Interactions between subtotal nephrectomy and salt: effects on blood pressure and renal function in pregnant and non-pregnant ewes.

Karen J Gibson, Amanda C Boyce, Clare L Thomson, Sarah Chinchen and Eugenie R Lumbers. Department of Physiology and Pharmacology, School of Medical Sciences, University of New South Wales, Sydney, Australia, 2052.

Running title: Remnant kidney, salt intake and pregnancy

Keywords: glomerular filtration, hypernatremia, fluid balance, salt balance

Address for correspondence:
Dr Karen Gibson
Department of Physiology & Pharmacology
School of Medical Sciences
University of New South Wales
Sydney, NSW, 2052
Australia.

Phone: +61 2 9385 3650
Fax: +61 2 9385 1059
Email: k.gibson@unsw.edu.au
Abstract

The effects of high salt intake on blood pressure and renal function were studied in 9 subtotally nephrectomised pregnant (STNxP) and 7 intact pregnant (IntP) ewes in late gestation, and in 8 subtotally nephrectomized non-pregnant (STNxNP) and 7 intact non-pregnant (IntNP) ewes. STNxP had higher mean arterial pressures ($P<0.02$) and plasma creatinine levels ($P<0.001$) than IntP. High salt (0.17 M NaCl as drinking water for 5 days) did not change blood pressure in either STNxP or IntP. STNxNP had higher mean arterial pressures ($P=0.03$) and plasma creatinine levels ($P<0.001$) than IntNP. In STNxNP, blood pressure increased with high salt intake and there was a positive relationship between diastolic pressure and sodium balance ($r=0.497$, $P=0.05$). This relationship was not present in IntNP, STNxP or IntP. Because high salt intake did not cause an increase in blood pressure of STNxP ewes, it is concluded that they were protected by pregnancy from further rises in blood pressure. The observed increase in GFR ($P<0.03$) and depression of fractional proximal sodium reabsorption ($P=0.003$) which occurred in STNxP, but not in STNxNP, in response to high salt may have contributed to this protection. As well, the increased production of vasorelaxants in pregnancy may selectively protect against the occurrence of salt sensitive hypertension in pregnancy.
Introduction

In the past we have shown that several well established models for hypertension in the non-pregnant sheep also cause hypertension when induced acutely in the pregnant ewe. These models include one clip-one kidney hypertension (13) and infusion of ACTH (12).

Recently we have studied the effects of chronic renal insufficiency, caused by subtotal nephrectomy (STNx; unilateral nephrectomy and ligation of a branch of the renal artery to the remaining kidney) on maternal cardiovascular and renal function (6) and on fetal development (5). Reduction in renal mass is carried out some months before the ewes are mated. These STNx pregnant ewes have higher blood pressures and bigger hearts compared with intact ewes from the same flock, living on the same pasture (6).

It is well recognized in a number of species that high salt intake results in hypertension in non-pregnant animals with reduced kidney mass (3). Similarly, in oophorectomized ewes, it has been reported that hypertension in response to ingestion of about 15 mmol/kg/day of salt is exacerbated by prior surgical removal of approximately 70% of renal mass (20).

The Dahl strain of rat can be salt sensitive or salt resistant, depending on their blood pressure response to a high salt diet. Interestingly while Dahl salt sensitive (SS) virgin rats had higher blood pressures than Dahl resistant (R) virgin rats, there was no difference in the blood pressures of Dahl SS and Dahl R pregnant rats measured at mid gestation (19). Thus pregnancy appears to protect this strain of rat from salt sensitive hypertension. Therefore we decided to expose our pregnant intact (IntP) and STNx (STNxP) ewes to a high salt intake (about 20 mmol/kg/day) to see if their blood
pressures increased and if the effect was more severe in STNx pregnant ewes. We also studied a cohort of non-pregnant intact (IntNP) and STNx (STNxNP) ewes.
Methods

These experiments were approved by the UNSW Animal Care and Ethics Committee.

Surgery

All surgeries were carried out under general anesthesia, induced by i.v. injection of 1-2g sodium thiopentone (Pentothal, Abbott Australasia Pty Ltd, Australia) and maintained with 2-3% halothane (Fluothane, Zeneca Ltd, UK) in oxygen. At the end of surgery, incisions were infiltrated with 0.5% bupivacaine HCl (Marcain; Astra Pharmaceuticals). Ewes were given 600mg penicillin (Ilium Propen, Troy Laboratories Pty Ltd, NSW, Australia) and 288mg oxytetracycline (Alamycin, Norbrook Laboratories Ltd, UK) i.m. at induction of anesthesia, and at completion of surgery. After catheter surgery, ewes also received these antibiotics (i.m. or i.v.) for at least 2 days post-operatively.

Unilateral nephrectomy and ligation of a branch of a renal artery. Non pregnant ewes underwent subtotal nephrectomy (STNx) as described previously (6). Briefly, one kidney was removed through a paravertebral incision. Through another paravertebral incision, at least one branch of a renal artery in the hilus of the other kidney was ligated so that there was a colour change in 30-50% of the kidney surface. Animals were held until recovery from surgery and then returned to pasture a week later. STNx and age matched intact ewes from the same flock were then time-mated in groups at various times over the next 2 years. STNx ewes (STNxP) became pregnant at 9.5-17 months after nephrectomy. In a separate study, another group of non-pregnant STNx ewes (STNxNP) were studied at 2-29 months (mean 9 ± 12 months) after nephrectomy. An age matched group of intact ewes (IntNP) were also studied.

Catheter surgery in pregnant and non-pregnant ewes. At 108 - 114 days gestation (term = 150 days), STNxP and IntP ewes were anesthetized and polyvinyl catheters
(2.7mm OD, 1.5mm ID) inserted into a maternal femoral artery and vein as described previously (18). Similarly one femoral artery and both femoral veins were catheterized in STNxNP and IntNP ewes. All vascular catheters were flushed daily with 0.15M heparinised saline (100 IU heparin/mL, Heparin Injection; Pharmacia & Upjohn Pty Ltd, NSW, Australia). Ewes were housed in metabolic cages and room temperature was maintained between 18 and 23°C. They were given free access to 8L of water, 1.2kg of lucerne chaff and 300g of oats per day. No experiments were carried out for 6 or more days after surgery. After an initial experiment carried out while ewes were on a normal salt intake (i.e. there was no salt added to their diet), ewes were given a high salt diet (up to 8 L/day of 0.17M NaCl in place of their normal drinking water) for 5 days and the experiment was then repeated. In pregnant ewes these experiments were carried out at 122 ± 0.1 days on a low salt diet and at 127 ± 0.1 days on a high salt intake.

**Experimental Protocol**

Food and fluid intake and urine and feces output were measured every day. Samples of urine were collected and stored at –20°C for further analysis. Ewes were removed from their cages on the mornings of the experiments and a 14G Foley catheter was inserted into the bladder via the urethra. They were returned to their cages and given an i.v. dose of 150μmol/kg lithium chloride and 4.8mg/kg of para-amino hippurate (PAH, Merck, Sharpe & Dohme (Australia) Pty Ltd or Sigma-Aldrich Inc (Australia)). During the course of the experiment, i.v. infusions of 10 μmol/kg/h lithium and 810mg/h PAH were delivered through a 0.2μm filter (Minisart), at a rate of 6.3mL/h (pregnant ewes) or 10 mL/h (non-pregnant ewes). After at least 45 min, urine was collected at 30 min intervals for 1.5-2h.
Arterial blood samples (5mL) were taken at the midpoint of the 2nd and 3rd or 4th periods and 20 IU/ml of heparin were added. Blood pressures were recorded throughout the course of the experiment using a pressure transducer (Easy Vent Deadender Cap, Ohmeda BOC) connected to a Grass polygraph (Quincy, MA) and recorded on an IBM-compatible PC.

After the high salt experiment, ewes were weighed and sacrificed by i.v. injection of 4-5g sodium pentobarbitone (Lethabarb, Virbac, NSW, Australia). Kidneys and hearts were removed and weighed.

**Biochemical Analysis**

**Arterial Blood Samples**

Arterial sodium, potassium and chloride concentrations were measured using a blood gas analyzer (ABL 715, Radiometer Pacific Pty Ltd). Hematocrits were determined in duplicate using a microhematocrit centrifuge and reader (Hettich, Tuttlingen, Germany). The remaining blood was centrifuged for 10 min at 1100 g and 4°C. Plasma and urine samples were stored at -20°C until biochemical analysis.

**Plasma and Urine**

Effective renal plasma flow was measured as the renal clearance of PAH using methods already described (1, 6). Urinary levels of sodium and potassium were measured using an FLM3 Flame Photometer (Radiometer). Plasma and urinary osmolalities were measured using a Fiske One-Ten Osmometer (Fiske Associates, Massachusetts).

The clearance of lithium was used to determine the amounts of sodium reabsorbed by the proximal and distal nephron (14). Lithium is freely reabsorbed by the proximal tubule but not by the distal tubule. Urinary and plasma lithium concentrations were
determined using a Varian-Techtron AA5 Atomic Absorption Spectrophotometer (Melbourne, Australia).

GFRs were measured as the renal clearance of endogenous creatinine. Creatinine levels in urine and plasma were measured by Laverty Pathology (Sydney) or using methods described by Haeckel (7). In pregnant ewes, urinary protein concentrations were determined using the Lowry method (9). In pregnant ewes, plasma renin levels were measured as the rate of formation of angiotensin I in ng/mL/h at 37°C and pH 7.5 in the presence of added nephrectomised sheep substrate (15). Plasma angiotensinogen levels were measured as the amount of angiotensin I formed in plasma in the presence of an excess of human renin (15). Angiotensin I was measured by radioimmunoassay (11).

Data Analysis and Statistics

The sodium content of normal drinking water was zero. Sodium intake in food was calculated based on a sodium content in chaff of 33 mmol/kg (4). Twenty-four hour urine collections were used to determine salt and water balance over the day.

Data are expressed as means ± the standard error of the mean (SEM); n is the number of animals. SPSS (Statistical Package for the Social Sciences; SPSS Inc; Chicago, Il, USA) was used to determine means and SEM. Values for each variable measured in each experimental period over 1.5-2h were averaged. Data from pregnant ewes and non-pregnant ewes were analysed separately. For both pregnant ewes and non-pregnant ewes, simple factorial ANOVA was carried out using SPSS, with experimental treatment (high and low salt intake) and renal integrity (STNx and intact) as the two factors. We determined if there were differences between intact and STNx ewes, if salt intake had an effect and if the effects were different in STNx compared with intact ewes (ie if there was a two-way interaction between salt intake
and renal integrity). Significance was set at 5%. Linear regression analysis was performed using SPSS.
Results

Morphology.

Pregnant Ewes. STNxP were heavier than IntP (61.7 ± 2.0 kg, $n=9$ versus 53.1 ± 3.6 kg, $n=7$, $P<0.05$). They had bigger hearts (307 ± 12 g versus 266 ± 9 g, $P<0.05$) and left ventricles (114 ± 4 g versus 90 ± 6 g, $P<0.01$). However there were no differences between the two groups in heart weight to body weight ratios nor in the left ventricular to body weight ratios. The left ventricular to heart weight ratio was higher in STNxP than IntP (0.37 ± 0.01 versus 0.34 ± 0.01, $P<0.05$). Total kidney mass was less in STNxP than in IntP (122 ± 7 g versus 171 ± 10 g, $P<0.001$) as was the kidney to body mass ratio (g/kg; 2.0 ± 0.1 versus 3.3 ± 0.2, $P<0.001$).

Non-pregnant ewes. Body weight was similar in the two groups (STNxNP 59.6 ± 2.9 kg, $n=8$ and IntNP 57.4 ± 1.9 kg, $n=7$) as were heart and left ventricular weights and the left ventricular to heart weight ratio. Total kidney mass was lower in the STNxNP than IntNP (100 ± 7 g versus 161 ± 8 g, $P<0.001$) as was the kidney to body mass ratio (g/kg; 1.7 ± 0.1 versus 2.8 ± 0.1, $P<0.001$). There was no relationship between time since nephrectomy and the kidney to body mass ratio in STNxNP.

Arterial pressure and heart rate

Pregnant ewes. STNxP had higher systolic, mean and diastolic pressures than IntP ($P=0.02$, $P<0.02$, $P=0.005$, Figure 1 and Table 1). However, there was no effect of a high salt intake on arterial pressures or heart rates.

Non-pregnant ewes. STNxNP had higher mean and diastolic pressures than IntNP ($P=0.03$, $P=0.02$). They also tended to have higher systolic pressures ($P=0.07$). High
salt diet tended to increase diastolic pressure in STNxNP ($P=0.08$) and when data from both low and high salt diets were combined, there was a positive relationship between diastolic pressure and salt balance in STNxNP ($r = 0.497$, $n=16$, $P=0.05$; Fig 2). This relationship was not present in IntNP ($r=0.094$, $n=14$, $P=0.75$), IntP ($r=0.009$, $n=11$, $P=0.98$) or STNxP ($r=0.038$, $n=15$, $P=0.894$).

One of the STNxNP sheep consumed only $10.7$ mmol/kg/day of salt when on the high salt diet, whereas the average of the other STNxNP animals was $21.0 \pm 1.9$ mmol/kg/day ($n=7$). If this animal is excluded because its salt intake was too low, the rise in blood pressure with high salt diet in STNxNP was significant. Systolic pressure rose from $133 \pm 5$ on low salt to $146 \pm 4$ mmHg on high salt ($n=7$, $P=0.03$ using a paired $t$-test); diastolic pressure rose from $86 \pm 3$ to $97 \pm 3$ mmHg ($P<0.02$); mean arterial pressure rose from $104 \pm 4$ to $116 \pm 3$ ($P<0.02$). There was no relationship between time since nephrectomy and arterial pressure on either the low or high salt diet, whether or not this sheep was included.

**Fluid balance**

*Pregnant ewes.* STNxP drank more fluid ($P<0.001$) than IntP (STNxP low salt $4.9 \pm 0.4$, $n=9$ and high salt, $7.8 \pm 0.2$ L/day, $n=7$; IntP low salt $3.7 \pm 0.4$, $n=7$ and high salt $5.8 \pm 0.6$ L/day, $n=7$). The addition of salt to the drinking water stimulated drinking ($P<0.001$) by the same extent in each group (a $1.8 \pm 0.2$ fold increase, $n=7$, in STNxP versus a $1.6 \pm 0.1$ fold increase, $n=7$, in IntP). The difference between daily fluid intake and the amount of fluid excreted as urine (fluid balance) was remarkably similar in STNxP and IntP and was not affected by high salt intake (STNxP, low salt
2.8 ± 0.3, n=9 and high salt 2.6 ± 0.3 L/day, n=7; IntP, low salt 2.2 ± 0.2, n=7 and 2.7 ± 0.3 L/day, n=7).

Non-pregnant ewes. STNxNP drank more fluid (P<0.01) than IntNP (STNxNP low salt 4.2 ± 0.4 and high salt 6.7 ± 0.7 L/day, n=8; IntNP low salt 2.5 ± 0.21 L/day and high salt 4.9 ± 0.8 L/day, n=7). The addition of salt stimulated drinking (P<0.001) by a similar extent in both groups (1.6 ± 0.1 fold, n=8 in STNxNP compared with 2.0 ± 0.3 fold, n=7 in IntNP). Fluid balance was greater (P<0.001) in STNxNP than IntNP but was not altered by salt intake (STNxNP low salt 2.7 ± 0.3 and high salt 2.7 ± 0.4 L/day, n=8; IntNP low salt 1.1 ± 0.1 and high salt 1.7 ± 0.1 L/day, n=7).

Salt balance.

Pregnant ewes. On a low salt diet, the intake of sodium was similar in STNxP and IntP (0.59 ± 0.04, n=9 and 0.62 ± 0.07 mmol/kg/day, n=7 respectively). With addition of salt to the water, it increased (P<0.001) by a similar extent in both groups, so that on the high salt diet it was 23 ± 1 mmol/kg/day (n=7) in STNxP and 20 ± 3 mmol/kg/day (n=7) in IntP. Although on a low salt diet, sodium balance (measured as the difference over a 24h period between sodium ingested and sodium excreted in the urine) was close to zero in STNxP (-0.01 ± 0.19 mmol/kg/day, n=9) but positive in IntP (0.41 ± 0.14 mmol/kg/day, n=7), this difference was not significant. On the high salt diet, salt balance increased (P<0.001) and became strongly positive in both groups (STNxP 2.88 ± 0.88 mmol/kg/day, n=7; IntP 3.24 ± 65 mmol/kg/day, n=7). It should be noted that these “balance” measurements do not account for any faecal sodium losses, so the degree of positive balance is likely to be overestimated.
Non-pregnant ewes. Salt intake was not affected by renal integrity but salt intake rose 
(P<0.001) when salt was added to the drinking water (STNxNP low salt 0.61 ± 0.04 
and high salt 19.7 ± 2.1 mmol/kg/day, n=8; IntNP low salt 0.32 ± 0.03 and high salt 
15.2 ± 2.6 mmol/kg/day, n=7). Similarly salt balance (measured as described above) 
rose (P<0.001) on high salt (STNxNP low salt 0.13 ± 0.14 and high salt 4.35 ± 0.64 
mmol/kg/day, n=8; IntNP low salt 0.21 ± 0.08 and high salt 3.14 ± 0.18 mmol/kg/d, 
n=7).

Blood Composition - plasma electrolytes and osmolality

Pregnant ewes. Plasma sodium was higher in STNxP than IntP (Figure 3, P=0.001). 
High salt intake was associated with increased plasma sodium and chloride levels 
(P<0.001) and there was a significant interaction between renal integrity and dietary 
salt intake on sodium and chloride concentrations (P=0.002, P<0.03) such that the 
highest levels were present in STNxP on high salt (Figure 3, Table 1). There was an 
interaction between renal integrity and salt intake on plasma osmolality (P=0.04, 
Figure 3) so that plasma osmolality was higher in STNxP than IntP (P=0.009). Plasma 
potassium levels and the plasma Na:K ratio were similar in STNxP and IntP and were 
not affected by high salt. STNxP had higher plasma bicarbonate levels than IntP 
(P=0.008, Table 1). Bicarbonate levels fell with high salt intake (P=0.001).

Non-pregnant ewes. STNxNP ewes had higher plasma sodium (P=0.001) and 
osmolality (P<0.001) but lower plasma chloride (P=0.05) than IntNP (Figure 3, Table 
2). The levels of plasma potassium and bicarbonate and the plasma Na/K ratio were 
similar in the two groups. High salt diet was associated with an increase in sodium 
(P=0.003), potassium (P=0.001), chloride (P<0.001) and osmolality levels (P=0.013),
but a fall in plasma bicarbonate ($P<0.001$) and the Na/K ratio ($P=0.004$). There was an interaction between renal integrity and salt intake on plasma chloride levels ($P=0.05$), so that on high salt, levels were similar in STNxNP and IntNP (Table 2).

**Blood composition - haemoglobin, haematocrit, creatinine, renin and angiotensinogen.**

*Pregnant ewes.* Similar declines in hemoglobin levels ($P<0.05$) and hematocrit ($P=0.02$) occurred in both STNxP and IntP on high salt (Table 1). Plasma creatinine levels were greater in STNxP than IntP ($P<0.001$) and did not change with salt. Plasma renin levels were suppressed in each group by high salt ($P<0.03$) but angiotensinogen levels did not change (Table 1).

*Non-pregnant ewes.* Both haemoglobin levels and haematocrit were lower in STNxNP than IntNP ($P=0.006$ and $P=0.03$) but there was no significant effect of high salt intake (Table 2). Plasma creatinine levels were greater in STNxNP than IntNP ($P<0.001$) and did not change with salt.

**Renal function**

*Pregnant ewes.* STNxP had higher urine flows and sodium and osmolar excretions than IntP ($P<0.004$, $P=0.003$, $P=0.002$; Table 3). High salt intake increased these values in both groups ($P<0.001$) but to a greater extent in STNxP ($P<0.02$). Free water clearance became more negative on a high salt diet ($P<0.001$). Urinary potassium excretion did not change. The urinary sodium to potassium ratio was higher in STNxP ($P<0.001$), increased in both groups ($P<0.001$) with high salt but increased to a greater extent in STNxP ($P<0.002$). Urinary osmolality was lower in STNxP.
regardless of salt intake ($P=0.007$). Urinary protein excretion was higher in STNxP ($P=0.04$).

*Non-pregnant ewes.* Urine flow rate, electrolyte and osmolar excretion rates, urinary Na/K ratio, urinary osmolality and free water clearance were remarkably similar in STNxNP and IntNP (Table 4). Sodium excretion ($P<0.001$), osmolar excretion ($P=0.001$), free water clearance ($P<0.001$) and the urinary Na/K ratio ($P=0.001$) increased with high salt diet (Table 4).

**Glomerular function**

*Pregnant ewes.* Effective renal plasma flow (ERPF) was lower in STNxP than IntP ($P<0.001$) and was not altered by high salt intake (Table 3). GFR was lower in STNxP than IntP ($P<0.001$) and increased in both groups with high salt intake (Table 3, Figure 4).

*Non-pregnant ewes.* ERPF was lower in STNxNP than IntNP ($P<0.001$) and was not altered by high salt intake (Table 4). Similarly GFR was lower in STNxNP than IntNP ($P<0.001$). Unlike in the pregnant ewes, GFR did not increase with high salt intake (Table 4, Figure 4).

**Tubular handling of sodium**

*Pregnant ewes.* The fraction of the filtered sodium load reabsorbed was lower in STNxP than IntP ($P<0.001$; Table 3). This was due mainly to lower fractional reabsorption of sodium in the proximal tubule in STNxP ($P<0.001$); indeed, fractional reabsorption of sodium by the distal tubule was higher in STNxP than in IntP ($P=0.002$). Fractional reabsorption of sodium and fractional reabsorption of sodium
by the proximal tubule decreased with high salt diet in both STNxP and IntP ($P<0.001$, $P=0.003$). There was an interaction between renal integrity and salt intake for fractional reabsorption of sodium ($P=0.04$), such that the fall was greater in STNxP than IntP (Table 3).

*Non-pregnant ewes.* Like in the pregnant ewes, the fraction of the filtered sodium load reabsorbed was lower in STNxNP than IntNP ($P=0.01$), as was the fraction reabsorbed proximally ($P=0.002$), while the fraction reabsorbed distally was higher ($P=0.006$; Table 4). High salt diet caused a reduction in fractional sodium reabsorption ($P<0.001$) and there was an interaction with renal integrity ($P=0.02$) such that the reduction was greater in the STNxNP group (Table 4). However, unlike in the pregnant ewes, there was no significant effect of high salt diet on fractional reabsorption of sodium by the proximal tubule.
Discussion

On a high salt intake (~ 20 mmol/kg/day) neither IntP nor STNxP ewes had any change in blood pressure. This was despite the fact that STNxP ewes had higher arterial pressures than IntP ewes on a normal salt intake (Figure 1). There was strong evidence that a high salt intake was associated with expansion of the extracellular volume in both STNxP and IntP as both hemoglobin and hematocrit fell, as did plasma renin levels (P<0.03) and the difference between salt intake and salt excreted in the urine increased markedly (P<0.001). The lack of any potentiating effect of high salt intake on the blood pressures of STNxP ewes was especially striking because their plasma sodium levels and osmolalities were particularly increased on a high salt intake (Figure 3) compared with levels measured in IntP. Furthermore, in STNxNP animals there was an increase in blood pressure when salt intake was high and there was a positive relationship between diastolic pressure and salt balance (Figure 2).

The combination of a remnant kidney and a high salt intake is a well established model of hypertension in species like the rat (3) and is cited in standard physiological texts as the experimental paradigm that underpins the long standing theory that hypertension is caused by sustained extracellular volume expansion (17). Sheep have been used to demonstrate that the hypertension that occurs with remnant kidney and high salt intake is due to volume expansion rather than to any vasoactive properties of sodium itself (17). In our non-pregnant sheep we had clear evidence that a high sodium intake increased blood pressure in the presence of reduced renal function (Table 2 and Figure 2). This finding is similar to that of Whitworth et al. (20) who also found that in oophorectomized ewes the combination of high salt diet and reduced renal mass had an additive effect on blood pressure. Although the reduction in renal mass alone did not cause hypertension in that study (20), GFR was not
measured so it is not clear whether the amount of renal impairment was as severe as in the STNxNP ewes in the current study.

Therefore our observation that STNxP ewes on a high salt intake did not show any further increase in arterial pressure was completely unexpected. The amount of sodium ingested by our pregnant ewes (~ 20 mmol/kg/day) was similar to that ingested by our non-pregnant ewes, and greater than that which caused a rise in blood pressure in intact and subtotally nephrectomised non pregnant sheep (20) and dogs (about 15 mmol/kg/day, 2).

The failure of the combination of subtotal nephrectomy and high salt to cause hypertension in pregnant sheep would therefore appear to be related to the fact that the sheep are pregnant rather than to the species used. We cannot find any reports of the effects of remnant kidneys on blood pressure in pregnancy nor can we find any reports on the combined effects of remnant kidney and high salt on the blood pressure of pregnant animals. Although the STNxP had been nephrectomized for a longer time period than many of the ewes in the STNxNP group (2-3 months), this is unlikely to account for why their arterial pressures were not salt sensitive because two ewes in the STNxNP group had been nephrectomized for even longer (over two years). Furthermore, there was no relationship between time from nephrectomy and arterial pressure on either the low or high salt diet.

There are several reasons why expansion of the extracellular volume of pregnant sheep may not cause a rise in blood pressure and why severe hypertension did not develop even in STNxP ewes on high salt. Firstly, the capacitance of the cardiovascular system is enormously increased in pregnancy (16), with growth and development of the uteroplacental circulation (12), the increase in renal blood flow (10), the increased vascularity associated with mammary gland development and the
relaxation of the venous circulation through the action of hormones like progesterone (16).

Secondly, both IntP and STNxP ewes reduced their plasma renin levels in response to high salt. The consequent fall in angiotensin II levels would have led to a reduction in aldosterone secretion and increased sodium excretion and potassium retention, as evidenced by the rise in the urinary Na:K ratio (Table 3). These changes, plus the increase in GFR (Figure 4) and fall in fractional reabsorption of sodium by the proximal tubule (Table 3), thus limited the degree to which the extra ingested salt was retained. Together with reduced levels of circulating vasoactive factors (such as angiotensin II) this would have limited the degree of extracellular volume expansion and the amount of vasoconstriction within the circulation.

Thirdly, the production of vascular endothelial factors like NO are increased in pregnancy ensuring relaxation of the peripheral vasculature (8). Pregnancy has marked effects on the cardiovascular system of salt sensitive rat strains (Dahl SS). Virgin Dahl SS rats have higher arterial pressures than virgin Dahl resistant strains (Dahl R) but in midgestation, pregnant Dahl SS rats have blood pressures similar to Dahl R pregnant rats. In addition, pregnancy restores the renal vasodilator response to glycine in the Dahl SS rat. Dahl SS virgin rats only had a 12% fall in renal vascular resistance (RVR) in response to glycine; Dahl R rats had a 30% fall, as did pregnant Dahl SS (19). It is claimed that in part this renal vasodilation in response to a high salt diet protects the Dahl R rat from hypertension (19). Both IntP and STNxP ewes had a rise in GFR on a high salt diet (Figure 4). By contrast, there was no increase in GFR in STNxNP (Figure 4).

Interestingly we can cause hypertension in pregnant sheep by inflation of an occluder around the renal artery in a remaining kidney and by infusion of ACTH, both well
known animal models of hypertension in the nonpregnant animal (12,13). Therefore the protection conferred by pregnancy against salt sensitive hypertension in rats (19) and STNx sheep, may be specific for salt sensitive hypertension.

As stated above there was strong evidence that both groups of pregnant ewes retained more sodium when on high salt as the difference between salt intake and urinary sodium loss increased and haematocrit, haemoglobin and plasma renin levels fell. The greater increase in maternal plasma sodium and osmolality and the interaction between subtotal nephrectomy and high salt (Figure 3) in STNxP ewes suggested that they were less able (compared to IntP) to maintain osmolar balance (Figure 3, lower panel) possibly because they had insufficient nephrons. Since STNx ewes had one kidney removed and at least 30% of the remaining kidney infarcted, nephron number must have been less than 50%.

In conclusion, a high salt intake did not have any adverse effects on the blood pressures and renal function of IntP nor STNxP. Thus it appears that pregnancy protects the mother from the hypertensinogenic effects of a high salt intake in the sheep as is the case in other animals with salt sensitive hypertension.

_Perspectives_

These findings may have implications for the pharmacological management of hypertension in human pregnancy. We suggest that because volume loading does not cause high blood pressure in pregnant ewes, even when they have renal impairment, antihypertensive therapies directed at vasodilation might be more effective than antihypertensive therapies directed at volume depletion (eg diuretics) during pregnancy.
Acknowledgements

We would like to acknowledge the technical assistance of Ms Pamela Bode, Ms June Wu and Ms Vasumathy Kumarasamy.

Grants

This work was supported by a grant to Scientia Professor E.R. Lumbers from the National Health and Medical Research Council of Australia.
References


Figure Legends

Figure 1. Effects of low and high salt intake on systolic (upper panel) and diastolic pressure (lower panel) of intact pregnant (IntP, Low \( n=6 \), High \( n=5 \)), STNx pregnant (STNxP, Low \( n=9 \), High \( n=6 \)), intact non-pregnant (IntNP, \( n=7 \)) and STNx non-pregnant (STNxNP, \( n=8 \)) ewes.

For systolic pressure in pregnant ewes, \( P=0.02 \) for effect of renal integrity.

For diastolic pressure in pregnant ewes, \( P=0.005 \) for effect of renal integrity.

For diastolic pressure in non-pregnant ewes, \( P=0.02 \) for effect of renal integrity.

Figure 2. Salt dependency of diastolic pressure in STNx non-pregnant ewes.

Diastolic pressure was directly related to Na balance (measured as daily sodium intake minus urinary sodium excretion). Each animal (\( n=8 \)) was studied on both a low salt and high salt diet. \( r=0.497, \ n=16, \ P=0.05 \).

Figure 3. Effects of low and high salt intake on plasma sodium (upper panel) and osmolality (lower panel) in intact pregnant (IntP, Low \( n=7 \), High \( n=6 \)), STNx pregnant (STNxP, Low \( n=9 \), High \( n=6 \)), intact non-pregnant (IntNP, \( n=7 \)) and STNx non-pregnant (STNxNP, \( n=8 \)) ewes.

For sodium in pregnant ewes, \( P=0.001 \) for effect of renal integrity, \( P<0.001 \) for effect of salt and \( P=0.002 \) for interaction between renal integrity and salt.

For osmolality in pregnant ewes, \( P=0.009 \) for effect of renal integrity and \( P=0.04 \) for interaction between renal integrity and salt.

For sodium in non-pregnant ewes, \( P<0.05 \) for effect of renal integrity and \( P=0.003 \) for effect of salt.
For osmolality in non-pregnant ewes, $P<0.001$ for effect of renal integrity and $P=0.01$ for effect of salt.

**Figure 4. Effects of low and high salt intake on glomerular filtration rate (GFR)** in intact pregnant (IntP, Low $n=7$, High $n=6$), STNx pregnant (STNxP, Low $n=9$, High $n=6$), intact non-pregnant (IntNP, $n=7$) and STNx non-pregnant (STNxNP, $n=8$) ewes.

In pregnant ewes, $P<0.001$ for effect of renal integrity, $P<0.03$ for effect of salt.

In non-pregnant ewes, $P<0.001$ for effect of renal integrity.
Table 1. Mean arterial pressure, heart rate and the composition of maternal blood in intact pregnant (IntP) and subtotally nephrectomised pregnant (STNxP) ewes on low and high salt intake. Values are mean ± SE with the number of animals given in parenthesis if it differs from that stated at the top of the column. Δ P<0.05 for differences between IntP and STNxP ewes, † P<0.05 for effects of altered salt intake, § P<0.05 for interaction between STNx and salt. Actual P values are cited in the text. MAP, mean arterial pressure.

<table>
<thead>
<tr>
<th></th>
<th>IntP Low salt n=7</th>
<th>IntP High salt n=6</th>
<th>STNxP Low salt n=9</th>
<th>STNxP High salt n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg) Δ</td>
<td>90 ± 7 (6)</td>
<td>84 ± 5 (5)</td>
<td>103 ± 6</td>
<td>104 ± 4</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>126 ± 9 (6)</td>
<td>121 ± 6 (5)</td>
<td>112 ± 4</td>
<td>110 ± 8</td>
</tr>
<tr>
<td>Chloride (mmol/L) †§</td>
<td>112 ± 2 (4)</td>
<td>115 ± 2 (4)</td>
<td>110 ± 1 (7)</td>
<td>120 ± 2 (4)</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>3.8 ± 0.2</td>
<td>3.7 ± 0.2</td>
<td>3.8 ± 0.1</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>Plasma Na:K ratio</td>
<td>39.0 ± 1.9</td>
<td>41.9 ± 3.3</td>
<td>39.1 ± 1.4</td>
<td>39.7 ± 1.0</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L) Δ†</td>
<td>25.9 ± 0.8</td>
<td>24.0 ± 1.0</td>
<td>28.5 ± 0.3</td>
<td>25.4 ± 0.6</td>
</tr>
<tr>
<td>Hemoglobin (g/L) †</td>
<td>83 ± 3</td>
<td>75 ± 4</td>
<td>85 ± 4</td>
<td>75 ± 6</td>
</tr>
<tr>
<td>Hematocrit (%) †</td>
<td>24.3 ± 1.0</td>
<td>21.7 ± 1.0</td>
<td>24.6 ± 1.1</td>
<td>21.5 ± 1.5</td>
</tr>
<tr>
<td>Creatinine (mmol/L) Δ</td>
<td>0.05 ± 0.003</td>
<td>0.04 ± 0.003</td>
<td>0.07 ± 0.003</td>
<td>0.07 ± 0.005</td>
</tr>
<tr>
<td>Plasma renin levels (ng Ang I/ml/h) †</td>
<td>1.7 ± 0.4</td>
<td>0.6 ± 0.2</td>
<td>1.3 ± 0.6 (8)</td>
<td>0.4 ± 0.1 (6)</td>
</tr>
<tr>
<td>Angiotensinogen (µg/ml)</td>
<td>1.8 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>1.8 ± 0.1 (8)</td>
<td>1.8 ± 0.2 (5)</td>
</tr>
</tbody>
</table>
Table 2. Mean arterial pressure, heart rate and the composition of maternal blood in intact non-pregnant (IntNP) and subtotally nephrectomised non-pregnant (STNxNP) ewes on low and high salt intake. Δ P<0.05 for differences between IntNP and STNxNP ewes, † P<0.05 for effects of altered salt intake, § P<0.05 for interaction between STNtx and salt. Actual P values are cited in the text. MAP, mean arterial pressure.

<table>
<thead>
<tr>
<th></th>
<th>IntNP Low salt n=7</th>
<th>IntNP High salt n=7</th>
<th>STNxNP Low salt n=8</th>
<th>STNxNP High salt n=8</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg) Δ</td>
<td>101 ± 5</td>
<td>100 ± 7</td>
<td>106 ± 4</td>
<td>115 ± 3</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>80 ± 3</td>
<td>80 ± 6</td>
<td>89 ± 6</td>
<td>82 ± 4</td>
</tr>
<tr>
<td>Chloride (mmol/L) Δ</td>
<td>114 ± 1</td>
<td>116 ± 2</td>
<td>109 ± 1</td>
<td>116 ± 1</td>
</tr>
<tr>
<td>Potassium (mmol/L) †</td>
<td>3.7 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>3.7 ± 0.1</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>Plasma Na:K ratio †</td>
<td>39.5 ± 1.4</td>
<td>37.2 ± 1.0</td>
<td>39.5 ± 0.5</td>
<td>35.9 ± 0.7</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L)†</td>
<td>28.0 ± 0.2</td>
<td>25.9 ± 0.4</td>
<td>28.4 ± 0.5</td>
<td>24.7 ± 0.5</td>
</tr>
<tr>
<td>Hemoglobin (g/L) Δ</td>
<td>104 ± 4</td>
<td>98 ± 6</td>
<td>92 ± 4</td>
<td>86 ± 6</td>
</tr>
<tr>
<td>Hematocrit (%)Δ</td>
<td>29.2 ± 1.0</td>
<td>27.4 ± 1.8</td>
<td>24.9 ± 1.1</td>
<td>23.3 ± 1.6</td>
</tr>
<tr>
<td>Creatinine (mmol/L) Δ</td>
<td>0.04 ± 0.003</td>
<td>0.04 ± 0.004</td>
<td>0.09 ± 0.01</td>
<td>0.09 ± 0.01</td>
</tr>
</tbody>
</table>
Table 3. Renal function in intact pregnant (IntP) and subtotally nephrectomised pregnant (STNxP) ewes on low and high salt intake. ΔP<0.05 for differences between IntP and STNxP ewes, † P<0.05 for effects of altered salt intake, § P<0.05 for interaction between STNx and salt. Actual P values are cited in the text.

<table>
<thead>
<tr>
<th></th>
<th>IntP Low salt n=7</th>
<th>IntP High salt n=6</th>
<th>STNxP Low salt n=9</th>
<th>STNxP High salt n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine flow (ml/min)</td>
<td>2.4 ± 0.9</td>
<td>3.5 ± 0.8</td>
<td>2.9 ± 0.5</td>
<td>8.2 ± 1.2</td>
</tr>
<tr>
<td>Na⁺ excretion (µmol/min)</td>
<td>9 ± 6</td>
<td>1044 ± 227</td>
<td>131 ± 52</td>
<td>1944 ± 263</td>
</tr>
<tr>
<td>K⁺ excretion (µmol/min)</td>
<td>511 ± 39</td>
<td>454 ± 44</td>
<td>435 ± 71</td>
<td>371 ± 56</td>
</tr>
<tr>
<td>Osmolar excretion (µosm/min)</td>
<td>1468 ± 107</td>
<td>3283 ± 468</td>
<td>1776 ± 159</td>
<td>5059 ± 456</td>
</tr>
<tr>
<td>Free water clearance (ml/min)</td>
<td>-2.6 ± 1.2</td>
<td>-7.7 ± 0.8</td>
<td>-2.6 ± 0.4</td>
<td>-7.9 ± 0.8</td>
</tr>
<tr>
<td>Urinary osmolality (mosm/kg)</td>
<td>984 ± 196</td>
<td>1155 ± 166</td>
<td>697 ± 69</td>
<td>663 ± 57</td>
</tr>
<tr>
<td>Urinary NaK ratio</td>
<td>0.02 ± 0.01</td>
<td>2.3 ± 0.4</td>
<td>0.5 ± 0.2</td>
<td>6.4 ± 1.1</td>
</tr>
<tr>
<td>Protein excretion (µg/min)</td>
<td>10.8 ± 0.5</td>
<td>17.9 ± 2.8</td>
<td>25.3 ± 4.4</td>
<td>19.0 ± 4.3</td>
</tr>
<tr>
<td>ERPF (ml/min/kg body wgt)</td>
<td>15.4 ± 2.9</td>
<td>17.0 ± 1.0</td>
<td>8.3 ± 0.7</td>
<td>9.6 ± 0.7</td>
</tr>
<tr>
<td>GFR (ml/min/kg body wgt)</td>
<td>3.1 ± 0.3</td>
<td>3.5 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>Fraction of filtered Na load reabsorbed (%)</td>
<td>99.97 ± 0.02</td>
<td>95.58 ± 0.96</td>
<td>99.14 ± 0.36</td>
<td>89.12 ± 1.62</td>
</tr>
<tr>
<td>Fraction of Na reabsorbed proximally (%)</td>
<td>90.3 ± 1.3</td>
<td>80.6 ± 2.3</td>
<td>77.0 ± 2.5</td>
<td>69.7 ± 3.7</td>
</tr>
<tr>
<td>Fraction of Na reabsorbed distally (%)</td>
<td>9.2 ± 1.5</td>
<td>16.0 ± 2.0</td>
<td>22.1 ± 2.5</td>
<td>20.1 ± 3.3</td>
</tr>
</tbody>
</table>
## Table 4. Renal function in intact non-pregnant (IntNP) and subtotally nephrectomised non-pregnant (STNxP) ewes on low and high salt intake.

ΔP<0.05 for differences between IntNP and STNxNP ewes, † P<0.05 for effects of altered salt intake, § P<0.05 for interaction between STNx and salt. Actual P values are cited in the text.

<table>
<thead>
<tr>
<th></th>
<th>IntNP Low salt n=7</th>
<th>IntNP High salt n=7</th>
<th>STNxNP Low salt n=8</th>
<th>STNxNP High salt n=8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine flow (ml/min)</td>
<td>2.6 ± 0.6</td>
<td>2.8 ± 0.8</td>
<td>1.9 ± 0.3</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td>Na⁺ excretion (µmol/min) †</td>
<td>29 ± 11</td>
<td>606 ± 151</td>
<td>35 ± 10</td>
<td>895 ± 194</td>
</tr>
<tr>
<td>K⁺ excretion (µmol/min)</td>
<td>440 ± 50</td>
<td>371 ± 71</td>
<td>435 ± 44</td>
<td>382 ± 40</td>
</tr>
<tr>
<td>Osmolar excretion (µosm/min) †</td>
<td>1531 ± 205</td>
<td>2443 ± 364</td>
<td>1525 ± 137</td>
<td>3069 ± 505</td>
</tr>
<tr>
<td>Free water clearance (ml/min) †</td>
<td>-2.3 ± 0.5</td>
<td>-5.4 ± 0.6</td>
<td>-2.9 ± 0.3</td>
<td>-5.8 ± 0.9</td>
</tr>
<tr>
<td>Urinary osmolality (mosm/kg)</td>
<td>724 ± 134</td>
<td>1076 ± 124</td>
<td>893 ± 89</td>
<td>883 ± 98</td>
</tr>
<tr>
<td>Urinary NaK ratio †</td>
<td>0.06 ± 0.02</td>
<td>2.9 ± 1.3</td>
<td>0.09 ± 0.03</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>ERPF (ml/min/kg body wgt) Δ</td>
<td>13.9 ± 1.9</td>
<td>15.4 ± 1.4</td>
<td>8.0 ± 0.6</td>
<td>8.8 ± 0.7</td>
</tr>
<tr>
<td>GFR (ml/min/kg body wgt) Δ</td>
<td>3.4 ± 0.3</td>
<td>3.7 ± 0.3</td>
<td>1.6 ± 0.2</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Fraction of filtered Na load reabsorbed (%) Δ†§</td>
<td>99.89 ± 0.04</td>
<td>97.68 ± 0.60</td>
<td>99.67 ± 0.13</td>
<td>93.74 ± 1.32</td>
</tr>
<tr>
<td>Fraction of Na reabsorbed proximally (%) Δ</td>
<td>86.2 ± 1.4</td>
<td>79.9 ± 1.8</td>
<td>74.7 ± 3.0</td>
<td>73.3 ± 3.4</td>
</tr>
<tr>
<td>Fraction of Na reabsorbed distally (%) Δ</td>
<td>13.7 ± 1.4</td>
<td>18.0 ± 1.0</td>
<td>25.0 ± 2.9</td>
<td>20.4 ± 2.5</td>
</tr>
</tbody>
</table>
Figure 1. Effects of low and high salt intake on systolic (upper panel) and diastolic pressure (lower panel) of intact pregnant (IntP, Low n=6, High n=5), STNx pregnant (STNxP, Low n=9, High n=6), intact non-pregnant (IntNP, n=7) and STNx non-pregnant (STNxNP, n=8) ewes. For systolic pressure in pregnant ewes, P=0.02 for effect of renal integrity. For diastolic pressure in pregnant ewes, P=0.005 for effect of renal integrity. For diastolic pressure in non-pregnant ewes, P=0.02 for effect of renal integrity.
Figure 2. Salt dependency of diastolic pressure in STNx non-pregnant ewes. Diastolic pressure was directly related to Na balance (measured as daily sodium intake minus urinary sodium excretion). Each animal (n=8) was studied on both a low salt and high salt diet. $r=0.497$, $n=16$, $P=0.05$. 
Figure 3. Effects of low and high salt intake on plasma sodium (upper panel) and osmolality (lower panel) in intact pregnant (IntP, Low n=7, High n=6), STNx pregnant (STNxP, Low n=9, High n=6), intact non-pregnant (IntNP, n=7) and STNx non-pregnant (STNxNP, n=8) ewes. For sodium in pregnant ewes, P=0.001 for effect of renal integrity, P<0.001 for effect of salt and P=0.002 for interaction between renal integrity and salt. For osmolality in pregnant ewes, P=0.009 for effect of renal integrity and P=0.04 for interaction between renal integrity and salt. For sodium in non-pregnant ewes, P<0.001 for effect of renal integrity and P=0.003 for effect of salt. For osmolality in non-pregnant ewes, P<0.001 for effect of renal integrity and P=0.01 for effect of salt.
Figure 4. Effects of low and high salt intake on glomerular filtration rate (GFR) in intact pregnant (IntP, Low n=7, High n=6), STNx pregnant (STNxP, Low n=9, High n=6), intact non-pregnant (IntNP, n=7) and STNx non-pregnant (STNxNP, n=8) ewes. In pregnant ewes, P<0.001 for effect of renal integrity, P<0.03 for effect of salt. In non-pregnant ewes, P<0.001 for effect of renal integrity.