ESPEN Congress Istanbul 2006

ESPEN - ESPGHAN
Malnutrition and future infant development

Fetal origin of metabolic syndrome
- experimental evidence

C. Remacle (Belgium)
Fetal origin of metabolic syndrome – experimental evidence

Claude REMACLE

University of Louvain
Institute of Life Sciences
Metabolic Syndrome

- Glucose intolerance
- Hyperlipidemia
- Hypertension
- Type 2 diabetes
- Obesity
- Cardiovascular disease
## Prevalence of Insulin Resistance Syndrome, Glucose Intolerance and Diabetes in Men 59-70 Years of Age according to Birth Weight (Hertfordshire)

<table>
<thead>
<tr>
<th>Birth weight lb (kg)</th>
<th>% with insulin resistance syndrome</th>
<th>% with glucose intolerance and NIDDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5.5 (2.50)</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>6.5 (2.95)</td>
<td>19</td>
<td>34</td>
</tr>
<tr>
<td>7.5 (3.41)</td>
<td>17</td>
<td>31</td>
</tr>
<tr>
<td>8.5 (3.86)</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>9.5 (4.31)</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>&gt; 9.5 (4.31)</td>
<td>6</td>
<td>14</td>
</tr>
</tbody>
</table>

*Hales and Barker, BMJ, 1991*
### Prevalence of Coronary Heart Disease by Ponderal Index at Birth among Finnish Men 60 Years of Age

<table>
<thead>
<tr>
<th>Ponderal index at birth (kg/m³)</th>
<th>Standardized mortality ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25</td>
<td>116</td>
</tr>
<tr>
<td>25-27</td>
<td>105</td>
</tr>
<tr>
<td>27-29</td>
<td>72</td>
</tr>
<tr>
<td>&gt; 29.4</td>
<td>56</td>
</tr>
<tr>
<td><strong>All</strong></td>
<td><strong>86</strong></td>
</tr>
</tbody>
</table>

p value for trend : 0.001

Forsen et al., 1997
A foetal origin of adult disease?

“Programming”:
the process whereby a stimulus or input during a sensitive period of development has permanent effects on the structure, physiology and metabolism of the organ.

(Lucas, 1991)
Programming of the metabolic syndrome

= 

Programming of a thrifty phenotype

• Nutrients to critical organs
• Metabolic adaptations
An early origin of adult disease?

“Predictive Adaptive Response”: Allows a species to survive to reproduce in a compromised environment. The programming may be appropriate or not, owing to the consistency of both fetal and postnatal experiences.

(Gluckman & Hanson, 2004)
Maternal stress
infection, malnutrition, placental
dysfunction, smoking, alcohol, …

Changes in growth,
metabolism
and vasculature

Muscle, liver,
adipose tissue

HPA &
neuroendocrine
axes

Kidney

Inspired from Fernandez-Twinn & Ozanne, 2006
Maternal stress
infection, malnutrition, placental dysfunction, smoking, alcohol, …

In-utero programming

Inter-generational effects

Metabolic syndrome

- adult ß cell function
- ß cells
- Muscle, liver, adipose tissue
- HPA & neuroendocrine axes
- Kidney
- Changes in growth, metabolism and vasculature

Inspired from Fernandez-Twinn & Ozanne, 2006

Obesity

- Over-nutrition

Hypertension
Renal disease

Insulin resistance
Animal Models

Protein Restriction
General Food Restriction
Uterine Artery Ligation
Gestational Diabetes
High Fat Diet
Other specific dietary manipulation
The Programming of Diabetes
Maternal malnutrition

Low protein diet
- 20%
- 8%
- 8%

Low calorie diet
- 50%

gestation  lactation  weaning  adulthood  gestation

birth
$\beta$-cell mass in the offspring

Low protein diet

Last day of gestation

Low calorie diet

First day of life

Boujendar et al, 2002

Garofano et al, 1997
Maternal low-protein diet on fetal endocrine pancreas

**in vivo**
- 0.6 : Insulin content
- 0.7 : Islet size
- 0.6 : Islet cell proliferation
- 5.0 : Apoptosis
- 0.6 : IGFs positive islet cells
- 0.7 : Islet vascularisation

**in vitro**
- 0.1 – 0.7 : Insulin secretion
- 0.4 : Islet cell proliferation
- 2.4 : Apoptosis
- 1.5 : Susceptibility to IL-1
Maternal low calorie diet on glucose and insulin levels

3 months - OGTT

Plasma glucose (mg/dl)

Plasma insulin (ng/ml)

Early Low Calorie Diet
Maternal low protein diet on glucose and insulin levels

17 months - IVGTT

Early Low Protein Diet

Petry et al, 2001
Maternal low protein diet on insulin sensitivity

- Shepherd et al., 1997
- Hales et al., 1996
- Petry et al., 2001
- Fernandez-Twinn et al., 2005
Maternal low protein diet on insulin sensitivity

In muscle and adipose tissue

Shepherd et al., 1998, Ozanne et al., 1999
Maternal low protein diet on insulin sensitivity

In muscle

Ozanne et al., 2005
The Programming of Obesity
The Programming of Obesity

Epidemiology

Does birthweight affect:
- lean or fat mass
- type of obesity
- Importance of catch-up growth
- Composition of mother’s diet
- Confounding factors

Rogers and EURO-BLCS, 2001
The Programming of Obesity

Animal models
## Experimental groups (1)

<table>
<thead>
<tr>
<th></th>
<th>Gestation</th>
<th>Lactation</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C</strong></td>
<td>20%</td>
<td>20%</td>
<td>16%</td>
</tr>
<tr>
<td><strong>C-Caf</strong></td>
<td>20%</td>
<td>20%</td>
<td>16% + Caf</td>
</tr>
<tr>
<td><strong>R</strong></td>
<td>8%</td>
<td>8%</td>
<td>16%</td>
</tr>
<tr>
<td><strong>R-Caf</strong></td>
<td>8%</td>
<td>8%</td>
<td>16 % Caf</td>
</tr>
</tbody>
</table>

- **Birth**: Week 3
- **Week 3**: Week 7
# Experimental groups (2)

<table>
<thead>
<tr>
<th>Group</th>
<th>Gestation</th>
<th>Lactation</th>
<th>Adult males (6 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>20%</td>
<td>20%</td>
<td>16%</td>
</tr>
<tr>
<td>C-Caf</td>
<td>20%</td>
<td>20%</td>
<td>16% + Cafeteria</td>
</tr>
<tr>
<td>LP</td>
<td>8%</td>
<td>20%</td>
<td>16%</td>
</tr>
<tr>
<td>LP-Caf</td>
<td>8%</td>
<td>20%</td>
<td>16% + Cafeteria</td>
</tr>
<tr>
<td>LC</td>
<td>20%</td>
<td>20%</td>
<td>16%</td>
</tr>
<tr>
<td>LC-Caf</td>
<td>20%</td>
<td>20%</td>
<td>16% + Cafeteria</td>
</tr>
</tbody>
</table>

- Birth: 8 Pups
- Weaning: 4 Pups
- Cafeteria: 16%
Catch up growth during lactation

**Birth weight**
- C: 6.9 ± 0.2
- LP: 5.7 ± 0.2**
- LC: 5.7 ± 0.1**
Catch up growth during lactation

Body weight at 42 weeks

No Catch-up growth

Catch-up growth

Body Weight (gr)

Caf
Catch up growth during lactation

Weight of visceral fat depots

No Catch-up growth

Catch-up growth
Four Pathways for Programming Obesity

- Appetite regulation
- Endocrine control of metabolism
- Fat cell precursors
- Energy expenditure
Four Pathways for Programming Obesity

- Appetite regulation
- Endocrine control of metabolism
- Fat cell precursors
- Energy expenditure
Interactions among Hormonal and Neural Pathways that regulate Food Intake and Body-Fat Mass

Control of appetite

Blood leptin

Leptin (µg/ml)

Caf

C  LP  LC  C  LP  LC

§  §  $$$  ***  ***  ***
Control of appetite

- High fat
- High sucrose
- Lab chow
- Water

Daily Energy intake (kJ)

- C
- LP
- LC
- C-Caf
- LP-Caf
- LC-Caf
Calorie intake per day in offspring food-restricted during gestation

Leptin appears to play a crucial neurotrophic role in the development of the hypothalamic circuits regulating food intake and adiposity.

The neurodevelopmental actions of leptin appear specifically to be restricted to a neonatal critical period that coincides with the naturally occurring surge in leptin.

Bouret & Simerly, 2004
Neonatal leptin treatment reverses developmental programming

Vickers et al. (2005)

Caloric intake
Locomotor activity
Body weight
Fat mass

Glucose
Insulin
Leptin

Vickers et al. (2005)
Four Pathways for Programming Obesity

- Appetite regulation
- Endocrine control of metabolism
- Fat cell precursors
- Energy expenditure
Inspired from Fowden and Forhead, 2004

Nutritional state

Tissue accretion

Tissue differentiation

Programming of Endocrine Control

Insulin

T4, T3

IGFs

GH

Cortisol

Metabolism

Inspired from Fowden and Forhead, 2004
IGFs
- Low IGF-I in rat
- Normal IGF-I & II in guinea pig, but low IGF-II in placenta

IGF-BPs (mainly –1 and –2)
- Normal in rat
- High in rat

Regulation
- by insulin, glucocorticoids, & placental lactogens

References:
- Woodall et al., 1996
- Muaku et al., 1995
- El Khattabi et al., 2003
- Olausson & Sohlström, 2003
- Muaku et al., 1995
- Woodall et al., 1996
- El Khattabi et al., 2003
- El Khattabi et al., 2003, 2006
IGF-I treatment alleviates hyperphagia, obesity, hyperinsulinemia, hyperleptinemia, and hypertension in rats programmed to develop metabolic syndrome.

*Vickers et al., 2001*
Glucocorticoid axis

Undernutrition → Stress → Glucocorticoids → Mother → Placental 11βOHSD → Fetal brain → Increased HPA function

Fetus → Metabolic (syndrome X) → Adult

Fetus → Structural (muscle, fat, bone) → Adult

Fetus → Behavioral (brain) → Adult

Fetus → Gonadal function → Adult

Matthews et al., 2001
Four Pathways for Programming Obesity

- Appetite regulation
- Endocrine control of metabolism
- Fat cell precursors
- Energy expenditure
Adipose conversion

- Pluripotent stem determination
- Multipotent stem exponential growth
- Adipoblast clonal expansion
- Preadipocyte growth arrest
- Preadipocyte clonal expansion
- Preadipocyte growth arrest
- Immature adipocyte early and late differentiation
- Adipocyte
## Adipose conversion : signals

<table>
<thead>
<tr>
<th></th>
<th>Cell lines</th>
<th>Primary cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>+ / 0</td>
<td>+</td>
</tr>
<tr>
<td>T3</td>
<td>0 / +</td>
<td>0</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>+ / -</td>
<td>+ / -</td>
</tr>
<tr>
<td>GH</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>IGF-I</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EGF, TGF-α</td>
<td>- / +</td>
<td>- / +</td>
</tr>
<tr>
<td>TGF-β</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FGF</td>
<td>- / 0</td>
<td>0</td>
</tr>
<tr>
<td>PDGF</td>
<td>- / +</td>
<td>0</td>
</tr>
<tr>
<td>IL-1, IFN-γ, TNF-α</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PGF$_2α$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PGI$_2$</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Fat cell precursors
Fat cell precursors

Adipocyte precursors capacity for proliferation and differentiation in case of growth retardation

NO
Fat cell precursors

Adipocyte precursors capacity for proliferation and differentiation in case of growth retardation followed by catch up growth

YES
General Nutritional Molecular Programming?

- Epigenetic programming
- Mitochondrial programming
DNA methylation

Histone methylation
acetylation
phosphorylation

... si RNA
Nutritional Epigenetic Programming?

Inspired from Gallou-Kabani & Junien, 2005
Nutritional Epigenetic Programming?

- Carcinogens
- TGE
- FBS medium amino ac.
- M16 medium Agouti
- Development
- Maternal care litter size
- Crop diet-obesity folates

Nutritional factors and their influence on methylation levels during development:

- Genes
- Transposable elements
- Imprinted genes

Inspired from Gallou-Kabani & Junien, 2005
Nutritional Mitochondrial Programming?

IUGR after uterine artery ligation (Islets)

ATP production

Mitochondrial copy number

Simmons & al., 2005
Nutritional Mitochondrial Programming?

IUGR after high fat feeding of the mother (Liver)

Mitochondrial copy number

3 month-old rats

Taylor & al., 2004
Nutritional Mitochondrial Programming?

20% C Fetal pancreata  8% LP

7 days in vitro in standard culture medium

Neoformed islets

Genes Microarray, Affymetrix
GeneChip Rat Expression 230A probe array

Significance Analysis of Microarray (SAM)

1,999 / 10,343 genes
11% of the genes of which the expression was modified encode for mitochondrial proteins
Conclusion (1)

- Fetal and early postnatal environment plays a critical role in determining future susceptibility to the metabolic syndrome.

- Fetal malnutrition targets the endocrine pancreas, alters its structure and function and induces mitochondrial dysfunction in the beta cells.

- Alteration in insulin secretion appears first in young offspring. Later, modification in the insulin signalling pathway is obvious leading to frank diabetes.
Conclusion (2)

- For the programming of central obesity may be implicated a definitive dysregulation of appetite, the generation of an hormonal context which is favourable to adipogenesis and which may include high levels of corticosteroids, hyperinsulinemia and anomalies in IGF axis, as well as the genesis of an abnormal population of fat cell precursors in the depots.

- Epigenetic programming and mitochondrial programming are possible general molecular mechanisms.