The role of fetal programming in pathogenesis of arterial hypertension and kidney disease in the adult life

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Animal experiments: blood pressure “goes with the kidney”

- Kidney genetically programmed for hypertension, transplanted into a normotensive recipient animal with no immune rejection

persistent hypertension

Primary hypertension – role of the kidney

Blood pressure increase in kidney allograft recipients from hypertensive donor (SHR)
The origin of Barker’s hypothesis
- Fetal programming hypothesis

THE LANCET, MAY 10, 1986

Epidemiology

INFANT MORTALITY, CHILDHOOD NUTRITION, AND ISCHAEMIC HEART DISEASE IN ENGLAND AND WALES

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Summary

Although the rise in ischaemic heart disease in England and Wales has been associated with increasing prosperity, mortality rates are highest in the least affluent areas. On division of the country into two hundred and twelve local authority areas a strong geographical relation was found between ischaemic heart disease mortality rates in 1968–78 and infant mortality in 1921–25. Of the twenty-four other common causes of death only bronchitis, stomach cancer, and rheumatic heart disease were similarly related to infant mortality. These diseases are associated with poor living conditions and mortality from them is declining. Ischaemic heart disease is strongly correlated with both neonatal and postneonatal mortality. It is suggested that poor nutrition in early life increases susceptibility to the effects of an affluent diet.
Barker hypothesis

Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease

D J P Barker, C Osmond, J Golding, D Kuh, M E J Wadsworth

Abstract

In national samples of 9921 10 year olds and 3259 adults in Britain systolic blood pressure was inversely related to birth weight. The association was independent of gestational age and may therefore be attributed to reduced fetal growth. This suggests that the intrauterine environment influences blood pressure during adult life. It is further evidence that the geographical differences in average blood pressure and mortality from cardiovascular disease in Britain partly reflect past differences in the intrauterine environment.

Within England and Wales 10 year olds living in areas with high cardiovascular mortality were shorter and had higher resting pulse rates than those living in other areas. Their mothers were also shorter and had higher diastolic blood pressures. This suggests that there are persisting geographical differences in the childhood environment that predispose to differences in cardiovascular mortality.

disease is more closely related to neonatal and maternal death rates in the past than to postneonatal rates. This points to the importance of the environment during intrauterine rather than early postnatal life.

Blood pressure has been suggested as one link between the intrauterine environment and risk of cardiovascular disease. We have therefore examined the relations among blood pressure, pulse rate, and intrauterine influences, as measured by birth weight, gestational period, mother’s height, and mother’s blood pressure. To do this we have used data from two large national samples, one of children aged 10 and another of adults aged 36.

We used geographical comparisons within England and Wales to examine the relation between intrauterine influences and cardiovascular disease. We compared geographical variations in mothers’ heights and blood pressures, and in the birth weights of their children, with differences in cardiovascular mortality.
Initiation of hypertension in utero and its amplification throughout life

C M Law, M de Swiet, C Osmond, P M Fayers, DJP Barker, AM Cruddas, CHD I'all

Abstract

Objective—To determine whether the relation between high blood pressure and low birth weight is initiated in utero or during infancy, and whether it changes with age.

Design—A longitudinal study of children and three follow up studies of adults.

Setting—Farnborough, Preston, and Hertfordshire, England, and a national sample in Britain.

Subjects—1895 children aged 0-10 years, 3240 men and women aged 36 years, 459 men and women aged 46-54 years, and 1231 men and women aged 59-71 years. The birth weight of all subjects had been recorded.

Main outcome measure—Systolic blood pressure.

Results—At all ages beyond infancy people who had lower birth weight had higher systolic blood pressure. Systolic blood pressure was not related to growth during infancy independently of birth weight. The relation between systolic pressure and birth sanction can persist even after the primary cause, the tumour or stenosis, has been removed.

Recent work has shown that raised blood pressure in adults is associated with reduced growth in early life. Mean systolic and diastolic pressures of men and women in middle and late life fall progressively from those with the lowest birth weights to those with the highest. Blood pressure is also higher in those whose birth weights were low in relation to that expected from their placental weights. Children show similar relations. These findings could indicate that high adult blood pressure is initiated in utero. Alternatively, healthy infants of low birth weight may have accelerated growth, so called catch up growth, during the first six months of life. If this accelerated early growth were accompanied by an accelerated rise in blood pressure, then the values would be set on a higher than expected plane when tracking of blood pressure becomes established after 6 months of age.

We examined the timing of initiation of high blood
Initiation of hypertension in utero and its amplification throughout life

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We examined the timing of initiation of high blood

Low birth weight as a marker of impaired fetal development

**WHO definition of low birth weight (LBW)**

- is defined as the weight of live born infants less than 2,500 g
- Birth weight below the 10th percentile for that gestational age – a marker for intrauterine growth restriction (IUGR)
- Low birth weight in prematurity children – birth weight appropriate for gestational age, but small in comparison to children born on time
Prenatal programming
Abnormalities of kidney structure
(“nephron underdosing”):

may predispose to hypertension and increase the susceptibility to renal damage

_in the adult life_

Brenner, Am J Hypert ; 1988, 1, 335
Correlation between birth weight, number (A) and volume (B) of glomeruli

\[ r = 0.870, \ P < 0.0001 \]

\[ r = 0.840, \ P < 0.0001 \]

Correlation between birth weight and nephron number

Hughson, Kidney Int. 2003, 2113
<table>
<thead>
<tr>
<th></th>
<th>Hypertensive individuals (n=10)</th>
<th>Normotensive individuals (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of glomeruli</strong></td>
<td>890,869 ± 158,110</td>
<td>1,666,805 ± 411,690</td>
</tr>
<tr>
<td><strong>Volume of glomeruli</strong></td>
<td>5.67 ± 0.85</td>
<td>2.41 ± 0.71</td>
</tr>
</tbody>
</table>

► no evidence of obsolescent glomeruli as evidence of hypertension induced loss of glomeruli

“Oligomeganephrony”

Hypertension

Normotension

Low birth weight $\Rightarrow$ higher adolescent blood pressure (2 - 3mmHg)

Rotterdam study: Uiterwaal, Hypertension; 1997, 30:267

Higher catch up growth $\Rightarrow$ adult fatness $\Rightarrow$ obesity related blood pressure

Horta, J. Epidemiol. Community Health; 2003, 57: 226
Huxley, J. Hypertens.; 2000, 18:815
## Low birth weight and hypertension – Helsinki study

<table>
<thead>
<tr>
<th>Birth weight, kg</th>
<th>Cumulative incidence of hypertension</th>
<th>p for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 3.0</td>
<td>20.2 (18.3–22.1)</td>
<td></td>
</tr>
<tr>
<td>Up to 3.5</td>
<td>16.7 (15.4–17.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Up to 4.0</td>
<td>13.6 (12.4–14.9)</td>
<td></td>
</tr>
<tr>
<td>&gt;4.0</td>
<td>12.3 (10.1–14.6)</td>
<td></td>
</tr>
</tbody>
</table>
Outcome after unilateral renal agenesis (pre/perinatal)

- 157 patients with unilateral agenesis and normal contralateral kidney
- Mean age 37 years

# proteinuria 150 mg/day 19%
# high blood pressure 47%
# reduced renal function 13%
# 6 deaths from renal failure

Argueso, Ped. Nephrology; 1992, 6:412
No gross excess hypertension in live kidney donors

- 402 alive kidney donors 1964-1995
- proteinuria 12 %
- hypertension 38%
  → not exceeding rate in matched background population

Fehrman-Ekholm, Transplantation; 2001, 72:444
Less glomeruli, but glomeruli bigger calculated index of filtration surface was normal

Hypothesis

- High blood pressure is **not** a result of low glomerular number per se
  (less, but bigger glomeruli → normal filtration surface)

- **but** is a result of developmental changes in postglomerular segments
  (→ higher sodium reabsorption)
Main mechanisms contributing to hypertension from prenatal programming

- Upregulation of sodium channels
- Upregulation of RAS (AT1-R)
- Cortisol (11βHSD2↓) → mineralocorticoid receptor
- Sympathetic overactivity
Low birth weight – salt sensitivity of blood pressure in healthy adults

de Boer, Hypertension; 2008, 51: 928
Decreased nephron number shifts pressure natriuresis relationship to the right

Urinary Sodium Output ($x$ normal)

Mean Arterial Pressure (mmHg)
Causes of reduced glomerular numbers
(in animal experiments)

- maternal pathology → impaired nephrogenesis of offspring

- Low protein intake
  

- Uterine underperfusion
  
  *Wlodek, Kidney Int.; 2008, 74:187*

- Maternal hyperglycemia
  
  *Amri. Diabetes; 2001, 50:1069*

- Maternal hyperinsulinemia
  
  *Bursztyn, Hypertension; 2006, 48:717*

- High and low maternal salt intake
  
  *Balbi, Pediatr. Nephrol; 2004, 19:1212*
  
  *Koleganova N et al. Am J Physiol Renal Physiol. 2011, 301, F344-F354*

- Corticosteroids
  

- Alcohol (ethanol) consumption
  

- Smoking and CyA
  
  *Zarzecki M at al. Słabiak-Błaż et al. (Submitted)*
Maternal protein restriction

- Normal Protein (NP)
- Low Protein (LP)

*p < 0.05 compared with control

Maternal protein restriction reduces nephron number in multiple species

<table>
<thead>
<tr>
<th>Species</th>
<th>Maternal diet</th>
<th>Exposure in gestation</th>
<th>Nephron number</th>
<th>Basal HTN</th>
<th>Stim’d HTN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zeman</td>
<td>Low protein</td>
<td>0.0–1.0</td>
<td>↓ (NA)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Merlet-Benichou et al.</td>
<td>Low protein</td>
<td>0.0–1.0</td>
<td>↓ (NA)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Woods et al.</td>
<td>Low protein</td>
<td>0.0–1.0</td>
<td>↓ 25%</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Vehaskari et al.</td>
<td>Low protein</td>
<td>0.5–1.0</td>
<td>↓ 28%</td>
<td>Yes</td>
<td>—</td>
</tr>
<tr>
<td>Jones et al.</td>
<td>Low protein</td>
<td>0.0–1.0</td>
<td>↓ 30%</td>
<td>Yes</td>
<td>—</td>
</tr>
<tr>
<td>McMullen et al.</td>
<td>Low protein</td>
<td>0.0–1.0</td>
<td>↓ 25–50%</td>
<td>Yes</td>
<td>—</td>
</tr>
<tr>
<td>Sanders et al.</td>
<td>Placental insuff.</td>
<td>0.8–1.0</td>
<td>↓ 30%</td>
<td>No</td>
<td>Yes*</td>
</tr>
<tr>
<td>Sheep</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gilbert et al.</td>
<td>50% Low calorie</td>
<td>0.1–0.5</td>
<td>↓ 11%</td>
<td>Yes</td>
<td>—</td>
</tr>
<tr>
<td>Gopalakrishnan et al.</td>
<td>50% Low calorie</td>
<td>0.2–0.5</td>
<td>↓ 30%</td>
<td>No</td>
<td>—</td>
</tr>
<tr>
<td>Pig</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bauer et al.</td>
<td>Spontaneous</td>
<td>Runting</td>
<td>↓</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Rabbit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bassan et al.</td>
<td>Placental Insuff.</td>
<td>0.7–1.0</td>
<td>↓</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hinchliffe et al.</td>
<td>(IUGR)</td>
<td>—</td>
<td>↓</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Leroy et al.</td>
<td>(IUGR)</td>
<td>—</td>
<td>↓</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Manalich et al.</td>
<td>(IUGR)</td>
<td>—</td>
<td>↓</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Under stimulation of 2% NaCl drinking water.
Maternal hyperglycemia

Maternal diabetes programs hypertension and kidney injury in offspring

- Murine study, 3 groups:
  - offspring from non-diabetic mothers (Con-offsprings)
  - offspring from diabetic mothers (Dia-Offsprings)
  - offspring of diabetic mothers with insulin treatment (Ins-Treated Offspring)

**p≤0.01, ***p≤0.001

Maternal diabetes programs hypertension and kidney injury in offspring

Urinary albumin/creatinine ratio

ECM (extracellular matrix) protein expression in glomeruli by PAS staining of

PAI 1 and TGF-β expression in kidney

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001
Maternal diabetes programs hypertension and kidney injury in offspring

Real-time quantitative polymerase chain reaction (RT-qPCR) of intrarenal renin–angiotensin system (RAS) components.

The relative densities of angiotensinogen (Agt), angiotensin II (subtype 1) receptor (AT1R), angiotensin-converting enzyme (ACE), and angiotensin-converting enzyme-2 (ACE2) in the renal cortex of 20-week-old male offspring

\*p≤0.05, \**p≤0.01, \***p≤0.00

Both high and low maternal salt intake in pregnancy alters kidney development in the offspring.

Both high and low maternal salt intake in pregnancy alters kidney development in the offspring.

Mean arterial blood pressure (MAP) measured by intraaortal telemetry in offspring.

* $p<0.05$ HS or LS vs IS

HS - high sodium
LS - low sodium
IS - intermediate sodium

High salt diet in pregnant rats → high albuminuria in offspring

Maternal plasma vitamin A level

- vitamin A and all-trans retinoic acid are potent stimulators of nephrogenesis
- influence on ureteric bud branching capacity (i.e. by glial cell-line-derived neurotrophic factor –GDNF)

Smoking during pregnancy
Smoking during pregnancy

- In UK
  17% pregnant women are smoking
  45% pregnant women <20 years are smoking

- In USA
  20% pregnant women <25 years are smoking
  9% pregnant women >35 years are smoking

- In Poland
  33% pregnant women are smoking
  12% are smoking 3 months before delivery
Exposure to cigarette smoke condensate during pregnancy influence on kidney morphology in offspring

Exposure to cigarette smoke condensate during pregnancy influence on blood pressure and natuuresis in offspring

Systolic blood pressure at 12 weeks of age

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean ± SD</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarette smoke condensate</td>
<td>122±7</td>
<td>53</td>
</tr>
<tr>
<td>Control</td>
<td>116±8</td>
<td>51</td>
</tr>
</tbody>
</table>

p<0.001

Urinary sodium excretion

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarette smoke condensate</td>
<td>531±242</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Control</td>
<td>846±384</td>
<td></td>
</tr>
</tbody>
</table>

Urinary sodium/creatinine ratio

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarette smoke condensate</td>
<td>8.1±4.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Control</td>
<td>9.6±4.2</td>
<td></td>
</tr>
</tbody>
</table>

Effects of maternal smoking during pregnancy on offspring blood pressure in late adolescence

Lovisa Högberg\textsuperscript{a}, Sven Cnattingius\textsuperscript{b}, Cecilia Lundholm\textsuperscript{a}, Brian M. D’Onofrio\textsuperscript{c}, Niklas Långström\textsuperscript{a}, and Anastasia N. Iliadou\textsuperscript{a}

\textbf{Conclusion:} Maternal smoking during pregnancy is associated with a small but statistically significant increase in offspring blood pressure in late adolescence. Because the association does not appear to be explained by familial confounding, our results support an intrauterine effect of prenatal smoking exposure on blood pressure in late adolescence.
Prenatal exposure to ethanol results in a reduction in nephron number

MALE

FEMALE

***p<0.001

**p<0.01

Prenatal exposure to ethanol results in a larger mean glomerular volume

* $p<0.005$
** $p<0.01$

Prenatal exposure to ethanol increases mean arterial pressure

* $p < 0.005$
** $p < 0.01$

Prenatal exposure to ethanol results in increased proteinuria

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Sham</td>
</tr>
<tr>
<td>Protein (g/min)</td>
<td>313.04 ± 48.70a</td>
<td>202.65 ± 63.19e</td>
</tr>
<tr>
<td>Osmolality excretion (mOsmol/min)</td>
<td>21.33 ± 3.80</td>
<td>17.08 ± 3.23</td>
</tr>
<tr>
<td>Na⁺ excretion (mmol/min)</td>
<td>1.67 ± 0.94</td>
<td>1.55 ± 0.36</td>
</tr>
<tr>
<td>K⁺ excretion (mmol/min)</td>
<td>1.13 ± 0.26</td>
<td>0.45 ± 0.20</td>
</tr>
<tr>
<td>Cl⁻ excretion (mmol/min)</td>
<td>3.15 ± 0.82</td>
<td>2.98 ± 0.45</td>
</tr>
</tbody>
</table>

The male and female data were analyzed separately using one-way ANOVA with a Tukey’s post hoc test. The data are the means ± SEM, n = 8 rats per group, derived from six litters per treatment. The values that do not share a common letter are significantly different from each other (P < 0.05).
Influence of ethanol on culture of embryonic metanephroi

Immunofluorescence images showing the ureteric trees in whole rat metanephroi after 48 hours of culture in 0% ethanol (A and B) and 0.2% ethanol (C and D).

# List of drugs influencing renal development

<table>
<thead>
<tr>
<th>Drug</th>
<th>Effect of Maternal Treatment during Pregnancy on Offspring Kidney Development</th>
<th>Effect of Treatment during Postnatal Kidney Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td>Tubular alterations (16), low nephron number (17–19)</td>
<td>Tubular damage (21), low nephron number (19)</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>Low nephron number (22)</td>
<td>Glomerular and tubular injury (21), similar nephron number (21,26,27)</td>
</tr>
<tr>
<td>Prostaglandin synthetase inhibitors</td>
<td>Tubular alterations (21), similar nephron number (28)</td>
<td>Atrophy of the renal papilla, tubular alterations (32), low nephron number (33)</td>
</tr>
<tr>
<td>ACEIs/ARBs</td>
<td>Renal insufficiency (31)</td>
<td>Low nephron number (5,35)</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Altered tubular transporters (36,37), low nephron number (5), similar nephron number (38)</td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>Renal concentrating defect (40)</td>
<td></td>
</tr>
<tr>
<td>Antiepileptic drugs</td>
<td>More congenital malformations, specifically MCDK (44)</td>
<td></td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>Renal agenesis/ectopia (45,46)</td>
<td></td>
</tr>
<tr>
<td>Adriamycin</td>
<td>Bladder agenesis, hydrenephrosis (48)</td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Hydro(uretero)nephrosis (49)</td>
<td></td>
</tr>
</tbody>
</table>

MCDK, multicystic dysplastic kidney.

Effect of cyclosporine A administration in pregnant rats on number and volume of glomeruli in their offspring

![Bar chart showing the number of glomeruli and their volume with statistical significance values.]

Effect of cyclosporine A administration in pregnant rats on blood pressure in their offspring

SYSTOLIC BLOOD PRESSURE measured in offspring at 7th and 11th weeks of age

DIASTOLIC BLOOD PRESSURE measured in offspring at 7th and 11th weeks of age

Fetal exposure to steroides

Normal situation

Severe maternal stress, external steroids i.e. dexamethasone

11β-HSD2 barrier • Active cortisol ○ Inactive cortisone ◆ Dexamethasone

Fetal exposure to steroids

6 months

Birth weight (g) → Blood pressure (mmHg) → Blood glucose (mmol/L)

0 1 2 3 4 5 6
Control CBX

0 1 2 3 4 5 6 7 8 9
Control CBX

0 30 60 90 120
Time, minutes

Seckl J. et al. KI 2000; 57: 1412-1417
## Fetal exposure to steroids

<table>
<thead>
<tr>
<th>Species (reference)</th>
<th>Treatment</th>
<th>Time of treatment</th>
<th>Time of analysis</th>
<th>Blood pressure of offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat (McMullen and Langley-Evans 2005)</td>
<td>Protein restriction ± metyrapone (for drug) 1–22 for diet</td>
<td>1–14 days pregnancy</td>
<td>4 weeks PP</td>
<td>Increase in systolic BP male, female; dependent on GC-male only</td>
</tr>
<tr>
<td>Rat (Langley-Evans 1997)</td>
<td>Protein restriction ± metyrapone (1–14)</td>
<td>Low protein (1–22) ± metyrapone</td>
<td>7 weeks PP</td>
<td>Increase in systolic BP both sexes; GC-dependent in both sexes</td>
</tr>
<tr>
<td>Rat (Ortiz et al. 2003)</td>
<td>Dexamethasone</td>
<td>Days 15,16</td>
<td>3 weeks PP</td>
<td>Increase in systolic BP female, not male</td>
</tr>
<tr>
<td>Rat (Woods et al. 2004)</td>
<td>Modest protein restriction</td>
<td>Days 13–14, 15–16, 17–18</td>
<td>6 months</td>
<td>Increase in systolic BP male, not female</td>
</tr>
<tr>
<td></td>
<td>Severe protein restriction</td>
<td>All pregnancy</td>
<td>22 weeks PP</td>
<td>Hypertension male only</td>
</tr>
<tr>
<td>Sheep (Dodic et al. 2002a)</td>
<td>Dexamethasone</td>
<td>26–28 days</td>
<td>16–24 months</td>
<td>Hypertension in both male and female</td>
</tr>
<tr>
<td>Sheep (Dodic et al. 2002b)</td>
<td>Cortisol</td>
<td>26–28 days</td>
<td>18 months</td>
<td>Hypertension in both male and female</td>
</tr>
<tr>
<td>Guinea pig (Liu et al. 2001)</td>
<td>Dexamethasone</td>
<td>40–41, 50–51, 60–61 days</td>
<td>9–10 weeks</td>
<td>No change in MAP in either sex; reduced basal and activated HPA axis in males only</td>
</tr>
<tr>
<td>Guinea pig (Banjanin et al. 2004)</td>
<td>Dexamethasone</td>
<td>40–41, 50–51, 60–61 days</td>
<td>21–22 weeks</td>
<td>Increase in MAP in males only; NC in basal HPA axis in males</td>
</tr>
</tbody>
</table>

IUGR is associated with persistent aortic wall thickening and glomerular proteinuria during infancy

- 44 mothers with single-fetus pregnancies at 32 weeks gestation:
  - 23 growth restricted (IUGR) and
  - 21 of appropriate gestational age (AGA) as controls

<table>
<thead>
<tr>
<th>Prenatal measurements</th>
<th>IUGR (n=23)</th>
<th>AGA (n=21)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>32.1 (29.9–33.7)</td>
<td>32 (30–34)</td>
<td>0.99</td>
</tr>
<tr>
<td>Estimated fetal weight (g)</td>
<td>1750 (1450–2050)</td>
<td>2200 (1930–2470)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fetal aIMT (mm)</td>
<td>2.00 (1.78–2.23)</td>
<td>1.05 (0.95–1.15)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neonatal measurements</th>
<th>IUGR (n=23)</th>
<th>AGA (n=21)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>33 (31–36)</td>
<td>38 (35–41)</td>
<td>0.001</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1850 (1850–2200)</td>
<td>2975 (2700–3250)</td>
<td>0.001</td>
</tr>
<tr>
<td>Gender: male</td>
<td>12 (52.1)</td>
<td>10 (47.6)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Postnatal measurements</th>
<th>IUGR (n=23)</th>
<th>AGA (n=21)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected postnatal age (months)</td>
<td>18 (12.8–25.2)</td>
<td>19 (13.3–26.7)</td>
<td>0.48</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>12.3 (11.2–13.4)</td>
<td>12.5 (11.3–14)</td>
<td>0.23</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>87 (80–90)</td>
<td>86.5 (79–92)</td>
<td>0.34</td>
</tr>
<tr>
<td>Infant aIMT (mm)</td>
<td>2.1 (1.4–3.0)</td>
<td>1.05 (0.95–1.25)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>123 (107–139)</td>
<td>103 (95.5–112.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>65 (57.6–72.4)</td>
<td>64 (59–71)</td>
<td>0.99</td>
</tr>
<tr>
<td>Urine microalbumin (mg/l)</td>
<td>11.1 (1.9–19.5)</td>
<td>4.4 (0.0–8.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>A/CR (mg/g)</td>
<td>26 (9.8–41.4)</td>
<td>14.6 (8.2–21.2)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Abbreviations: A/CR, albumin/creatinine ratio; AGA, appropriate for gestational age; aIMT, aortic intima-media thickness; BP, blood pressure; IUGR, intrauterine growth restriction.
Values are shown as number (%) or as median (IQR).

Zandaro V. et al. Kidney Int., 2011, 80, 119–123
High salt intake causes adverse fetal programming—vascular effects beyond blood pressure

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¹Department of Internal Medicine, University of Heidelberg, Heidelberg, Germany, ²Institute of Pathology, University of Heidelberg, Heidelberg, Germany, ³Department of Nephrology, Endocrinology and Metabolic Diseases, Medical University of Silesia, Katowice, Poland and ⁴Laboratory of Cardiovascular Science, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA

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*Both authors contributed equally to this work.

Abstract

Background. High salt intake causes hypertension, adverse cardiovascular outcomes and potentially also blood pressure (BP)-independent target organ damage. Excess salt intake in pregnancy is known to affect BP in the offspring. The present study was designed to assess whether high salt intake in pregnancy affects BP and vascular morphology in the offspring.

Methods. Sprague-Dawley rats were fed a standard rodent diet with low-normal (0.15%) or high (8.0%) salt content during pregnancy and lactation. After weaning at 4 weeks of age, the offspring were maintained on their respective environmental diets and killed at 10 weeks of age.

Results. High salt intake in pregnancy was associated with higher BP in the offspring. Moreover, high salt intake in pregnancy altered vascular morphology, including increased intima-media thickness and reduced nitric oxide availability as indicated by lower levels of nitrotyrosine and higher levels of asymmetric dimethyl arginine (ADMA).

Conclusions. High salt intake in pregnant rats has long-lasting effects on the modeling of central and muscular arteries in the offspring independent of postnatal salt intake and BP. Circulating MBG and ADMA and local oxidative stress correlate with the adverse vascular modeling.

Keywords: blood pressure; fetal programming; nitric oxide; salt; vessel development
Fig. 6. Representative microphotographs of PCNA staining in the aorta from LL (left) and HL (right) offspring at Week 7. PCNA-positive cells are marked with arrows.
Urine microalbumin in intrauterine growth restricted (IUGR) and appropriate for gestational age (AGA) infants

Median (interquartile range)

11.1 vs 4.4 mg/l

P<0.001

Association between low birth weight (<2500g) and CKD in adult life: meta-analysis

<table>
<thead>
<tr>
<th>Author</th>
<th>Country of origin</th>
<th>Year of publication</th>
<th>Participant sex</th>
<th>OR (95% CI)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haysom</td>
<td>Australia</td>
<td>NA</td>
<td>M &amp; F</td>
<td>0.95 (0.21, 4.37)**</td>
<td>6.27</td>
</tr>
<tr>
<td>Ramirez²</td>
<td>Singapore</td>
<td>2001</td>
<td>M &amp; F</td>
<td>2.09 (0.46, 9.56)**</td>
<td>6.31</td>
</tr>
<tr>
<td>Rudborg</td>
<td>Sweden</td>
<td>1998</td>
<td>M &amp; F</td>
<td>2.77 (0.77, 9.95)*</td>
<td>8.29</td>
</tr>
<tr>
<td>Vastarhelyi</td>
<td>Hungary</td>
<td>2000</td>
<td>M &amp; F</td>
<td>0.71 (0.20, 2.55)*</td>
<td>8.35</td>
</tr>
<tr>
<td>Yuzkin²</td>
<td>UK</td>
<td>2001</td>
<td>M &amp; F</td>
<td>3.10 (0.87, 10.98)**</td>
<td>8.42</td>
</tr>
<tr>
<td>Nelson</td>
<td>USA</td>
<td>1996</td>
<td>M &amp; F</td>
<td>2.30 (0.73, 7.27)**</td>
<td>9.68</td>
</tr>
<tr>
<td>Painter²</td>
<td>Netherlands</td>
<td>2005</td>
<td>M &amp; F</td>
<td>3.22 (1.35, 7.89)**</td>
<td>13.95</td>
</tr>
<tr>
<td>Hoy</td>
<td>Australia</td>
<td>1999</td>
<td>M &amp; F</td>
<td>2.82 (1.26, 6.31)**</td>
<td>15.26</td>
</tr>
<tr>
<td>Fagerudd²</td>
<td>Finland</td>
<td>2006</td>
<td>M &amp; F</td>
<td>0.99 (0.61, 1.61)**</td>
<td>23.47</td>
</tr>
</tbody>
</table>

**Subtotal (I² = 35.1%, p = 0.1)**

<table>
<thead>
<tr>
<th>Author</th>
<th>Country of origin</th>
<th>Year of publication</th>
<th>Participant sex</th>
<th>OR (95% CI)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyck</td>
<td>Canada</td>
<td>2003</td>
<td>M &amp; F</td>
<td>1.62 (0.88, 2.97)*</td>
<td>8.22</td>
</tr>
<tr>
<td>Fan</td>
<td>USA</td>
<td>2006</td>
<td>M &amp; F</td>
<td>1.56 (1.02, 2.39)**</td>
<td>16.69</td>
</tr>
<tr>
<td>Vikse</td>
<td>Norway</td>
<td>2006</td>
<td>M &amp; F</td>
<td>2.00 (1.41, 2.83)**</td>
<td>25.19</td>
</tr>
<tr>
<td>Lackland</td>
<td>USA</td>
<td>2000</td>
<td>M &amp; F</td>
<td>1.40 (1.09, 1.79)*</td>
<td>49.90</td>
</tr>
</tbody>
</table>

**Subtotal (I² = 0.0%, p = 0.4)**

<table>
<thead>
<tr>
<th>Author</th>
<th>Country of origin</th>
<th>Year of publication</th>
<th>Participant sex</th>
<th>OR (95% CI)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al Salim²</td>
<td>Australia</td>
<td>2007</td>
<td>M &amp; F</td>
<td>3.66 (1.50, 8.43)*</td>
<td>8.96</td>
</tr>
<tr>
<td>Hallam³</td>
<td>Norway</td>
<td>2006</td>
<td>Females</td>
<td>1.08 (0.55, 2.12)**</td>
<td>9.39</td>
</tr>
<tr>
<td>Hallam²</td>
<td>Norway</td>
<td>2006</td>
<td>Males</td>
<td>2.35 (1.30, 4.24)**</td>
<td>16.43</td>
</tr>
<tr>
<td>Al Salim³</td>
<td>Australia</td>
<td>2007</td>
<td>Males</td>
<td>3.40 (2.13, 5.42)**</td>
<td>12.16</td>
</tr>
<tr>
<td>Al Salim⁴</td>
<td>Australia</td>
<td>2007</td>
<td>Females</td>
<td>2.04 (1.45, 2.88)*</td>
<td>13.88</td>
</tr>
<tr>
<td>Poulter²</td>
<td>UK</td>
<td>NA</td>
<td>Females</td>
<td>1.31 (0.97, 1.76)**</td>
<td>14.51</td>
</tr>
<tr>
<td>Li⁴</td>
<td>USA</td>
<td>2006</td>
<td>Males</td>
<td>1.65 (1.24, 2.20)**</td>
<td>14.62</td>
</tr>
<tr>
<td>Li⁵</td>
<td>USA</td>
<td>2008</td>
<td>Females</td>
<td>1.07 (0.92, 1.25)*</td>
<td>16.04</td>
</tr>
</tbody>
</table>

**Subtotal (I² = 83.5%, p = 0.001)**

Heterogeneity between groups: p = 0.4
Overall (I² = 66.3%, p < 0.001)

NOTE: Weights are from random effects analysis.
**Albuminuria (microalbuminuria, age 50)**

- **Effect of the Dutch Famine** (During World War II for a 6 month period the rations fell to below 1000 kcal in one part of the Netherlands) on albuminuria 50 years later

- Babies who were exposed to this starvation during the midterm period had a 2-3 times increased risk for albuminuria 50 years later

<table>
<thead>
<tr>
<th>Born before (Ref.)</th>
<th>Late</th>
<th>Mid</th>
<th>Early</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR ≥ 2.5 (%)</td>
<td>8</td>
<td>7</td>
<td>12e</td>
</tr>
<tr>
<td>OR (95% CI)c</td>
<td>1.14 (0.50–2.59)</td>
<td>2.07 (1.01–4.27)e</td>
<td>1.61 (0.64–4.08)</td>
</tr>
<tr>
<td>OR adjusted (95% CI)d</td>
<td>1.27 (0.49–3.26)</td>
<td>3.22 (1.34–7.65)e</td>
<td>1.89 (0.59–6.11)</td>
</tr>
</tbody>
</table>

- This study included 700 people and they also adjusted for cardiovascular risk factors in adult age

ESDR (risk for RRT during first 38 years of life)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All</th>
<th>Adjusted&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>P</td>
<td>RR</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td></td>
<td></td>
<td>RR</td>
<td>P</td>
</tr>
<tr>
<td>Birth weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10th percentile</td>
<td>81</td>
<td>1.7 (1.4 to 2.2)</td>
<td>&lt;0.001</td>
<td><strong>1.8 (1.3 to 2.4)</strong></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10 to 90th percentile</td>
<td>402</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥90th percentile</td>
<td>39</td>
<td>0.86 (0.62 to 1.2)</td>
<td>0.4</td>
<td><strong>0.79 (0.51 to 1.2)</strong></td>
<td>0.3</td>
</tr>
<tr>
<td>Birth weight for gestational age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10th percentile</td>
<td>81</td>
<td>1.5 (1.2 to 1.9)</td>
<td>0.002</td>
<td><strong>1.6 (1.1 to 2.1)</strong></td>
<td>0.006</td>
</tr>
<tr>
<td>10 to 90th percentile</td>
<td>369</td>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>≥90th percentile</td>
<td>38</td>
<td>0.94 (0.67 to 1.3)</td>
<td>0.7</td>
<td><strong>0.87 (0.55 to 1.4)</strong></td>
<td>0.5</td>
</tr>
</tbody>
</table>

Low eGFR (< sex–specific 10th percentile age 20-30)

- 7500 people from the population based HUNT 2 cohort
- Birth weight <2500g was associated with a 2.4 times increased risk for low eGFR in men, also after adjustment for both maternal and for adult risk factors
- The risk in women was not increased at all

<table>
<thead>
<tr>
<th>Multiadjusted birth weight</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3rd percentile (&lt;2,450 g)</td>
<td>2.35 (1.30-4.24)</td>
<td>1.08 (0.55-2.11)</td>
</tr>
<tr>
<td>3rd-10th percentile (2,450-2,870 g)</td>
<td>1.06 (0.62-1.82)</td>
<td>1.23 (0.81-1.86)</td>
</tr>
<tr>
<td>10th-90th percentile (2,870-4,190 g)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Hallan S. Am J Kidney Dis 2007; 51: 10-20
Prenatal programming
Epigenetics:

- Nutritional deprivation
- Placental malfunction
- Hyperglycemia
- Smoking...

in pregnancy

- Hypertension,
- Kidney disease,
- Metabolic syndrome

in the adult life

Mechanisms of Programming

Nutritional Influence

Altered Cell Number or intracellular organization

Reorganisation of organ structure
Abnormal early cell-cell interactions?

Metabolic Differentiation
DNA Control?
(altered cell specific gene regulation)
DNA Environment?
(altered DNA binding proteins)
Altered DNA methylation?
Mechanisms of epigenetic changes during fetal development
Changes in gene expression by epigenetic modifications

Changes in gene expression by epigenetic modifications—different phenotype
Cardiovascular and renal diseases: The “multi-hits” hypothesis

Cardiovascular and renal diseases: The “multi-hits” hypothesis

Cardiovascular and renal diseases: The “multi-hits” hypothesis

Cardiovascular and renal diseases: The “multi-hits” hypothesis

Factors that directly or indirectly affect fetal development and may thus favour programming of diseases that occur in later life

- preeclampsia
- maternal or fetal malnutrition
- smoking or alcoholism
- high or low salt intake
- certain drugs (i.e., steroids, CSA)
- hypo- or hyper-vitaminosis

Adverse conditions during pregnancy lead to epigenetic modification of DNA, resulting in impaired fetal development (birth weight ↓ or ↑, low nephron number, arterial remodelling).

Impaired fetal development leads to:
- renal disease (i.e., glomerulonephritis)
- cardiovascular disease (i.e., hypertension, coronary heart disease)
- metabolic disease (i.e., insulin resistance, dyslipidaemia)

How can we identify patients with potentially impaired intrauterine development who are at risk for prenatally programmed diseases in later life?

---

Babies with intrauterine growth retardation/small for gestational age (due to under-/malnutrition or under-/malperfusion)
Babies with low birth weight (as a surrogate parameter of impaired intrauterine development)
Babies who are large for gestational age (due to maternal diabetes)
Babies who had a history of intrauterine exposure to drugs (i.e. corticosteroids, gentamicin, ACE-inhibitors, cyclosporine A etc.)

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Thank you for your attention!

Andrzej Wiececk
Katowice