Early Metabolic Programming and Calorie Restriction

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Outline

1. Review and Introduction
2. Methods
3. Results
4. Conclusions
Diet and Development

- Pancreatic islets and the hypothalamus mature after birth.
- Previous work suggests that early overnutrition leads to metabolic problems in adult rats.

What is the effect of calorie restriction on rats that consumed a high-carbohydrate diet early in development?
NPY and POMC

- Increased NPY encourages more food intake
- Increased POMC causes satiation
- Both of these hormones are regulated by leptin
- Located in the arcuate nucleus of the hypothalamus

Schwartz et al. (2000)
Critical Periods of Development

- Gestation
- Birth
- Postnatal-weaning
Brain Development

Brains develop in stages

Development of the Hypothalamus

24-32 weeks

Earliest evidence of NPY neurons at 21 weeks in humans

Koutcherov et al. (2002)

34 weeks to birth
Development of the Hypothalamus

Development of the hypothalamus in wild-type (WT) and leptin-deficient mice

Bouret and Simerly (2004)
Pancreatic Islets

- **Parasympathetic nervous system** increases insulin secretion
  - Parasympathetic nerves are cholinergic
  - Beta cells within the islet secrete insulin

- **Sympathetic nervous system** inhibits glucose-related insulin secretion
  - Sympathetic nerves are adrenergic

Ahren (2000)
Development of the Pancreatic Islets in Mice

Cabrera-Vasquez et al. (2009)
Development of the Pancreatic Islets

Expression of pro-NGF (a Nerve Growth Factor Precursor) in Mouse Pancreatic Islets

Cabrera-Vasquez et al. (2009)
Development of the Pancreatic Islets

Definitive endodermal progenitor
- Sox17
- Fox2a
- Pdx1

Pancreatic progenitor
- Ngn3
- Pdx1
- Ptf1a
- Nkx6.1
- Nkx2.2

α cells
- CD133+
- CD49f+/low
- CD24+
- Ecadherin+
- CD55+
- CXCR4+/−

β Cells and IPCs (in vitro)
- Insulin, IAPP

δ cells
- Somatostatin

PP cells
- PP

Self-renewal

Pancreatic stem cell?
- Ngn3?
- Pdx1?
Signalling Pathways

**JAK-STAT Pathway**

- **Stat3** has effects on gene transcription and downregulates the effect of leptin via Socs3
- **Socs3** is also triggered by inflammation, which can be triggered by diet

Morton and Schwartz (2011)
IRS-PI3K Pathway

- **Stat3** effects on gene transcription can also be activated through this pathway
- **Socs3** can affect leptin and insulin signalling

Morton and Schwartz (2011)
Early Diet Influences on Metabolism

STEP 1: Dietary assignments

- Postnatal Day 4
- **Group 1: Motherfed (MF)**
  - natural rearing on mother’s high fat milk
- **Group 2: High Carbohydrate (HC)**
  - artificial rearing on high carb milk

**AL - Ad libitum**

- Unlimited access to food and allowed to eat at will
Postnatal Diets During Suckling Period

**Composition of Control Milk**
- MF: Mother-fed (Control)
- HC: High-Carbohydrate

**Composition of High-Carbohydrate Milk**
- MF: Mother-fed (Control)
- HC: High-Carbohydrate
Early Diet Influences on Metabolism

STEP 2: Change in diet

- Postnatal Day 24
- **Group 1:** MF weaned off milk
  - standard rat chow ad libitum
- **Group 2:** HC weaned off milk
  - standard rat chow ad libitum
- **Group 3:** High Carbohydrate/Pair-fed (HC/PF)
  - pair-fed standard rat chow

**PF - Paired-feeding**

HC/PF pups were force fed standard rat chow to match the caloric intake of their paired MF pup

**Purpose:** to control for caloric intake between the rat pairs
STEP 3: Freedom in feeding

- Postnatal Day 90
- **Group 4: High Carbohydrate/Pair-fed → Ad libitum (HC/PF/AL)**
  - switch from pair-feeding to free-feeding

**Purpose:** to observe the effects of caloric restriction on adult-onset obesity
End of Experiment Measures

- Postnatal Day 140
- Brains and pancreases dissections
- Serum collection for insulin and leptin concentrations at basal (5.5mM) and max stimulatory (16.7mM) levels
- Insulin secretion 5.5mM glucose alone, +Acetylcholine, and +Oxymetazoline
- mRNA levels of *Npy, Pomc, Lepr, Socs3, Stat3*
Body Weight Gains of Postnatal Days 24-140

Day 24-52: consistent *increase*, but *no significant difference* in body weight change between groups

Day 52-94: consistent *increase*, HC rats gained *significantly more* weight than MF and HC/PF

Day 94-108: MF and HC/PF start to *level off*, HC/PF/AL and HC both *increasing*

Day 108-140: HC/PF/AL *catch up* to HC

Srinivasan (2013)
Food Intake by Week

Significant increase at week 12 in HC rats allowed to feed ad libitum
No difference between HC and HC/PF/AL

Srinivasan (2013)
Results: Serum Insulin and Leptin

Calorie restriction in adulthood can help maintain a normal metabolic profile.
Despite a normal metabolic profile, response of the HC\PF islets to glucose is not normal.
Islet Responses

-Acetylcholine application increases insulin production more in the three experimental groups
-Oxymetazoline inhibited insulin secretion less in the three experimental groups
-Calcium deprivation shows insulin signaling in pancreatic islets is disrupted in experimental groups
## mRNA Expression

<table>
<thead>
<tr>
<th>Expressed Protein</th>
<th>Pattern</th>
<th>Interpretation</th>
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<tbody>
<tr>
<td>NPY</td>
<td>HC, HC Pair-fed, and HC/PF/AL pattern together</td>
<td>Metabolic programming</td>
</tr>
<tr>
<td>Pomc</td>
<td>HC, HC Pair-fed, and HC/PF/AL pattern together</td>
<td>Metabolic programming</td>
</tr>
<tr>
<td>Lepr</td>
<td>HC and HC/PF/AL pattern together, Mother-fed and pair-fed pattern together</td>
<td>Caloric intake</td>
</tr>
<tr>
<td>Stat3</td>
<td>No significant differences between groups with possible trends</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>Socs3</td>
<td>HC, HC Pair-fed and HC/PF/AL pattern together</td>
<td>Metabolic Programming</td>
</tr>
</tbody>
</table>
Conclusions

• Calorie restriction creates a similar metabolic profile between MF and HC rats
• Calorie restriction does not change hypothalamic and pancreatic islet signals that lead to obesity in HC rats
• Early high-carb feeding leads to permanent changes in metabolic regulation
• These changes can be modulated by calorie restriction, but not reversed


