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High Blood Pressure in Children with Hydronephrosis

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Abstract

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The most common cause of secondary hypertension is intrinsic renal disease, but little is known about the influence of hydronephrosis on blood pressure. In this thesis, the risk of development of hypertension in children with hydronephrosis was studied.

Experimental and clinical studies were combined in order to investigate the risk of developing elevated blood pressure following conservative treatment of hydronephrosis, and to further explore underlying mechanisms. We started with a clinical study in children (study I), which in agreement with previous experimental studies, showed that blood pressure was lowered by surgical management of hydronephrosis. In parallel, an experimental study was conducted (study II) to investigate the involvement of renal sympathetic nerve activity in development of hypertension following induction of hydronephrosis caused by pelvo-ureteric junction obstruction. Renal denervation of the obstructed kidney attenuated hypertension and restored the renal excretion pattern, effects that were associated with reduced activity of both renal NADPH oxidase derived oxidative stress and components of the renin-angiotensin-aldosterone system.

Based on the findings in studies I and II, we continued our studies in children with hydronephrosis, and including two control groups as comparisons with the hydronephrotic group (study III). In the same study, we further investigated potential mechanism(s) of hypertension by analyzing markers of oxidative stress and nitric oxide homeostasis in both urine and blood samples. We demonstrated increased arterial pressure and oxidative stress in children with hydronephrosis compared with healthy controls, which was restored to normal levels by surgical correction of the obstruction. Finally, in a retrospective cohort study, blood pressure of adult patients undergoing surgical management of hydronephrosis due to pelvo-ureteric junction obstruction was assessed (study IV). Similar to that demonstrated in the pediatric hydronephrotic population, blood pressure was significantly reduced by relief of the obstruction. In addition, blood pressure was increased again if the hydronephrosis recurred, and was reduced again following re-operation.

It is concluded that conservative management of hydronephrosis in children is associated with a risk for development of high blood pressure, which can be reduced or even normalized by relief of the obstruction. The mechanism(s), at least in part, is coupled to increased oxidative stress.

Keywords: Blood pressure, hydronephrosis, hypertension, ambulatory blood pressure monitoring, nitric oxide, oxidative stress, pelvo-ureteric junction obstruction.

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*To my Parents, my lovely wife
Liqaa and my flowers:
Ruqayah, Fadhl and Taim*

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I. **Al-Mashhadi, A.**, Nevéus, T., Stenberg, A., Karanikas, B., Persson, AEG., Carlström, M.[#], Wåhlin, N.[#] Surgical treatment reduces blood pressure in children with unilateral congenital hydronephrosis. *Journal of Pediatric Urology* 11(91):e91-e96. (2015)
[#] Shared senior authors.
- II. Peleli, M. *, **Al-Mashhadi, A. ***, Yang, T., Larsson, E., Wåhlin, N., Jensen, LP., Persson, AEG., Carlström, M. Renal denervation attenuates NADPH oxidase-mediated oxidative stress and hypertension in rats with hydronephrosis. *Am J Physiol Renal Physiol* 310: F43–F56. (2016)
*Equal contribution
- III. **Al-Mashhadi, A.**, Checa, A., Wåhlin, N., Nevéus, T., Fossum, M., Wheelock, CE., Karanikas, B., Stenberg, A., Persson, AEG., Carlström, M. Changes in arterial pressure and markers of nitric oxide homeostasis and oxidative stress following surgical correction of hydronephrosis in children. *Journal of Pediatric Nephrology*. (2017)
- IV. **Al-Mashhadi, A.**, Häggman, M., Läckgren, G., Ladjevardi, S., Nevéus, T., Stenberg, A., Persson, AEG., Carlström, M. Reduction of arterial pressure following relief of obstruction in patients with hydronephrosis. (manuscript) (2018)

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Abbreviations

ABPM	Ambulatory blood pressure monitoring
ADMA	Asymmetric dimethyl arginine
ANG I	Angiotensin I
ANG II	Angiotensin II
cGMP	Cyclic guanosine monophosphate
CT	Computerized tomography
DNx	Unilateral renal denervation
DTPA	Diethyltriaminepentaacetic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
GFR	Glomerular filtration rate
GTP	Guanosine triphosphate
HC	Healthy control
HN	Hydronephrosis
HN Post	Hydronephrosis postoperative
HN Pre	Hydronephrosis preoperative
HPLC	High-performance liquid chromatography
IBMX	Isobutylmethylxanthine
iNOS	Inducible nitric oxide synthase
JGA	Juxta glomerular apparatus
L-NMMA	L-NG-monomethyl arginine
LP	Laparoscopic pyeloplasty
LS	Low salt
MAG3	Mercaptoacetyltriglycine3
MAP	Mean arterial pressure
MIS	Minimally invasive surgery
MRI	Magnetic resonance imaging
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide

NO₂
NO₃
NOS
NOX
NS
NS2
OC
PCR
PUUO
PUJO
RAAS
RBF
ROS
SDMA
SNA
SOD
SOD1
TGF
UPJ
VCUG
VUR
WCH

Nitrite
Nitrate
Nitric oxide synthase
NADPH oxidase
Normal salt
Normal salt diet once again
Operated control
Polymerase chain reaction
Partial unilateral ureteral obstruction
Pelvo-ureteric junction obstruction
Renin angiotensin aldosterone system
Renal blood flow
Reactive oxygen species
Symmetric dimethylarginine
Sympathetic nerve activity
Superoxide dismutases
Superoxide dismutases1
Tubuloglomerular feedback
Uretero pelvic junction
Voiding cystourotherogram
Vesicoureteral reflux
White coat hypertension

Introduction

Hydronephrosis

Background

The kidneys play a key role in whole body fluid and electrolyte homeostasis, and hence in long-term blood pressure control. Abnormal renal autoregulation of glomerular perfusion and filtration as well as intrinsic renal diseases can cause hypertension [1].

Hydronephrosis is a condition with dilatation of the renal pelvis which is fairly common in children. With increasing use of ultrasound, the incidence of hydronephrosis due to pelvo-ureteric junction obstruction (PUJO) among newborn infants has been found to be about 1-2% [2, 3].

Hypertension is one of the largest growing health problems in the Western world. The large health risks associated with hypertension include increased incidence of stroke.

The most common cause of secondary hypertension is intrinsic renal disease, but virtually any renal pathological condition may lead to hypertension [4]. The mechanism is either renovascular, occurring through the action of vasoactive substances, or more commonly chronic hypervolemia due to a reduced ability to regulate sodium excretion. Increased activity of the renin angiotensin aldosterone system (RAAS) has been demonstrated in renal hypertension in both humans and experimental animal models. There is also increasing evidence of a close connection between increased oxidative stress and/or reduced nitric oxide (NO) availability in the development and maintenance of hypertension [5, 6].

Less is known about the influence of hydronephrosis on blood pressure. Although hypertensive effects of hydronephrosis have been suggested in experimental studies and clinical case reports [7-9], this has not been substantiated by prospective studies in humans. It has been shown that the function of the hydronephrotic kidney in many cases remains surprisingly well preserved for several years [10, 11]. This observation has led to a worldwide trend towards non-operative treatment, but the long-term effects of this policy on the cardiovascular and renal function are not known [3].

Experimental studies on rats and mice with partial unilateral ureteral obstruction (PUUO) have shown that animals with induced or congenital hydronephrosis develop salt-sensitive hypertension [12] which strongly corre-

lates to the degree of obstruction [13-16]. Moreover, relief of the obstruction normalized blood pressure [17].

Etiology

Nearly 50% of the cases of prenatally detected hydronephrosis are caused by partial ureteropelvic junction (UPJ) obstruction (Figure 1) [18].

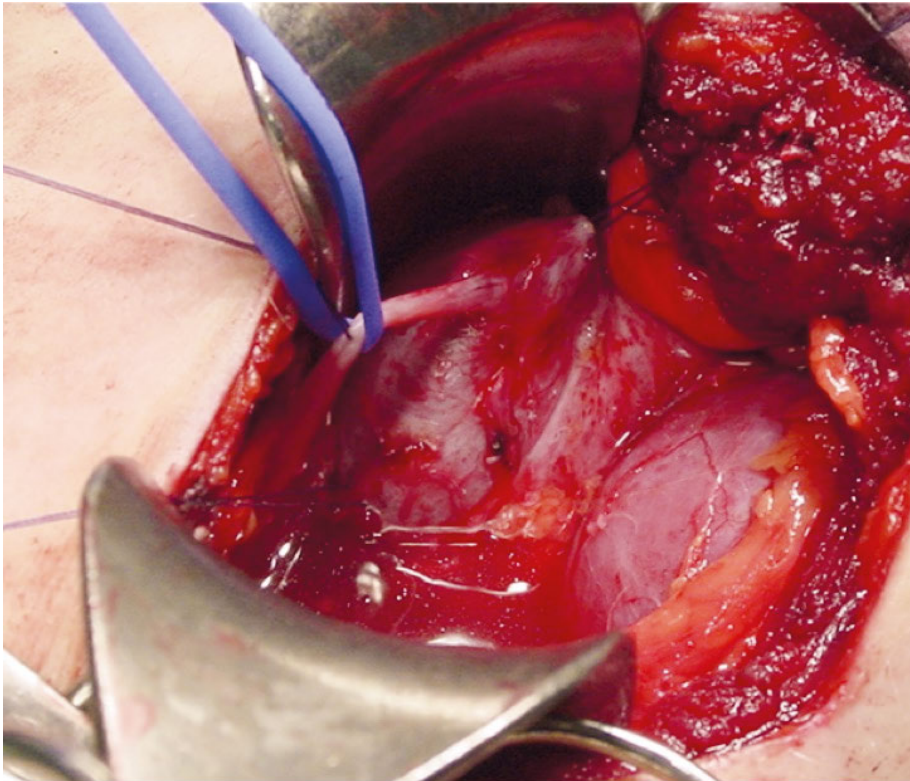


Figure 1. Hydronephrosis due to partial plevo-ureteric junction obstruction.

The etiology of the UPJ obstruction is still unclear, but several intrinsic and extrinsic factors have been proposed. Among the former are muscle disorientation [19], collagen excess [20] or absence of smooth muscle cells [21] while extrinsic factors include overlying aberrant vessels (Figure 2) [22, 23], pelvic or abdominal tumors [24-27], retroperitoneal fibrosis, or neurological deficits [28].

Antenatal hydronephrosis may also be a sign of dilating vesicoureteral reflux (VUR). The overall incidence of VUR in a population with antenatal hydronephrosis range from 8% to 38% [29].

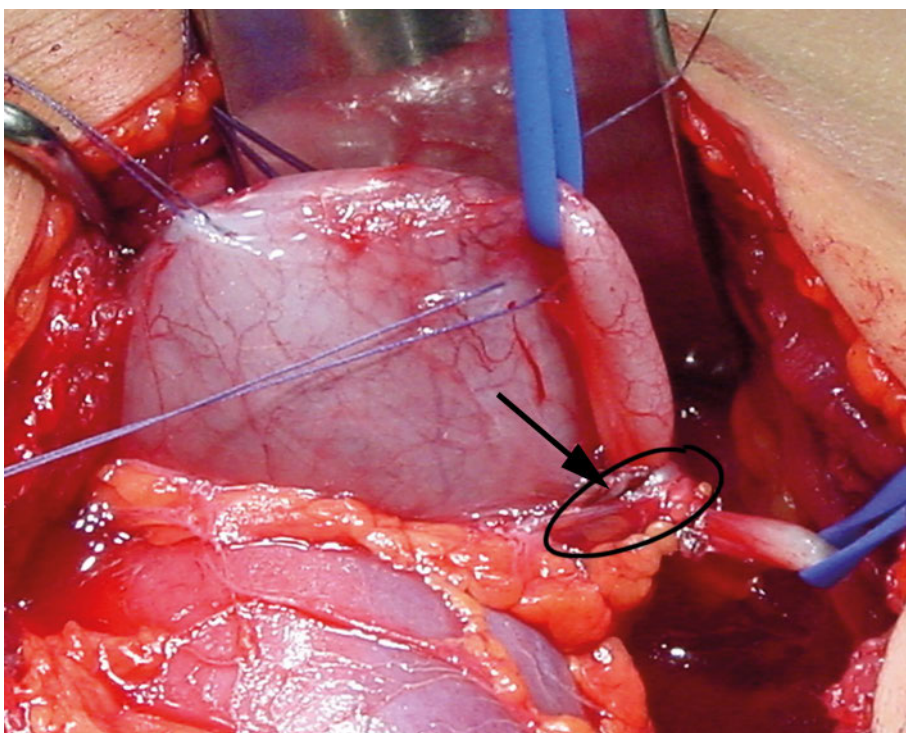


Figure 2. Hydronephrosis due to overlying aberrant vessel (black arrow).

Diagnosis

Ultrasound

Prenatal ultrasound is now widely used. As a result, fetal renal pelvic dilatation is reported to be present in 4.5% of pregnancies [30, 31].

The hydronephrosis is usually diagnosed and quantified by measurement of the anteroposterior diameter of the renal pelvis, although this measurement does not take into consideration calyceal or ureteral dilation or parenchymal changes, and therefore may not precisely reflect the severity of the condition.

Antenatal hydronephrosis is defined as an anteroposterior diameter of the renal pelvis of ≥ 4 mm in the second trimester or ≥ 7 mm in the third trimester. It can be further graded as mild, moderate or severe according to a set of anteroposterior diameter thresholds that have provided the best prognostic information based on available evidence (Table 1). According to this grading system, about two thirds of cases are regarded as mild.

Table 1. Classification of antenatal hydronephrosis based on the anteroposterior diameter of the renal pelvis.

Degree of antenatal hydronephrosis	Second trimester (mm)	Third trimester (mm)
Mild	4 to <7	7 to <9
Moderate	7 to 10	9 to 15
Severe	>10	>15

It should be remembered that the finding of antenatal hydronephrosis is not necessarily a sign of obstruction, nor does it necessarily reflect altered renal function. Nonetheless, there is a positive association between the degree of antenatal hydronephrosis and the incidence of significant postnatal pathology. In general, the greater the extent of dilatation of the renal pelvis, the greater the risk for significant anomalies. Serial prenatal and postnatal ultrasound evaluations are recommended in all cases of antenatal hydronephrosis.

All newborns with a history of antenatal hydronephrosis should undergo a postnatal ultrasound (Figure 3) evaluation within the first week of life, even if the renal pelvic dilation was resolved prenatally. Ultrasound should not be performed until 4 days after birth because the relative dehydration and decreased glomerular filtration rate (GFR) that are present immediately after delivery may lead to false-negative results or underestimation of the severity of hydronephrosis. On the other hand, early neonatal ultrasound, within 1 or 2 days of birth, is required when there is bilateral hydronephrosis, severe hydronephrosis in a solitary kidney, or suspicion of posterior urethral valves [32].

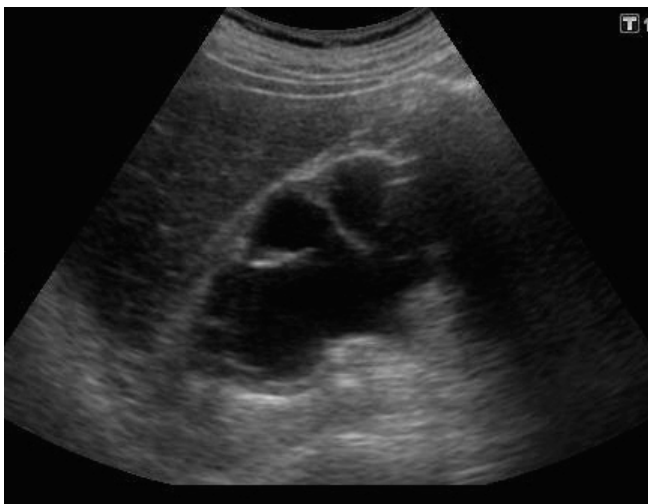


Figure 3. Postnatal hydronephrosis.

Voiding cystourethrography (VCUG)

During VCUG radiological contrast is introduced into the bladder via a urethral catheter. This examination is performed to detect VUR and, in boys, to evaluate the posterior urethra. If major hydronephrosis is present on the postnatal ultrasound, then VCUG should be performed, usually within 4 weeks. However, it must be obtained within 48 hours of birth in any infant suspected to have posterior urethral valve or bladder outlet obstruction for other reasons. The examination is quite invasive and involves a non-negligible amount of radiation [31, 33-35].

Diuretic renography

Diuretic renography is used to detect signs of urinary tract obstruction in infants with persistent or large hydronephrosis, and is usually ordered after a VCUG has failed to demonstrate VUR. This examination, which is relatively noninvasive, is usually performed at 1-3 months of age, and gives quantitative data on function and drainage. The radionuclide of choice, which is injected intravenously, is 99m-technetium mercaptoacetyltriglycine (MAG3) due to its high initial renal uptake, although 99m-technetium diethyltriamine pentaacetic acid (DTPA) can be used also [36]. Renography does not provide fine details of renal anatomy.

Renal glomerular function is often described in terms of glomerular filtration rate (GFR), and it has been noted that changes in filtration rate are closely related to changes in renal blood flow (RBF). The functional status of the hydronephrotic kidney, determined as GFR, usually remains well preserved for several years in newborn [10, 11, 37]. However, a poor correlation between the severity or duration of symptoms and the degree of renal function has been demonstrated [38, 39]. Several methods have been used to

estimate kidney function. Measurement of GFR combined with a renal scan to determine relative uptake (i.e. split function), is widely used as well as assessment of the elimination characteristics (i.e. renography). The washout curve at renography in response to furosemide administration, as described by O'Reilly [40], can be used as an indicator of the degree of outflow obstruction (Figure 4).

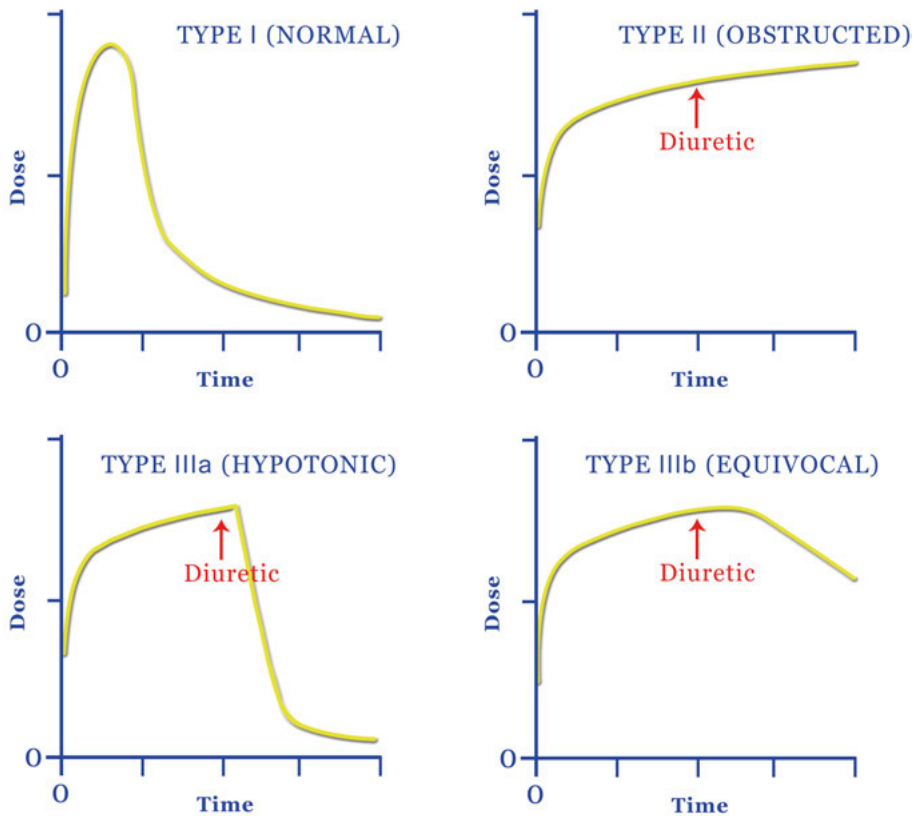


Figure 4. Drainage patterns according to O'Reilly.

Drainage Patterns – O'Reilly curves.

Type 1 – Normal: Normal uptake with prompt washout. Immediate rise of the curve with a peak at 2-5 minutes and with a normal rapid washout (curve falls quickly).

Type 2 – Obstructed: Rising uptake curve but no response to furosemide, i.e. curve continues to rise. Anything but an exponentially falling curve could be considered evidence of obstruction. False positive results due to dehydration, poor renal function, massive dilatation or bladder effects are common.

Type 3a – Hypotonic: An initially rising curve that falls rapidly in response to furosemide (non-obstructive dilatation). Dilatation is a result of stasis rather than obstruction.

Type 3b – Equivocal: An initially rising curve which neither falls promptly following injection of furosemide nor continues to rise [41, 42].

Other examinations

In addition, CT or MRI can be of value, as also the renal resistive index determined by Doppler ultrasound [41, 42].

Treatment

Although UPJ obstruction is common, the clinical management has been debated among urologists for many years. Studies demonstrating that the renal function is rather well preserved for several years [10, 11, 37] have led to a worldwide trend towards a non-operative management of neonatal hydronephrosis. However, the long-term physiological consequences of this new strategy are not known.

The treatment of symptomatic hydronephrosis (i.e. hydronephrosis causing pain) is surgical. The usual repair of UPJ obstruction involves removal of the obstruction and then a reconstruction of the continuity by pyeloplasty (Figure 5).

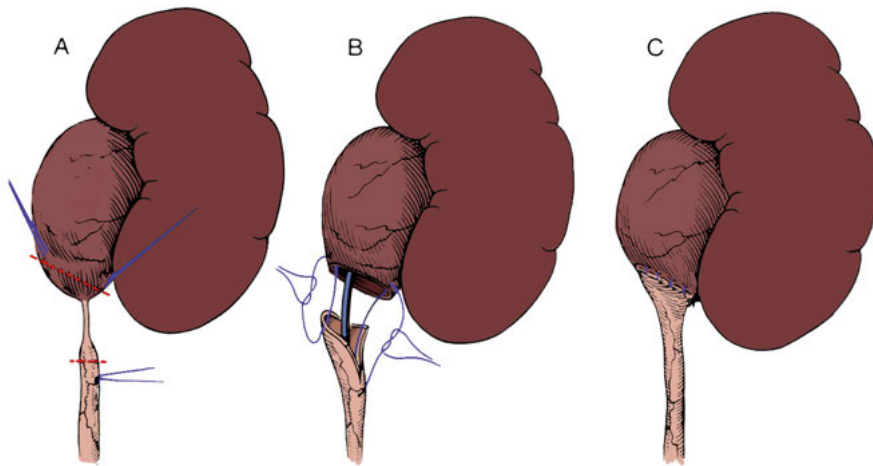


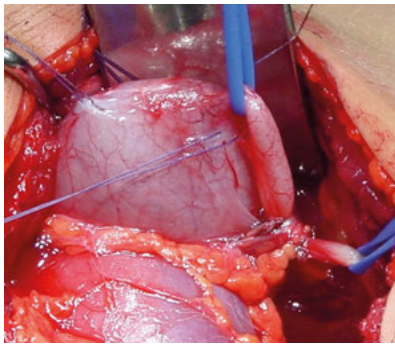
Figure 5. Hydronephrosis repair.

Since the first pyeloplasty was described in 1939, when Foley introduced the Y-V technique, several techniques have been applied to correct UPJ obstruction, but Anderson-Hynes dismembered pyeloplasty [43] is established as

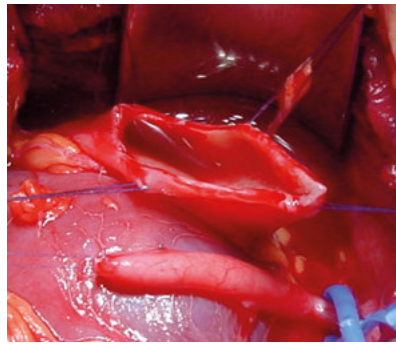
the gold standard (Figure 6), to date also in minimally invasive surgery (MIS) technique [44].

Several studies confirm the safety and efficacy of the MIS for both trans- and retroperitoneal routes, with a success rate between 81 and 100% and an operation time of 90-228 min. These studies have demonstrated the safety and efficacy of this procedure in the management of UPJ obstruction in children. It is still debated whether the transperitoneal or the retroperitoneal approach is to be preferred [44].

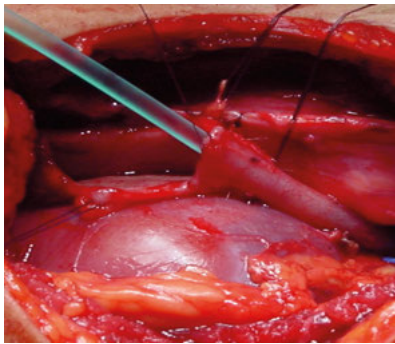
The first laparoscopic pyeloplasty (LP) was described by Kavoussi et al. in 1993 who used the Anderson-Hynes technique [45] on a young female (24 years old). In 1995 this technique was applied on a child [46, 47].



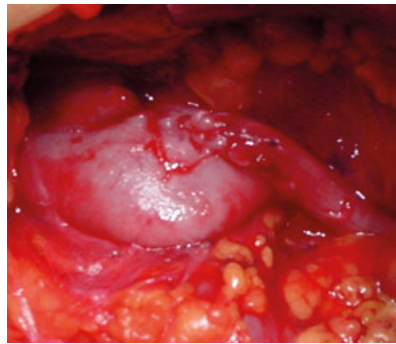
A. Exposure of the UPJO



B. Excision of the UPJO



C. Suturing of posterior layer of anastomosis



D. Complete anastomosis

Figure 6. Stages of pyeloplasty operation (panel A-D).

Hydronephrosis and hypertension

Our research group in Uppsala recently discovered that there is a causal link between both experimental and congenital hydronephrosis, and the development of obstructive nephropathy and hypertension in later life [13, 14, 17]. Oxidative stress and reduced nitric oxide (NO) bioavailability in the affected kidney appear to play an important role.

To our knowledge hypertension in children with UPJ obstruction has not been reported in the literature as an indication for surgery. Furthermore, there has been no systematic research into the effect of relief of obstruction on existing hypertension. Most young children with hydronephrosis are not reported to be hypertensive, but there are several case reports of hypertension obviously caused by hydronephrosis, since in these reports the patients became normotensive following relief of the obstruction [7-9].

An increased activity of the RAAS has been demonstrated, but the participation of renin in this type of hypertension appears to be influenced by the duration of the obstruction, the presence or absence of a contralateral normal kidney, as well as other intrarenal factors. Other investigators have been unable to show any blood pressure effects of hydronephrosis [48, 49]. Furthermore, in large surveys on causes of secondary hypertension, hydronephrosis does not appear to be common [50]. However, this relationship is difficult to interpret, as the prevalence of hydronephrosis in humans is lower than that of hypertension.

Ambulatory blood pressure monitoring

Unfortunately, accurate measurement of blood pressure is often not easy to obtain, particularly in younger children, and this has led to increased use of automated devices. Despite various drawbacks, these are easier to use and do eliminate observer bias. The appreciation that multiple measurements over a 24-hour period is a better reflection of a continuously variable blood pressure has resulted in the development of ambulatory blood pressure monitoring (ABPM) as a standard procedure. A portable monitor that can be worn on the belt or in the pocket can be programmed to undertake multiple blood pressure readings during normal daytime and nighttime activities.

Recently there have been great advances in the use of ABPM in children. Office blood pressure measurements can lead to an overestimation of blood pressure owing to the so-called white coat phenomenon. This can lead to an erroneous diagnosis of hypertension when office blood pressure is high but ambulatory blood pressure is below conventional thresholds, a phenomenon known as white coat hypertension (WCH), or the more appropriately termed isolated clinical hypertension [51].

A major boost has been the publication of normative data for blood pressure in children. ABPM has been able to detect significant differences in blood pressure in many conditions including chronic renal failure, polycystic kidney disease and status post renal transplantation and has helped in identifying both WCH and the opposite phenomenon masked hypertension. Current evidence suggests that sole reliance on office blood pressure is not always appropriate. It is now routine clinical practice to do ABPM before prescribing any long-term antihypertensive treatment [52].

According to the recommendations of the European Society of Hypertension the use of ABPM in all children and adolescents with hypertension may be even more important than in adults [53, 54].

Experimental studies

For several years, our research group has been working with an animal model in which hydronephrosis is induced by a partial unilateral ureteral obstruction (PUUO) at young age (3 weeks). Studies using this model have demonstrated that increased oxidative stress and NO deficiency in the affected kidney, together with increased TGF sensitivity, are associated with hypertension in later life [15, 55]. Based on the mechanistic similarities with other experimental models for renal hypertension, we considered it important to investigate if there was a link between hydronephrosis and the development of hypertension in animals and patients with this condition.

Causal link between experimental hydronephrosis and hypertension

Studies on the long-term physiological consequences of hydronephrosis in our model demonstrated that both rats and mice developed renal dysfunction and salt-sensitive hypertension in adult life (Figure 7). The hypertension correlated with the degree of hydronephrosis, and the mechanisms are thought to be primarily located in the affected kidney since relief of the obstruction attenuated blood pressure within hours. In contrast, removal of the contralateral kidney increased blood pressure [13, 14].

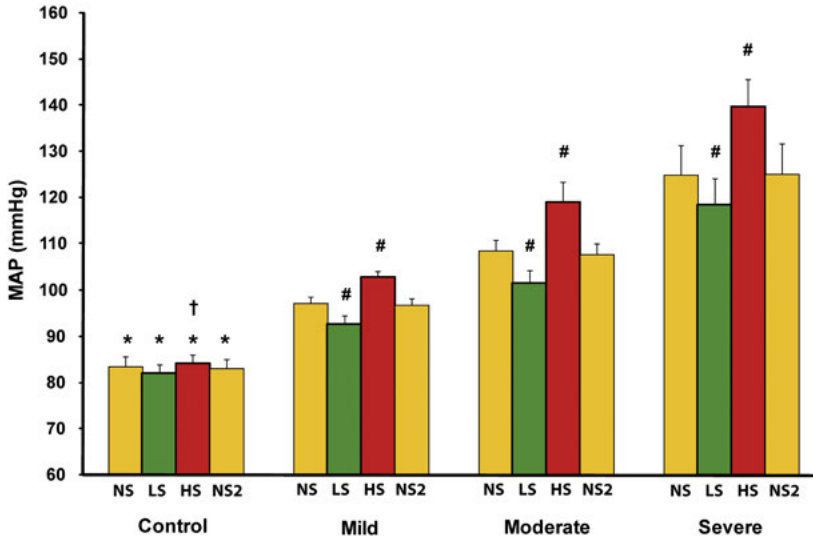


Figure 7. Mean arterial blood pressure (MAP) in controls and hydronephrotic rats with different hydronephrotic degrees, treated with normal salt (NS), low salt (LS), high salt (HS) and normal salt diet once again (NS2)

* $P < 0.05$ 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.

Nitric oxide

In the late 1970s, Dr. Robert Furchgott observed that acetylcholine caused the release of a substance that produced vascular relaxation. By the mid 1980s, Louis J. Ignarro and Ferid Murad, identified this substance as NO. This observation opened a new field of research and eventually led to a Nobel Prize in 1998.

NO is produced by many cells in the body; however, its production by vascular endothelium is particularly important in the regulation of blood flow and pressure. Abnormal production of NO, as occurs in different disease states, can adversely affect cardiovascular and renal function [56]. Furthermore, blocking of NO production results in increased blood pressure [6].

Research on the biological roles of NO has revealed that it acts as an important signal and effector molecule in a variety of physiological and pathological settings [57].

NO is produced by a group of enzymes called NO synthases (NOS). These enzymes convert L-arginine to citrulline, producing NO in the process. Oxygen and NADPH are necessary co-factors. There are three isoforms of NOS named according to their activity or the tissue type in which they were first described. The isoforms of NOS are neuronal NOS (nNOS), inducible

NOS (iNOS), and endothelial NOS (eNOS). These three isoforms can be found in a variety of tissues and cell types [58]. The general mechanism of NO production is illustrated below (Figure 8).

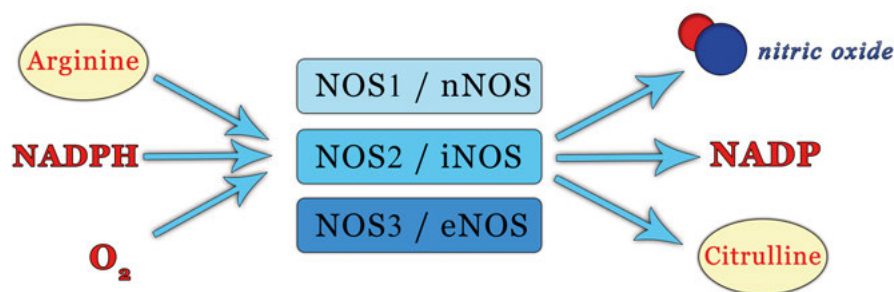


Figure 8. The general mechanism of NO production by NOS.

Three forms of methylated arginine, which can be considered arginine analogues, have been identified in eukaryotes: L-NG-monomethyl-arginine (L-NMMA); asymmetric dimethylarginine (ADMA); and symmetric dimethylarginine (SDMA). NMMA and ADMA are inhibitors of NOS. All three methylated arginines are inhibitors of arginine transport at super physiological concentrations, although the physiological relevance of this inhibition remains unclear.

Circulating ADMA is present at higher concentrations than L-NMMA and is often considered to be the principal inhibitor of NOS activity. However, it is important to note that the relative concentrations of ADMA and L-NMMA may differ between tissues and organ systems and hence the contribution of endogenously produced L-NMMA to the regulation of NO bioavailability may be of more importance in certain tissues and in various disease states [59].

Intracellular mechanisms

When NO forms, it has a half-life of only a few seconds, in large part because superoxide anions have a high affinity for NO (both molecules have an unpaired electron making them highly reactive). Therefore, the superoxide anion reduces NO bioavailability. NO also avidly binds to the heme moiety of hemoglobin in red blood cells and guanylyl cyclase, which is found in vascular smooth muscle cells as well as most other cells of the body. There-

fore, when NO is formed by vascular endothelium, it rapidly diffuses into the blood where it binds to hemoglobin and is subsequently broken down. It also diffuses into the vascular smooth muscle cells adjacent to the endothelium where it binds to and activates guanylyl cyclase. This enzyme catalyzes the dephosphorylation of GTP to cGMP, which serves as a second messenger for many important cellular functions, particularly smooth muscle relaxation.

Cyclic GMP induces smooth muscle relaxation by multiple mechanisms including

1. Increased intracellular cGMP, which decreases intracellular calcium concentration by inhibiting calcium entry into the cells.
2. Activation of K^+ channels, which leads to hyperpolarization and relaxation.
3. Stimulation of a cGMP-dependent protein kinase that activates myosin light chain phosphatase, which in turn, dephosphorylates myosin light chains and leads to smooth muscle relaxation [56].

Oxidative stress

Oxidative stress is defined as an imbalance between increased levels of reactive oxygen species (ROS) and a low activity of antioxidant mechanisms. An increased oxidative stress can induce cellular damage and potentially tissue injury. However, physiological levels of ROS are needed for adequate cell function, including mitochondrial energy production. Increased oxidative stress has been implicated in situations such as aging and exercise, and in several pathological conditions (e.g. cancer, neurodegenerative diseases, cardiovascular disease, diabetes, inflammatory diseases). Still, interventions with currently available antioxidants (vitamin C and E) have been mostly inefficient [60], possibly due to low bioavailability.

In vivo, ROS are produced by multiple pathways and released from several cell types, with important differences in the amount produced upon stimulation [60-62]. The phagocytes (monocytes, macrophages and polymorphonuclear neutrophils) are the most important producers of ROS in acute conditions [60, 63], as a component of the immune response designed to neutralize invading particles and microorganisms. A continuous production of low amounts of ROS is present in cells equipped with active mitochondria, and ROS are by-products of energy production by the respiratory chain [60-62, 64].

In the past decade, a new family of highly regulated ROS-producing enzymes has been identified, and named the NADPH oxidase (NOX) family proteins because of their structural similarity to the phagocyte NADPH oxidase. It is evident that these proteins are crucial in various biological events. Among 7 NOX family proteins, NOX1, NOX2 and/or NOX4 are expressed

in relevant amounts by vascular cells, and account for ROS production in vascular walls. These three NOX proteins seem to contribute cooperatively to vascular pathophysiological events, such as hypertension, atherosclerosis, angiogenesis, and ischemia/reperfusion injury.

It is thought that ROS, particularly superoxide, and hypertension are closely related: superoxide contributes to the development of hypertension by decreasing NO bioavailability, whereas increased shear stress caused by the hypertension augments vascular superoxide production in endothelial cells. Thus, ROS constitute both causes and consequences of hypertension, and may provide the basis for a vicious cycle [65].

NOX are widely expressed in the vasculature and in the kidney. NOX-derived superoxide is the main ROS in the vasculature both in animals and humans and is either being metabolized by superoxide dismutases (SOD) or by NO scavenging [66].

Role of oxidative stress and NO deficiency in hydronephrosis

Emerging evidence suggests that there is a critical link between oxidative stress and NO deficiency in the renal vasculature and the development of renal and cardiovascular disease [5].

Hydronephrotic animals have a reduced NO availability in the affected kidney, which is associated with increased TGF response and development of hypertension [15]. However, the mechanisms behind the NO deficiency are not clear, but increased renal production of free radicals (i.e. superoxide) has been suggested.

As mentioned above, oxidative stress is considered to be crucially involved in the development or progression of cardiovascular and renal disease. Patients with mild to moderate renal insufficiency, as well as those with end-stage renal disease, have been demonstrated to suffer from increased oxidative stress. Transgenic mice overexpressing SOD do not develop hypertension from hydronephrosis, which could be explained by higher concentration of NO in the JGA [16].

Renal sympathetic denervation

Renal sympathetic denervation (RSD) using state-of-the-art technique (percutaneous, catheter-based radiofrequency ablation) has been shown to be beneficial in patients with resistant hypertension. The procedure presents several significant advantages compared with radical sympathectomy, a therapy that was commonly used five decades ago. It is a localized, minimal-

ly invasive procedure with no systematic side effects, and its procedural and recovery times are very short.

Sympathetic nerve activation enhances noradrenaline production. When renal sympathetic nerves are activated, $\beta 1$ adrenergic receptors mediate renin secretion, sodium reabsorption occurs via $\alpha 1$ adrenoceptors, and renal vessel vasoconstriction takes place via $\alpha 1$ receptors, with a reduction of RBF as the end result [67].

Renal sympathetic varicosities release noradrenaline directly to renal epithelial cells and promote the reabsorption of water and sodium from the tubular lumen. It has become apparent that RSD attenuates sodium and water reabsorption, independently of GFR and RBF, confirming the direct effects of renal innervation on tubular function [67].

There is sound evidence that renal sympathetic activation results in a significant decrease of RBF. Sympathetic nerve activation results in vascular smooth muscle cell contraction of the resistance vessels and therefore reduces the blood flow through the kidneys. Sympathetic-induced vasoconstriction is more profound in preglomerular than postglomerular microvessels. This imbalance in the vasoconstrictive effects on renal microcirculation represents the main contributor of RBF reduction [67].

Increased renal sympathetic nerve activity from efferent and sensory afferent nerve fibers have also been associated with activation of the immune system and progressive inflammation, stimulation of renin release and activation of the RAAS and also activation of NOX and increased oxidative stress. These multiple mechanisms may interact and contribute to the development of both cardiovascular disease and renal dysfunction or injury (Figure 9), which in turn may activate the central sympathetic nervous system (via afferent nerves) and hence cause a vicious circle [68].

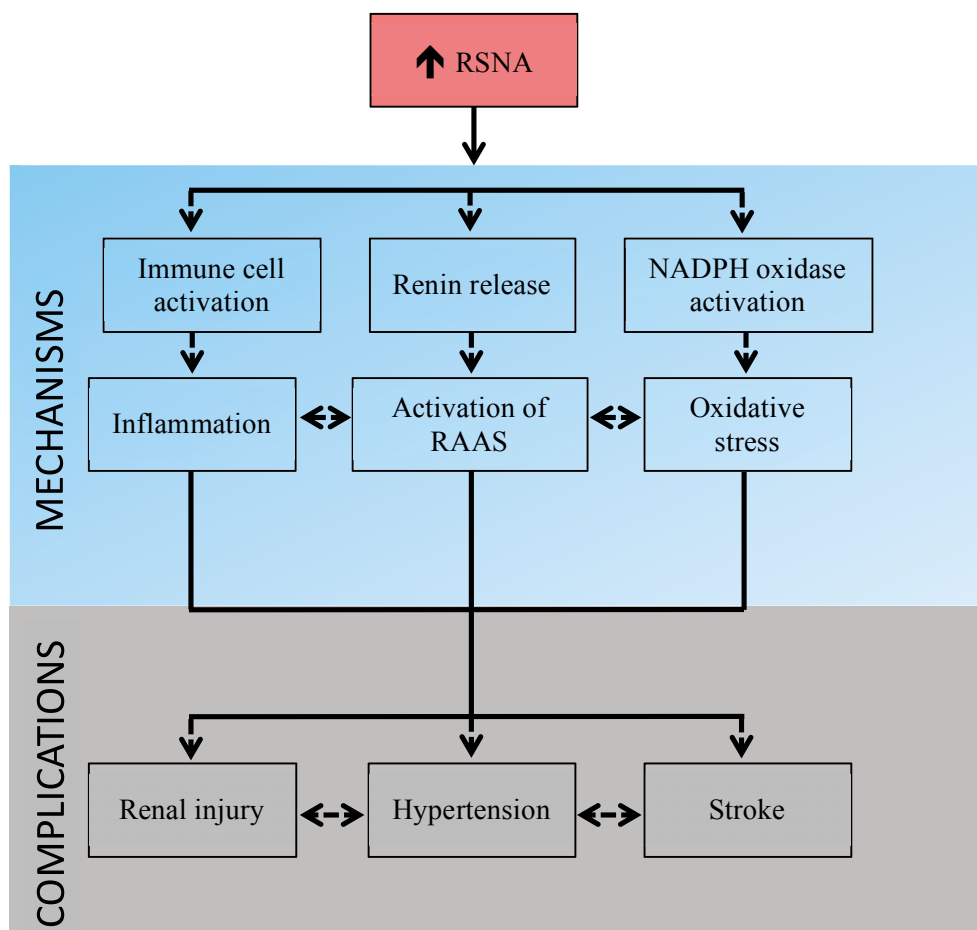


Figure 9. Pathophysiological mechanisms and complications associated with increased renal sympathetic nerve activity. RSNA = renal sympathetic nerve activity. RAAS = renin angiotensin aldosterone system.

Obstructive nephropathy

It has been demonstrated that obstructive nephropathy, which is the most important cause of renal insufficiency in children, is not a simple result of mechanical impairment to urine flow but represents a complex syndrome resulting in alterations of both glomerular hemodynamics and tubular function. The mechanism behind this complex syndrome is the interaction of vasoactive factors and immunological components that are activated in response to ureteral obstruction [1].

Rats with experimental PUUO is associated with infiltration of inflammatory cells and interstitial fibrosis in the obstructed kidney, with consequent impairment of both tubular and glomerular function [69]. Oxidative stress

may activate the secretion of inflammatory molecules that, in turn, may exert effects mediated by ROS, giving rise to a vicious circle perpetuating renal pathological changes [5].

Aims

The overall aim was to evaluate if there is a link between hydronephrosis in children and development of hypertension. Specific aims for the different studies are described below.

Study I

To study the blood pressure pattern in pediatric patients with hydronephrosis before and after surgical correction of the ureteral obstruction. Specifically, we investigated if preoperative blood pressure is reduced after surgery and if split renal function and renographic excretion curves provide any prognostic information.

Study II

To investigate the role of renal sympathetic nerve activity, its link to oxidative stress, and the development of hypertension in rats with hydronephrosis.

Study III

To investigate if preoperative blood pressure is higher in children with hydronephrosis compared with healthy controls, and if the blood pressure can be reduced by surgical management. Moreover, to investigate the association between blood pressure and oxidative stress as well as NO homeostasis.

Study IV

To further investigate the proposed link between hydronephrosis due to UPJ obstruction and elevated arterial pressure in adults.

Materials and Methods

Study protocols

Study I (clinical study)

In this prospective study 12 patients with unilateral congenital hydronephrosis were included from 2007 to 2014. Patient age ranged from infancy to 13 years. Ambulatory blood pressure was measured for 20-24 hours before surgical correction of hydronephrosis and six months postoperatively. MAG3 scintigraphy was performed preoperatively to assess bilateral renal function. The renography curves were classified according to O'Reilly.

Study II (experimental animal study)

PUUO was created in 3 week-old rats to induce hydronephrosis. Surgical denervation, or sham procedure, of the PUUO kidney was performed at the time of PUUO, and 4 weeks later during implantation of a telemetry device. Blood pressure was measured during normal, high and low salt diets, and renal excretion and NOX function were assessed.

Study III (clinical study)

In this prospective clinical study, ABPM for 20-24 hours was performed in pediatric patients (n=15) with congenital hydronephrosis before and after surgical correction. The results were compared with blood pressure levels in two sex and age matched control groups. Patients were included during the period 2007-2016. Patient age ranged from infancy to 13 years. Markers of oxidative stress and NO homeostasis were analyzed in matched urine and plasma samples of both the hydronephrosis patients and the same two control groups. The hydronephrosis diagnosis was confirmed by ultrasonographic measurements of the anteroposterior diameter of the renal pelvis.

Study IV (clinical study)

In this retrospective cohort study, medical records of 212 adult patients undergoing surgical management of hydronephrosis due to UPJ obstruction between 2000-2016 were assessed. After excluding patients with chronic diseases and those with antihypertensive treatment, paired arterial pressures (i.e. before and after surgery) were compared in 48 patients (35 years old; 95% CI 29-39). Split renal function was evaluated by MAG3 renography before surgery.

Animals

Male Sprague-Dawley rats (Scanbur, Charles River) were divided into the following four experimental groups: sham-operated non-hydronephrotic rats (control), non-hydronephrotic rats with unilateral renal denervation (control + DNx), rats with PUUO, and rats with PUUO and ipsilateral denervation (PUUO + DNx). All animals were given a standardized normal salt diet (0.7% NaCl, SD389-R36, Lactamin, Kimstad, Sweden) for 4 weeks before cardiovascular function was assessed. In one series, salt sensitivity was evaluated by measuring blood pressure and heart rate changes during the normal salt diet followed by high-salt diet treatment (4% NaCl, SD312-R36, Lactamin) and low-salt diet treatment (0.02% NaCl, Lactamin; see details below). In a second series, all animals were euthanized after cardiovascular measurements on the normal salt diet, and blood and tissue samples were processed for further analyses.

Creation of PUUO (Study II)

PUUO was created in 3 weeks-old rats to induce hydronephrosis as previously described [14]. In brief, anesthesia with spontaneous inhalation of isoflurane (2% in air, Forene, Abbot Scandinavia, Kista, Sweden) was used. The abdomen was opened under sterile conditions through a midline incision, and the left ureter was identified and isolated. The underlying psoas muscle was split longitudinally to form an approximately 15 mm-long groove in which the ureter was placed. The muscle edges were then sutured above the ureter with two 6/0 silk sutures, thus embedding the ureter in the muscle. The abdomen was closed, and animals were allowed to wake up under a heating lamp. Sham operations were performed in the same way but without dissecting the ureter. All animals were then left to grow with free access to the normal salt diet for 4 weeks.

Renal denervation (Study II)

During the procedure to induce hydronephrosis (i.e. 3 weeks of age), the left kidney of control or PUUO rats was denervated or exposed to sham denervation. Renal denervation was accomplished by previously validated surgical-pharmacological procedures [70]. In brief, the left kidney artery and vein were exposed through the abdominal incision and isolated from the surrounding connective tissue. Mechanical denervation was performed by stripping all visible nerves along the renal arteries and veins from the aorta to the hilum of the kidney. Chemical denervation was performed by applying phe-

nol (20% in ethanol) to the renal artery for 2 minutes. The artery was then carefully washed with isotonic saline. For sham denervation, the surgical procedure was the same, but the renal artery and vein were not isolated and the nerves were left intact.

Telemetric measurements (Study II)

A telemetric device (PA-C40, Data Sciences, St. Paul, MN) was implanted in adult animals, and blood pressure and heart rate were measured as previously described [71]. Inhalation anesthesia was used as described above, the skin was sterilized and an abdominal midline incision made. A 20mm long segment of the abdominal aorta was exposed and the catheter of the telemetric probe was inserted into the aortic lumen. The entry site was sealed by application of n-butyl-cyanoacrylate tissue adhesive (Vetbond TM, 3M Animal Care Products, St Paul, MN, USA). The transmitter was placed in the peritoneal cavity and sutured to the inside of the abdominal wall, after which the abdomen was closed.

For measurements of blood pressure and heart rate, the telemetric device was activated and the cage placed on a receiver plate that transferred the signals to a computer, where calibrated blood pressure values were measured. Data were collected for five seconds every two minutes for at least 48 hours at a time. The recorded data were continuously analyzed by a computer program (PC-Lab 5.0, AstraZeneca, Mölndal, Sweden).

Telemetric measurements during 48 h were conducted during normal, high-, and low-salt diet conditions. Animals were kept on different salt diets for 7 days, respectively, before cardiovascular data were collected.

Renal excretion measurements (Study II)

Rats were housed individually in metabolism cages for 24 h with free access to food and water. Water consumption and urine production were measured gravimetrically. Na^+ and K^+ concentrations were determined by flame photometry (FLM3, Radiometer, Copenhagen, Denmark), and urine osmolality was determined by depression of the freezing point (Fiske 210 Micro-Sample Osmometer, Fiske Associates, Norwood, MA). Urinary protein content was determined by the colorimetric method of the Detergent Compatible Protein Assay (Bio-Rad Laboratories, Hercules, CA). Plates were read using a microplate reader (model Safire II, Tecan Austria, Grödig, Austria) at 750-nm absorbance, as previously described [71].

Determination of the hydronephrotic ratio and collection of tissues and plasma (Study II)

Once the renal and cardiovascular experiments had been conducted, animals were anesthetized by an intraperitoneal injection of thiobutabarbital sodium [Inactin (120 mg/kg body weight)], whereupon the abdomen was opened using a midline incision. A macroscopic examination of both kidneys was performed. Blood was collected from the vena cava, transferred to tubes containing EDTA (final concentration: 2 mM), immediately centrifuged at 4°C (2,000 rpm, 5 min), and stored at -80°C for later analysis. The kidneys and heart were rapidly removed, weighed, rinsed, snap frozen in liquid nitrogen, and stored at -80°C for later analysis or prepared for histology (as described below). Hydronephrotic ratios (i.e. residual urine weight/renal parenchyma weight) were calculated before samples were frozen in the same way as previously described [72]. The renal cortex and medulla were dissected on ice and frozen separately (-80°C) for later analysis.

NOX activity (Study II)

Chemiluminescence techniques were used to determine NOX mediated superoxide formation.

Quantitative real-time RT-PCR (Study II)

Total RNA was isolated from the kidney cortex or heart using the RNeasy Mini Kit (Qiagen, Valencia, CA), and cDNA was synthesized with the High Capacity cDNA Reverse transcription kit (Applied Biosystems) according to the manufacturer's protocol with some modifications. The final results are expressed as percentages of the respective controls.

Plasma analysis (Study II)

Na⁺ and K⁺: Electrolyte concentrations were determined by flame photometry (FLM3, Radiometer, Copenhagen, Denmark).

Renin: The plasma renin concentration was measured by the rate of angiotensin I (ANG I) formation, and ANG I was detected by radioimmunoassay.

Aldosterone: An ELISA kit (MS E-5200, human aldosterone, Labor Diagnostika, Nord, Germany) was used.

Histology (Study II)

Sagittal slices from both the kidney and heart were placed in 4% paraformaldehyde solution immediately after the animals were sacrificed. All slices were stored at 4°C and transferred to 70% ethanol solution the next day. Sections from 6 animals/group were histopathologically evaluated for fibrosis and inflammation (i.e. infiltration of plasma cells and lymphocytes) in a blinded fashion. A score of 0–3 was given depending on the severity of changes (0 = no observable changes, 1 = mild changes, 2 = moderate changes, and 3 = severe changes), as previously described [16, 71].

Blood pressure measurement and evaluation (Study I and III)

In both studies I and III, ABPM was performed for hydronephrosis patients preoperatively during 20-24 hours, including the full nocturnal sleeping period, with readings obtained every 20 min during daytime and every hour during nighttime. ABPM was then performed again for 20-24 hours 6 months postoperatively. This was done as an outpatient procedure without hospital admission.

Study population (Study I and III)

In study I we included twelve children, all of them boys. The children's age ranged from infancy to 13 years of age.

In study III we included two control groups. The first control group consisted of eight completely healthy children, who had not undergone any general anesthesia or surgery. Another control group of age- and sex-matched children (n=8) was also included, who underwent general anesthesia for a minor day ward operation, but were otherwise considered healthy. The children's age in all three groups ranged from infancy to 12 years of age. The children were fourteen boys and one girl in the hydronephrotic group and eight children in the two different control groups. Both control groups consist of seven boys and one girl.

In study III, we also performed ABPM in both healthy controls and the operated control group (preoperatively), using exactly the same method, as for the hydronephrotic group.

Pyeloplasty (Study I and III)

The PUJO was relieved surgically by pyeloplasty, either laparoscopically or the traditional open procedure.

Preoperative renography (Study I and III)

Preoperative MAG3 renography with forced diuresis was also performed, in a standardized fashion, in all patients as part of routine evaluation. The investigation gives information about the split function of the kidneys and the elimination of the tracer, the renography curve. These curves were classified according to O'Reilly as described above.

Preoperative renal ultrasound (Study III)

Anteroposterior diameter of the renal pelvis was measured by ultrasound a few weeks to few days preoperatively in the hydronephrosis patient in order to confirm the diagnosis of hydronephrosis.

Analyses of blood and urine samples (Study III)

Blood samples were immediately centrifuged (4700 g, 5 min, 4°C) and collected aliquots of both plasma and urine were stored at -80°C for later analyses of markers of NO homeostasis and oxidative stress, as described below.

Nitrate and nitrite: Levels in plasma were analyzed by high-performance liquid chromatography (HPLC) (ENO-20) connected to an auto-sampler (840, EiCom, Kyoto, Japan).

cGMP: An ELISA kit was purchased from GE Healthcare (Uppsala, Sweden), and run according to the manufacturer's instructions. Plasma was collected in IBMX containing tubes (10 µmol/L) to prevent degradation of cGMP.

Amino acids: Plasma levels of arginine, citrulline, ornithine, ADMA and SDMA were measured by (HPLC) tandem mass spectrometry (LC-MS/MS) as previously described with minor modifications [71, 73].

Creatinine: Creatinine was quantified in 10 µl of urine as previously described [74].

Oxidative stress: Isoprostanes were quantified in 300 µl of urine using an urinary eicosanoid LC-MS/MS platform as previously described [75].

Blood pressure measurements (Study IV)

Medical records and hospital charts of 218 patients who were operated due to hydronephrosis between 2000 and 2016 at the Urology Department of Uppsala University Hospital were studied. In total, 212 patients had hydronephrosis due to UPJ obstruction. Patients with other chronic disorders, bilateral hydronephrosis, patients that is already receiving antihypertensive treatment and whose blood pressure data (before and/or after surgery) were missing were not included in the analysis (Figure10).

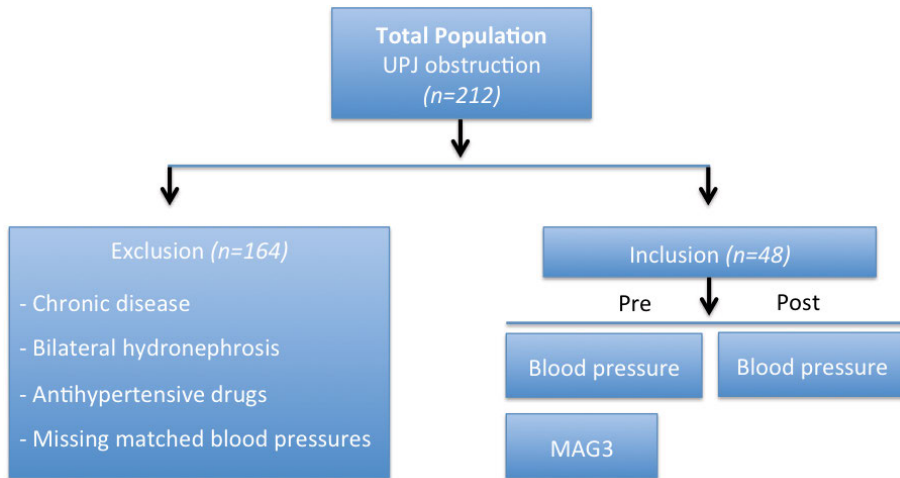


Figure 10. Schematic illustration of the study population.

From the total population with operated UPJ obstruction 48 patients fulfilled the inclusion criteria. Their systolic, diastolic and MAP were analyzed before and after surgery, and linear regression analysis were made comparing changes in arterial pressure with split renal function. We included blood pressure measurements only if the patients were not in pain or any other known stressful situation. Office blood pressure measurements were used for all the patients. Pre-relief arterial pressure was measured one day to one year before temporary relief of obstruction in 40 patients, while pre-relief arterial pressure for the rest of patients was measured one day before pyeloplasty surgery. Post-relief arterial pressures were measured between two weeks and two years after relief of the obstruction.

Preoperative renography (Study IV)

Preoperative evaluations of bilateral renal function were made using MAG3 renography with forced diuresis. This examination was performed in a standardized fashion as described above.

Pyeloplasty and relief of renal obstruction (Study IV)

Renal obstruction was relieved temporarily, because of pain or pyelonephritis, with either a double J stent and/or percutaneous nephropylostomy before pyeloplasty in 40 patients. The duration of this preoperative relief of obstruction ranged between two weeks and one year.

Thirty-eight patients were operated by laparoscopic pyeloplasty, while nine patients were operated by robot assisted pyeloplasty and one patient underwent open surgery.

Statistical analysis

Calculations were performed using GraphPad Prism 6 for Mac OS X (version 6.0b; San Diego, CA, USA). Statistical significance was defined as $p < 0.05$.

Study I

Nonparametric Wilcoxon matched-pairs signed rank tests were used to test for changes in systolic and diastolic blood pressure after surgery. Pearson's R analysis was used for linear regression and to determine any potential association between variables. Values are presented as mean and 95% CI.

Study II

Values are presented as mean \pm SEM. For multiple comparisons among groups, analysis of variance (ANOVA), followed by the Fisher's post-test, was used. Scored data for the histological evaluation was analyzed by the non-parametric Kruskal-Wallis test followed by the Mann-Whitney U-test.

Study III

The comparisons of the arterial pressure and urine and plasma markers between groups were analyzed using ANOVA (or the non-parametric Kruskal-Wallis test) followed by Dunn's multiple comparisons test. Wilcoxon matched-pairs signed rank test (two-tailed) was used to analyze the effect of

surgical management on the hydronephrotic group. Data are shown as box and whiskers (5-95 percentile) plots. Linear regression analysis and Pearson r correlation was used to test for the association between split renal function and mean arterial pressure.

Study IV

Nonparametric Kruskal-Wallis test, followed by Dunn's test, was used for multiple comparisons among groups. Matched blood pressure values (before and after relief) were analyzed by Wilcoxon test (i.e. matched-pairs signed rank test). Linear regression analysis (least squares ordinary fit) was used to compare age-grouped subpopulations (i.e. total population, ≤ 30 and > 30 years of age). Computed p-values are indicated, but $P < 0.05$ denotes statistical significance.

Ethics

Study I and III were approved by the regional ethical review board in Uppsala, Sweden (Protocol Number 2011/267). Every child's guardian gave informed consent. The study adhered to the principles of the Declaration of Helsinki.

Study II was approved by the institutional ethics review board in Stockholm (N314/12). All animal procedures performed conform with guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes or National Institutes of Health guidelines.

Study IV was performed in accordance with the ethical standards of the institutional and/or national research committee (Protocol Number 2017/017, Uppsala, Sweden), and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Results

Study I

Blood pressure

Both systolic and diastolic blood pressure levels, daytime as well as nighttime values, became significantly lower postoperatively as compared to preoperative levels (Figure 11). In all patients, there was a normal circadian blood pressure variation both pre- and postoperatively in which both systolic and diastolic blood pressure levels were lower during the night. The day-to-nighttime difference remained the same for the whole group. The mean arterial pressure was also lowered postoperatively. No significant difference was noted in the pulse pressure.

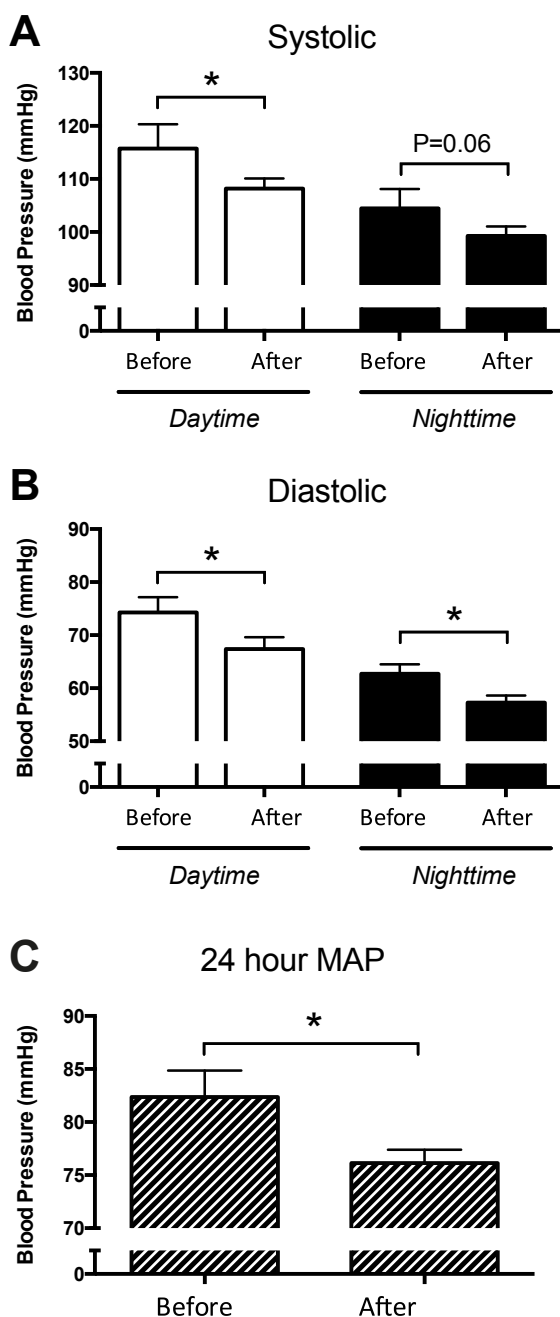


Figure 11. Systolic (A) and diastolic (B) blood pressure in children (n=12) with PUJO. Matched 24-h ambulatory blood pressure measurements were conducted preoperatively (Before) and again 6 months following surgical management of the PUJO (After) (C). Both during daytime and nighttime, systolic and diastolic blood pressures were lower compared with preoperative values. Note. Data presented are mean \pm 95% CI. PUJO = pelvo-ureteric junction obstruction. *p < 0.05

Renal function

The left kidney was more often hydronephrotic than the right (eight vs four patients; 66.7% vs 33.3 %). The renal functional share of the hydronephrotic kidney ranged from 11 to 55% (median = 45%). There was a correlation between the degree of reduced functional share in the affected kidney and the outcome of surgery in terms of difference between the pre- and postoperative blood pressure levels. The evaluation of the excretory pattern showed that seven patients had hydronephrosis grade II (obstructed) while five patients had grade IIb (equivocal). There was no correlation neither between the degree of renal function impairment and preoperative excretory pattern, nor between the preoperative excretory pattern and the reduction of blood pressure after surgery.

Study II

Animal characteristics and renal excretory function

Rats with PUUO developed elevated water intake and urine production, decreased urine Na^+ and K^+ concentrations, increased K^+ excretion, similar Na^+ excretion, and reduced urine osmolality as compared to control rats. The renal excretion pattern in rats with PUUO + DNx was similar to that of control rats. The Na^+ -to- K^+ concentration ratio was significantly decreased in PUUO animals, and this was comparable with both control animals and PUUO+DNx animals. Denervated control animals presented similar renal excretion pattern as sham-operated control animals.

Telemetric measurements of blood pressure and heart rate

Mean arterial blood pressure in the PUUO group was significantly higher under normal (Figure 12A), low, and high-salt diets compared with the control group. Rats in the PUUO + DNx group had significantly lower blood pressure compared with the PUUO group during all salt diet periods, but blood pressure was still elevated compared with rats in the control group. Salt sensitivity, as determined by blood pressure changes in response to different salt diets, was more profound in the PUUO group compared with the control group (Figure 12B). In PUUO rats with denervation, the salt sensitivity was similar to that of control rats. In a subset of control animals, we also looked at potential effects of renal denervation. However, control + DNx rats had similar blood pressure levels and salt sensitivity compared to sham-operated control rats

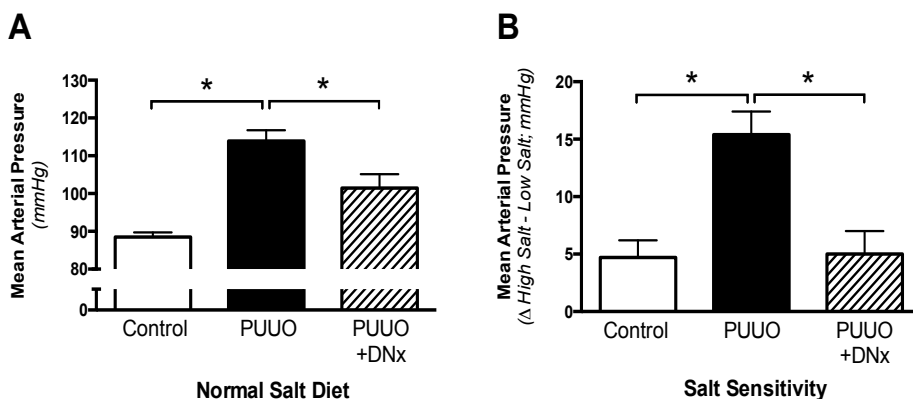


Figure 12. Mean arterial pressure measured during normal salt diet (A) and salt sensitivity (B) in sham operated rats (Controls, n=10), rats with partial unilateral ureteral obstruction (PUUO, n=15), and rats with PUUO and renal denervation (PUUO+DNx, n=10). Blood pressure and salt sensitivity was significantly higher in the PUUO group compared with controls. Animals with PUUO+DNx had significantly lower blood pressure compared with PUUO rats, yet higher blood pressure than controls. Salt sensitivity, i.e. difference in blood pressure level between high and low salt conditions, was more pronounced in PUUO compared with control rats. In the PUUO+DNx group the salt sensitivity of blood pressure was similar to that of controls.

In addition to blood pressure, we also measured heart rate under normal, low, and high-salt diets. Interestingly, the hydronephrotic group had lower heart rate under all dietary conditions. Renally denervated PUUO rats had similar heart rates as control rats under normal and high-salt diets but presented the opposite trend when given the low-salt diet. Again, control + DNx rats had similar heart rates as sham-operated control rats. Salt sensitivity in terms of heart rate changes under the different diets was significantly lower in the PUUO + DNx group compared with the other groups.

NOX activity

NOX activity was measured in the renal cortex (Figure 13A), medulla, and heart (Figure 13B) after the normal salt diet period. Superoxide production in the cortex from hydronephrotic kidneys ($4,794 \pm 391$ units/min/mg, n=15) was significantly higher compared with control kidneys ($3,139 \pm 69$ units/min/mg, n=10, $p < 0.05$). Denervation of PUUO kidneys reversed this effect, with similar values as control kidneys (Figure 13A). These differences in NOX activity were observed only in the renal cortex and not in the medulla. Hydronephrosis was also associated with higher NOX activity in the heart (772 ± 110 units/min/mg, n=15) compared with the control group (476 ± 55 units/min/mg, n=10, $p < 0.05$). However, in the presence of hy-

dronephrosis, renal denervation was associated with much lower superoxide generation in the heart (Figure 13B).

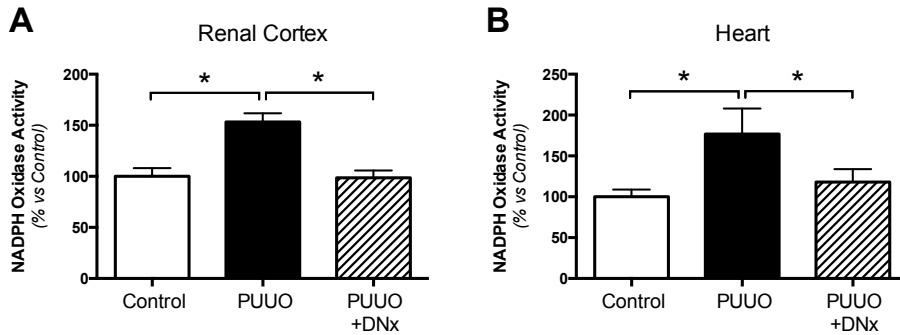


Figure 13. NADPH oxidase (NOX) activity in the renal cortex (A), and heart (B) from control rats (n=10), PUUO rats (n=15), and PUUO + DNx rats (n=10). The renal cortex from rats with PUUO displayed increased NOX activity in the hydronephrotic (left) kidney compared with that of kidneys from control rats. In rats with renal denervation, NOX activity was significantly reduced and not different from that of control rats. In the heart, hydronephrotic animals displayed higher NOX activity, which was significantly reduced but not normalized in PUUO + DNx animals. Values are presented as means \pm SE. *p < 0.05 between the indicated groups.

mRNA expression of NOX in the renal cortex

We analyzed the mRNA expression of NOX subunits (NOX2, NOX4, p22^{phox}, p47^{phox}, and p67^{phox}) in the renal cortex. The hydronephrotic kidney (PUUO, left side) displayed higher NOX2 (Figure 14A), NOX4 (Figure 14B), and p22^{phox} (Figure 14C) levels compared with control kidneys, whereas the expression of p47^{phox} and p67^{phox} (Figure 14, D and E) were not significantly changed. Renal denervation in PUUO rats was associated with similar or even lower expression of NOX2, NOX4, p22^{phox}, and p47^{phox}, than in control rats (Figure 14, A–D). In the contralateral kidney (PUUO, right side), the expression of all tested isoforms was similar to that of control kidneys and significantly lower compared with hydronephrotic left kidneys.

mRNA expression of NOX in the heart

Hydronephrosis affected NOX expression not only in the kidney but also in the heart. Expression levels of all NOX isoforms in the left ventricular area were significantly higher in hydronephrotic animals (Figure 14 F–J).

Interestingly, renal denervation was linked to reduced or even normalized levels of NOX2, p22^{phox}, p47^{phox}, p67^{phox}, and there was also a trend towards reduced NOX4 expression (Figure 14 F–J).

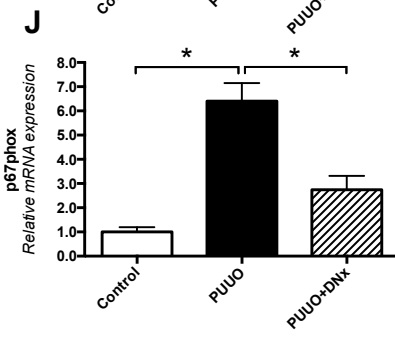
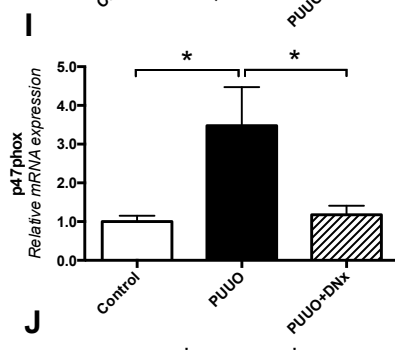
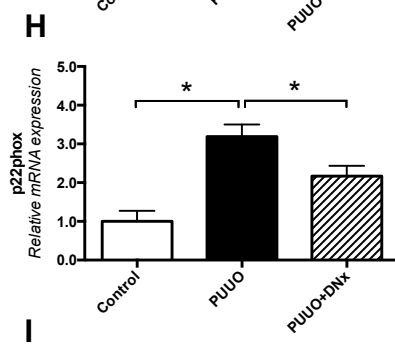
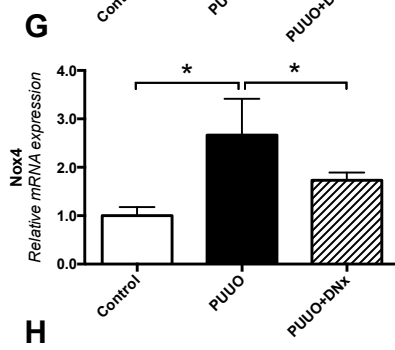
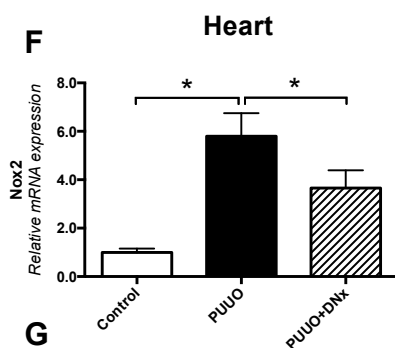
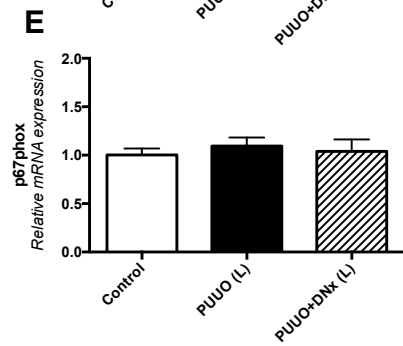
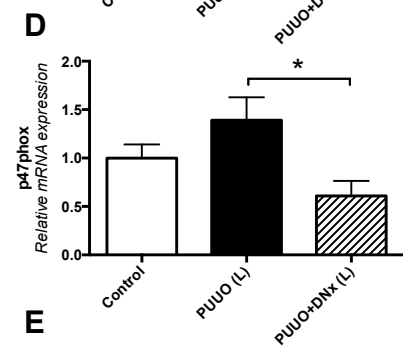
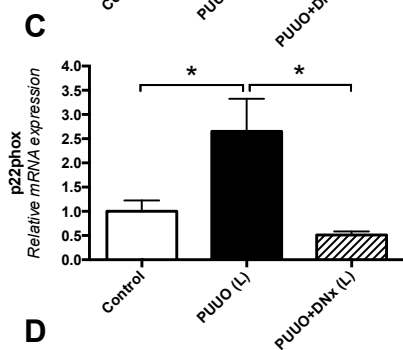
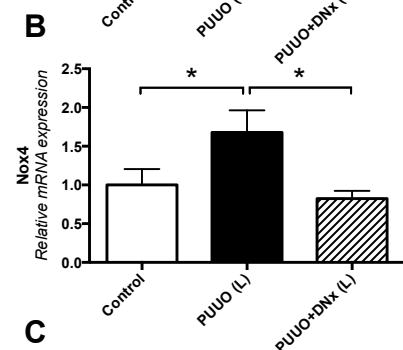
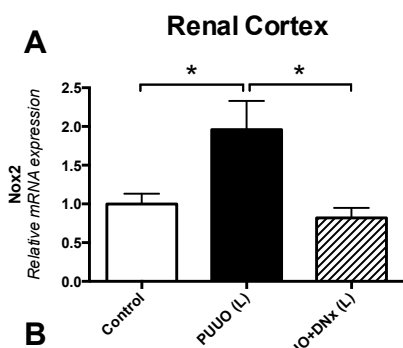


Figure 14. mRNA expression of NOX in the renal cortex and the heart from control rats, PUUO rats, and PUUO + DNx rats. The renal cortex from rats with PUUO displayed increased expression of NOX2 (A), NOX4 (B), and p22^{phox} (C), whereas expression of p47^{phox} (D) and p67^{phox} (E) was not significantly increased in the hydronephrotic left kidney. In PUUO rats with renal denervation, mRNA expression of NOX2 (A), NOX4 (B), p22^{phox} (C), and p47^{phox} (D) was significantly reduced. Denervated PUUO kidneys even had reduced p22^{phox} (C) and p47^{phox} (D) expression compared with control kidneys. Cardiac tissue from rats with PUUO displayed increased expression of NOX2 (F), NOX4 (G), p22^{phox} (H), p47^{phox} (I), and p67^{phox} (J). In the PUUO + DNx group, reduced or even normalized levels of NOX2 (F), p22^{phox} (H), p47^{phox} (I), and p67^{phox} (J), there was also a trend towards reduced NOX4 expression. Values are presented as means \pm SE; n=6 animals/group. *P<0.05 between the indicated groups; #P < 0.05 compared with the control group.

Effect of PUUO on components of the RAAS

Hydronephrotic kidneys had elevated mRNA expression of renin compared with control kidneys. Interestingly, renal denervation in PUUO rats markedly suppressed renin expression, down to levels even lower than in control rats (Figure 15A). Expression of the AT_{1A} receptor was significantly elevated in the hydronephrotic left kidney, which was reduced to control levels with denervation (Figure 15B). In similar to what was observed with NOX subunits, renal denervation in control rats did not affect the expression of renin or the AT_{1A} receptor. Plasma Na⁺ and K⁺ levels were elevated in PUUO rats, but were normalized by renal denervation. In contrast to the kidney, plasma renin and angiotensin II (ANG II) levels were not different between the four groups. Finally, plasma aldosterone was significantly higher in hydronephrotic rats but was not significantly reduced by renal denervation. Surprisingly, denervation in control rats was associated with elevated K⁺ and aldosterone levels.

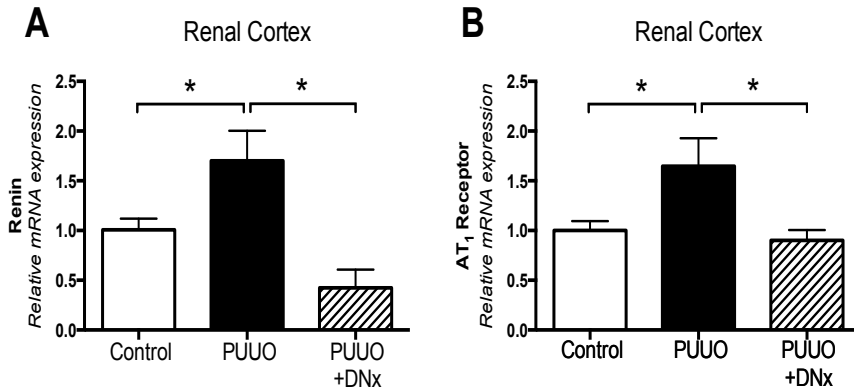


Figure 15. mRNA expression of renin (A) and the ANG II type 1A (AT_{1A}; B) receptor in the renal cortex of control rats, PUUO rats, and PUUO + DNx rats (B). The renal cortex of rats with PUUO displayed increased expression of both renin (A) and the AT_{1A} receptor (B). In rats with renal denervation, expression of renin was markedly suppressed in the hydronephrotic left kidney (A), while AT_{1A} expression was comparable with control values (B).

Renal injury and inflammation

Renal injury was detected by the presence of proteinuria and renal inflammation and fibrosis. Interestingly, hydronephrotic animals presented significantly higher protein excretion than controls, a phenomenon which was normalized by renal denervation (Figure 16A).

Renal denervation had no effect in control rats. Compared with the normal renal histological architecture in control kidneys, hydronephrotic left kidneys displayed dilated pelvic areas with flattening of the papilla, associated with mild to moderate fibrosis (Figure 16B) and inflammation (Figure 16C), changes which were not significantly reduced by denervation.

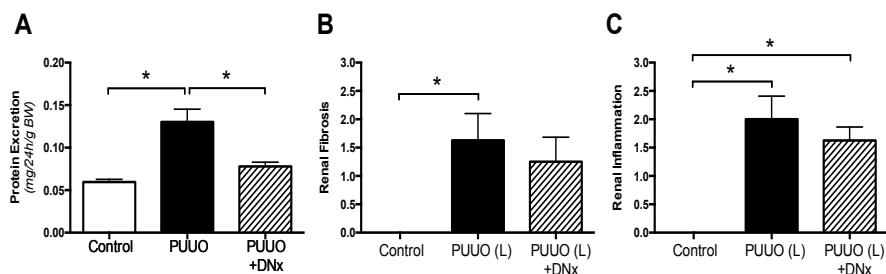


Figure 16. Urinary protein excretion (A), interstitial fibrosis (B) and histological signs of inflammation (C) in the renal cortex. Hydronephrotic rats had increased proteinuria, which was normalized by denervation (A). Hydronephrosis was also associated with increased fibrosis, which was still present in PUUO rats with renal denervation but was not significantly differing from control rats (B). Hydro-nephrotic rats had increased inflammation, which was not significantly reduced by denervation (C). Values are presented as means \pm SE; $n=6$ animals/group * $P < 0.05$ between the indicated groups; # $P < 0.05$ compared with the control group.

Cardiac injury

Hydronephrosis was associated with a higher heart-to-body weight ratio, suggesting cardiac hypertrophy. This was almost normalized in PUUO rats with renal denervation (Figure 17A). Histological analysis revealed mild to moderate cardiac fibrosis in rats with hydronephrosis, which was reduced to minimal levels in denervated PUUO rats (Figure 17B). Denervated control animals were similar to sham-operated animals in all investigated parameters.

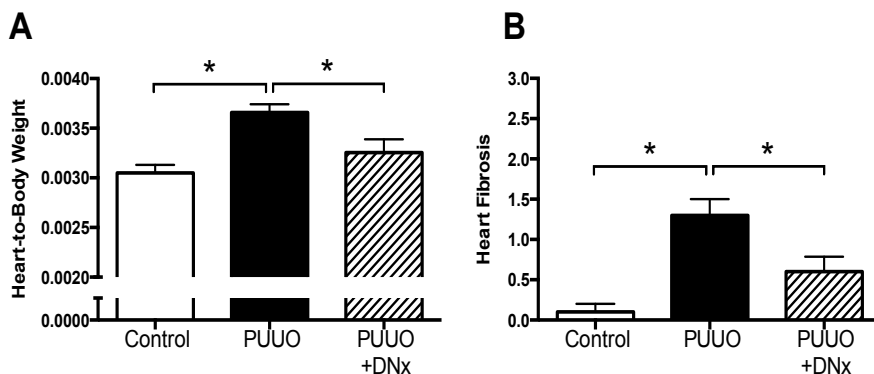


Figure 17. Heart-to-body weight ratio (A) and histological evaluation of left ventricular fibrosis (B). Hydronephrosis was associated with a higher heart-to-body weight ratio compared with control rats, but was almost normalized in denervated PUUO rats (A). PUUO was also associated with mild to moderate cardiac fibrosis, which was significantly reduced in PUUO + DNx animals (B). Scale bars = 60 μ m. Values are presented as means \pm SE; $n=6$ animals/group. * $P < 0.05$ between the indicated groups.

Study III

Blood pressure

Ambulatory 24 h blood pressure monitoring MAP was significantly higher in the hydronephrotic group compared with the healthy control group, but not significantly ($p=0.08$) higher than the operated control group (preoperatively). Moreover, there was no difference in arterial pressure between the two control groups (Figure 18A). Surgical correction of the hydronephrosis was associated with significantly lower arterial pressure (Figure 18B-C). Postoperatively, there were no differences in arterial pressure among the investigated groups; Healthy controls (HC) 74 mmHg (95% CI, 69-80 mmHg), operated controls (OC) 78 mmHg (95% CI, 73-83 mmHg), HN (Post) 76 mmHg (95% CI, 74-79 mmHg). Both systolic and diastolic pressures were significantly reduced following surgical management of the obstruction in children with hydronephrosis.

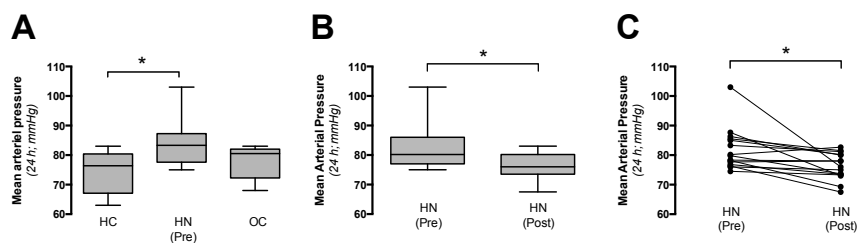


Figure 18. Arterial pressure in hydronephrotic children and controls. Panel A: Mean arterial pressure (MAP) in two control groups, healthy controls (HC) and operated controls (OC), and hydronephrotic children before surgical management of the obstruction (HN Pre). MAP is presented for 24 h. Panel B: Mean arterial pressure in hydronephrotic children before (HN Pre) and after surgical management of the obstruction (HN Post). Data are presented as median with first and third quartiles (box) and range (whiskers). Panel C: Mean arterial pressure (24 hours) in hydronephrotic children before (HN Pre) and after surgical management of the obstruction (HN Post). HC, $n=8$; OC, $n=8$; HN Pre, $n=15$; HN Post, $n=15$. * denotes $P<0.05$

Renal ultrasound

Anteroposterior diameter of the hydronephrotic kidney before surgery was ranging from 15 to 50 mm (median = 27.5 mm).

Renal function

The left kidney was more often hydronephrotic than the right (nine vs. six patients; 60% vs. 40%). The renal functional share of the hydronephrotic kidney ranged from 11% to 55% (median = 47%). Nine patients had hydronephrosis grade II (obstructed) while five patients had grade IIb (equivocal).

A significant and strong negative correlation was found between split renal function and 24 h MAP before surgical management of the hydronephrosis (Figure 19A), but not 6 months after surgery (Figure 19B). A significant and strong negative correlation was also found between split renal function and the change in blood pressure following surgery.

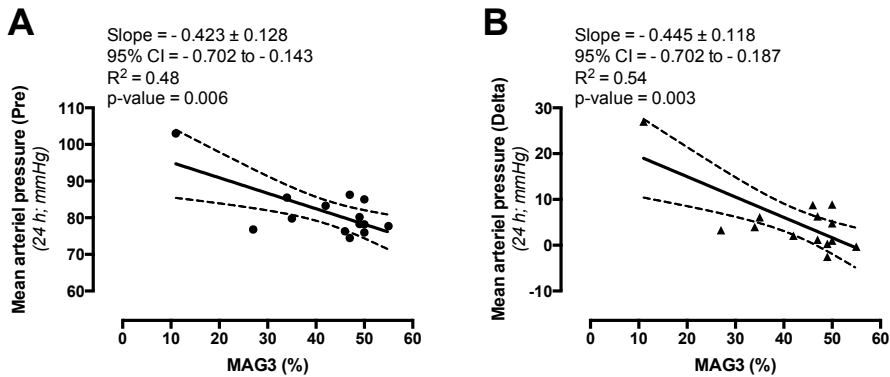


Figure 19. Correlation between split renal function according to MAG3 scintigraphy and MAP. Panel A: There was a significant and strong negative linear relationship ($r = -0.69$) between split renal function and 24-mean arterial pressure before surgical management of the hydronephrosis. Panel B: A significant and strong negative linear relationship ($r = -0.74$) was also found between split renal function and the degree of blood pressure reduction following surgery. Data are presented as mean and error with 95% CI.

Markers in blood and urine

Several different markers of NO homeostasis and oxidative stress level were measured in plasma and in urine before and after surgical correction of hydronephrosis, as well as in children in both control groups.

Nitrite, nitrate & cGMP

NO can be oxidized to nitrite (NO_2^-) and nitrate (NO_3^-), and hence the levels of these inorganic anions have been widely used as an index of NO generation. There were no differences in the plasma nitrate, whereas plasma nitrite levels were significantly higher in patients with hydronephrosis, before and after surgical management, compared with controls. In addition, there were no differences in NO signaling among the groups as indicated by similar plasma levels of cGMP. These results suggest that overall NO homeostasis is not impaired in patients with hydronephrosis, but that the activity of NO generating systems may be increased. At the same time, local renal NO concentration is reduced, as indicated by a shift in the TGF response.

Amino acids

Analyses of plasma arginine, citrulline and ornithine were performed. The ratio for citrulline/arginine (NOS activity), ornithine/arginine (arginase activity) and citrulline/ornithine (relative NOS vs. arginase activity) were calculated. Taken together, our results suggest increased NOS activity in patients with hydronephrosis, an increase that is normalized following surgery.

There were no differences in ADMA or SDMA levels between the groups. However, in support of the suggested increased NOS activity data, the levels of the endogenous NOS inhibitor NMMA were significantly lower in hydronephrotic patients compared with healthy controls, and these were normalized following management of the obstruction.

Oxidative stress

We analyzed several lipid markers associated with oxidative stress in urine samples before and after surgical management. Four markers (11-dehydroTXB₂, PGF₂ α , 8-iso-PGF₂ α , 8,12-iso-iPF₂ α -VI) were significantly elevated in hydronephrotic patients compared with healthy controls and these were normalized following surgery (Figure 20A-D). In addition, both PGE₂ and 2,3-dinor-8-iso-PGF₂ α (Figure 20C) were significantly lowered following surgical correction of the obstruction.

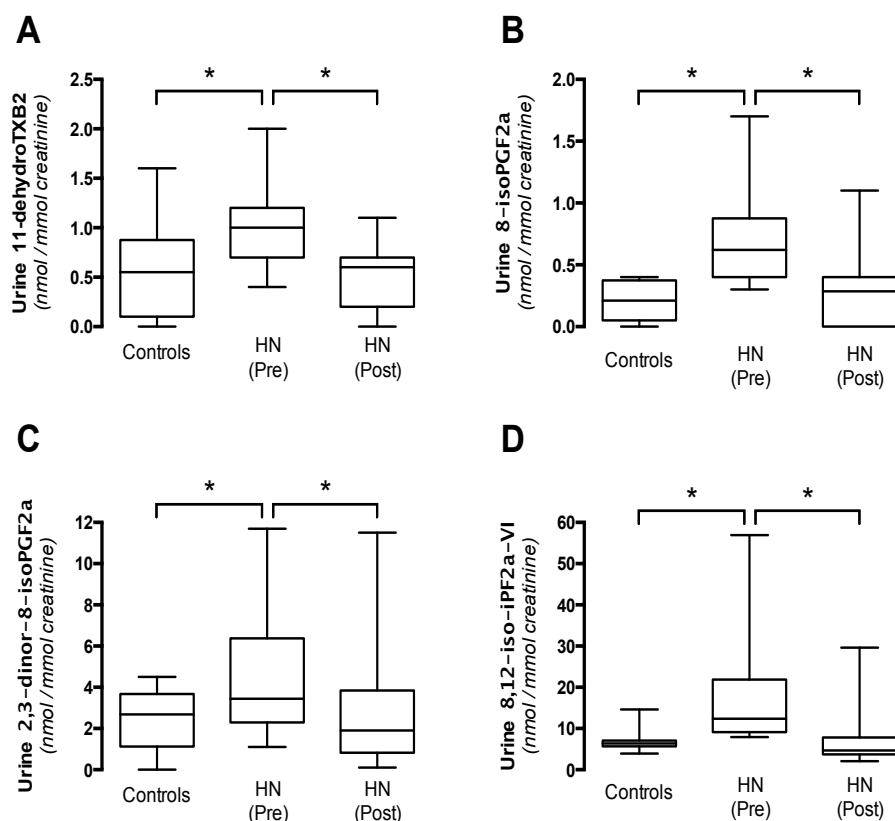


Figure 20. Pathways and markers associated with oxidative stress. Urine markers of prostaglandin and thromboxane signaling that has been associated with oxidative stress (A-B) as well as markers of oxidative stress (C-D). Urine samples were obtained from healthy controls (HC, n=8), operated controls (OC, n=8) and hydronephrotic children before (HN Pre, n=8) and after surgical management of the obstruction (HN Post, n=8). The levels were estimated as creatinine ratios. Data are presented as median with first and third quartiles (box) and range (whiskers). * denotes $p < 0.05$

Study IV

Blood pressure

Unpaired analysis performed for the total population of hydronephrotic patients suggested that systolic, diastolic and MAP were higher before surgical management of UPJ. After excluding those patients that not matched our inclusions criteria, paired analysis of blood pressure values (i.e. pre and post relief) were performed on the remaining 48 patients (Figure 21).

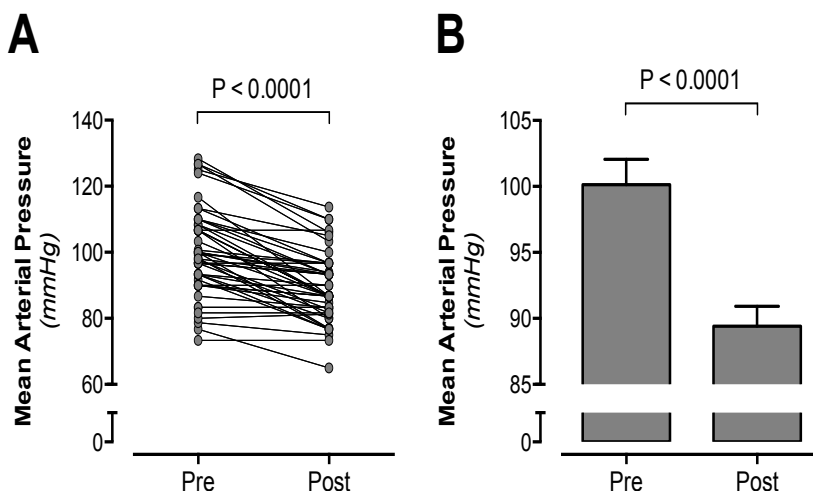


Figure 21. Mean arterial pressure (MAP) before and after relief of obstruction. Panel A-B: Matched MAP in hydronephrotic patients (n=48; male 52%; female 48%) before and after surgical management.

In agreement with the total population, systolic (Δ : -14 mmHg), diastolic (Δ : -10 mmHg) and MAP (Δ : -11 mmHg) were all significantly reduced after relief of the obstruction ($p < 0.001$ for all variables).

Seven patients had recurrence of UPJ obstruction two to four months after surgery and were thus re-operated. In these patients systolic, diastolic and MAP were higher compared with that after the original surgical management of the UPJ. Blood pressure was reduced again after reoperation of the recurrent hydronephrosis.

We performed a sub-analysis in order to evaluate if the arterial pressure elevation and the reduction of blood pressure following surgery were influenced by age. The study population was divided into patients younger or older than 30 years at the time of surgery. Systolic, diastolic and MAP were all significantly higher in the older patient group. The blood pressure was significantly reduced following surgery in both age groups, and the magnitude of blood pressure was not significantly different, although there was a clear trend towards greater reduction in systolic arterial pressure in older patients ($P = 0.08$).

Split renal function

Linear regression analysis did not reveal any correlation between blood pressure (systolic, diastolic or MAP) before surgery and the split renal function of the hydronephrotic kidney according to MAG3 scintigraphy. In addition, there was no correlation between the changes in blood pressure following surgery and split renal function. Subdivision into age groups (≤ 30 and > 30 years of age) at the time of surgical management did not change these findings.

Discussion

Hydronephrosis due to PUJO is a fairly common condition in newborns. Secondary hypertension caused by renal abnormalities is present in 5-20% of the hypertensive population [76, 77]. Published reports of hypertension due to hydronephrosis are scarce, and the numbers of patients included in these reports are low [7, 9].

In the present study, experimental hydronephrosis, induced by a PUJO at young age rats, caused renal injury and salt sensitive hypertension which was normalized by renal denervation. The clinical studies in children also showed a reduction in the blood pressure and oxidative stress after surgical treatment of the hydronephrosis. The study on adults confirmed both the experimental and pediatric studies, as the blood pressure was reduced after relief of the obstruction.

The findings in this thesis suggest that there is a risk of high blood pressure in children with hydronephrosis due to UPJ obstruction. Based on these findings, we recommend to the pediatric urologist and pediatric nephrologist to perform routine measurements of the blood pressure in children with hydronephrosis and consider the hypertension as an indication for surgical treatment, in order to avoid potential cardiovascular long-term risks.

Discussion of the findings

Blood pressure changes after renal sympathetic nerve denervation

There is evidence suggesting that increased SNA and oxidative stress promote the development of hypertension (17, 18). We demonstrated that there is an important link between renal nerve activity and the regulation of NO in the PUJO kidney, which may explain why denervation attenuates the development of hypertension.

In agreement with previous studies [12-16, 78-81], we found that hydronephrosis was associated with increased renal oxidative stress, reduced renal NO concentration [6] and the development of salt-sensitive hypertension. This, in turn, was coupled to renal fibrosis, inflammation and glomerular

injuries with proteinuria and increased diuresis likely due to impaired renal concentration capacity. The concentrating defect, i.e. increased urine production and reduced urine osmolality in animals with PUUO, has previously been explained by atrophy of the papilla [12-14, 16], which was also seen in our study. Here, we show that denervation of the PUUO kidney normalized urine flow, urine osmolality, and the Na^+ -to- K^+ ratio despite a similar degree of hydronephrosis, renal fibrosis, and inflammation.

Role of oxidative stress in hydronephrosis

A causal link between the degree of oxidative stress and the development of hypertension in hydronephrosis has been suggested [15, 16]. Reduced scavenging of superoxide, due to SOD1 deficiency, aggravates salt sensitivity and hypertension, whereas overexpression of SOD1 or treatment with the SOD mimic tempol halts hypertension [16]. Taken together, these previous studies suggest a crucial role of oxidative stress in the development of hypertension.

To investigate the link between renal denervation and a reduction in oxidative stress as a mechanism for blood pressure reduction in rats with hydronephrosis, we investigated NOX activity and expression. In the cortex, but not in the medulla, NOX was significantly higher in the hydronephrotic kidney. Enhanced NOX-derived superoxide production was associated with higher expression of the membrane-bound subunits NOX2, NOX4, and p22^{phox} , whereas intracellular p47^{phox} and p67^{phox} were not significantly changed. Moreover, the hydronephrotic kidney displayed more inflammation and interstitial fibrosis, which in turn, could contribute to increased oxidative stress [81, 82]. Despite a similar degree of renal inflammation and fibrosis, denervated PUUO kidneys displayed normal NOX activity, which was associated with significant reductions in NOX2, NOX4, p22^{phox} , and p47^{phox} expression. This is in agreement with previous findings, which demonstrated that mice with increased antioxidant defense had lower levels of renal oxidative stress and were protected from PUUO-induced hypertension, but not from the development of renal inflammation and fibrosis [15, 16].

These results suggest that oxidative stress, in particular increased superoxide generation from NOX, is an important contributing factor to the development of hypertension in the PUUO model. Since NOX activity and expression were reduced by renal denervation, without significant changes in renal inflammation, we assume that this effect is mainly derived from renal vascular or tubular cells rather than from inflammatory cells.

Other studies have shown that renal denervation can prevent the inflammatory cascade after complete ureteral obstruction [83] or ANG II-induced hypertension [84]. However, to our knowledge there is no evidence for a

causative link between renal inflammation and hypertension in our model of PUUO, which is much more subtle and clinically relevant compared with the complete ureteral obstruction model, and is not only dependent on increased circulating levels of ANG II.

Role of the RAAS in hydronephrosis

Previous studies using the PUUO model have also suggested enhanced RAAS activity during the development of hypertension and renal damage [14, 17]. A suggested mechanism for the blood pressure lowering effect of renal denervation is inhibition of the RAAS in the kidney [85]. NOX activity in the kidney, and blood pressure regulation, may be coupled to ANG II levels and activation of the AT_{1A} receptor [86-89].

We found that plasma Na⁺ and K⁺ levels were elevated in PUUO rats, but normalized by renal denervation. Aldosterone was increased in PUUO rats, but none of the other RAAS components in plasma were significantly reduced by renal denervation [90].

Instead of systemic changes in RAAS components, there is accumulating evidence that intrarenal ANG II regulation plays a major role in the pathogenesis of hypertension and renal injury [91]. In the cortex of PUUO kidneys, both renin and AT_{1A} receptor expression were higher compared with control kidneys, and denervation reduced or even normalized their expression. Moreover, it has been shown that a decreased urinary Na⁺-to-K⁺ ratio can be associated with increased RAAS activity [92, 93]. In agreement with increased intrarenal RAAS activity, this ratio was reduced in hydronephrotic rats and normalized by renal denervation.

Several studies have shown that proteinuria can be promoted by activation of the intrarenal RAAS and NOX-mediated oxidative stress, which, in turn, can trigger a vicious circle with further activation of the RAAS and NOX [94-97]. In our study, we found that renal denervation diminished PUUO-induced proteinuria, which may suggest renoprotection.

Role of NOX in hydronephrosis

Previous experimental studies have shown that renal denervation can reduce expression of NOX isoforms and superoxide production in the renal cortex [89], but also reduce oxidative stress-induced brain and heart injury [98, 99].

Our findings demonstrate that renal denervation does not only affect the hydronephrotic kidney, but also the contralateral kidney as well as the heart. We observed that hydronephrotic animals displayed an increased heart-to-body weight ratio and cardiac fibrosis, which was associated with increased

NOX activity and expression. All of these parameters were reduced or even normalized by renal denervation.

Blood pressure results in children

A key finding in this thesis is that surgical correction significantly reduced ambulatory systolic and diastolic blood pressure, both during the day and during the night, in children with hydronephrosis. This effect was seen 6 months after surgical correction of the hydronephrosis in spite of the fact that the children could have been expected to increase their blood pressure during that time interval. These results confirmed that even unilateral hydronephrosis with well-preserved renal and upper urinary tract function is associated with higher blood pressure, a finding which should be taken into consideration in the management of the patient. This is in agreement with early findings from Abramson et al who suggested that unilateral hydronephrosis appeared to be sufficient to cause elevated blood pressure, regardless of the presence of a normal contralateral kidney [7].

We included two age- and sex-matched control groups in order to evaluate if the preoperative stress factor play a role in the elevation of the preoperative blood pressure in hydronephrotic patient. The first control group consisted of healthy children, while the other control group included also healthy children whom underwent 24-hour ambulatory arterial pressure monitoring one day before undergoing minor surgery for unrelated reasons. Our aim was to evaluate preoperative stress as a factor in the elevation of the blood pressure. We found no difference in arterial pressure between the two control groups. Based on our results we conclude that higher arterial pressure in children with hydronephrosis cannot be explained by preoperative stress.

None of our patients was hypertensive preoperatively (i.e. none needed antihypertensive treatment). We should expect to find a subgroup needing medication in big studies such as the big retrospective study by de Waard et al [8] in which 11 of 277 patients needed such therapy.

Our results confirmed that surgical relief of the obstruction decreased blood pressure in children. Some authors describe resolution of hypertension following removal of the affected kidney, while others, in agreement with our study, show that relief of obstruction may also lead to normalization of blood pressure. It therefore appears that the intrarenal mechanism leading to hypertension is reversible [7].

Blood pressure results in adults

We studied all adult hydronephrotic patients that were operated in Uppsala University Hospital between 2000 and 2016. We included in the final analysis only those patients with unilateral hydronephrosis due to UPJ obstruction who were not previously on antihypertensive therapy and had no underlying chronic disease, since it would be difficult to evaluate whether there is any relationship between hydronephrosis and hypertension in such patients. We find it unlikely that this exclusion biases the results in a way that invalidates the conclusions drawn.

Our results are in agreement with previous experimental studies, case reports [13, 14, 17] and clinical studies [100, 101], demonstrating that hydronephrosis is associated with elevated arterial pressure, which can be reduced by surgical relief.

Our results also indicate that recurrence of obstruction, following surgical management of the hydronephrosis, is associated with increased arterial pressure, and that the pressure can again be reduced after relief of the obstruction. This finding gives further support to our hypothesis about the causal link between UPJ obstruction and elevation of the arterial pressure.

Oxidative stress and nitric oxide in children

As mentioned above, oxidative stress is characterized by increased production of ROS and/or reduced antioxidant capacity. This oxidative stress is prevalent in patients with kidney disease and has been suggested as an important pathogenic mechanism underlying cardiovascular disease [102, 103].

Previous studies demonstrated that hydronephrosis, induced by PUUO, leads to development of hypertension in both rats and mice [13, 14]. Underlying pathological mechanisms in this model include altered prostaglandin and thromboxane signaling [104, 105] in the affected kidney, together with oxidative stress and reduced NO bioavailability [15, 16]. The above mentioned pathological mechanisms have never been investigated in patients with hydronephrosis.

Measurement of oxidative stress in body fluids is complicated because of the very short half-life of ROS. Isoprostanes, which are mainly formed through free-radical catalyzation of arachidonic acid, are now considered to be reliable biomarkers of oxidative stress [106]. Here we show that pathways associated oxidative stress due to impaired prostaglandin and thromboxane signaling [107, 108], as well as biomarkers of oxidative stress and lipid peroxidation (isoprostanes) [106, 109] were significantly elevated in patients with hydronephrosis, and they were all reduced following surgery.

Oxidative stress due to increased generation of ROS from vascular NOX and mitochondria is often associated with reduced NO levels due to scaveng-

ing (i.e. reaction with superoxide leading to peroxynitrite formation) [110]. However, in contrast to the observations in previous experimental studies [15, 78] using adult rodents we did not find any signs of reduced NO bioavailability or signaling in young patients with hydronephrosis. Actually, NOS activity appeared to be increased before surgery and normalized following management of the obstruction. This finding warrants future investigations, but we speculate that in patients characterized by oxidative stress a compensatory up-regulation of NOS activity may partially maintain vascular NO homeostasis in order to balance the increased generation of ROS. This study only included children, but it is possible that this compensation of NO generating system due to oxidative stress may get exhausted over time, and hence lead to progressively increased arterial pressure with higher renal and cardiovascular risks. If this hypothesis of imbalance between oxidant and antioxidant systems hold true, as described in patients with chronic kidney disease [5, 102, 103], surgical management of hydronephrosis should be considered in all cases, even if the arterial pressure is normal and without other symptoms or complications.

Renal function in hydronephrosis

In our pediatric study, there was a good correlation between the degree of functional reduction and the outcome of surgery, but no correlation between the excretory pattern and the surgical outcome or between the excretory pattern and split renal function. Thus, in the setting of hypertension, it appears that the functional share of the hydronephrotic kidney should be considered an indicator of the need for surgery, whereas the renography curve is less reliable in this respect. Renographic results from many studies indicate that the function of the hydronephrotic kidney is often surprisingly well preserved despite a pelvic dilatation of large dimensions or long duration [38, 39].

In the present as well as other studies the finding of a low split renal function indicates that a considerable obstruction is or has been present [111, 112]. However, other studies have suggested that split renal function detected by renography may not always accurately predict the recovery of poorly functioning kidneys after relief of the obstruction [113]. One might argue that a reduced degree of split renal function may represent maldevelopment/hypoplasia rather than acquired damage. Total recovery of reduced renal function is rarely seen after surgery for hydronephrosis. When comparing a malfunctioning kidney with a hyperfunctioning one, and especially if contralateral hypertrophy has occurred, a return to 50/50 functional share will rarely occur. However, a progressive difference in functional share preoperatively, that stops insofar as the affected kidney keeps its functional share during growth postoperatively, meaning that both kidneys are growing

proportionally, would generally be accepted as a preoperative obstructive insult that is eliminated by surgery.

In our adult study, it was clear that in spite of preserved split renal function of the hydronephrotic kidney, the arterial pressure was elevated. Moreover, we found no correlation between split renal function and the reduction in arterial pressure after relief of the obstruction, while our results in children suggested the presence of such a relationship. It is, however, previously known that children with reflux nephropathy, i.e. renal parenchymal defects associated with vesicoureteral reflux, have an increased risk for hypertension [114]. What is not known is whether this risk pertains to all scintigraphic uptake defects or only those that are due to acquired renal damage (“scarring”) as opposed to congenital hypoplasia – since these subgroups are difficult or impossible to differentiate clinically. The same ambiguities pertain to children with hydronephrosis.

Conclusions

Study I

This novel prospective study in pediatric patients with congenital hydronephrosis demonstrates a reduction in blood pressure following relief of the obstruction.

Study II

Renal denervation in rats with hydronephrosis attenuates the development of hypertension and restores renal excretory pattern, which is associated with reduced renal NOX activity and components of the RAAS. A link between renal nerves, the development of hypertension, and modulation of NOX function is demonstrated.

Study III

Children with hydronephrosis have increased arterial pressure and oxidative stress compared with healthy controls, disturbances which can be restored to normal levels by surgical correction.

Study IV

This retrospective study shows that adults with hydronephrosis have elevated blood pressure, which was reduced or even normalized by relief of the obstruction.

Future perspectives

This thesis has contributed to the understanding of the relationship between hydronephrosis due to PUJO and hypertension in children, and has further investigated the underlying mechanisms.

Depending on the results of this thesis our recommendation is to measure blood pressure routinely in children with hydronephrosis and to treat those patients surgically to avoid development of hypertension in later life.

Currently, this routine is not present in most of the pediatric surgery clinics around the world. We consider this thesis as a basic study that confirmed the risk of developing of hypertension in children with hydronephrosis. Therefore, it will be very important for the pediatric urologist and nephrologist to give this issue more attention in order to avoid missing or delaying the diagnosis of hypertension in hydronephrotic patients.

For the future, it is important to follow these patients over time in order to detect any elevation of blood pressure.

A prospective multicenter study, including a large number of patients with hydronephrosis, measuring ambulatory 24 hours blood pressure pre-operatively and several years after relief of the obstruction is warranted to provide clinicians with blood pressure tables and cut-off values that can help when determining if surgical management should be performed or not.

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