The placenta, adverse pregnancy outcomes and long term consequences for cardiovascular health

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• I have no disclosures
Mortality from Coronary Heart Disease in Hertfordshire

Barker DJP 1995
Programming of Cardiovascular Disease

- Main cause of mortality and morbidity in 21st century
- CVD accounted for 18.6 million deaths in 2019
- In utero and postnatal gene-environment interactions
- IUGR offspring have highest rate of CHD, myocardial dysfunction, type 2 diabetes, hypertension and stroke as adults.
- Incidence of ischemic heart disease and death 3x higher in men with low birth weight vs high birth weight.
- Dutch famine winter 1944/45 – association of maternal starvation, low birth weight and high incidence of hypertension and CHD as adults.
Developmental (Fetal) Programming
(Barker Hypothesis)
(Fetal Origins of Adult Disease)
(Developmental Origins of Health and Disease [DOHaD])

- Life in utero determines risk of development of disease in adult life
  - Cardiovascular
  - Diabetes (Insulin resistance/Metabolic syndrome)
  - Obesity
  - Stroke
  - Osteoporosis
  - Obstructive Airway Disease
  - Cancer
  - Disordered HPAA axis
  - Behavioral abnormalities

- Sexual dimorphism in effect
- Epigenetic mechanisms
  - Histone modification, DNA methylation
Pregnancies with adverse outcomes

- Pregnancy-induced hypertension, preeclampsia - 5-7%
- Fetal growth restriction - 7%
- Macrosomia - 6-10%
- Pre-gestational diabetes - 1%
- Gestational diabetes - 2-10%
- Multifetal gestation - 3%
- Preterm birth - 11%
- Miscarriage - 10-20%
- Stillbirth - 0.6%
- Congenital malformation – 2-4%
Long Term Consequences of Adverse Pregnancy Outcome (Preeclampsia) - Mother

Increased risk of:
- Hypertension
- Coronary artery disease
- Stroke
- Type 2 diabetes mellitus

CVD risk in relation to time of occurrence
- Preeclampsia at term - 2x risk
- Preeclampsia <37 weeks) - 5x risk
- Preeclampsia <34 weeks - 10x risk
- Fetal growth restriction – 2x risk
- Preeclampsia + FGR - 8x risk

? Pregnancy as a stress test (exposes subclinical disease)
? Does preeclampsia damage vascular system
Long Term Consequences of Adverse Pregnancy Outcomes (Preeclampsia) - Offspring

Fetal programming for subsequent disease due to exposure to adverse intrauterine environment (Developmental Origins of Health and Disease, DOHaD)

At risk for subsequent development of

- Cardiovascular disease (↑BP, ↑cardiac wall thickness)
- Metabolic syndrome (↑BMI)
- Neurodevelopmental disorders (↑ASD [50%], ↑ADHD [28%])
- Congenital heart defects (↑50%)
- Epilepsy

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Roles of the Placenta

UTERUS
- Uterine vasculature
- Oxygen

PLACENTA
- Nutrient Transport
- ATP
- Peptide/Steroid Production/Metabolism
- Maternal substrates (Glucose, Fatty Acids, Amino Acids)

FETUS
- Carbon Dioxide

FETAL GROWTH, DEVELOPMENT & PROGRAMMING

Plane of Nutrition
- Obesity
- Diabetes

Immune Barrier

Maternal metabolism

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Placenta – Not Just a Conduit

• Second only to the brain in the number of gene transcripts

• In human placenta consumes 50% of oxygen and 30% of glucose supplied to uterus

• Metabolic activity 4-6 fold higher per unit weight than fetus

• One third of placental oxygen consumption used for de novo generation of peptides, one third to maintain cation gradient across membrane for transport

• Not simply a conduit, it regulates nutrient composition and supply from mother to fetus. Is it a selfish organ?
Different evolutionary strategies for males and females. Male fetuses appear to keep growing, are larger but have more adverse outcomes due to less placental adaptability:

- preterm birth, PPROM, placenta previa, preeclampsia, lagging lung development, macrosomia, late stillbirths, poorer maternal B cell function and increased risk of GDM.

Females adapt growth rate to optimize survival in a poor environment. Also reflected in differences in fetal programming males vs females.
Evidence for Sexual Dimorphism in Placental Function

- Differences in gene expression, 1st trimester and term, linked to escape from X chromosome inactivation
- Inflammatory, hypoxia, apoptosis and autophagy responses
- Expression of antioxidant defense enzymes
- Fatty acid transporters
- Fatty acid oxidation
- Response to maternal adiposity and inflammatory status
- microRNA expression in normal pregnancy
- Steroid and peptide hormone synthesis
- All linked to difference in outcomes male vs female
# Placental Growth and Development Throughout Gestation

<table>
<thead>
<tr>
<th>Metric</th>
<th>6 weeks</th>
<th>Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental Weight (g)</td>
<td>6.0</td>
<td>470</td>
</tr>
<tr>
<td>Fetal Weight (g)</td>
<td>1.1</td>
<td>3500</td>
</tr>
<tr>
<td>Fetal/Placental Weight Ratio (efficiency)</td>
<td>0.18</td>
<td>7.23</td>
</tr>
<tr>
<td>Villous volume occupied by vessels (%)</td>
<td>2.7</td>
<td>28.4</td>
</tr>
<tr>
<td>Trophoblast Surface area (m²)</td>
<td>0.08</td>
<td>12.5</td>
</tr>
<tr>
<td>Mean Trophoblast Thickness (µm)</td>
<td>18.9</td>
<td>4.1</td>
</tr>
<tr>
<td>Maternofetal Diffusion Distance (µm)</td>
<td>55.9</td>
<td>4.8</td>
</tr>
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</table>
Placental development can be affected by type, severity, timing and duration of a challenge – clearly seen in animal models [structure/function]. An insult e.g. nutritional, applied at a specific time will have a specific effect on placental development /function. The same insult at a different times may have different effect.

E.g. IDDM plus LGA gives increased Glut 1 in BM and increased system A aa transporter, whereas GDM plus LGA no change in Glut 1 in BM but increased system A.
Influences on placental development, function and programming

- Type and plane of nutrition
- Oxygen availability (altitude, anemia)
- Developmental timing, severity, duration of challenge
- Micronutrient availability
- Environmental toxicants - exposome
- Maternal stress
(Mal)adaptive responses of the placenta

- Alteration in size, shape, surface area and structure
  - Vascularization (maternal, fetal)
  - Barrier surface area, thickness (diffusion distance)
- Epigenetic changes, gene expression
- Function
  - Hormone production
  - Expression of type and quantity of transporters
  - Buffering/storage/metabolism of nutrients to alter/limit transfer to fetus
  - Lipid accumulation, inflammation, oxidative stress
  - Barrier - metabolism of glucocorticoids

Seen with maternal obesity, gestational diabetes, preeclampsia, fetal growth restriction, large for gestational age, preterm birth
Fetal programming of the heart

- Direct correlation of oxygen and nutrient supply to fetus and cardiomyocyte development and function
- Cardiac remodeling – changes in size, shape, function resulting from cardiac load and injury
- Cardiac hypertrophy - globular shape, higher intraventricular septum thickness, increased pressure overload leading to systolic and diastolic dysfunction
- Signs of ventricular electrical remodeling
- Changes in cardiac gene expression *HIF-1α, PARP1, HSP70, PKCε, SIRT3, FOXO1*
- Genes and miRNA expression for energy metabolism
- Epigenetic changes - DNA methylation, histone modification
Villous angiogenesis in normal and IUGR pregnancy

Kingdom et al 2000

Barut et al 2010
Cardiac Shape in Normal and IUGR Fetus

A. Control

B. IUGR

-Increased placental resistance & fetal hypertension
-Increased pressure
-Decreased oxygen & nutrients

C. Normal cardiac shape

D. IUGR cardiac shape
Influence of Nutrition or the Metabolic Environment on Epigenetic Modifications

- Epigenetics – heritable changes in gene expression (active/inactive) that do not involve changes in DNA sequence but changes in physical structure (methylation/demethylation)

- Epigenome responds to changes in nutrients including methyl donors, folate supplementation, fat, glucose and caloric restriction

- Differences in DNA methylation reported in individuals exposed to the Dutch Hunger Winter

- Variations in DNA methylation associated with many aspects of diabetes mellitus and metabolic/inflammatory milieu of obesity
Micronutrients with a Role in the Placenta

- One carbon metabolism regulated by folic acid, choline, vitamin B12, and ω-3 fatty acids, results in biosynthesis of lipids, nucleotides, proteins, maintenance of redox status and methylation

- Transfer of zinc, iron, copper, calcium, selenium and Vitamins A, D and E may have a role in programming

- Micronutrient deficiency affects placental function, 
  - see upregulation of transporters to maintain fetal supply and demand against concentration gradient e.g. Vit B6, B12, C, folate, iron, zinc

- Maternal iron restriction affects placental structure in rats, Zn deficiency can reduce trophoblast differentiation, placental weight and change protein expression

- Antioxidants, Zinc - Cu/ZnSOD, selenium – selenoproteins GPx, TrxR
Nutrients that affect Epigenetic Modification

- Folate
- Vitamin B12
- Methionine
- Choline
- Betaine
- Biotin
- Niacin
- Pantothenic acid
- Resveratrol
- Butyrate
- Curcumin

Genistein
Polyphenols
Tea catechin

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Maternal Metabolic Milieu with Obesity and GDM

- Insulin resistance
- Maternal hyperglycemia, hyperlipidemia
- Inflammation
- Oxidative stress
- Associated with adverse outcomes including stillbirth
- Both program the offspring for disease in later life
- Sexually dimorphic responses
- Increasing maternal adiposity associated with decreased placental mitochondrial respiration and further exacerbated with gestational diabetes.

(Mele et al 2014, Muralimanoharan et al 2016)
Oxidative Stress In Pregnancy

- Antioxidants protect cells from oxidative stress which causes cellular damage of DNA, lipids and protein

- Normal pregnancy is a state of increased oxidative stress which is increased further in pathologic pregnancies e.g. PE, GDM

- The placenta is a source of oxidative stress due to its high metabolic activity with mitochondria being a major source

- The inflammatory conditions of obesity and gestational diabetes heighten oxidative stress and deplete antioxidant defenses often in a sexually dimorphic manner (Evans and Myatt 2017)

- Nutritional and supplemental sources of antioxidants
  - Vitamin C, Vitamin E, Resveratrol, N acetylcysteine (NAC), Omega 3 fatty acids, vegetables, selenium, zinc
Effect of Obesity on Placental DNA Methylation

Methylated regions identified by NimbleGen 2.1 M arrays (NimbleScan)

<table>
<thead>
<tr>
<th>Number of methylated regions (peak score&gt;3)</th>
<th>5mC</th>
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<tbody>
<tr>
<td></td>
<td>Normal</td>
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<tr>
<td>All tiled regions</td>
<td>12,319</td>
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<tr>
<td>TSS1500</td>
<td>3,187</td>
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<tr>
<td>(1500 bp upstream to 500 bp downstream of TSS)</td>
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</tr>
<tr>
<td>TSS100</td>
<td>1,459</td>
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<tr>
<td>(100 bp upstream - 100 bp downstream of TSS)</td>
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<tr>
<td>CpG islands</td>
<td>3,294</td>
</tr>
<tr>
<td>CpG island shores (2 kb flanking CpG islands)</td>
<td>3,127</td>
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<tr>
<td>CpG island shelves (2kb flanking shores)</td>
<td>1,502</td>
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<tr>
<td>Gene body</td>
<td>8,560</td>
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<tr>
<td>microRNA (-15 kb to +1 kb)</td>
<td>429</td>
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</table>

Mitsuya et al 2017
Genes showing significant differences in methylation in the region -100 to +100bp from TSS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Locus</th>
<th>Gene</th>
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<tr>
<td></td>
<td></td>
<td><strong>methylated in obese placentas (18 genes)</strong></td>
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<tr>
<td>MIA</td>
<td>19q13</td>
<td>Melanoma inhibitory activity</td>
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<tr>
<td>PSG1</td>
<td>19q13</td>
<td>Pregnancy specific glycoprotein</td>
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<tr>
<td>PSG4</td>
<td>19q13</td>
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<tr>
<td>PSG5</td>
<td>19q13</td>
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<td>PSG8</td>
<td>19q13</td>
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<tr>
<td>ABCG5</td>
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<td>ATP-binding cassette, sub-family G</td>
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<tr>
<td>ABCG8</td>
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<tr>
<td>BEST4</td>
<td>1p32</td>
<td>Bestrophin 4</td>
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<tr>
<td>HSPB3</td>
<td>5q11</td>
<td>Heat shock 27kDa protein 3</td>
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<tr>
<td>CSH2</td>
<td>17q22</td>
<td>Chorionic somatomammotropin hormone (placental lactogen)</td>
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<td>CSH1</td>
<td>17q22</td>
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<tr>
<td>GH1</td>
<td>17q22</td>
<td>Growth hormone 1</td>
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<tr>
<td>TET3</td>
<td>2p13</td>
<td>TET methylcytosine dioxygenase 3</td>
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<tr>
<td>ADAM6</td>
<td>14q32</td>
<td>ADAM metallopeptidase domain 6</td>
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<tr>
<td>ZFP92</td>
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<td>SEC16 homolog B (S. cerevisiae)</td>
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<td>IL19</td>
<td>1q32</td>
<td>Interleukin 19</td>
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<td><strong>methylated in normal placentas (3 genes)</strong></td>
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<td>CMTM1</td>
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<td>CKLF-like MARVEL transmembrane domain containing</td>
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<tr>
<td>CMTM2</td>
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<tr>
<td>SERPINB13</td>
<td>18q21</td>
<td>Serpin peptidase inhibitor</td>
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</table>

placental development (6)
cellular invasion (2)
cancer metastasis (2)
vascular remodeling (4)
increased BMI (1)
lipid transport (2)
energy metabolism (1)
immune and/or inflammatory responses (7)
Placental Fuel Substrates

Fatty acids

Glucose

LCA-CoA

Pyruvate

Glycolysis

Acetyl-CoA

B-oxidation

CPT1

Placental function

ATP

ETC

TCA cycle

α-KG

Glutamate

Glutamine
Effect of Obesity and GDM on Fuel Usage by Trophoblast

• In lean women there was no difference in dependency on or flexibility for these three fuels for baseline respiration between male and female trophoblast.

• With obesity and A2GDM (hyperglycemia and hyperlipidemia) we find increased dependency on glucose and fatty acids for baseline respiration but only in male placenta.
  • Accompanied by significantly decreased flexibility for both glucose and fatty acids, but also glutamine, i.e. male trophoblast cannot easily switch between fuels.

• Changes in placental metabolism may affect amount of each substrate available for transfer to fetus and hence fetal growth and development

• Is this related to the increased risk for adverse outcomes in males?

Wang et al JCEM 2019
Role of the placenta in fetal programming

Maternal environment
Type/plane of nutrition, obesity, diabetes, stress, exposome

Placental function/adaptation?
Vascular development/perfusion, oxygenation, transport, metabolism, hormones, inflammation, oxidative stress, epigenetics

Physical forces
Molecular mechanisms

Fetal growth, development, epigenetics
Heart, vasculature, kidney, pancreas, liver, brain, HPA axis
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K Ahuna     L Kadam