</td>
<td>114 ± 2 (23)</td>
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<tr>
<td></td>
<td>Diastolic</td>
<td>99 ± 2 (23)</td>
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<td></td>
<td>Systolic</td>
<td>139 ± 2 (23)</td>
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<tr>
<td>Body weight, g</td>
<td>439 ± 12 (24)</td>
<td>411 ± 9 (35)</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>343 ± 7 (23)</td>
<td>344 ± 5 (33)</td>
</tr>
<tr>
<td>Calcium content, μmol/g dry wt</td>
<td>Kidney</td>
<td>19 ± 1 (20)</td>
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<td></td>
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<td>8.1 ± 0.7 (23)</td>
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Values are means ± SE. VDN, vitamin D and nicotine treatment. *P < 0.05 vs. control; n = number of values.

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No calcium deposits were found by histological analysis in control rat kidneys (Fig. 3C). In VDN rats, large calcium deposits were visible throughout the renal cortex (Fig. 3A, D, E, and F); none were found in the medulla or papilla. Calcium was mainly located in the blood vessel wall. Deposits were observed in the media of all segments of the preglomerular vasculature, including early branches of the renal arteries (Fig. 3F), interlobular arteries (Fig. 3E), and afferent arterioles (Fig. 3E). Deposits were also observed within the media of the arteries feeding the pelvic mucosa (Fig. 3F). Calcification also occurred within the areas of interstitial fibrosis (Fig. 3D–E) and less frequently inside tubules (Fig. 3F). Calcium deposits were occasionally found within the glomerular tuft (data not shown) or Bowman’s capsule (Fig. 3D).

Histological indexes of renal damage were minimal in control rats (5.2 ± 0.3% of total glomeruli with slight signs of anatomical alteration; 2.2 ± 0.6% of focal, cortical areas exhibiting interstitial fibrosis per section; n = 5).

In VDN rats, the cortex showed moderate to extensive glomerular damage with atrophic and cystic glomeruli leading to an increased variability in glomerular size compared with control kidneys. The percentage of total glomeruli with signs of anatomical alteration was significantly higher in VDN (mean value: 26 ± 5%; n = 13) than in control rats. The histology of a severely damaged VDN and a kidney from a control rat is illustrated in Fig. 4, A–D. In the least severe cases, the percentage of damaged glomeruli was 10–25%, with atrophic or fibrotic glomeruli mainly in outer and midcortex. In severe cases, damage occurred throughout the cortex, with crescentic glomeruli and glomerular cysts (percentage of altered glomeruli was 30–55%). The cortex, particularly the outer cortex, showed extensive areas of interstitial and perivascular fibrosis, expansion of interstitium with collapsed tubules, and mononuclear cell infiltration (Fig. 4A). In some sections, the cortex corticis was absent and glomerular cysts were observed on the kidney surface (data not shown).
The number of areas exhibiting interstitial fibrosis was significantly higher in VDN (mean value: 61 ± 12) than in control rats. This number varied from 15–50 per midsagittal section in the least severe cases to 50–150 in the more severely damaged VDN kidneys. The latter corresponds to extensive interstitial fibrosis throughout the cortex and extending along vasa recta (Fig. 4, B and C). Tubular casts affected about six zones of the deep cortex and outer medulla in the least severe cases and up to 60 zones, extending from the outermost cortex to the outer medulla, in the more severely damaged kidneys.

Figure 4 shows dilated tubules filled with proteinaceous casts extending from the outer cortex (Fig. 4A) to the limit between the inner stripe of the outer medulla and the inner medulla (Fig. 4, B and C). The papilla remained unaffected in VDN rat kidney (Fig. 4C).

There were significant positive relationships between albuminuria (dependent variable) and the percentage of altered glomeruli (independent variable, \( r^2 = 0.316; n = 18; P < 0.05 \)) and between albuminuria and the number of areas exhibiting interstitial fibrosis (independent variable, \( r^2 = 0.591; n = 18; P < 0.05 \)).

There were also significant positive relationships between the percentage of altered glomeruli (dependent variable) and central aortic pulse pressure (independent variable, \( r^2 = 0.405; n = 14; P < 0.05 \)), and between the number of areas exhibiting interstitial fibrosis (dependent variable) and renal calcium content (independent variable, \( r^2 = 0.689; n = 16; P < 0.05 \)), but not between the percentage of altered glomeruli and renal calcium content (\( r^2 = 0.343; n = 16; P > 0.05 \)) nor between the number of areas exhibiting interstitial fibrosis and central aortic pulse pressure (\( r^2 = 0.323; n = 14; P > 0.05 \)).

**DISCUSSION**

Renal dysfunction in the chronic VDN rat model (90 days after VDN treatment) is characterized by two of the hallmarks of renal failure in humans, a fall in glomerular filtration rate and an increase in albuminuria, the latter being associated with histological indexes of renal damage but not by the other clinical symptoms seen in humans such as the increases in plasma levels of urea and creatinine. The degree of fall in glomerular filtration rate and the increase in albuminuria are, however, far lower than those observed in other rat models of renal failure. The 5/6 nephrectomized rat model shows an eight to ten times increase in albuminuria and proteinuria and a fall of −40 to −80% in the glomerular filtration rate (16, 24, 45).

In the puromycin amino-nucleoside model, albumin and proteinuria increase by 26–100 times and glomerular filtration rate fell by 50 to −90% (3, 12, 31).

Tissue calcification produced by VDN treatment is associated with both aortic (increase in wall stiffness and central blood pressure pulsatility) and renal (albuminuria and a fall in glomerular filtration rate) dysfunction. In patients suffering from ESRD, the London group described extensive aortic wall calcification (calcification score × 100 × 1,000, quantified in situ by the recent electron-beam computed tomography technique; Refs. 28, 29). In ESRD, the pattern of calcification of the aortic wall is similar to that we described in the VDN model (19, 20, 34) and is characterized by mineral deposition in the tunica media, involving an active bone-related process and recruitment of calcioproteins (8, 28, 29). Renal calcium content is not reported in ESRD patients, as it is impossible to measure in humans in vivo. Several studies from London and coworkers (17, 18, 27–29) showed a strong correlation between “aortic wall calcification and stiffening responsible for hyperpulsatility” and chronic kidney disease or ESRD, but the authors did not conclude on the causal or consequences link between the renal and aortic dysfunction. Therefore, in both the human and the VDN rat model of renal dysfunction described here, arteriopathy and nephropathy may have the same sys-
Tissue calcification as a cause for renal and aortic dysfunction in the VDN rat model. Renal tissue calcification (total kidney calcium content) is pronounced in VDN rats (16 times vs. control). Histological analysis showed extensive calcification in interstitial fibrosis and inside tubules. Nephrocalcinosis was accompanied by histological evidence of extensive damage to glomeruli and was strongly associated with interstitial fibrosis, with total renal calcium content explaining 83% (r = 0.83) of the changes in number of areas exhibiting interstitial fibrosis. Such tubular and peritubular calcifications, associated with renal tissue damage, may explain in part the increase in albuminuria. The positive linear regression observed between albuminuria and the increased number of areas exhibiting interstitial fibrosis (r = 0.77).

Concerning tissue calcification, a question is whether renovascular calcification plays a role in the development of renal dysfunction. Although VDN-induced vascular calcification is mainly restricted to the elastic fiber network of large diameter compliance arteries (25, 34), it does occur in small diameter muscular resistance arteries (19, 25). Renovascular calcification was observed in the present study within the media of intrarenal arteries and afferent arterioles (Fig. 4). However, it is unlikely that marked preglomerular arterial calcification does modify renovascular resistance, as we have shown several times that peripheral resistance is unchanged in the VDN model (e.g., see Refs. 2, 34) and this even after more than a 1-yr exposure to arterial wall calcification (2). If calcification of intrarenal arteries produces wall stiffness (as observed for the aorta; Refs. 2, 26) this would presumably lower the ability of those arteries to damp hyperpulsatility and thus damage glomerular filter [according to the hypothesis of Loutzenhiser et al. (30), see above].

Central aortic hyperpulsatility as a cause of renal dysfunction. In a recent study, O’Rourke and Safar (35), after revisiting the works of Byrom, suggested that organs with low

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Fig. 3. Intrarenal calcium distribution (day 90). For A and B, scale bar = 500 μm, and for C–F = 300 μm. A: distribution of calcium apatite crystals as revealed by von Kossa’s procedure (black deposits) in a VDN rat. Calcium deposits are found throughout the cortex; black arrowhead indicates the kidney surface. B: lack of calcium deposits when the von Kossa’s procedure was performed after acid treatment of a section contiguous to that shown in A. Black star points to the same structure appearing on both sections. C: lack of calcium deposits in a control rat kidney; gl, glomerulus. D–F: calcium distribution in a VDN rat after highlighting renal structures with safranin counterstain. D: calcium (black) deposits are mostly located within zones of expanded interstitium but can also be found within a Bowman’s capsule (arrowhead). Note the presence of 2 cystic glomeruli and a crescentic glomerular tuft (white star). E: the presence of calcium within fibro-interstitial zones. In addition, heavy calcium deposition is visible within the media of an interlobular artery (ila) and within the media of an afferent arteriolar (black arrow); arca, arcuate arteries. F: cortical zone located near the renal hilus, immediately underneath the pelvic mucosa (pm). Black arrow points to an area with calcium deposits around and within tubules. Note the presence of calcium (black arrowhead) within the media of a large arcuate artery and within the media (white star) of an artery of the pelvic mucosa.
upstream vascular resistance, such as the kidney, are vulnerable to the pulsations of pressure and flow, which, via increased circumferential and shear stress, damage endothelial and smooth muscle cells and so produce vascular disruption. The starting point of their argumentation is the close relationship between pulse pressure and cardiovascular events especially in the case of patients with ESRD on hemodialysis. They draw a parallel to the development of damage to the pulmonary circulation after increased pulsatility induced by congenital arteriovenous shunts.

Some of our results can be interpreted as supportive of this argument. In VDN, there is pronounced aortic wall calcification with increased wall stiffness and central pressure pulsatility. Linear regression analysis showed significant relationships between 1) albuminuria and central aortic pulse pressure (with pulse pressure explaining 64% of the changes in albuminuria), 2) percentage of altered glomeruli and central aortic pulse pressure (with pulse pressure explaining 64% of the glomerular alteration), and 3) glomerular filtration rate and central aortic pulse pressure (with pulse pressure explaining 41% of the changes in glomerular filtration rate).

To evaluate whether the increase in pulse pressure by itself is a critical factor in the etiology of renal dysfunction, we tried to induce tissue calcification and at the same time prevent the increase in pulse pressure by partially clipping one renal artery, using the contralateral kidney as control. The left renal artery was clipped in young male Wistar rats \((n = 5)\), vitamin D\(_3\) and nicotine were administrated 21 days later, and hemodynamic renal function was evaluated 3 mo after VDN treatment. The left (clipped) and right (nonclipped) kidneys were explored separately (left ureter and bladder canulation), and the pulse arterial blood pressure was measured in the left (between the clip and the kidney) and right renal artery. Renal pulsatility (expressed as pulse/mean renal arterial pressures) was not prevented (clipped: 63 ± 4; nonclipped: 63 ± 8%; \(P > 0.05\)), but mean renal arterial pressure decreased (90 ± 17 mmHg, −20%, \(P < 0.05\) vs. nonclipped: 114 ± 2) and kidney weight decreased (clipped: 4.0 ± 0.2, \(P < 0.05\) vs. nonclipped: 4.6 ± 0.4 g/g). The glomerular filtration rate (clipped: 678 ± 15; nonclipped: 698 ± 68 µl·min\(^{-1}\)·g\(^{-1}\)), renal plasma flow (clipped: 1.9 ± 0.2; nonclipped: 1.9 ± 0.2 ml·min\(^{-1}\)·g\(^{-1}\)), and filtration fraction (clipped: 34 ± 4; nonclipped: 37 ± 4%) were similar for both kidneys.

It seems therefore difficult to obtain prevention of pulse with no change in mean pressure in VDN rats. Other physiological models such as induction of hyperpulsatility by arterial/venous fistula (in non-VDN rats) may be useful to evaluate whether the increase in pulse pressure by itself is a critical factor for renal dysfunction. However, long-term increase in pulsatility would probably be difficult to maintain with such an arterial/venous fistula model.

Were the increase in pulse pressure to be a critical factor for renal dysfunction, then the VDN model would be very useful to the further study of several physiological hypotheses that arise from this observation. It would be interesting to evaluate whether the increased central pressure pulsatility is transmitted to the afferent arteriole and whether this induces myogenically mediated vasoconstriction as suggested by Loutzenhiser et al. (30). Moreover, as renal plasma flow remained inside the normal range and glomerular filtration rate fell only by 17% in VDN, there was thus only a minor fall (−6%) in filtration fraction. This suggests simultaneous, proportionally similar
increases in pre- and postglomerular resistances of remnant nephrons stemming from an arteriolar myogenic vasoconstrictor response evoked by increased pressure pulsatility. Finally, VDN rats provide a useful model to evaluate whether increased pulse pressure amplifies the age-related decline in renal function (43).

Other factors responsible for the link between tissue calcification and pressure hyperpulsatility and chronic renal dysfunction. The interaction between arterio- and nephropathy may operate in the opposite direction, i.e., nephropathy could alter phosphocalcium metabolism and thus cause calcium deposition in arteries. It has been reported (6) that a plasma factor from uremic patients provokes calcium precipitation in cell culture (6). In cultured vascular smooth muscle cells, the addition of serum from dialysis patients produced mineralization, possibly by transforming the cells into osteoblast-like cells (32). Furthermore, in uremic rats fed a high phosphorus diet for 3 to 6 mo, the aortic wall calcium content increases (7). In previous studies (42), we have shown that plasma urea doubles and plasma creatinine goes up by 50% in the 4 days after VDN treatment but that 16 days after treatment values are normal. As calcification of the aorta wall increases progressively from the day after VDN treatment for several weeks (25), it is possible that early vascular and renal calcification is linked to the presence of a factor in the uremic plasma. This hypothesis would not hold at a later stage (3 mo).

In summary, to our knowledge, this study provides the first evidence (in an experimental model of renal dysfunction, the VDN) of a link between 1) increased central aortic pulsatility and renal dysfunction, and 2) renal calcification and renal dysfunction. Its preclinical predictive value in terms of proof of concept opens new perspectives. The group of Mimran et al. (13, 43) has proposed a similar link between hyperpulsatility and albuminuria in the elderly. However, direct causality, namely increased pulsatility provokes renal failure, may not provide the only explanation of the link between these two pathophysiological events. Vascular and renal calcification occurs concomitantly with renal dysfunction in the VDN rat model, thus arterio- and nephropathy may have similar etiologies without being causally related. Thus it is possible that VDN (especially vitamin D) provokes both aortic and renal calcification and that this leads, independently, to concomitant pressure hyperpulsatility and renal dysfunction.

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