Development of Salt-Sensitive Hypertension in Hydronephrosis

MATTIAS CARLSTRÖM
Dissertation presented at Uppsala University to be publicly examined in BMC / B21, Uppsala Biomedical Center, Husargatan 3, Uppsala, Friday, April 18, 2008 at 13:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English.

Abstract

Hydronephrosis, due to ureteropelvic junction obstruction, is a common condition in infants with an incidence of approximately 0.5-1%. During the last decade, the surgical management of non-symmetric hydronephrosis has become more conservative, and the long-term physiological consequences of this new policy are unclear. The overall aim of this thesis was to determine whether there is a link between hydronephrosis and the development of hypertension. Hydronephrosis was induced by partial ureteral obstruction in 3-week old rats or mice. In the adult animals, blood pressure was measured telemetrically during different sodium conditions and the renal function was evaluated. Both species developed salt-sensitive hypertension and histopathological changes (i.e. fibrosis, inflammation, glomerular and tubular changes) that correlated with the degree of hydronephrosis. An abnormal renal excretion pattern with increased diuresis and impaired urine concentrating ability was observed in hydronephrosis. The mechanisms were primarily located to the diseased kidney, as relief of the obstruction attenuated blood pressure and salt-sensitivity. Increased renin angiotensin system activity, due to ureteral obstruction, might be involved in the development but not necessary the maintenance of hypertension. Hydronephrotic animals displayed reduced nitric oxide availability, which might be due to increased oxidative stress in the diseased kidney. Renal nitric oxide deficiency and subsequent resetting of the tubuloglomerular feedback mechanism, appeared to have an important role in the development of hypertension. In conclusion, experimental hydronephrosis, induced by partial ureteral obstruction, provides a new model for studies of salt-sensitive hypertension. Furthermore, the new findings imply that the current conservative treatment strategy in hydronephrosis should be reconsidered in favor of treatment that is more active, in order to prevent the development of renal injury and hypertension in later life.

Keywords: blood pressure, nephrectomy, nitric oxide, oxidative stress, renal function, renin angiotensin system, salt-sensitivity, telemetry, tubuloglomerular feedback, ureteral obstruction

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To my family
I. Hydronephrosis causes salt-sensitive hypertension in rats.

*Carlström M*, Wåhlin N*, Sällström J, Skött O, Brown R & Persson AEG.

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*Equal contribution

II. Relief of chronic partial ureteral obstruction attenuates salt-sensitive hypertension in rats.

*Carlström M, Wåhlin N, Skött O & Persson AEG.*


III. Hydronephrosis causes salt-sensitive hypertension and impaired renal concentrating ability in mice.


*Equal contribution

IV. Role of nitric oxide deficiency in the development of hypertension in hydronephrotic animals.

*Carlström M, Brown R, Edlund J, Sällström J, Larsson E, Teerlink T, Palm F, Wåhlin N & Persson AEG.*

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADMA</td>
<td>Asymmetrical dimethylarginine</td>
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<tr>
<td>c-UNX</td>
<td>Contralateral nephrectomy</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
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<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
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<td>GU</td>
<td>Goldblatt units</td>
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<tr>
<td>HN</td>
<td>Hydronephrotic</td>
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<td>HNR</td>
<td>Hydronephrotic ratio</td>
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<td>HS</td>
<td>High salt</td>
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<tr>
<td>i-UNX</td>
<td>Ipsilateral nephrectomy</td>
</tr>
<tr>
<td>L-NAME</td>
<td>(N^G)-nitro-L-arginine methyl ester</td>
</tr>
<tr>
<td>LS</td>
<td>Low salt</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial blood pressure</td>
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<tr>
<td>nNOS</td>
<td>Neuronal nitric oxide synthase</td>
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<tr>
<td>NO</td>
<td>Nitric oxide</td>
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<tr>
<td>NS</td>
<td>Normal salt</td>
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<tr>
<td>NS2</td>
<td>Normal salt (second time)</td>
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<tr>
<td>PRC</td>
<td>Plasma renin concentration</td>
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<td>PUUO</td>
<td>Partial unilateral ureteral obstruction</td>
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<td>PBUO</td>
<td>Partial bilateral ureteral obstruction</td>
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<tr>
<td>P_{FF}</td>
<td>Free-flow pressure</td>
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<td>P_{SF}</td>
<td>Stop-flow pressure</td>
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<tr>
<td>\Delta P_{SF}</td>
<td>Maximal TGF response</td>
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<td>RBF</td>
<td>Renal blood flow</td>
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<td>SDMA</td>
<td>Symmetrical dimethylarginine</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>SHR</td>
<td>Spontaneous hypertensive rats</td>
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<td>7-NI</td>
<td>7-Nitroindazole</td>
</tr>
<tr>
<td>TGF</td>
<td>Tubuloglomerular feedback</td>
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<td>UPJ</td>
<td>Ureteropelvic junction</td>
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INTRODUCTION

Kidney function and Autoregulation

The kidneys play a key role in the homeostatic regulation of body fluid status and electrolyte balance, and consequently have a dominant role in long-term blood pressure control.\(^1\) One of the mechanisms for achieving a stable fluid balance is renal autoregulation. Via autoregulation, the kidneys are able to maintain constant renal blood flow (RBF) and glomerular filtration rate (GFR) despite wide changes in the arterial perfusion pressure.\(^2-4\) The mechanisms responsible for autoregulation are the myogenic response and the tubuloglomerular feedback (TGF) systems. Myogenic response is an inherent ability of the afferent arterioles to respond to changes in vessel-wall tension by contracting or relaxing. The TGF mechanism is a negative feedback system that operates within the juxtaglomerular apparatus (Figure 1). An increased flow in the thick ascending limb is sensed by the macula densa cells, which release a mediator that is transferred to the preglomerular vessels causing vasoconstriction. Adenosine is the mediator of the TGF, whereas, nitric oxide (NO) and angiotensin II are modulators.\(^5,6\)
Figure 1. The juxtaglomerular apparatus with the macula densa cells in the wall of the distal tubule, glomerular arterioles (afferent and efferent), and between these structures the extraglomerular mesangial cells.

Renal hypertension

Hypertension is a common chronic disorder worldwide and secondary forms of hypertension are found in a large proportion of the hypertensive population. The most common cause of secondary hypertension is intrinsic renal disease, but virtually any form of renal pathological condition may lead to hypertension. The mechanism is either renovascular, occurring through the action of vasoactive substances, or more commonly an inability to regulate sodium excretion with resulting chronic hypervolaemia. Increased activity of the renin angiotensin system has been demonstrated in renal hypertension in both humans and in experimental animal models. There is also increasing evidence of a close connection between increased oxidative stress and reduced NO availability in the development and maintenance of hypertension.
Hydronephrosis

**Incidence and aetiology**

Hydronephrosis is a common condition and the incidence of detectable urinary tract dilatation in utero is reported to be 1-1.4% of all foetuses, and confirmed postnatally in approximately 0.5-1%.\textsuperscript{10-12} Hydronephrosis is a condition with a distended kidney or a dilatation of the renal pelvis. The presenting symptoms in neonates and young children are palpable mass, failure to thrive, urinary tract infections and pain in the flank.\textsuperscript{13} There are different forms of hydronephrosis and ureteropelvic junction (UPJ) obstruction is the most common cause of antenatal and neonatal hydronephrosis. In this condition, there is an obstruction of the urine flow from the renal pelvis to the proximal ureter. This obstruction may lead to progressive renal damage and deterioration. The aetiology of hydronephrosis due to UPJ obstruction is still obscure and can be caused by several different mechanisms, including both intrinsic (i.e. muscle disorientation,\textsuperscript{14} excessive collagen\textsuperscript{15} or absence of smooth muscle cells\textsuperscript{16}) and extrinsic factors such as overlying aberrant vessel,\textsuperscript{17, 18} retroperitoneal fibrosis,\textsuperscript{19, 20} pelvic or abdominal tumours\textsuperscript{21-24} or neurological deficits.\textsuperscript{25} In general, the obstruction is partial, unilateral and congenital and also more frequently observed in boys than in girls.\textsuperscript{26}

**Diagnostics**

Hydronephrosis is often detected during an ultrasonography of the urinary system. This procedure reveals information about the enlargement of the kidney and the ureter related to the UPJ obstruction, however, diagnosis is often confirmed later by other techniques. Intravenous urography is a method where contrast medium is injected intravenously and excreted by the kidneys. X-rays are then taken to follow the excretion of the contrast medium. The intravenous urography reveals information about both drainage
and functioning of the kidney. Historically, intravenous urography was the predominant method of evaluating patients with possible UPJ obstruction. However, in the evaluation of a child with a hydronephrotic kidney, diuretic renograms have replaced intravenous urography. The benefits are that iodine-based intravenous contrast is not used, radiation exposure is minimal, and renal function can be better quantified. The disadvantage of the nuclear medicine scan is that details about renal anatomy are not obtained.

**Hydronephrosis and changes in renal function**

Renal function is often described in terms of GFR, and it has been noted that changes in filtration rate are closely related to changes in RBF. The functional status of the hydronephrotic kidney, as measured by GFR, remains well preserved for several years in newborns. However, a poor correlation between the severity and duration of symptoms and the degree of renal function has been demonstrated. Several animal models have been established for studying ureteral obstruction. The model of partial unilateral ureteral obstruction (PUUO) is used to induce hydronephrosis in many animal species after birth. In experimental hydronephrosis, GFR is reported as increased, unchanged or decreased. This discrepancy may depend on the severity and duration of the obstruction and the diuretic state. However, in general, ureteral obstruction results in decreased RBF and GFR in the ipsilateral kidney, whereas, in the non-obstructed, contralateral kidney, a compensatory increase occurs. In addition, the literature on electrolyte and water excretion during PUUO is conflicting.
Treatment
The treatment of symptomatic hydronephrosis is surgical and uncomplicated. The usual repair of UPJ obstruction involves removal of the obstruction and then a reconstruction of the continuity by a pyeloplasty (Figure 2).

Figure 2. Schematic illustration of pyeloplasty in a hydronephrotic kidney. The obstruction is removed to drain and decompress the kidney and finally the ureter is reconnected with the pelvic region.

Patients who demonstrated with a dilated collecting system and a non-visible ureter on intravenous urography were considered as having an obstruction and were operated, irrespective of symptoms. With the introduction of scintigraphic methods for estimating renal function, knowledge concerning kidney function in the presence of outlet obstruction increased. As a well preserved renal function in hydronephrosis has been demonstrated, the management of asymptomatic ureteral obstruction has become more conservative. This worldwide policy is new and the long-term physiological consequences are still unknown.
Hydronephrosis and Hypertension

Most children with hydronephrosis are not hypertensive, but there are several reports on limited numbers of patients with hypertension obviously caused by hydronephrosis, as the patients became normotensive after nephrectomy or pyeloplasty. An increased activity of the renin angiotensin system has been demonstrated, but the participation of renin in this hypertension appears to be influenced by the duration of the obstruction, the presence or absence of a contralateral normal kidney, and other intrarenal factors. Other investigations are unable to show any differences in blood pressure in hydronephrosis. Furthermore, in large surveys on causes of secondary hypertension, hydronephrosis does not appear overrepresented. However, this relationship is difficult to interpret, as the prevalence of hydronephrosis among the human population is lower than that of hypertension.

Experimental findings

In previous experimental studies in our laboratory in Uppsala, weanling rats were submitted to PUUO at three weeks of age, leading to considerable hydronephrosis. Experiments were performed three weeks later. Functional parameters such as RBF and GFR under baseline conditions were at the same level as in controls. However, during volume expansion, major changes in blood flow and filtration occurred.
Figure 3. Schematic drawing of a nephron, with the pipettes used for determining TGF-characteristics. A wax block is placed in the proximal tubule. Distal to this block, a perfusion pipette is inserted so perfusion rate can be changed. Proximal to the wax block, the stop flow pressure ($P_{SF}$) can be measured at different perfusion rates.

With micropuncture techniques (Figure 3), volume expansion causes a paradoxical resetting of the TGF mechanism to a much higher sensitivity and activity in the hydronephrotic kidney, instead of making the TGF insensitive to flow (Figure 4). Consequently, the single nephron GFR and the urinary output are maintained at low levels. These effects are interpreted as possibly serving to protect the hydronephrotic kidney from further damage caused by elevated intrarenal pressures. However, in the contralateral kidney, desensitisation of the TGF mechanism occurs, similar to in healthy animals. The same paradoxical resetting of the TGF, towards higher sensitivity and activ-
ity, is seen in rats of the Milan hypertensive strain rats, before the animals develop hypertension, and in spontaneous hypertensive rats (SHR). 

Figure 4. TGF-responses before (blue curve) and after volume expansion. In control animals (green curve), there is a resetting of the TGF mechanism to decreased sensitivity and activity. In the hydronephrotic kidney (red curve), there is a paradoxical shift towards higher sensitivity and activity.

The regulation of the TGF sensitivity is intimately coupled to NO production in the macula densa cells. Chronic, selective blockade of neuronal nitric oxide synthase (nNOS) increases TGF sensitivity, reduces GFR and salt and water excretion, and leads to hypertension. Taken together, it appears as if the functional adaptations to PUUO could lead to development of renal hypertension.
AIMS

The overall aim was to determine if there is a link between hydronephrosis and the development of hypertension.

Study I
- To determine whether hydronephrosis and hypertension are causally related and to evaluate the short and long term effects of hydronephrosis on blood pressure level.
- To elucidate the effects of different sodium diets on the blood pressure and the renin-angiotensin system in hydronephrotic animals.

Study II
- To examine the effects of ipsilateral nephrectomy, contralateral nephrectomy and ureterovesicostomy on blood pressure in hydronephrotic animals treated with different sodium diets, to determine whether salt-sensitive hypertension is of renal origin and if the mechanisms are located primarily to the hydronephrotic kidney.
- To study the effects of relief from partial ureteral obstruction (ipsilateral nephrectomy) on the plasma renin concentrations (PRC) in hydronephrotic animals.

Study III
- To develop a model for partial ureteral obstruction in mice.
- To investigate if hydronephrotic mice develop renal injury and salt-sensitive hypertension similar to that found in rats.

Study IV
- To investigate if NO deficiency is involved in the development of salt-sensitive hypertension in hydronephrosis.
MATERIAL AND METHODS

Animals
The experiments were conducted on male Sprague-Dawley rats (*Studies I, II and IV*) and C57bl/6J mice (*Study III*) from the M&B Research and Breeding Centre (Ry, Denmark).

A ureteral obstruction was created at young age (described below) and the animals were then left to grow with free access to standard rat chow (TD96329) and tap water. The experiments were conducted on adult animals treated with different sodium diets: normal salt (NS) (0.7% NaCl; TD96329), low salt (LS) (0.012% NaCl; TD90228) or high salt (HS) (4% NaCl; TD92034) (Harland Scandinavia, Allerød, Denmark). All animals were allowed to recover for at least one week after any surgical procedures and an equilibration period of 10 days was used on the different sodium diets before any measurements were taken.

Study protocols
*Study I.* Blood pressure in rats with PUUO was measured at different time points during a 5-month period. The effects of different sodium loads on blood pressure were investigated and the PRC were analysed.
**Study II.** The effects of relief from obstruction (ipsilateral nephrectomy or ureterovesicostomy) or contralateral nephrectomy on blood pressure and plasma renin levels were studied in hydronephrotic rats (PUUO) with salt-sensitive hypertension.

**Study III.** A model for PUUO in mice was developed. Blood pressure, renal excretion and PRC were measured during different sodium conditions, and renal histological and stereological changes were evaluated.

**Study IV.** Animals with both unilateral (PUUO) and bilateral (PBUO) hydronephrosis were investigated. Blood pressure and renal excretion were measured during different sodium conditions, before and after chronic N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) (0.5 mg/ml in drinking water for one week) or L-arginine treatment (1mg/ml in drinking water for 30 days). Plasma levels of renin, L-arginine, asymmetrical dimethylarginine (ADMA) and symmetrical dimethylarginine (SDMA) were analysed. The TGF characteristics were determined before and after administration of 7-Nitroindazole (7-NI) or L-arginine. The protein expression of NOS-isoforms in the cortex and medulla was analysed, and the renal histological changes were evaluated.

**Creation of partial ureteral obstruction (Studies I-IV)**
A partial unilateral (PUUO – Studies I-IV) or bilateral (PBUO – Study IV) ureteral obstruction was created to induce hydronephrosis. Male rats (~50 g) and mice (~7 g) underwent surgical obstruction at three weeks of age in which the left, or both, ureters were embedded in the psoas muscle by a modified technique to that originally described by Ulm and Miller in dogs.\textsuperscript{67} Anaesthesia was induced by spontaneous inhalation of isoflurane (Forene\textsuperscript{©}, Abbot Scandinavia AB, Kista, Sweden) and continued during surgery by
inhalation of ~2% isoflurane in air. Gas flow rate was set to ~150 ml/min. The animals were placed on a servo regulated heating pad to maintain body temperature between 37 and 38°C. The surgical field was sterilized with 70% ethanol and sterile NaCl and the abdominal wall was opened through a mid-line incision. The intestines were retracted to gain access to the left ureter, which was dissected free under the microscope (Figure 5).

Figure 5. Creation of partial unilateral ureteral obstruction (PUUO).

The underlying psoas muscle was split longitudinally to form a groove (rats: ~15 mm; mice ~5 mm) and the ureter was placed in it. The muscle edges were approximated above the ureter with two non-absorbable 6/0 (rats) or 7/0 (mice) silk sutures (Silk®, Ethicon, Johnson & Johnson Intl, USA), and the ureter was thus embedded in a tunnel. The abdominal incision was then closed in one layer with interrupted sutures. Sham operations were performed in the same manner, but without dissection of the ureter or the psoas muscle. After the operation, the animals were allowed to wake up under a heating lamp, and were not returned to their cages until fully awake.
Telemetry - Implantation (Studies I-IV)

**Rats (Studies I, II and IV):** Four to six weeks after PUUO, the animals were prepared for blood pressure measurements. Gas anaesthesia as described above, but with a higher concentration of isoflurane (2-2.5% in air) and also a higher gas flow rate (180-220 ml/min), was used. The skin was sterilized and the abdominal wall was opened, as described above. The intestines were retracted to achieve access to the deep vessels and kidneys. A macroscopic examination of both kidneys (see below) was performed in both sham operated and obstructed animals, and subsequently a 20 mm long segment of the abdominal aorta was dissected free from surrounding fat and connective tissue. The blood flow was then occluded by clamping the aorta (caudal to the left dorsal renal muscular branch and cranial to the iliac bifurcation of the aorta). A needle (21G X 1½"), bent 90° at the bevelled end, was used to puncture the aorta 5mm cranial to the iliac bifurcation. The catheter of the telemetric blood pressure device, PA-C40 (DSITM, Transoma Medical, St Paul, MN, USA) was then inserted into the aortic lumen (Figure 6) and the entry site was sealed by application of n-butyl-cyanoacrylate tissue adhesive (Vetbond™, 3M Animal Care Products, St Paul, MN, USA). The clamps were removed, the intestines were replaced in their original position: the transmitter was placed in the peritoneal cavity on top of the intestines.

![Figure 6. Implantation of the telemetric blood pressure device (PA-C40, DSI™) in rats.](image)
Mice (Study III): Six to eight weeks after PUUO, the telemetric device (PA-C10 (DSI™, Transoma Medical, St Paul, MN, USA) was implanted. Gas anaesthesia was used in the same way as described above, and a midline incision was made between the lower mandible and sternum (Figure 7). The catheter of the telemetric blood pressure device was then inserted into the left carotid lumen and secured by 6/0 silk sutures (Silk®, Ethicon, Johnson & Johnson Intl, USA). The entry site was sealed by application of n-butylcyanoacrylate tissue adhesive (Vetbond™, 3M Animal Care Products, St Paul, MN, USA) and the body of the transmitter was placed subcutaneously in the right flank.

Finally, the muscle edges were sutured and the skin incision was closed. After surgery, the animals were placed in new cages and allowed to wake up under a heating lamp. The hydronephrotic and sham operated animals (controls) were treated exactly the same. To verify that the tip of the catheter was correctly positioned, the transducer was switched on and the recorded radio signal (AM 550 kHz) pulsation was confirmed.

Figure 7. Implantation of the telemetric blood pressure device (PA-C10, DSI™) in mice (left panel). Telemetric blood pressure measurement in mice (right panel).
Telemetry - Measurements (*Studies I-IV*)

After surgery, the animals were allowed to recover for at least one week before blood pressure recording was started. The animal cage was placed on a receiver and the transmitter switched on (Figure 7). The signals received were transferred to a computer where calibrated blood pressure values were sampled by a computer-based system for data acquisition; PC-lab version 5.0. Data were collected for five seconds every second minute for at least 48 hours at a time. The recorded data were continuously analysed by the computer program, as follows: by comparing the incoming signal with a template blood pressure curve, individual heartbeats could be identified and stored together with the distance in time between the curves. If the time interval from a heartbeat to the surrounding pressure curves differed more than 20% from the average interval in the sampling period, the beat was discarded together with its two neighbouring waves. Pulse waves that passed this test were used for calculation of mean arterial blood pressure (MAP) and heart rate, which was stored together with the number of accepted pulse curves during the 5-second collecting period in question. Blood pressure data were collected and further analysed with a computer program developed at the department and which discarded values considered unreasonable, i.e. lower than 50 and higher than 220 mmHg. Data were also discarded if the number of accepted pulse curves during a 5-second sampling period was less than eight, indicating a disturbed transmission. The evaluation of the data with this system provided a higher level of accuracy than conventional methods, in which a mean value is taken straight from raw data.

Classification of hydronephrosis (*Studies I-IV*)

The kidneys were examined macroscopically before insertion of the telemetric equipment (*Studies I, II and IV*) or once the experiments were conducted (*Study III*). The obstructed kidneys were categorized as having a normal
appearance (i) or as having mild (ii), moderate (iii) or severe (iv) hydronephrosis (Figure 8). Grossly enlarged, sacculated kidneys without any visible parenchyma were categorized as non-functioning (v).

![Figure 8. Normal kidney and hydronephrotic kidneys with mild, moderate and severe degree of hydronephrosis.](image)

In this thesis, only animals with unilateral hydronephrosis were used, i.e. animals allocated to categories (i) and (v), and those with abnormalities in the contralateral kidney were excluded. In the sham-operated animals, both kidneys were examined, and a telemetric device was implanted only in the absence of macroscopic changes.

After completion of all experiments, the animals were anaesthetized and the hydronephrotic kidney was again macroscopically examined. The degree of hydronephrosis was measured by weight in the following way: the ureter was ligated \textit{in situ} and the vessels were cut to exsanguinate the kidney, which was then taken out and weighed. Thereafter, the kidney was sliced and emptied of urine, and was then reweighed to calculate the parenchymal weight: the difference between total weight and parenchymal weight was the weight of the urine volume. To measure the degree of hydronephrosis, the simple equation: renal pelvic volume, as measured by weight, divided by
renal parenchymal weight, yielding the so-called *hydronephrotic ratio* (HNR) was used.

\[
HNR = \frac{\text{Pelvic volume}}{\text{Parenchymal weight}}
\]

Normal kidneys have an HNR of less than 0.1 (0.06–0.09). An HNR of 0.1–0.3 was classified as indicating mild hydronephrosis, 0.3 and 1.0 as moderate hydronephrosis, and above 1.0 as severe hydronephrosis. The macroscopic examination and weight grading corresponded well.

**Sampling and renin assay (Studies I-IV)**

Blood samples for renin analysis were taken from the tail tip (rats) or the orbital plexus (mice) immediately after anaesthesia at the end of each diet period. Samples were centrifuged and instantly frozen to -70°C for later assay. The PRC was measured by radioimmunoassay (RIA) of angiotensin I with the antibody-trapping technique. In short, 10 μl of plasma from each sample was serially diluted (25, 50, 100 and 200×). From dilution of plasma, 5 μl was incubated in duplicate for 24 hours, together with a mixture of rabbit angiotensin I antibody and renin substrate (Angiotensinogen equivalent to 1200 ng angiotensin I ml⁻¹) from rats nephrectomized 24 hours previously, from which renin had been extracted by affinity chromatography. After the incubation step, the reaction was stopped by addition of 1 ml cold barbital buffer, an Angiotensin I tracer was added and RIA was performed. Renin values were standardized by reference to renin standards obtained from the Institute for Medical Research (MRC, Holly Hill, London, UK), and the values were expressed in standard Goldblatt units (GU).
Ipsi- and contralateral nephrectomy (*Study II*)

The animals were anaesthetised with isoflurane as above and placed on a heating pad exposing their left flank. The site of the incision was shaved and sterilized and a flank incision exposed the left kidney. The renal artery and vein were carefully isolated and a single ligature was placed around them and tied tightly before making a distal cut. The ureter was exposed and two ligatures were placed close to the UPJ and cut in between. The kidney was removed, the incision closed and HNR calculated, as described above. The animals were allowed to recover and telemetric measurements were performed.

The procedure, for the contralateral nephrectomy, was the same as for the ipsilateral nephrectomy, except that the contralateral kidney was removed. Furthermore, the protocol for the telemetric measurements was identical to that in the ipsilateral nephrectomy group. In the control animals, a left side nephrectomy was performed.

Ureterovesicostomy (*Study II*)

The same kind of anaesthesia was used and the abdomen was opened through a sterile midline incision and the ipsilateral kidney, ureter and bladder were exposed and visually examined. To validate the outcome of the ureterovesicostomy, the cross-sectional area of the kidney (\(length \times width \ mm^2\)) was measured, before and after this procedure. A sterilized plastic catheter was inserted through a punctured hole within a purse-string suture in the bladder, and the purse-string was drawn up tightly and tied. Two sutures were then placed around the ureter distal to the pelvic area, the catheter was allowed to enter the exposed pelvic region through a punctured hole and finally secured. In control animals, the ureter was visually examined but not
catheterised. The incision was closed and the animals were allowed to recover before telemetric measurements were performed.

In animals with an increased cross-sectional area at the time of euthanasia, the ureterovesicostomy was considered unsuccessful and the data were discarded.

Renal excretion measurements (Studies III and IV)
Animals were housed individually for 24 hours in metabolism cages. Urine production and water consumption were determined gravimetrically. Sodium and potassium concentrations were measured by flame photometry (FLM3; Radiometer, Copenhagen, Denmark) and osmolality by depression of the freezing point (Fiske® Micro-sample Osmometer, Model 210; Fiske Associates, Norwood, MA, USA).

L-arginine, ADMA and SDMA measurements (Study IV)
From anaesthetised controls, PUUO and PBUO animals, 1 ml blood was withdrawn from the carotid artery and plasma concentrations of L-arginine, ADMA, and SDMA were determined simultaneously by high-performance liquid chromatography, as described previously but with modified chromatographic separation conditions.

Western blotting for nitric oxide synthases (Study IV)
The animals were anaesthetized and a catheter was placed in the left carotid artery. The infusion of cold phosphate buffered saline (PBS) was started once the right renal vein was cut and the kidneys were explanted for Western blotting. Renal cortex and medulla were separated and homogenized in lysis buffer (1.0% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 10 mM NaF, 80mM Tris, pH 7.5) containing enzyme inhibitors (Complete Mini; 1 tab-
let/1.5 ml; Roche Diagnostics, Mannheim, Germany). Samples were run on 7.5% Tris-HCl gels with Tris/glycine/SDS buffer. After transfer to nitrocellulose membranes, the proteins were detected with rabbit anti-nNOS (1 μg/ml; Zymed Laboratories; Invitrogen, Carlsbad, CA, USA), mouse anti-eNOS (1 μg/ml; Zymed Laboratories) and HRP-conjugated secondary antibodies (goat anti-rabbit and goat anti-mouse, 1:5000; Sigma Aldrich) by an ECL-camera (Kodak image station 2000; New Haven, CT, USA). β-actin was detected with mouse anti-rat β-actin antibody (1:10,000; Sigma Aldrich) and secondary HRP-conjugated goat-anti mouse antibody (1:60,000; Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA).

Histology (Studies III and IV)
The animals were anaesthetized and a catheter was placed in the left carotid artery to allow formalin (4% in PBS) infusion. The abdomen was opened and the right renal vein was cut at the same time as the infusion started. The infusion continued for 10 minutes and the kidneys were then removed. Sagittal slices of the renal tissue were fixed in buffered formalin and embedded in paraffin. Embedded tissue blocks were cut into 5-μm thick sections and stained with Haematoxylin and Eosin (HE), Periodic Acid-Schiff (PAS) stain and Picro-Sirius stain for a blind histological evaluation.

Stereology (Study III)
To assess glomerular area and volume, a light microscope (Leitz DMRB, Leica Microsystems, Wetzlar, Germany) with a CCD camera (AxioCam color, Carl Zeiss, Oberkochen, Germany) was used. The cortical area of one section was photographed and a computer program (Scion image, Scion Corporation, MD, USA) was then used to determine the area of all visible glomeruli in the cross-section. From the measured glomerular area, an approximation of the real area and volume was calculated with the equation D=
$4/\pi \times d$, where $D$ is the real diameter and $d$ is the measured mean glomerular diameter.\textsuperscript{72}

Stop-flow pressure measurements (Study IV)
Characteristics of the TGF were determined with stop-flow pressure techniques as described earlier.\textsuperscript{73} The animals were anaesthetized, a flank incision was made and the kidney was prepared for micropuncture experiments (Figure 9).

![Figure 9. Set-up for renal micropuncture experiment.](image)

Randomly chosen proximal tubular segments on the surface were punctured with a sharpened glass pipette (OD 3-5 μm) filled with a lissamine green-stained 1M NaCl solution. The pipette was connected to a servo-nulling pressure system (World Precision Instruments, New Haven, CT, USA) to determine the proximal tubular free-flow pressure ($P_{\text{FF}}$). A second pipette (OD 7-9 μm), filled with artificial ultrafiltrate (140 mM NaCl, 5 mM KCl, 2 mM CaCl$_2$, 1 mM MgCl$_2$, 4 mM NaHCO$_3$, 7 mM urea, and 2 g/L Lissamine green, pH 7.4) and connected to a microperfusion pump (Hampel, Frankfurt,
Germany), was inserted in the last accessible segment of the proximal tu-
bule. A solid wax block was placed in between with a third pipette (OD 7-9
μm). The proximal tubular stop-flow pressure (PSF) upstream to the block
was determined at different perfusion rates (0-35 nl/min) in the loop of
Henle. The maximal change in stop-flow pressure (ΔPSF), was used to indi-
cate the TGF reactivity and the tubular flow rate eliciting half-maximal ΔPSF,
(i.e. the turning point), indicated the TGF sensitivity. To create TGF-
response curves, normalized data were used in a non-linear least squares
curve-fitting program.73 The TGF-characteristics were determined in normo-
volemic animals during control conditions and after either nNOS inhibition
with 7-NI (25 mg/kg i.p.) or intratubular infusion of L-arginine (10⁻² M).

Statistical analysis
Values are presented as means ± SEM. Single comparisons between nor-
mally distributed parameters were tested for significance with Student’s
paired or unpaired t-test. For multiple comparisons, analysis of variance
(ANOVA) followed by the Bonferroni or Fisher’s post-hoc test, were used.
Scored data for the histological evaluation were analysed by the Kruskal-
Wallis test followed by the Mann-Whitney-U-test. Statistical significance
was defined as p<0.05.

Ethics
All experiments were approved by the Uppsala Ethical Committee for Ani-
mal Experiments and performed in accordance with national guidelines for
care and use of laboratory animals.
RESULTS

In all studies (I-IV) the animals were in good condition, and at the beginning of the experimental protocols there was no difference in body weight between the sham operated controls and the hydronephrotic animals. All hydronephrotic animals developed salt-sensitive hypertension that correlated to their degree of hydronephrosis. No differences were determined in heart rate between the groups.
Study I

*Long-term effects on blood pressure*

Blood pressure level was higher in the obstructed animals (106±5 mmHg) than in the controls (87±1 mmHg). Furthermore, blood pressure increased slowly with time in the hydronephrotic animals but not in the controls (Figure 10).

**Figure 10.** Long term effect on mean arterial blood pressure (MAP) in hydronephrotic animals and controls.

* P<0.05 compared with the initial value (within same group)

# P<0.05 compared with the controls
Effects of different salt loads on blood pressure

All hydronephrotic groups displayed elevated blood pressures compared with the controls on NS, LS and HS diets (Figure 11). There were no differences in blood pressure levels between the first and second NS period. Furthermore, all hydronephrotic animals displayed salt-sensitive blood pressure that became more pronounced with the degree of hydronephrosis.

Figure 11. Mean arterial blood pressure (MAP) in controls and hydronephrotic animals with different hydronephrotic degrees, treated with normal salt (NS), low salt (LS), high salt (HS) and normal salt diet once again (NS2).

* P<0.05 compared with hydronephrotic groups on same diet.
# P<0.05 compared with NS and NS2 diet within the same group
† P<0.05 compared with LS diet within the same group.
Typical 24-hour circadian variations in blood pressure were observed in all groups, with a higher blood pressure during active periods (night time). However, the circadian variation in blood pressure pattern was less pronounced in obstructed animals, with a mean blood pressure increase of 9.3±1.1% in the control animals but only 7.0±0.4% in the hydronephrotic ones (p<0.05). Fluctuations in MAP during the day and night also appeared less pronounced with increasing degree of hydronephrosis (Figure 12).

**Figure 12.** 48-hour mean arterial blood pressure (MAP) recordings in representative animals with different degrees of hydronephrosis and in sham operated control. Shaded areas represent nighttime.
As shown in the scatter plot (Figure 13), there was a correlation between blood pressure and the degree of hydronephrosis, expressed as HNR.

**Figure 13.** Mean arterial blood pressure (MAP) in controls and hydronephrotic animals (HN) with different hydronephrotic ratios (HNR).
Plasma levels were higher in the hydronephrotic rats on all diets. However, it was only found significant on the NS diet (Figure 14). In all groups given the LS diet, PRC increased, compared with baseline level. Animals with severe hydronephrosis displayed lower PRC levels than both mild and moderate groups on all diets, however, this was only significant on the LS diet.

**Figure 14.** Plasma renin concentration (PRC) in controls and hydronephrotic animals with different hydronephrotic degrees, treated with normal salt (NS), low salt (LS), high salt (HS) diet.

* P<0.05 compared with hydronephrotic groups on same diet.
# P<0.05 compared with both NS and HS diets
† P<0.05 compared with those with moderate and mild hydronephrosis on same diet
Study II

*Ipsilateral nephrectomy*

In the hydrenephrotic animals, ipsilateral nephrectomy attenuated both hypertension and salt-sensitivity, whereas, in the controls there was a transient increase in blood pressure (Figure 15).

![Graph showing mean arterial blood pressure (MAP) in controls and hydrenephrotic animals with different hydrenephrotic degrees, treated with NS and HS diets, before and after ipsilateral nephrectomy (i-UNX).]

* Figure 15. Mean arterial blood pressure (MAP) in controls and hydrenephrotic animals with different hydrenephrotic degrees, treated with NS and HS diets, before and after ipsilateral nephrectomy (i-UNX).

* P<0.05 compared with controls on same diet and with the same surgical treatment.

# P<0.05 compared with NS diet within the same group and with the same surgical treatment

† P<0.05 compared with same diet within the same group before nephrectomy.
Before nephrectomy, PRC was elevated in the hydronephrotic group, compared with the controls, but after removal of the obstructed kidney, these differences were eliminated (Figure 16).

**Figure 16.** Plasma renin concentration (PRC) in controls and hydronephrotic animals with different degrees of hydronephrosis treated with normal salt (NS) and high salt (HS) diets, before and after ipsilateral nephrectomy (i-UNX). GU=Goldblatt units.

* P<0.05 compared with controls on same diet and with the same surgical treatment.
† P<0.05 compared with same diet within the same group before nephrectomy.

**Contralateral nephrectomy**

In contrast to ipsilateral nephrectomy, contralateral nephrectomy augmented the hypertension in the hydronephrotic animals (97±2 to 107±3 mmHg), whereas, in the controls, no persistent changes occurred (87±4 to 88±4 mmHg).
Ureterovesicostomy

As with ipsilateral nephrectomy, ureterovesicostomy decreased blood pressure in the hydronephrotic animals, and salt-sensitivity became less pronounced. These changes were not observed in the sham-operated controls. The reduction cross-sectional kidney area (1264±178 to 780±137 mm²) correlated with a concomitant fall in blood pressure (Figure 17).

![Graph](image)

**Figure 17.** Correlation between the reduction in cross-sectional area and the change in mean arterial blood pressure (MAP) in hydronephrotic animals after ureterovesicostomy (UV).
Study III

Similar the rats in Study I, the hydronephrotic mice developed salt-sensitive hypertension (Figure 18) that correlated well with their hydronephrotic degree ($R^2 = 0.91$).

![Figure 18](image)

**Figure 18.** 24-hour mean arterial blood pressure (MAP) in controls and hydronephrotic animals (HN), treated with NS and HS diets.

* $P<0.05$ compared with controls on same diet.

# $P<0.05$ compared with same diet within the same group.

**Renal excretion measurements**

Urine excretion rate was higher in the hydronephrotic animals on both NS (77±7 μl/24h/g BW) and HS diets (103±5 μl/24h/g BW), than in the controls (NS 58±4 μl/24h/g BW, and HS 69±12 μl/24h/g BW). This was associated with reduced urine osmolality in the hydronephrotic animals (1043±81 mOsm) on the NS diet, compared to the controls (1539±117 mOsm), but was not significant on the HS diet (hydronephrotic animals 1174±73 mOsm and controls 1215±64 mOsm).
**Plasma renin concentration**

No differences in PRC were found between the hydronephrotic (1043±119 $10^{-5}$ GU/ml) and controls animals (1312±316 $10^{-5}$ GU/ml) on the NS diet or the HS diet (582±77 $10^{-5}$ GU/ml hydronephrotic animals, 449±49 $10^{-5}$ GU/ml controls).

**Histology and stereology**

The parenchymal weight of the hydronephrotic kidneys decreased, whereas, compensatory hypertrophy and glomerular volume increase observed in non-obstructed contralateral kidneys. The obstructed animals displayed dilated pelvic areas with flattening of the papilla, which was associated with areas of fibrosis, inflammation and glomerular changes (Figure 19).

![Representative photomicrographs](image)

**Figure 19.** Representative photomicrographs of a control kidney (A) and a hydronephrotic kidney (B-D). (A) The control displays normal histoarchitecture with a distinct cortex, medulla and renal papilla. (B) In hydronephrosis,
the pelvic area is slightly dilated, with flattening of the renal papilla. (A) and (B) are stained with Haematoxylin and Eosin (HE). (C) With Periodic Acid-Schiff (PAS) staining, slight sclerotic glomeruli (black arrows) and infiltration of inflammatory cells (white arrow) are observed. (D) Picro-Sirius staining showed areas with subepithelial fibrosis (black arrows).
Study IV

Renal excretion measurements

During control conditions, urine excretion rate was higher and urine-concentrating ability reduced in the hydronephrotic groups for both diets (Table 1.). This was in accordance with observations from Study III.

Table 1. Renal excretion data on normal and high salt diets in controls and unilateral (PUUO) and bilateral (PBUO) hydronephrotic animals.

<table>
<thead>
<tr>
<th></th>
<th>Normal sodium diet</th>
<th></th>
<th></th>
<th>High sodium diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>PUUO</td>
<td>PBUO</td>
<td>Controls</td>
</tr>
<tr>
<td>Diuresis (µl/24h/g BW)</td>
<td>24 ± 2</td>
<td>35 ± 4*</td>
<td>59 ± 16*</td>
<td>80 ± 5</td>
</tr>
<tr>
<td>Osmolality (mM)</td>
<td>1629 ± 66</td>
<td>1389 ± 110*</td>
<td>849 ± 221*</td>
<td>1322 ± 33</td>
</tr>
</tbody>
</table>

* p<0.05 compared with controls on same diet
† p<0.05 compared with PUUO on same diet

After L-NAME treatment (0.5mg/ml; drinking water; one week), the controls had a reduced ability for excreting sodium, potassium and total osmoles, both during NS and HS conditions. The effects of L-NAME were less pronounced in the hydronephrotic animals and reduced excretion was only found for sodium and potassium during NS diet.

L-arginine treatment (1 mg/ml; drinking water; 30 days) did not affect renal excretion; however, the hydronephrotic groups tended to increase electrolyte and water excretion, which was not observed in the controls.
**Plasma renin analysis**

During both NS and HS conditions, PRC was higher in PUUO than in controls, but not in PBUO animals (Figure 20).

![Graph showing Plasma renin concentration (PRC) in controls and unilateral (PUUO) and bilateral (PBUO) hydronephrotic animals, treated with normal (NS) and high salt (HS) diets.](image)

* p<0.05 compared with controls on same diet.
† p<0.05 compared with PUUO on same diet.

**L-arginine, ADMA and SDMA measurements**

The ADMA levels were higher in both hydronephrotic groups than in controls. SDMA levels were only elevated in animals with bilateral hydronephrosis (Table 2). L-arginine levels were similar between the three groups (i.e. Controls, PUUO and PBUO).
Table 2. Plasma levels of L-arginine, ADMA and SDMA in controls and unilateral (PUUO) and bilateral (PBUO) hydronephrotic animals.

<table>
<thead>
<tr>
<th></th>
<th>L-Arginine (μmol/l)</th>
<th>ADMA (μmol/l)</th>
<th>SDMA (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>132.4 ± 5.9</td>
<td>0.482 ± 0.020</td>
<td>0.291 ± 0.007</td>
</tr>
<tr>
<td>PUUO</td>
<td>143.4 ± 8.8</td>
<td>0.560 ± 0.028*</td>
<td>0.319 ± 0.020</td>
</tr>
<tr>
<td>PBUO</td>
<td>128.2 ± 8.5</td>
<td>0.747 ± 0.063†</td>
<td>0.675 ± 0.146†</td>
</tr>
</tbody>
</table>

* p<0.05 compared with controls
† p<0.05 compared with PUUO

Effects of L-NAME on blood pressure

During both NS and HS conditions, blood pressure was higher in PBUO (NS 132±7, HS 158±8 mmHg) and PUUO (NS 114±4, HS 129±9 mmHg) animals than in the controls (NS 85±2, HS 89±2 mmHg).

On NS diet, blood pressure response to L-NAME treatment was more pronounced in the controls (26±1%) than in the PUUO (18±1%) and PBUO (16±2%) animals.

During HS conditions, this response became more pronounced in the controls (34±3%), whereas, no differences were found for the PUUO (18±2%) and PBUO (13±2%) animals, when compared with the NS diet. L-NAME treatment increased salt-sensitivity only in controls, and differences in salt-sensitivity were no longer present between the PBUO (18±5%), PUUO (13±3%) and control (12±2%) animals.
**Effects of L-arginine on blood pressure**

The hydronephrotic animals displayed salt-sensitive hypertension similar to that observed for the L-NAME treated groups (see above). In the non-treated hydronephrotic animals a progressive blood pressure increase was observed during the 30 days experimental period for PUUO during HS diet (+5 mmHg) and in PBUO animals during HS (+24 mmHg) and NS diet (+14 mmHg) (Figure 21; upper panel).

Chronic L-arginine supplementation (1 mg/ml) in the drinking water for 30 days reduced the blood pressure in PUUO during both NS (-11 mmHg) and high sodium conditions (-9 mmHg). In the PBUO animals, L-arginine treatment completely prevented further increase in blood pressure during NS conditions. During HS conditions L-arginine supplementation did not prevent blood pressure increase, however, the augmentation in blood pressure (+11 mmHg) was not as pronounced as in the non-treated group (+24 mmHg). In the control animals, L-arginine supplementation had no effect on blood pressure (Figure 21; lower panel).
Figure 21. Mean arterial blood pressure (MAP) in controls and unilateral (PUUO) and bilateral (PBUO) hydronephrotic animals, treated with NS (dashed line) or HS diets (solid line) for 30 days, with or without L-arginine supplementation (1 mg/ml in drinking water).

* p<0.05 compared with the control period, within same group and diet.
# p<0.05 decreased compared with the control period, within same group and diet.
**Stop-flow pressure measurements**

There were no differences in $P_{FF}$ or $P_{SF}$ between the controls and hydronephrotic animals during control conditions, or after 7-NI or L-arginine administration. During control conditions, the reactivity of the TGF response, as indicated by the $\Delta P_{SF}$, was greater in the hydronephrotic group (14.4±1.0 mmHg) than in control animals (9.4±0.8 mmHg). The turning point was lower in the hydronephrotic animals (14.9±1.4 nl/min) than in the controls (19.1±1.2 nl/min), indicating higher sensitivity of TGF response in hydronephrosis. Administration of 7-NI, increased the reactivity and sensitivity of the TGF response (leftward shift) in the control animals, but had no effect in the hydronephrotic animals. L-arginine administration reduced the reactivity and sensitivity of the TGF response (rightward shift) in the hydronephrotic animals, but had no effect in the control animals (Figure 22).

![Figure 22. TGF response curves in controls and hydronephrotic animals during control conditions (Baseline) and after administration of 7-NI or L-arginine.](image)

* $p<0.05$ compared with baseline values of same group

# $p<0.05$ compared with values of control animals under similar conditions
**Western Blotting for NOS**

There was a reduced protein expression of nNOS in the cortex and medulla for both hydronephrotic groups. The eNOS expression was only significantly reduced for the PBUO animals.

**Histology**

The hydronephrotic kidneys displayed variable degrees of dilatation of the pelvic area with flattening of the papilla. This was associated with areas of fibrosis, inflammation, and glomerular and tubular changes. The severity of the histopathological changes correlated with the degree of hydronephrosis. Fibrotic and glomerular changes were also observed in the contralateral kidney in PUUO animals (Figure 23).

![Figure 23](image)

**Figure 23.** Representative photomicrographs of hydronephrotic kidneys, with mild (A-C) and severe histopathological changes (D-F). (A) Overview of a mild hydronephrotic kidney with mild fibrosis and tubular dilatations. (B) Higher magnification with tubular dilatation (red arrow) and slight inflammation (black arrow). (C) Mild fibrosis in medulla and corticomedullary junction (black arrows). (D) Overview showing severe hydronephrosis with inflammation, tubular dilatation and fibrosis. (E) Detail from cortex demon-
strating dilated tubuli filled with PAS-positive material (white arrow), interstitial fibrosis, inflammation and tubular atrophy. Destructive glomerular changes are conspicuous with proliferate changes, necrosis, collapse and thickening of the bowman capsule (black arrows). (F) Areas with severe fibrosis (black arrow), atrophy and tubular dilatation (red arrow). Photomicrographs A, B, D and E are stained with Periodic Acid-Schiff (PAS) and C and F with Picro-Sirius. The control kidneys displayed normal histoarchitecture with distinct cortex, medulla and renal papilla, with no histopathological changes.
DISCUSSION

Hydronephrosis due to UPJ obstruction is a common condition in newborn infants and the renal function in hydronephrosis, as measured by RBF and GFR, has been demonstrated to be well preserved for a long time.\textsuperscript{27-29} The outcome of these observations has been a worldwide trend towards more conservative treatment of hydronephrosis. However, the long-term consequences of this new treatment strategy are unknown. In the present study, experimental hydronephrosis, induced by a PUUO at young age, caused renal injury and salt-sensitive hypertension in both rats and mice. The hypertension and salt-sensitivity correlated well with the degree of the hydronephrosis and increased slowly with time. The explanation for this time dependency is probably progressive hydronephrosis and renal pathological changes.

Relief of chronic partial ureteral obstruction

There are several case reports\textsuperscript{20, 34-58} of hypertension in hydronephrotic patients. The hypertension is obviously secondary to hydronephrosis as nephrectomy or pyeloplasty normalized the blood pressure. Even though the management policy of hydronephrosis has become more conservative, symptomatic patients are treated surgically. In Study II, relief from the obstruction by unilateral nephrectomy or ureterovisicostomy attenuated salt-sensitive hypertension in hydronephrotic animals, whereas, an augmentation occurred after removal of the non-obstructed, functioning kidney. The reduction in cross sectional area of the hydronephrotic kidney (i.e. the efficiency of internal urinary drainage) correlated with the concomitant drop in blood pressure.
Unilateral nephrectomy in the controls caused a transient blood pressure increase, which was not seen in hydronephrotic animals after ipsilateral nephrectomy. This could reflect that control animals had to adapt to the loss of a normally functioning kidney, a situation that was already present in hydronephrosis. Moreover, contralateral nephrectomy augmented salt-sensitive hypertension in hydronephrosis. The hypertensive mechanisms are primarily located to the diseased kidney, insofar as relief of the obstruction attenuated blood pressure. However, as the blood pressure levels following ipsilateral nephrectomy correlated with the previous degree of hydronephrosis, secondary mechanisms must be considered.

Renal excretion measurements
The renal function, in terms of GFR and RBF, are well preserved for a long time in hydronephrotic children. In animal models with PUUO, GFR increases, remains unchanged or decreases. However, in general, ureteral obstruction results in decreased RBF and GFR. The literature on electrolyte and water excretion during PUUO is also conflicting. This discrepancy may depend on the severity and duration of the obstruction, the diuretic state and the compensatory ability of the contralateral kidney. In the model for experimental hydronephrosis presented here, the results are consistent; both rats and mice with PUUO display increased diuresis and reduced ability to regulate electrolyte concentrations. The changes observed could possibly be explained by reduction of the renal medulla and down regulation of aquaporins-2 and sodium transporters in the obstructed kidney.

Renin Angiotensin System
Many forms of secondary hypertension display changes in the activity of the renin-angiotensin system, leading to increased concentration of circulating angiotensin II, a powerful vasoconstrictor that acts both systemically, by af-
fecting vascular tone, and intrarenally, by sensitising the TGF system. In hydronephrotic patients with hypertension, increased plasma renin levels have been demonstrated. However, the participation of renin appears to be influenced by the duration of the obstruction, the presence or absence of a contralateral normal kidney and other intrarenal factors. In the present studies, hydronephrotic rats, but not mice, displayed elevated PRC, compared to the controls. The elevated renin concentrations were normalized after removal of the hydronephrotic kidney. However, increased PRC cannot entirely explain the hypertension, as animals with severe unilateral hydronephrosis or bilateral hydronephrosis, (i.e. those with the highest blood pressures) were the one with the lowest PRC. These data could be interpreted as the severely hydronephrotic kidneys are damaged and cannot respond to different salt diets in a normal way, or, that renin is down regulated due to the higher blood pressure level. It has also been suggested that the angiotensin II concentration may be 60-100 times higher in the renal interstitium than in the plasma as a result of intrarenal formation. In this thesis changes of intra renal renin levels were not studied and remain to be investigated. Studies on the genetic model of rat hypertension (SHR), demonstrate an important role of renin angiotensin system in the development, but not maintenance, of hypertension. Taken together, the renin angiotensin system is considered involved, but changes in plasma renin levels cannot alone be responsible for hypertension in hydronephrosis.

Histopathological changes
Obstructive nephropathy is not a result of simple mechanical impairment of urine flow, but a complex condition resulting from alterations in the glomerular and tubular function. Complete unilateral ureteral obstruction causes rapid destruction of the kidney, but is rare in clinical practice. Experimental ureteral obstruction is an important model for studying the mechanisms of
renal fibrosis and inflammation, and for evaluating the impact of potential therapeutic approaches to ameliorate renal disease. Increased activity of the renin angiotensin system and oxidative stress with subsequent reduction in NO availability are involved in the development of renal pathological changes taking place in ureteral obstruction. The magnitude of renal injuries and renal function impairment (i.e. reduction in GFR and RBF) has been shown to be dependent on the angiotensin II type 1 receptor (AT1-R). Inhibition of AT1-R during nephrogenesis exacerbates renal injuries of the obstructed kidney, but inhibition during subsequent renal maturation has salutary effects.

In the model for chronic PUUO presented here, the hydronephrotic kidneys of rats and mice displayed variable degrees of dilatation of the pelvic area, with flattening of the renal papilla. Moreover, hydronephrotic kidneys exhibited areas with subepithelial fibrosis, infiltration of inflammatory cells (i.e. plasma cells and lymphocytes), predominantly localized to the medulla and pelvic region, and glomerular changes (i.e. sclerosis, mesangial matrix increase and collapsed glomeruli). Tubular changes (i.e. hyaline material in the lumen, atrophy and thickening of the basal membrane) and vascular changes (i.e. hypertrophy of the media, hyperplasia in arterioles and arteries) were only detected in animals with severe hydronephrosis. Mild fibrotic and glomerular changes and compensatory hyperthrophy (i.e. increased weight and glomerular volume) were identified in the contralateral kidneys of animals with PUUO. Based on this evidence, the model exhibited the characteristics for obstructive nephropathy and could therefore provide useful information for the clinician.
L-NAME and blood pressure

NO is a potent vasodilator that possesses an important role in blood pressure regulation. Long term blockade of the NO system with L-NAME results in salt-sensitive hypertension.88-92 The acute blood pressure response to NOS inhibition appears due to generalized arterial vasoconstriction, whereas, renal sodium and water handling are more important for sustained blood pressure elevation and salt-sensitivity. In the present study, the effects of chronic L-NAME administration on blood pressure and salt-sensitivity were more pronounced in controls than in hydronephrotic animals. After L-NAME administration, blood pressure levels were still higher in the hydronephrotic groups, but blood pressure elevation was highest in the controls. Salt-sensitivity increased only in control animals, and after NO blockade, no differences between the three groups (i.e. Controls, PUUO and PBUO) were identified. This indicated that animals with hydronephrosis had reduced NO availability, which could contribute to the development of salt-sensitive hypertension.

L-arginine and blood pressure

The L-arginine-NO pathway is ascribed an important role in the development of systemic hypertension and progressive renal disease.93-95 NOS substrate supplementation has beneficial effects on both blood pressure and renal disease in clinical trials96-98 and in experimental models.99-105 In animals with ureteral obstruction, the administration of L-arginine increases GFR and RBF to the post-obstructed kidney, and decreases infiltration of macrophages of the renal parenchyma106 and tubulointerstitial fibrosis.103 In the present study, chronic L-arginine supplementation reduced or prevented further increase in blood pressure in hydronephrotic animals.
Nitric oxide, renal excretion and salt-sensitivity

Reduced NO availability in hydronephrotic animals was apparent from renal excretion measurements. Inhibition of the NO system by chronic L-NAME administration did not change renal excretion properties in hydronephrosis, whereas, the controls displayed a reduction in osmolar excretion. Stimulation of the NO system by chronic L-arginine supplementation did not change renal excretion in the controls, whereas, the hydronephrotic groups tended to increase electrolytes and water excretion.

NO has an important role in the control of renal sodium excretion during physiologic conditions, and plays a critical role in the adaptation to high sodium intake.\textsuperscript{107-110} Evidently, high sodium conditions (i.e. high salt intake) result in high NO concentrations and a large NOS expression, especially in the renal medulla. An increased NO concentration will result in increased sodium and water excretion and deficient NO increase during these conditions will result in sodium retention and hypertension. The mechanisms for this are unclear, but oxidative stress has been demonstrated in the pathophysiology of salt-sensitive hypertension\textsuperscript{9, 111} and may also be involved in hydronephrosis.

Supplementation of antioxidant vitamin-E (\textit{\alpha}-DL-tocopherol) in rat chow (5\% w/w) for three weeks lowered blood pressure in hydronephrotic animals both during NS conditions (114±8 to 109±9 mmHg) and HS conditions (120±6 to 104±5 mmHg), but not in control animals [(NS: 90±9 to 88±9 mmHg; HS: 92±5 to 91±5 mmHg)] (unpublished data). Vitamin E is a potent, naturally occurring antioxidant that scavenges reactive oxygen species and lipid peroxyl radicals. In a model for chronic kidney disease (5/6 nephrectomy), long-term vitamin E treatment reduces superoxide production and preserves renal NO generation.\textsuperscript{112}
Enhanced production of free radicals, especially superoxide, will reduce NO availability with consequences, such as, increased TGF sensitivity, increased afferent arteriolar responsiveness, reduced medullary blood flow and increased tubular reabsorption. In the studies presented here, the suggestion that reduced NO availability explains salt-sensitive hypertension was further supported by reduced NOS protein expressions in the kidney cortex and medulla of hydronephrotic animals. The same feature of reduced NO synthase activity is observed in Dahl salt-sensitive rats.

Sympathetic nervous activity, renal excretion and salt-sensitivity
Increased renal sympathetic nerve activity can increase PRC, and has been demonstrated to lead to sodium retention and salt-sensitive hypertension. An impairment of the renorenal reflexes contributes to hypertension in both two-kidney one clip model, and in SHR. Renal denervation delays the onset of hypertension and is associated with increased diuresis and natriuresis. In the current model for hydronephrosis, renal denervation of the partially obstructed kidney (at three and seven weeks of age) attenuated both hypertension and salt-sensitivity (unpublished data).

L-arginine, ADMA and SDMA
The limitation of L-arginine and increased levels of endogenous NOS inhibitor (ADMA) are two possible causes of NO deficiency in renal and cardiovascular disease. An association between oxidative stress and increased ADMA levels has also been demonstrated. A closely related compound SDMA does not inhibit NOS; however, as arginine, ADMA and SDMA share a common pathway for enter into the cell, high plasma levels of SDMA may indirectly reduce NO production by competing with arginine in cellular uptake. In this study, plasma concentrations of ADMA and
SDMA were elevated, whereas, L-arginine levels were normal in hydronephrosis. Endogenous inhibition of NOS and competition between SDMA and arginine within the kidney may explain the reduced NO concentrations and thus, cause renal injury and hypertension in hydronephrosis.

**Characteristics of TGF**

There is a connection between renal NO deficiency, enhanced TGF responsiveness and development of hypertension.\(^6^6\), \(^1^1^9\), \(^1^2^0\) Micropuncture experiments have showed that the TGF mechanism, which is an important regulator of GFR, is reset towards higher responsiveness in hydronephrosis during volume expansion.\(^6^1\) This paradoxical change of the TGF is also manifested in Milan hypertensive strain rats before they develop hypertension by volume retention and in SHR.\(^1^1^9\) The resetting of TGF sensitivity is intimately coupled to nNOS derived NO from the macula densa cells in the juxtaglomerular apparatus. Increased NO availability desensitises TGF response (rightward shift), whereas decreased NO or increased angiotensin II increases responsiveness (leftward shift). Chronic blockade of nNOS by 7-NI increases TGF sensitivity in a similar way as in hydronephrosis, and leads to the development of hypertension.\(^6^6\)

Based on this, it appeared as if the functional adaptation to PUUO could lead to the development of NO dependent hypertension. The stop-flow pressure measurements demonstrated increased sensitivity and reactivity of TGF response in normovolemic hydronephrotic animals. Inhibition of nNOS increased responsiveness in controls, but not in hydronephrotic kidneys. However, intratubular infusion of L-arginine, attenuated TGF response in hydronephrosis, whereas, no changes were observed in the controls. The mechanisms for NO deficiency in the juxtaglomerular apparatus, with sub-
sequent resetting of the TGF response, are not clear, but oxidative stress in the diseased kidney should be considered. The increased TGF responsiveness in SHR is similar to that seen in hydronephrosis and increased responsiveness can be diminished by administration of tempol (SOD-mimetic).\textsuperscript{120}
Hydronephrosis, due to chronic PUUO, causes salt-sensitive hypertension in both rats and mice. Hypertension can be markedly attenuated by relief of the obstruction, thus, the mechanisms appear to be of renal origin and primarily located to the diseased kidney. Hydronephrotic animals display an abnormal renal excretion pattern with increased diuresis and impaired urine-concentrating ability. This is associated with histopathological changes similar to that observed in obstructive nephropathy (i.e. interstitial fibrosis, inflammation, glomerular and tubular changes). These new findings imply that the current conservative treatment strategy in hydronephrosis should be reconsidered in favour of treatment that is more active, in order to prevent the development of renal injury and hypertension in later life. The mechanisms for salt-sensitive hypertension in hydronephrosis are unclear and remain to be further investigated. Increased renin angiotensin system activity, due to ureteral obstruction, might be involved in the development but not necessary the maintenance of hypertension. Reduced NO availability in hydronephrosis may also be the result of increased oxidative stress in the diseased kidney. Renal NO deficiency, and subsequent resetting of the TGF mechanism, appear to play an important part in contributing to the development of hypertension in hydronephrosis.
**FUTURE PERSPECTIVES**

There is a causal relationship between experimental hydronephrosis and the development of salt-sensitive hypertension. Experimental hydronephrosis from chronic partial ureteral obstruction does not only provide useful information for the clinicians, but offers a new model for studies of the mechanisms behind salt-sensitive hypertension. Chronic kidney disease is accompanied by NO deficiency and oxidative stress, which contribute to the progression. It is possible that increased oxidative stress in the diseased kidney can also explain the reduced NO availability in hydronephrosis. Further investigation into the role of oxidative stress with the described model for PUUO in genetically modified mice (e.g. *SOD-transgenic* and *SOD-knock-out*) could be beneficial.
SAMMANFATTNING PÅ SVENSKA

Hydronefros är ett ganska vanligt förekommande fenomen hos nyfödda barn med en incidens på cirka 0.5-1%. Vanligtvis är orsaken en förträngning i övergången mellan uretär och njurbäcken. Under det senaste årtiondet har behandlingspolicyn för asymptomatisk hydronefros blivit alltmer konservativ, men de långsiktiga fysiologiska konsekvenserna av detta är inte klarlagda. Det är väl känt att det finns en tydlig koppling mellan en förändrad njurfunktion och utvecklingen av sekundär hypertension. I denna avhandling undersökte vi om det finns en koppling mellan hydronefros och hypertension. Hydronefros inducerades genom att skapa en partiell förträngning av uretären i tre veckor gamla råttor eller möss. I de vuxna djuren mättes blodtrycket med hjälp av telemetri och njurfunktionen studerades. Båda djurslagen utvecklade saltkänslig hypertension och histopatologiska förändringar såsom fibros, inflammation och glomerulära och tubulära förändringar. De observerade förändringarna korrelerade väl med hydronefrosgraden (mild, mättlig eller kraftig hydronefros). De hydronefrotiska djuren hade ett förändrat renalt utsöndringsmönster i form av en ökad diures och en reducerad förmåga att koncentrera urinen. Mekanismerna för hypertensionen anses huvudsakligen vara lokalisade till den hydronefrotiska njuren eftersom avlägsnande av förträngningen via nefrektomi eller pyeloplastik reducerade såväl blodtrycket som saltkänsligheten. Obstruktion av uretären kan leda till en ökad aktivitet av renin-angiotensin systemet, vilket skulle kunna vara involverat i utvecklingen av hypertensionen. Eftersom djuren med kraftigast hydronefros och högst blodtryck inte hade ökade reninnivåer behöver dock inte förändringar i detta system vara involverade i upprätthållandet av hypertensionen. De hydronefrotiska djuren hade en reducerad kväveoxidtillgäng-
lighet, vilket kan bero på en ökad oxidativ stress i den skadade njuren. Brist på kväveoxid i njuren och ökad känslighet av den tubuloglomerulära återkopplingsmekanismen verkar spela en betydande roll i utvecklingen av hypertensionen. De nya fynden indikerar att dagens konservativa behandlingsstrategi vid hydronefros borde förändras för att förhindra utvecklingen av njurskador och saltkänslig hypertension senare i livet.
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REFERENCES


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)