RENAL FUNCTION AND STRUCTURE IN A RAT MODEL OF ARTERIAL
CALCIFICATION AND INCREASED PULSE PRESSURE

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ABSTRACT

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 elastocalcinosis 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 (992±171 µmol.g⁻¹ dry weight, x50 control 19±1) and pulse pressure (61±4 mmHg, x1.5 control 40±2). Significant renal calcification (124±27 µmol.g⁻¹ dry weight, x16 control 8.1±0.7) occurred mainly within the media of the preglomerular vasculature and in the areas of interstitial fibrosis in VDN. Extensive renal damages (26±5% of collapsed-atrophic or sclerotic glomeruli, or glomerular cysts, x5 control 5.2±0.3; 61±12 areas of focal, cortical areas exhibiting interstitial fibrosis per section, x28 control 2.2±0.6) were observed histologically. Glomerular filtration rate significantly decreased (880±40 µL.min⁻¹.g⁻¹ kidney weight vs control 1058±44). Albuminuria increased 6x (1.6±0.4 mg.24h⁻¹, control 0.27±0.04). 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 (i) hyperpulsatility and renal dysfunction, and (ii) 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.

Key words
INTRODUCTION

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, 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 of 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 months 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 through 6 days later, aortic wall calcification followed by aortic wall stiffening then hyperpulsatility (19, 20, 25, 42). The latter remains elevated for more than one year (2). Given the long half-life of vitamin D₃ (16 hours in rats (23)), and the fact that high doses of vitamin D₃ initially decrease renal blood flow by constricting the renal vasculature (36), we cannot exclude an initial phase of renal failure in VDN rats as a result of vitamin D toxicity. In order to avoid this initial phase
of vitamin D toxicity, experiments on renal function have been performed in the present study 3 months 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 localisation 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 indices (5): an index of glomerular structural alteration (collapsed-atrophic glomeruli, sclerotic glomeruli, 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 a.m. to 6 p.m.) and given a rat diet (A04, Safe, Villemoisson-sur-Orge, France; calcium content: 150 mmol.kg⁻¹) and water (Aqua-clear®, Culligan, Northbrook, USA; calcium 28 µmol.L⁻¹) ad libitum for one week 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 (D₀), 38 rats (VDN rats, starting body weight 237±2 g) were injected with vitamin D₃ (300,000 IU.kg⁻¹, i.m.) and nicotine (2 x 25 mg/kg, 5 mL.kg⁻¹, p.o.) to induce elastocalcinosis as previously described (2, 15, 25, 26, 34, 40). Three VDN rats (8%) died between D₂ and D₆. We have previously reported a similar percentage mortality following VDN treatment (19, 20, 42). Control rats (n = 24, starting body weight 238±3 g) received 0.15
M NaCl, *i.m.* and 2 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 D$_{90}$, rats were anesthetized (sodium pentobarbital 60 mg.kg$^{-1}$, i.p.) 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, 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* following sacrifice) divided by the pulse wave transit time.

**Evaluation of renal function**

A femoral venous cannula was implanted for infusion of the radiolabelled 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 g 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/24h) was expressed as urinary albumin concentration (immuno-nephelemetric method (5, 9))x 24 hour urine volume (mean of 2 days).

Before sacrificing the animals, 1 milliliter of arterial blood was collected. Plasma and urinary concentrations of Na$^+$, K$^+$, Cl$^-$, Ca$^{2+}$, creatinine and urea were measured (CX3,
Synchron Clinical System, Beckman). Urinary excretion was expressed as urinary concentration x 24 hour urine volume.

**Measurement of total tissue calcium content**

After sacrifice, 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 determined by atomic absorption spectrophotometry following 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 sacrificed with a sodium pentobarbital overdose (100 mg.kg⁻¹, i.v.).

The right and left kidneys were removed and weighed. The left kidney was kept in fixative overnight, 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 previously described (5). Two histological indices 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, glomerular cycts, as illustrated in Figure 4A). Countings were performed in a blind fashion on three non-contiguous sections stained with picrosirius red and hematoxilin, 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
non-consecutive 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 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 following 1 hr exposure of the sections to 3% citric acid (14).

**Statistics**

Values are given as means ± S.E.M. 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), sodium pentobarbital from Ceva Santé Animale (33501 Libourne, France), and all other chemicals from Sigma Chemical Co (St Louis, MO, U.S.A).

**RESULTS**

Body weight fell in VDN from D₂ through D₆ then stabilized (results not shown). Following this initial period, VDN recovered normal growth and their body weight was similar to controls on D₉₀ (Table 1). VDN had no effect on heart rate.

Central aortic pulse pressure increased in VDN (x1.5 versus control, p < 0.05) (Figure 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 \( x16 \) versus control, \( p < 0.05 \)) and aortic wall calcification (calcium content \( x50 \) versus control, \( p < 0.05 \), Table 1) and stiffening (pulse wave velocity \( x1.4 \) versus control, \( p < 0.05 \), Figure 1).

The plasma calcium concentration (VDN 2.42±0.05, control 2.39±0.04 mmol.L\(^{-1}\)) and urinary excretion of calcium (VDN 14±3, control 8±2 µmol.24h\(^{-1}\)) were similar in both groups, as were plasma urea concentration (VDN 6.1±0.5, control 6.2±0.6 mmol.L\(^{-1}\)) and plasma creatinine concentration (VDN 37±3, control 26±2 µmol.L\(^{-1}\)) (\( p > 0.05 \)). Plasma concentrations and urinary excretions of Na\(^+\), K\(^+\), Cl\(^-\) were similar in both groups (results not shown).

Albuminuria was higher in VDN (\( x6 \) versus control, Figure 1). Glomerular filtration rate decreased (\( p < 0.05 \)) but renal plasma flow remained within the normal range (\( p > 0.05 \), Figure 1), as did filtration fraction (33±1%, \( p > 0.05 \) versus 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 (Figure 2), but not between glomerular filtration rate and renal calcium content (\( r^2 0.168, n = 27, p > 0.05 \)).

No calcium deposits were found by histological analysis in control rat kidneys (Figure 3C). In VDN rats, large calcium deposits were visible throughout the renal cortex (Figure 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 (Figure 3F), interlobular arteries (Figure 3E) and afferent arterioles (Figure 3E). Deposits were also observed within the media.
of the arteries feeding the pelvic mucosa (Figure 3F). Calcification also occurred within the areas of interstitial fibrosis (Figure 3D-E), and less frequently inside tubules (Figure 3F). Calcium deposits were occasionally found within the glomerular tuft (data not shown) or Bowman’s capsule (Figure 3D).

Histological indices 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 as compared to 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 are illustrated in Figure 4A-C, D). In the least severe cases, the percentage of damaged glomeruli was 10 to 25%, with atrophic or fibrotic glomeruli mainly in outer and mid-cortex. In severe cases, damage occurred throughout the cortex, with crescentic glomeruli and glomerular cysts (percentage of altered glomeruli was 30 to 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 (Figure 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 mid-sagittal 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 (Figure 4B and 4C). Tubular casts affected about 6 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 (Figure 4A) to the limit between the inner stripe of the outer medulla and the inner medulla (Figure 4B, 4C). The papilla remained unaffected in VDN rat kidney (Figure 4C).

There were significant positive relationships between albuminuria (dependent variable) and the percentage of altered glomeruli (independent variable, r² 0.316, n = 18, p < 0.05), and between albuminuria and the number of areas exhibiting interstitial fibrosis (independent variable, r² 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, r² 0.405, n = 14, p < 0.05), and between the number of areas exhibiting interstitial fibrosis (dependent variable) and renal calcium content (independent variable, r² 0.689, n = 16, p < 0.05), but not between the percentage of altered glomeruli and renal calcium content (r² 0.343, n = 16, p > 0.05) nor between the number of areas exhibiting interstitial fibrosis and central aortic pulse pressure (r² 0.323, n = 14, p > 0.05).

**DISCUSSION**

Renal dysfunction in the chronic VDN rat model (90 days following VDN treatment) is characterized by two of the hallmarks of renal failure in man – a fall in glomerular filtration rate and an increase in albuminuria, the latter being associated with histological indices of renal damage – but not by the other clinical symptoms seen in man such as increases in plasma levels of urea and creatinine. The degree of fall in glomerular filtration rate and increase in albuminuria is, however, far lower than that observed in other rat models of renal
failure. The 5/6 nephrectomized rat model shows a 8-10x increase in albuminuria and proteinuria and a fall of -40 to -80% in glomerular filtration rate (16, 24, 45). In the puromycin amino-nucleoside model, albumin and proteinuria increase by 26-100x and glomerular filtration rate falls 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 x100-x1000, quantified in situ by the recent electron-beam computed tomography technique) (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 active bone–related process and recruitment of calciproteins (8, 28, 29). Renal calcium content is not reported in ESRD patients as impossible to measure in human in vivo. Several reports from London and coworkers showed a strong correlation between “aortic wall calcification and stiffening responsible for hyperpulsatility” and chronic kidney disease or ESRD, but the authors do not conclude on the causal or consequences link between the renal and aortic dysfunction (17, 18, 27-29). Therefore, in both man and the VDN rat model of renal dysfunction described here, arteriopathy and nephropathy may have the same systemic physiopathological etiology (calcification) with causal or independent links between the two.

_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 (x16 versus 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, 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 \emph{per se} the increase in albuminuria. The positive linear regression observed between albuminuria and total calcium content in the kidney in VDN argues in favor of this hypothesis. This is not the only factors, however, as total renal calcium content explains only 57% of the changes in albuminuria \( (r = 0.57) \). Proteinuria is a global biomarker of progressive kidney disease \( (46) \) and is responsible for a vicious circle, as the presence of albumin inside tubules leads to damage of the proximal tubular and instertitial fibrosis \( (11, 21, 22, 41) \). This is confirmed in the present study by 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 \( (Figure 4) \). 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 \( (e.g. \ 2, 34) \) and this even after more than 1 year's exposure to arterial wall calcification \( (2) \). If calcification of intrarenal arteries produces wall stiffness \( (as \ observed \ for \ the \ aorta \ 2, 26) \) this would presumably lower the ability of those arteries to damp hyperpulsatility and thus damage glomerular filter \( (according \ to \ the \ hypothesis \ of \ Louthensizer \ et \ al. \ (30), \ see \ above). \)

\textit{Central aortic hyperpulsatility as a cause of renal dysfunction}
In a recent paper, O’Rourke and Safar (35), after revisiting the works of Byrom, suggested that organs with low 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 following 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 (i) albuminuria and central aortic pulse pressure (with pulse pressure explaining 64% of the changes in albuminuria), (ii) percentage of altered glomeruli and central aortic pulse pressure (with pulse pressure explaining 64% of the glomerular alteration) and (iii) glomerular filtration rate and central aortic pulse pressure (with pulse pressure explaining 41% of the changes in glomerular filtration rate).

In order to evaluate whether the increase in pulse pressure by itself is a critical factor in the etiology of renal dysfunction, we have tried to induce tissue calcification, and at the same time prevent the increase in pulse pressure by partially clipping one renal artery, using the controlateral kidney as control. The left renal artery was clipped in young male Wistar rats (n = 5), vitamin D₃ and nicotine was administrated 21 days later, and hemodynamic renal function were evaluated 3 months after VDN treatment. The left (clipped) and right (non-clipped) kidney were explored separately (left ureter and bladder canulation) and pulse arterial blood pressure 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, non-clipped 63±8%, p > 0.05), but mean renal arterial pressure decreased (90±17 mmHg, -20%, p < 0.05 vs non-clipped 114±2) and kidney weight decreased (clipped 4.0±0.2, p < 0.05 vs non-clipped 4.6±0.4 g.g⁻¹). Glomerular filtration rate (clipped 678±15, non-clipped 698±68 µL.min⁻¹.g⁻¹), renal plasma flow (clipped 1.9±0.2, non-clipped 1.9±0.2 mL.min⁻¹.g⁻¹) and filtration fraction (clipped 34±4, non-clipped 37±4 %) was 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 be a critical factor for renal dysfunction, then the VDN model would be very useful to further study of several physiological hypotheses which 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, 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 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 months, aortic wall calcium content increases (7). In previous publications we have shown that plasma urea doubles and plasma creatinine goes up by 50% in the 4 days following VDN treatment (42), but 16 days after treatment values are normal. As calcification of the aorta wall increases progressively from the day following 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 months).

In summary, to our knowledge, this study provides the first evidence (in an experimental model of renal dysfunction, the VDN) of a link between (i) increased central aortic pulsatility and renal dysfunction, and (ii) renal calcification and renal dysfunction. Its preclinical predictive value in terms of proof of concept opens new perspectives. The group of Mimran has proposed a similar link between hyperpulsatility and albuminuria in the elderly (13, 43). However direct causality *viz* 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 this leads - independently – to concomitant pressure hyperpulsatility and renal dysfunction.

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REFERENCES


FIGURE LEGENDS

Figure 1: Central aortic pulse pressure and pulse wave velocity, albuminuria, glomerular filtration rate and renal plasma flow in control (empty bars) and VDN (full bars) rats 90 days following VDN treatment. (means ± SEM, *: p < 0.05 versus control, (n) number of values).

Figure 2: Linear regressions between albuminuria (y axis) and central aortic pulse (top; n = 24) or kidney calcium content (middle; n = 24) (x axis), and between glomerular filtration rate (y axis) and central aortic pulse (top; n = 27) in VDN rats 90 days following VDN treatment.

Figure 3: Intrarenal calcium distribution (day 90). For panels A and B, the scale bar is 500 µm, C to F, 300 µm.

Panel 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; the black arrowhead indicates the kidney surface.

Panel 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. The black star points to the same structure appearing on both sections.

Panel C shows the lack of calcium deposits in a control rat kidney; gl: glomerulus.

Panels D-F: calcium distribution in a VDN rat after highlighting renal structures with safranin counterstain. In panel 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 two cystic glomeruli (gl) and a crescentic glomerular tuft (white star). Panel E illustrates 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). Panel F shows a cortical zone located near the renal hilus,
immediately underneath the pelvic mucosa (pm). The 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 (arca), and within the media (white star) of an artery of the pelvic mucosa.

Figure 4: Low-power light micrographs illustrating the histologic appearance of the renal tissue of a VDN (A-C) and a control rat (D) (day 90). Sections were stained with picrosirius red for collagen. Panels A to C were obtained on the same section. The scale bar (500 μm) applies to all panels.

Cortical alterations shown in panel A comprise atrophic/sclerotic glomeruli (open arrow) contrasting with expanded glomerular cysts with collapsed glomerular tuft (open star). Black arrows indicate zones of interstitial fibrosis that prevail near the renal surface and also affect the perivascular interstitium around the interlobular arteries (ila), arcuate arteries (arca) and veins (v). The black star indicates a zone with dilated tubules blocked by proteinaceous casts that appear in yellow.

Panel B shows an area spanning the outer stripe (os) and the inner stripe (is) of the outer medulla. Tubular expansions with luminal casts are shown by black stars; the black arrow points to collagen accumulation along vasa recta bundles.

Panel C spans the deepest portion of the inner stripe (is), the inner medulla (im) and the early papilla (p). Note that tubular casts (black stars) and fibrosis (black arrow) are not found beyond the is/im limit.

Panel D shows cortical structures of a control rat with no tubular or vascular alterations; gl: glomeruli.
### Table 1: Central blood pressures, body weight, left ventricle / body weight and tissue calcium content in control and VDN rats 90 days following VDN treatment (means ± SEM, *: p < 0.05 versus control, (n) number of values).

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<th>Control</th>
<th>VDN</th>
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<td><strong>Central aortic blood pressure, mmHg</strong></td>
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<td>Diastolic</td>
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<td>Aortic wall</td>
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<td>Kidney</td>
<td>8.1±0.7 (23)</td>
<td>124±27 * (34)</td>
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Figure 1

**Central aortic pulse blood pressure**

- **Thoraco-abdominal aortic pulse wave velocity**

**Albuminuria**

- **Glomerular filtration rate**

**Renal plasma flow**
Figure 2

Panel 1: Central aortic pulse blood pressure vs. albuminuria.
- Slope: $13.5 \pm 3.5 \times 10^{-3}$
- Intercept: $-0.31 \pm 0.21$
- $r^2$: 0.402
- $P_{\text{slope} \neq 0}$: 0.0008

Panel 2: Kidney calcium content vs. albuminuria.
- Slope: $4.6 \pm 1.4 \times 10^{-3}$
- Intercept: $0.11 \pm 0.13$
- $r^2$: 0.328
- $P_{\text{slope} \neq 0}$: 0.0034

Panel 3: Central aortic pulse blood pressure vs. glomerular filtration rate.
- Slope: $-3.8 \pm 1.8$
- Intercept: $1107 \pm 114$
- $r^2$: 0.168
- $P_{\text{slope} \neq 0}$: 0.0378
Figure 3
Figure 4