Fetal Programming
from Epidemiology to Epigenetics

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Coronary Heart Disease in 35-74 years old people (1968-1978)

Neonatal Mortality (1901-1910)
<table>
<thead>
<tr>
<th>Weight at Birth</th>
<th>Weight 1st Year</th>
<th>Food</th>
<th>No. of Visits</th>
<th>Condition, and Remarks of Health Visitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 1/2 lbs 24 1/2 lbs</td>
<td>12 yrs</td>
<td>Healthy &amp; well developed. Buckland School. Card to 5 yrs.</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>15 1/2 lbs</td>
<td>12 hrs.</td>
<td>9 yrs. 4 yrs. 8 yrs.</td>
<td>Moved to Bury Green. St. Chadham. Had measles, pneumonia.</td>
<td></td>
</tr>
<tr>
<td>20 lbs.</td>
<td>11 yrs.</td>
<td>Y. Y. Y. Y.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Details from one of Miss Burside`s ledgers in 1917

Ethel Margaret Burside
Standardised mortality ratios for Coronary Heart Disease in 15726 Hertfordshire adults under 65 years of age

Age-adjusted Relative Risk of Non-fatal Coronary Heart Disease and Stroke

121,700 American Nurses, self report study  BMJ 315:396,1997
Fetal programming

An event in a “critical”, early period which permanently alters structure and function leading to cardiovascular diseases.
Low Birth Weight as Risk Factor for Cardiovascular Diseases in Later Life
– Mechanisms

- Undernutrition
- Overnutrition
- 11-beta HSD Hypothesis
- Fetal Insulin Hypothesis
- Advanced Barker Hypothesis
- Nephron number
- Paternal Programming

- Epigenetic DNA Modification - the underlying molecular explanation?
Low protein (LP) intake during pregnancy increases blood pressure in later life of the F1 generation

Male

Female

Systolic blood pressures in offspring from low protein (●) and control ( □) pregnancies.

Vehaskari et al., Kidney Int. 59 (2001), pp. 238-245
Low protein (LP) intake during pregnancy increases plasma aldosterone (醛固酮) in later life of the F1 generation.
Low protein (LP) intake during pregnancy increases mortality in later life of the F1 generation

Survival of the offspring from control (dashed line) and low protein (LP; solid line) pregnancies.

Vehaskari et al., Kidney Int. 59 (2001), pp. 238-245
During World War II in 1944, food supplies were cut off to western Holland in retaliation for a railway strike. Over 10,000 people died from starvation, with food intake being 400-800 kcal/day.
Two-hour plasma glucose concentrations in 702 men and women exposed to famine in utero at different periods of gestation.

Raveli et al., Lancet 1990, 2(6653): 577-80
Birth weights, individually adjusted for sex and gestational age, by nutrient intake in early pregnancy (n=693)

<table>
<thead>
<tr>
<th>Daily intake approximate thirds</th>
<th>Results before adjustment for maternal characteristics</th>
<th>Results after adjustment for maternal height and smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean birth weight (g)*</td>
<td>P value for nutrient effect on birth weight*</td>
</tr>
<tr>
<td>Energy (kcal):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1855</td>
<td>3315</td>
<td>0.51</td>
</tr>
<tr>
<td>1855-2204</td>
<td>3364</td>
<td></td>
</tr>
<tr>
<td>≥ 2205</td>
<td>3360</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate (g):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;233</td>
<td>3334</td>
<td>0.33</td>
</tr>
<tr>
<td>233-276</td>
<td>3344</td>
<td></td>
</tr>
<tr>
<td>≥ 277</td>
<td>3350</td>
<td></td>
</tr>
<tr>
<td>Fat (g):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;75</td>
<td>3323</td>
<td>0.54</td>
</tr>
<tr>
<td>75-93</td>
<td>3385</td>
<td></td>
</tr>
<tr>
<td>≥ 94</td>
<td>3352</td>
<td></td>
</tr>
<tr>
<td>Protein (g):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;66</td>
<td>3372</td>
<td>0.97</td>
</tr>
<tr>
<td>66-79</td>
<td>3323</td>
<td></td>
</tr>
<tr>
<td>≥ 80</td>
<td>3361</td>
<td></td>
</tr>
<tr>
<td>Total vitamin C (mg)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;55</td>
<td>3310</td>
<td>0.002</td>
</tr>
<tr>
<td>55-77</td>
<td>3336</td>
<td></td>
</tr>
<tr>
<td>≥ 98</td>
<td>3410</td>
<td></td>
</tr>
<tr>
<td>Total vitamin E (mg)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;7</td>
<td>3297</td>
<td>0.0049</td>
</tr>
<tr>
<td>7-10.5</td>
<td>3386</td>
<td></td>
</tr>
<tr>
<td>≥ 10.5</td>
<td>3378</td>
<td></td>
</tr>
<tr>
<td>Total folate (g)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;222</td>
<td>3300</td>
<td>0.029</td>
</tr>
<tr>
<td>222-299</td>
<td>3334</td>
<td></td>
</tr>
<tr>
<td>≥ 300</td>
<td>3415</td>
<td></td>
</tr>
</tbody>
</table>

* Mean values derived after individual adjustment for sex and gestational age. P values based on univariate regression of birth weights, individually adjusted for sex and gestational age, on nutrient intake (entered as continuous variables).

¹ Mean values derived after individual adjustment for sex and gestational age, height, and smoking. Regression coefficients and P values based on multiple linear regression of birth weight, individually adjusted for sex and gestational age, on maternal height, smoking, and nutrient intake.

² Nutrients in transformed for regression analysis.

³ One outlier excluded.
Human Data; Maternal vitamin D deficiency is linked to Low Birthweight of the Offspring – a Risk Factor for Cardiovascular Diseases in Later Life
<table>
<thead>
<tr>
<th>Model (R$^2$) and Independent Variables</th>
<th>Partial Eta squared</th>
<th>Observed power</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model A (0.048)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grouped 25(OH)D [&lt;1 vs. ≥ 1 nmol/L]</td>
<td>0.035</td>
<td>0.990</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean systolic blood pressure 3rd trimester, mmHg</td>
<td>0.014</td>
<td>0.767</td>
<td>0.007</td>
</tr>
<tr>
<td>Body mass index before pregnancy, kg/m²</td>
<td>0.007</td>
<td>0.491</td>
<td>0.053</td>
</tr>
<tr>
<td>Mean weight 3rd trimester, kg</td>
<td>0.011</td>
<td>0.657</td>
<td>0.018</td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>0.000</td>
<td>0.059</td>
<td>0.780</td>
</tr>
</tbody>
</table>
Gestational week at birth was significantly and positively associated with math score: (F [4, 127 527] = 27.904; P < 3.4 × 10−23).
Gestational week at birth was significantly and positively associated with reading score: (F [4, 128 045] = 21.635; P < 7.2 × 10\(^{-18}\)).
Maternal Vitamin D Deficiency in Mice

Glucose tolerance

blood glucose [mg/dl]

Vitamin-D-sufficient
Vitamin-D-insufficient

0min 15min 30min 45min 60min 90min 120min
Maternal Vitamin D Deficiency in Mice

Survival proportions

P < 0.05
Low Birth Weight as Risk Factor for Cardiovascular Diseases in Later Life
– Mechanisms

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- **Overnutrition**
- 11-beta HSD Hypothesis
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- Nephron number
- Paternal Programming

- Epigenetic DNA Modification - the underlying molecular explanation?
High-Protein Nutrition during Pregnancy and Lactation Programs Blood Pressure, Food Efficiency and Body Weight of the Offspring in a Gender Dependent Manner

Female

Male

* Thöne-Reinike et al., Am J Physiol 2006
High-Protein Nutrition during Pregnancy and Lactation Programs
Blood Pressure, Food Efficiency and Body Weight of the Offspring
in a Gender Dependent Manner

Female

Male

Time (week)

Body weight (g) / Food-intake (g/week)

NP
HP

Thöne-Reinike et al., Am J Physiol 2006
Low Birth Weight as Risk Factor for Cardiovascular Diseases in Later Life – Mechanisms

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- Epigenetic DNA Modification - the underlying molecular expansion?
The 11-beta HSD2 and fetal programming

Maternal Plasma → CORTISOL → 11βHSD2 → Fetal Plasma → CORTISOL

Genes; Low Protein Diet
Prenatal carbenoxolone (CBX) treatment reduces birthweight (□) and programs permanent hypertension and hyperglycemia (fasting and post-prandial) in the adult offspring compared with control offspring (Con; ■) of vehicle-treated pregnancies.

Increased “in vivo” Cortisol Availability in preeclamptic human Placenta

Normal pregnancies are characterized by an 11b-HSD2 activity sufficient to almost completely Inactivated cortisol within the placenta. In contrast, reduced 11b-HSD2 activity in preeclampsia is unable to abolish placental cortisol. Placental 11b-HSD2 activity is associated with pregnancy related hypertension and low birth weight.

Late Gestational Maternal Serum Cortisol is Inversely Associated with Fetal Brain Growth

Relationship between maternal total cortisol and fetal head circumference
Right figure: Women with full labor when taking blood
Left figure: All participating women irrespectively of labor status.

Labor might mimique a stress test to the maternal hypothalamus-hypothesis-adrenal endocrine system unmasking the close inverse relationship between maternal cortisol secretion and fetal brain growth.

Li, ….Hocher, Neuroscience & Biobehav Rev, 2012
Breastfeeding duration and academic achievement at ten years.

*Interaction effects for boys and girls breastfed for 6 months or longer compared to those breastfed for less than 6 months for mathematics ($p=0.007$), reading ($p=0.353$), writing ($p=0.108$) and spelling ($p=0.047$) scores, following adjustment for maternal age, maternal education, maternal race, marital status, family income and looking at books with the child at age five. Error bars represent one standard error of the mean.

*Pediatrics* 2011;127;e137-e145.
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Alternative Hypothesis: Genetic Influences

Genetic Influences

Low Birth Weight Infants

Adulthood Insulin Resistance

Low birth weight is already associated with elevated fetal glycosylated hemoglobin (胎儿总糖化血红蛋白) at birth.

An increase in TGH by 1% in the child is significantly associated with a mean birthweight reduction of 135 g (p<0.0001)

whereas the same increase in the mother is associated with a mean birthweight increase of 88 g (p<0.0001)

These data suggest that the pathophysiological mechanisms linking prenatal growth and postnatal sensitivity to insulin are present as early as before birth.

Low Birth Weight and Elevated Head to Abdominal Circumference Ratio - Risk Factors for Cardiovascular Diseases in Later Life – are associated with Elevated Fetal Glycated Serum Protein (GSP) Concentrations Already at Birth

Fetal GSP was inversely associated with birth weight ($R^2=0.390$, $P<0.001$) and with the head to abdominal circumference ratio, whereas the maternal GSP was negatively correlated with the offspring’s head to abdominal circumference ratio ($R^2=0.282$, $P=0.010$ and $R^2=0.259$, $P=0.020$, respectively).

The disproportional intrauterine growth is in line with the concept brain sparing, a mechanism maintaining the intrauterine growth of the brain at the expense of trunk growth.

Our data suggest that the low birth weight phenotype, linked to cardiovascular diseases like hypertension in later life, might be a phenotype of disproportional intrauterine growth retardation and early life insulin resistance.

Li, ... Hocher, J Hypertension, 2011
In an extended genome-wide association study (up to 69,308 individuals from 43 studies), we identified five, and confirmed two, loci associated with birth weight (P<5x10^{-9}). Notably, two loci (ADCY5 and CDKAL1) are also associated with type 2 diabetes, one (ADRB1) is also associated with adult blood pressure and two (HMGA2 and LCORL) are associated with adult height. Our findings highlight genetic links between fetal growth and postnatal growth and metabolism. Nat Genetics, 2013
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The modified Barker Hypothesis

- Maternal genes and environment
- Nutritional or endocrine perturbations
- Low birthweight
  - Altered structure and function of endocrine pancreas, blood vessels and liver
  - Altered structure and function of insulin-sensitive target tissues
- Survival in early life
- Adulthood
  - Adult lifestyle risk factors (e.g. smoking, no sport)
  - Adult disease (metabolic syndrome: hypertension, type 2 diabetes, hypercholesteremia)

Hocher et al., NDT 16(6):1298-9 (2001)
Association of maternal G protein beta3 subunit 825T allele with low birthweight

Hocher et al., Lancet 2000
Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight


All Mothers

Maternal Genotype: CyP 1A1
AA

Maternal Genotype: CyP 1A1
Aa/aa

Quitter Smoker
Continuous Smoker

#: p<0.05
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- Epigenetic DNA Modification - the underlying molecular expansion?
Reduced Nephron number?

Offspring of IUGR rats (by reduced maternal dietary protein intake) have fewer nephrons (Woods et al, 2004)

Low birthweight babies have fewer nephrons - and essential hypertension in man associated with reduced nephron number (Luyckx and Brenner, 2005; review)
Low protein (LP) intake during pregnancy decreases glomerular number (肾小球数量) and increases glomerular size in later life of the F1 generation.
Nephron number in patients with primary hypertension


Number of Glomeruli per Kidney and Mean Glomerular Volume in 10 Patients with Hypertension and 10 Matched Normotensive Controls. The median value is shown for each group.
Low Birth Weight as Risk Factor for Cardiovascular Diseases in Later Life – Mechanisms

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- Epigenetic DNA Modification - the underlying molecular expansion?
Chronic high-fat diet in fathers programs b-cell dysfunction in female rat offspring

Ng et al., NATURE 2010
Biparietal diameter of male (A) and female (B) offspring in relationship to paternal BMI.

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0036329
Birth weight of offspring as a function of birth order for parents with or without diabetes; numbers relate to each birth order. Error bars are 95% confidence intervals.
Illustration of the non-genomic pathways through which paternal effects on offspring development can be achieved. Experiences of males (drugs, nutrition, toxin, age), particularly those experienced during early development, may lead to epigenetic alterations in the male germline (red circle) which are then transmitted to offspring with consequences for phenotypic variation.
Low Birth Weight as Risk Factor for Cardiovascular Diseases in Later Life
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DEVELOPMENTAL PROGRAMMING OR THE ‘FETAL ORIGINS’ HYPOTHESIS

Programming’ may be due to DNA modifications independent of alterations of the nucleotide sequence.

The totality of such marks on the genome is described as the epigenome and comprises 3 distinct, but closely inter-acting, mechanisms including:

1. DNA methylation
2. histone modifications
3. non-coding microRNAs
Epigenetics, environment and development

Environmental exposure

Maternal Factors

Diet and Life style

Germline epimutation
Genome-wide Demethylation

Developmental epigenetic reprogramming

Somatic epimutation

Gametes → Zygote → Embryo → Fetus → Baby → Adolescent → Adult → Elderly

Dysregulation of epigenetic processes → Disease development

Modified from Foley et al., 2008
Rat – Study protocol

Pregnancy

- Control diet (18%)
- 9% protein
- 9% protein + folic acid

Lactation

- 9% protein
- 9% protein + folic acid

28 days

Term

Weaning

D34

Gene expression and GR promoter methylation
The PR diet induces, and folic acid prevents, altered epigenetic regulation

**PPARα**

- **Methylation**
- **mRNA expression**
- **AOX expression**
- **β oxidation**

**GR**

- **Methylation**
- **mRNA expression**
- **PEPCK expression**
- **Gluconeogenesis**

Lillycrop et al. 2007, Burdge et al. 2007
The 4 R Fetal Programming Hypothesis

Environment (diet) → Receive and Record → Remember → Reveal
Integrating hypotheses

Prenatal Nutrition and other factors

Fetal programming

Genetics

Adult Chronic Diseases

Adulthood Nutrition and other Risk Factors
Review Article

The Fetal Origins of the Metabolic Syndrome: Can We Intervene?

Noelle Ma$^{1,2,3}$ and Daniel B. Hardy$^{1,2,3}$

1 The Department of Physiology and Pharmacology, The University of Western Ontario, London, ON, Canada N6A 5C1
2 The Department of Obstetrics & Gynecology, The University of Western Ontario, London, ON, Canada N6A 5C1
3 The Children’s Health Research Institute, The Lawson Health Research Institute, London, ON, Canada N6A 4V2

- Reduce Oxidative Stress
- Micronutrients
- Alter DNA Methylation
- Omega-3 Fatty Acids
Fetal Programming of Academic Achievements – Potential Projects

• Animal studies linking specific early life environmental stimuli (maternal/paternal over/under-nutrition, stress, deficiency of micronutrients) to learning skill of the offspring.
• These studies should aim to demonstrate the intersection of environmental stimuli, epigenetics, gene expression and functional readouts.
• Dietary/pharmacological intervention should be tested
Fetal Programming of Academic Achievements – Potential Projects

- Human epidemiological studies linking specific early life environmental stimuli (maternal/paternal over/under-nutrition, breast feeding, stress, deficiency of micronutrients) to learning skill of the offspring (age of first sitting, age of first walking, age of first two word sentences etc, reading- and math skills in school)
- We should focus on environmenta factors that are potentially changeable.
- Pathways need to be addressed by surrogate parameters -> measurement of BMI, micronutrients, hormones, toxins (blood/placenta)
- Dietary/pharmacological intervention might be considered. But, difficult processes, costly, ethical aspects -> so far only pilot studies in high risk populations might be feasible.
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J Li  
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M. Yanagisawa, Dallas, USA  
D. Webb, Edinburgh, UK
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121,700 American Nurses, self report study  BMJ 315:396,1997