Prenatal alcohol exposure and metabolic disease in adulthood

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Acknowledgments

**Alcohol project**
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Learning Objectives:

• What is ‘fetal/developmental’ programming” and is there a role for prenatal alcohol exposure?

• Explore the long term consequences for metabolic, health (and disease) following prenatal alcohol exposure
  • Effects on males and females may be different
  • Timing and dose of prenatal exposure is important

• Mechanisms causing ‘programming’ in the peri-conceptional period
  - circadian rhythm changes
  - Epigenetic changes
The DOHaD hypothesis

• Developmental Origins of Health and Disease
  – Developed from ‘Barker Hypothesis’
  – Dutch Famine Studies
• Concept of critical windows
• Strong programming of metabolic outcomes
DOHaD in human populations: Dutch Winter Famine

First trimester
- Glucose intolerance
- Cardiovascular disease
- Hypertension
- Dyslipidemia
- Obesity
- Affective disorders

Second trimester
- Glucose intolerance
- Pulmonary disease
- Renal disease

Third trimester
- Glucose intolerance
### Programming of Metabolic syndrome

<table>
<thead>
<tr>
<th>Birthweight (kg)</th>
<th>Total no.</th>
<th>No. with Syndrome X</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 2.5</td>
<td>20</td>
<td>6</td>
<td>18 (2.6 -118)</td>
</tr>
<tr>
<td>-2.95</td>
<td>54</td>
<td>10</td>
<td>8.4 (1.5 -49)</td>
</tr>
<tr>
<td>-3.41</td>
<td>114</td>
<td>19</td>
<td>8.5 (1.5 - 46)</td>
</tr>
<tr>
<td>-3.86</td>
<td>123</td>
<td>15</td>
<td>4.9 (0.9 - 27)</td>
</tr>
<tr>
<td>-4.31</td>
<td>64</td>
<td>4</td>
<td>2.2 (0.3 - 14)</td>
</tr>
<tr>
<td>&gt;4.31</td>
<td>32</td>
<td>2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Low birth weight adults (<2.95 kg) have a 10-fold increased risk of developing Syndrome X, whereas lifestyle factors (smoking, overeating), increased the risk up to 3-fold.

Why study prenatal alcohol in the context of DOHaD?
Australian Aborigines and programming

Prevalence of morbidities in one remote community in Australia.

Animal models for fetal alcohol exposure

- Chronic, low/moderate (daily consumption)
  - Liquid diet containing 6% v/v ethanol, consumed *ad lib*
  - BAC- ~0.03-0.04%

- Periconceptional exposure (E-4 until E4)
  - Liquid diet containing 12% ethanol consumed *ad lib*
  - Second hit – high fat diet from 3 months of age
What metabolic outcomes can be programmed by prenatal alcohol exposure?

• Fetal and offspring growth
• Metabolic outcomes
  – Basal blood glucose
  – Glucose tolerance test and insulin challenge
  – Food preference
  – Interaction with a lifestyle factor (high fat diet)
  – Hyperlipidemia? Fatty liver?

OBESITY IS NOW A GLOBAL EPIDEMIC!
Prenatal alcohol and fetal growth (1)

CLD: Low Birth Weight
Catch up growth during weaning,
Males have slowed growth in later life
Normal basal blood glucose
Elevated basal insulin
Elevated 1st phase insulin secretion
ONLY in males

What about exposure only around the time of conception?
Periconceptional alcohol exposure: - interaction with a postnatal high fat diet

Fetal growth
Placenta

Metabolic studies
CV
Renal, Behaviour
HPA
Prenatal alcohol and fetal growth (2)

PCE: Reduced fetal weight & body length in late gestation, Catch up growth during weaning, Normal body weight in adulthood
PCE causes elevations in fasting glucose and insulin in males at 8 months

PCE: Response to a GTT

PCE causes insulin resistance in males AND females at 8 months

PCE: changes to hepatic gene expression
Altered insulin signaling in adipose tissue

PCE: Altered hepatic gene expression and altered muscle and adipose (peripheral) insulin signaling
Programming of obesity

No differences in body weight
Abdominal fat – ‘fat-thin Indian’

Gardebjer et al, Am J Physiol, 2018
Plasma hormones

8 months of age

Day 30
Increased fat deposition (in males) – abdominal fat
Increased expression of inflammatory genes,
Increased early life leptin.
Programming of metabolic disease: a role for altered circadian clock?

A role for circadian clock in metabolic disease

Ippei Shimizu¹,², Yohko Yoshida¹,² and Tohru Minamino¹

Many human behaviors and physiological activities show circadian rhythms. Circadian rhythms generated by central and peripheral clocks maintain homeostasis, including the regulation of metabolic processes. Biological rhythmicity is important for metabolic health, but circadian rhythms are affected and impaired by nocturnal activities and irregular food intake in modern society. Disruption of sleep is an established risk factor for diabetes and is known to promote systemic metabolic dysfunction in both humans and rodents. Metabolic stress promotes circadian clock disorders and modulation of clock gene expression has a causal role in the development of metabolic dysfunction. Maintenance of a physiological circadian rhythm is crucial for metabolic health and is an important strategy for combating obesity.

Hypertension Research advance online publication, 18 February 2016; doi:10.1038/hr.2016.12
Circadian Rhythm

- Biological processes that oscillate over 24hr day
  - hormone secretion,
  - sleep/wake cycles,
  - glucose homeostasis
  - immune function
- Peripheral ‘clocks’ regulated by a ‘master clock’ housed within SCN of hypothalamus
Circadian regulation of gluconeogenesis and cortisol secretion
Programming of circadian rhythms

No differences in eating behaviour
Programming of circadian rhythms

Salivary cortisol levels are elevated in the afternoon and at bedtime in children with prenatal alcohol exposure

Kathy Keiver, Chris P. Bertram, Alison Pritchard Orr, Sterling Claren

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2 Centre for Community Child Health Research, Canada Northwest FASED Research Network, Vancouver, British Columbia, Canada
PCE alters circadian rhythms

<table>
<thead>
<tr>
<th>Corticosterone</th>
<th>Female Control</th>
<th>Female Ethanol</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesor</td>
<td>354.2 ± 16.3</td>
<td>352.6 ± 16.8</td>
<td>ns</td>
</tr>
<tr>
<td>Amplitude</td>
<td>36.9 ± 23.5</td>
<td>102.2 ± 24.1</td>
<td>p=0.05</td>
</tr>
<tr>
<td>Acrophase</td>
<td>9.47 ± 2.37</td>
<td>16.81 ± 0.87</td>
<td>p&lt;0.005</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Male Control</th>
<th>Male Ethanol</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesor</td>
<td>163.90 ± 8.36</td>
<td>159.3 ± 11.50</td>
<td>ns</td>
</tr>
<tr>
<td>Amplitude</td>
<td>66.80 ± 11.90</td>
<td>90.7 ± 16.6</td>
<td>ns</td>
</tr>
<tr>
<td>Acrophase</td>
<td>14.212 ± 0.664</td>
<td>12.183 ± 0.699</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>
G6pc Gene Expression - Females

\[ p_{time} < 0.05 \]
\[ p_{treatment} = 0.06 \]
\[ p_{interaction} = NS \]

\[ p_{time} < 0.01 \]
\[ p_{treatment} = NS \]
\[ p_{interaction} = NS \]
Potential Mechanism

PCEtOH → Epigenetic change → Altered CNS ‘clock’

Altered Circadian Rhythm

Corticosterone secretion shifted forward

→ Altered HPA axis regulation

↑ Corticosterone secretion

↑ Blood glucose

Metabolic Disease

Corticosterone secretion

Blood glucose
How are these effects mediated?

- Epigenetic effects?
- Effects on the blastocyst?
- Effects on the uterus?
- Effects on the placenta?
The periconceptional period is susceptible to epigenetic modification

DNA methylation
Histone modifications
miRNAs
Alter DNA methylation?

Nuclear 5MC labelling (green) was increased in flushed day 5 blastocysts exposed to PCE. Blue labelling (DAPI) indicates cell nuclei whilst red labelling (pan-cytokeratin) labels cell membranes.
PCE results in changes in DNMT’s

Fetal Liver – E20

Gardebjer et al, FASEB J, 2015
PCE alters miRNA's regulating leptin and inflammatory genes (adult)

Gardebjer et al, Am J Physiol, 2018
Prenatal alcohol exposure may ‘program’ metabolic syndrome/disease?

Fig. 1 A hypothetical scheme of the main mechanisms linking prenatal exposure to substance of abuse to development of metabolic syndrome and T2D in adult life

Take home messages and questions going forward:

• Exposure to alcohol in rat models can later glucose homeostasis and cause insulin resistance, a risk for increased fat deposition and changes in circadian rhythms.

Does this also occur in adults with FASD? Checking of fasting glucose at an early age? Guidance on interaction with lifestyle factors.