Programming metabolic and neurological diseases in the NHP: Impact of poor maternal health and diet

Kevin L. Grove
Director of Metabolic Disease
Oregon National Primate Research Center
Oregon Health & Science University

Childhood Obesity

Fetal/Neonatal Imprinting?
Maternal Obesity

• Rates of obesity in pregnant women are rapidly increasing (Lu et al. 2001).

• Currently, at least a third of all pregnant women in the US are obese (King 2006).

• Goal: Use a Nonhuman primate model to examine the impact of maternal obesity on offspring’s risk of developing obesity and energy balance regulation.
Maternal Obesity & disease risk in offspring

• Obesity – associated complications
  – inflammation
  – Hyperinsulinemia – insulin resistance
• Pregnancy weight gain
• Hyperglycemia (GDM)
• Diet – sugars, carbohydrates and/or fats
Experimental Groups

Maternal Diet

CTR (15% Calories from Fat)

HFD (32% of Calories from Fat)

McCurdy et al. 2009
Placental function is key to a healthy pregnancy and normal fetal development

- Hyperinsulinemia and hyperglycemia (GDM) cause complications in placental function.

- What are the potential impacts of HFD consumption?
  - Inflammation
  - Vascular dysfunction

Ragavendra et al., Placenta 2001
Impact of maternal diet on placental health

C Uterine Artery Volume Blood Flow

Frias et al, Endocrinology, 2010
These are actually decreases in inflammatory markers. Sex differences in inflammation associated with obesity. These two significant differences were not observed in fetal offspring.

<table>
<thead>
<tr>
<th>Target</th>
<th>Alternate</th>
<th>Control</th>
<th>HF Diet</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5a</td>
<td>CD154</td>
<td>2.64 ± 1.06</td>
<td>2.15 ± 0.32</td>
<td>0.68</td>
</tr>
<tr>
<td>CD40 ligand</td>
<td>CD154</td>
<td>0.51 ± 0.31</td>
<td>0.57 ± 0.12</td>
<td>0.87</td>
</tr>
<tr>
<td>G-CSF</td>
<td>CSFβ</td>
<td>1.09 ± 0.50</td>
<td>0.72 ± 0.13</td>
<td>0.52</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>CSFα</td>
<td>1.33 ± 0.47</td>
<td>0.64 ± 0.09</td>
<td>0.22</td>
</tr>
<tr>
<td>GROα</td>
<td>CXCL1</td>
<td>2.50 ± 0.41</td>
<td>0.98 ± 0.19</td>
<td>0.03*</td>
</tr>
<tr>
<td>I-309</td>
<td>CCL-1</td>
<td>0.84 ± 0.33</td>
<td>0.27 ± 0.12</td>
<td>0.18</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>CD54</td>
<td>0.46 ± 0.23</td>
<td>0.14 ± 0.05</td>
<td>0.25</td>
</tr>
<tr>
<td>IFNγ</td>
<td></td>
<td>0.69 ± 0.14</td>
<td>0.16 ± 0.06</td>
<td>0.02*</td>
</tr>
<tr>
<td>IL-1α</td>
<td>IL-1F1</td>
<td>0.68 ± 0.39</td>
<td>0.74 ± 0.13</td>
<td>0.89</td>
</tr>
<tr>
<td>IL-1β</td>
<td>IL-1F2</td>
<td>0.39 ± 0.26</td>
<td>0.58 ± 0.05</td>
<td>0.51</td>
</tr>
<tr>
<td>IL-β</td>
<td>IL-IF3</td>
<td>2.76 ± 0.69</td>
<td>2.09 ± 0.28</td>
<td>0.42</td>
</tr>
<tr>
<td>IL-2</td>
<td></td>
<td>0.86 ± 0.31</td>
<td>0.38 ± 0.13</td>
<td>0.22</td>
</tr>
<tr>
<td>IL-4</td>
<td></td>
<td>0.55 ± 0.17</td>
<td>0.21 ± 0.10</td>
<td>0.17</td>
</tr>
<tr>
<td>IL-5</td>
<td></td>
<td>0.38 ± 0.16</td>
<td>0.11 ± 0.06</td>
<td>0.19</td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td>0.34 ± 0.19</td>
<td>0.17 ± 0.05</td>
<td>0.45</td>
</tr>
<tr>
<td>IL-8</td>
<td></td>
<td>2.38 ± 0.26</td>
<td>3.17 ± 2.0</td>
<td>0.72</td>
</tr>
<tr>
<td>IL-10</td>
<td></td>
<td>0.49 ± 0.32</td>
<td>0.61 ± 0.10</td>
<td>0.73</td>
</tr>
<tr>
<td>IL-12p70</td>
<td></td>
<td>0.40 ± 0.28</td>
<td>0.58 ± 0.07</td>
<td>0.58</td>
</tr>
<tr>
<td>IL-13</td>
<td></td>
<td>2.89 ± 0.75</td>
<td>1.92 ± 0.22</td>
<td>0.28</td>
</tr>
<tr>
<td>IL-16</td>
<td></td>
<td>1.79 ± 0.47</td>
<td>0.96 ± 0.24</td>
<td>0.19</td>
</tr>
<tr>
<td>IL-17</td>
<td></td>
<td>0.86 ± 0.24</td>
<td>0.32 ± 0.15</td>
<td>0.13</td>
</tr>
<tr>
<td>IL-17E</td>
<td></td>
<td>0.84 ± 0.35</td>
<td>0.29 ± 0.13</td>
<td>0.21</td>
</tr>
<tr>
<td>IL-23</td>
<td></td>
<td>0.58 ± 0.26</td>
<td>0.32 ± 0.15</td>
<td>0.44</td>
</tr>
<tr>
<td>IL-27</td>
<td></td>
<td>1.17 ± 0.06</td>
<td>0.58 ± 0.30</td>
<td>0.13</td>
</tr>
<tr>
<td>IL-32α</td>
<td></td>
<td>1.28 ± 0.71</td>
<td>0.96 ± 0.06</td>
<td>0.68</td>
</tr>
<tr>
<td>IP-10</td>
<td>CXCL10</td>
<td>0.70 ± 0.15</td>
<td>0.92 ± 0.01</td>
<td>0.23</td>
</tr>
<tr>
<td>I-TAC</td>
<td>CXCL11</td>
<td>4.14 ± 0.78</td>
<td>5.28 ± 0.82</td>
<td>0.37</td>
</tr>
<tr>
<td>MCP-1</td>
<td>CCL2</td>
<td>0.95 ± 0.30</td>
<td>0.57 ± 0.26</td>
<td>0.40</td>
</tr>
<tr>
<td>MIF</td>
<td>GIF</td>
<td>12.37 ± 1.71</td>
<td>6.42 ± 1.59</td>
<td>0.06</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>CCL3</td>
<td>1.05 ± 0.44</td>
<td>0.40 ± 0.19</td>
<td>0.24</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>CCL4</td>
<td>0.62 ± 0.26</td>
<td>0.32 ± 0.14</td>
<td>0.37</td>
</tr>
<tr>
<td>Serpin E1</td>
<td>PAI-1</td>
<td>9.24 ± 0.29</td>
<td>6.88 ± 3.84</td>
<td>0.57</td>
</tr>
<tr>
<td>RANTES</td>
<td>CCL5</td>
<td>20.76 ± 9.48</td>
<td>20.33 ± 8.55</td>
<td>0.98</td>
</tr>
<tr>
<td>SDF-1</td>
<td>CXCL12</td>
<td>1.72 ± 0.98</td>
<td>1.24 ± 0.07</td>
<td>0.65</td>
</tr>
<tr>
<td>TNFα</td>
<td></td>
<td>1.16 ± 0.47</td>
<td>0.97 ± 0.31</td>
<td>0.74</td>
</tr>
<tr>
<td>sTREM-1</td>
<td></td>
<td>1.01 ± 0.43</td>
<td>0.56 ± 0.30</td>
<td>0.44</td>
</tr>
</tbody>
</table>

HFD vs CTR (n = 3 per group)  
Frias et al, Endo 2011
Suggests that fetal inflammatory cytokines coming from placenta, not maternal circulation

Table 3: Plasma expression of Cytokines using the Proteome Profiler™ Array in CTR and HFD G130 fetuses

<table>
<thead>
<tr>
<th>Target</th>
<th>Alternate</th>
<th>O-CTR</th>
<th>O-HFD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5a</td>
<td></td>
<td>0.14 ± 0.01</td>
<td>0.43 ± 0.15</td>
<td>0.198</td>
</tr>
<tr>
<td>CD40 Ligand</td>
<td>CD154</td>
<td>0.11 ± 0.02</td>
<td>0.30 ± 0.06</td>
<td>0.031</td>
</tr>
<tr>
<td>G-CSF</td>
<td>CSFβ</td>
<td>0.14 ± 0.01</td>
<td>0.28 ± 0.05</td>
<td>0.045</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>CSFα</td>
<td>0.14 ± 0.02</td>
<td>0.27 ± 0.05</td>
<td>0.079</td>
</tr>
<tr>
<td>GROα</td>
<td>CXCL1</td>
<td>0.16 ± 0.02</td>
<td>0.47 ± 0.18</td>
<td>0.222</td>
</tr>
<tr>
<td>I-309</td>
<td>CCL1</td>
<td>0.11 ± 0.01</td>
<td>0.28 ± 0.07</td>
<td>0.132</td>
</tr>
<tr>
<td>IFNγ</td>
<td></td>
<td>0.11 ± 0.02</td>
<td>0.28 ± 0.07</td>
<td>0.153</td>
</tr>
<tr>
<td>IL-1α</td>
<td>IL-1F1</td>
<td>0.10 ± 0.02</td>
<td>0.29 ± 0.04</td>
<td>0.014</td>
</tr>
<tr>
<td>IL-1β</td>
<td>IL-1F2</td>
<td>0.08 ± 0.02</td>
<td>0.25 ± 0.06</td>
<td>0.051</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>IL-1F3</td>
<td>0.36 ± 0.13</td>
<td>0.68 ± 0.13</td>
<td>0.161</td>
</tr>
<tr>
<td>IL-2</td>
<td></td>
<td>0.14 ± 0.03</td>
<td>0.25 ± 0.06</td>
<td>0.173</td>
</tr>
<tr>
<td>IL-4</td>
<td></td>
<td>0.13 ± 0.03</td>
<td>0.25 ± 0.08</td>
<td>0.219</td>
</tr>
<tr>
<td>IL-5</td>
<td></td>
<td>0.09 ± 0.02</td>
<td>0.27 ± 0.05</td>
<td>0.027</td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td>0.07 ± 0.01</td>
<td>0.27 ± 0.07</td>
<td>0.106</td>
</tr>
<tr>
<td>IL-8</td>
<td>CXCL8</td>
<td>0.09 ± 0.01</td>
<td>0.50 ± 0.21</td>
<td>0.186</td>
</tr>
<tr>
<td>IL-10</td>
<td></td>
<td>0.07 ± 0.02</td>
<td>0.22 ± 0.03</td>
<td>0.017</td>
</tr>
<tr>
<td>IL-12 p70</td>
<td></td>
<td>0.11 ± 0.03</td>
<td>0.25 ± 0.03</td>
<td>0.019</td>
</tr>
<tr>
<td>IL-13</td>
<td></td>
<td>0.30 ± 0.07</td>
<td>0.31 ± 0.06</td>
<td>0.857</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Target</th>
<th>Alternate</th>
<th>O-CTR</th>
<th>O-HFD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-16</td>
<td>LCF</td>
<td>0.17 ± 0.03</td>
<td>0.41 ± 0.12</td>
<td>0.113</td>
</tr>
<tr>
<td>IL-17</td>
<td></td>
<td>0.13 ± 0.03</td>
<td>0.28 ± 0.07</td>
<td>0.117</td>
</tr>
<tr>
<td>IL-17E</td>
<td></td>
<td>0.09 ± 0.02</td>
<td>0.31 ± 0.06</td>
<td>0.023</td>
</tr>
<tr>
<td>IL-23</td>
<td></td>
<td>0.09 ± 0.02</td>
<td>0.27 ± 0.07</td>
<td>0.141</td>
</tr>
<tr>
<td>IL-27</td>
<td></td>
<td>0.11 ± 0.01</td>
<td>0.28 ± 0.05</td>
<td>0.033</td>
</tr>
<tr>
<td>IL-32α</td>
<td></td>
<td>0.09 ± 0.02</td>
<td>0.28 ± 0.05</td>
<td>0.022</td>
</tr>
<tr>
<td>IP-10</td>
<td>CXCL10</td>
<td>0.12 ± 0.03</td>
<td>0.30 ± 0.03</td>
<td>0.013</td>
</tr>
<tr>
<td>I-TAC</td>
<td>CXCL11</td>
<td>0.17 ± 0.03</td>
<td>0.39 ± 0.07</td>
<td>0.051</td>
</tr>
<tr>
<td>MCP-1</td>
<td>CCL2</td>
<td>0.15 ± 0.02</td>
<td>0.30 ± 0.05</td>
<td>0.069</td>
</tr>
<tr>
<td>MIF</td>
<td>GIF</td>
<td>2.63 ± 0.51</td>
<td>4.85 ± 0.86</td>
<td>0.091</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>CCL3</td>
<td>0.26 ± 0.06</td>
<td>1.00 ± 0.20</td>
<td>0.024</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>CCL4</td>
<td>0.11 ± 0.00</td>
<td>0.27 ± 0.06</td>
<td>0.123</td>
</tr>
<tr>
<td>RANTES</td>
<td>CCL5</td>
<td>0.50 ± 0.08</td>
<td>3.92 ± 1.02</td>
<td>0.079</td>
</tr>
<tr>
<td>SDF-1</td>
<td>CXCL12</td>
<td>0.13 ± 0.02</td>
<td>0.30 ± 0.06</td>
<td>0.053</td>
</tr>
<tr>
<td>Serpin E1</td>
<td>PAI-1</td>
<td>1.36 ± 0.22</td>
<td>3.44 ± 0.77</td>
<td>0.059</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>CD54</td>
<td>0.09 ± 0.01</td>
<td>0.25 ± 0.06</td>
<td>0.108</td>
</tr>
<tr>
<td>sTREM-1</td>
<td>CD54</td>
<td>0.12 ± 0.02</td>
<td>0.30 ± 0.06</td>
<td>0.036</td>
</tr>
<tr>
<td>TNFα</td>
<td>TNFSF1A</td>
<td>0.12 ± 0.02</td>
<td>0.27 ± 0.05</td>
<td>0.042</td>
</tr>
</tbody>
</table>
Summary - Placenta

MATERNAL

- Dietary Lipids
- Insulin resistant obesity
- Postprandial sat. FFA
- N3-FFA
- N6/N3 ratio

Fetal

- Cytokines
  - Oxidative damage
  - Insulin resistance
  - Metabolic mal-programming
  - Pregnancy complications

Placenta

- Cytokines
- TLR4 (?)
Summary: Maternal HFD and Fetal development

• Placental insufficiency (Frias)
  – Increased cytokine production
  – Decreased N3 fatty acids & increased N6/N3 ratio (Gillingham)

• Small fetal weights
  – Catch up growth and risk of obesity

• Increased liver TGs (McCurdy/Thorn/Friedman; Grant/Marks)
  – Increased apoptosis
  – Risk of NAFLD

• Abnormal development of melanocortin & 5HT systems (Grayson and Sullivan)
  – Likely due to increased inflammatory cytokines
  – Hyperphagia and obesity
  – Anxiety / depression disorders

• Suppression of islet differentiation factors (Comstock)
  – Islet development
Inflammatory Cytokines modulate melanocortins

Grayson et al, Endocrinology 2010
Maternal HFD Consumption Results in Abnormal Development of the Fetal Melanocortin System

Maternal HFD $\rightarrow$ ↑ POMC mRNA

Maternal HFD $\rightarrow$ ↓ AGRP mRNA & protein

Fetal HFD Offspring Display Perturbations in the Central 5-HT System

Sullivan et al. 2010 J. Neuroscience 2010;30 3826-3830
Melanocortin and Serotonin Circuits are Important Regulators of Energy Homeostasis

Dorsal Raphe 5HT

Lipids Cytokines

Grayson et al. Endocrin. 2010
Sullivan et al. J. Neurosci. 2010
Fetal Consequences

- Not dependent on maternal obesity or insulin resistant
- Reversible with switching obese animals to a chow diet.
Why is the developing fetus so vulnerable?

- Placental deficiency is associated with numerous health risks in offspring.
  - Increased risk of cardiovascular disease: humans, sheep and rodents
  - Restricted fetal growth—catch up growth and obesity
- Nutrient restriction or inappropriate nutrients
  - Fetus is preferentially glycolytic
- Susceptibility to lipotoxicity
  - Adipose tissue doesn’t develop until late 3rd trimester
- Placental inflammatory cytokines
  - Many conditions result in placental cytokine production (GDM, Maternal obesity, etc)
Postnatal Groups

Weaned at approximately 7 months and euthanized at 13 months

Critical Periods:
- Preconception
- Conception
- Placentation
- Organogenesis
- Early/late Brain
- Early/late Pancreas
- Early postnatal
- Post weaning
Hypothalamic melanocortin system in juvenile offspring

AgRP stimulates food intake

αMSH inhibits food intake

offspring at 13 mo of age

No long term change in POMC, AgRP mRNA expression in the ARH of juvenile offspring
Maternal HFD Consumption leads to a *Decrease* in Dorsal Raphe TPH2 mRNA Expression *Female* Juvenile Offspring

Female offspring at 13 mo of age

CTR/CTR

HFD/CTR

Increased 5HT 1A N/C SERT

**DR TPH2 mRNA**

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR/CTR</td>
<td><img src="CTR.png" alt="CTR/CTR" /></td>
<td><img src="CTR.png" alt="CTR/CTR" /></td>
</tr>
<tr>
<td>HFD/CTR</td>
<td><img src="HFD.png" alt="HFD/CTR" /></td>
<td><img src="HFD.png" alt="HFD/CTR" /></td>
</tr>
</tbody>
</table>

Area (Relative units)

- **CTR/CTR**
- **HFD/CTR**
Maternal HFD Results in a Decrease in CSF 5-HT in Juvenile Offspring
What are the long-term effects of fetal lipotoxicity?

• Abnormal development of the melanocortin and 5HT systems
  – Predictive effects on body weight homeostasis
    • Food intake
    • Energy expenditure
    • Glucose homeostasis
  – Stress responsiveness (HPA)
  – Anxiety
Food Preference Test - HFD
HFD animals eat more normal chow throughout the day.
HFD animals display differences in food preferences and binge eating.
Summary: Maternal + postnatal HFD

• Abnormal development of melanocortin & 5HT systems
  – Likely due to increased inflammatory cytokines

• Increased adiposity
  – Glucose intolerance and insulin resistance
  – Pancreatic islet hyperplasia
  – Hyperphagia of palatable diets
  – Fatty liver
  – Cardiac hypertrophy – endothelial dysfunction
In Humans, Maternal Energy Status Influences Risk for Psychiatric & Behavioral Diseases

- Adults whose mothers experienced *malnutrition during pregnancy* are at increased risk for developing schizophrenia (St Clair, Xu et al. 2005).

- **Maternal obesity** is associated with children’s risk of developing attention deficit hyperactivity disorder (Ray GT, Croen LA, et al. 2009).
Maternal HFD Consumption Leads to Increased Latency to Explore Novel Objects in Female Offspring Suggesting Increased Anxiety

(Pre-weaning; 4 mo of age)

Sullivan et al. 2010  J. Neuroscience 2010; 30 3826-3830
HFD Offspring Display Social Withdrawal
(10 mo of age)

**Percent of Time Spent Alone**

- **CTR**: 20%
- **HFD**: 60%

**Percent of Time Engaged in Social Play**

- **CTR**: 4%
- **HFD**: 8%

**Time Spent in Contact (sec)**

- **CTR**:
  - Initiate: 50 sec
  - Receive: 100 sec
- **HFD**:
  - Initiate: 200 sec
  - Receive: 150 sec
Broad abnormalities in metabolic efficiency

The true impact may be later in life – premature aging
Conclusions: *Behavior*

- Offspring from HFD mothers are at increased risk for developing behavioral disorders:
  - **Female** HFD offspring display increased anxiety
  - *Male & female* HFD offspring display social withdrawal (associated with depression)
  - *Male* HFD offspring display increased aggression

- These results are consistent with studies in humans, but identify an important underlying cause.

- These broad behavioral abnormalities have relevance to ADHD, learning disorders, and even Autism

- These studies will change how we think of and potentially treat behavioral disorders in children.
Maternal HFD Consumption Has an Enduring Impact on the Developing Offspring

Sullivan and Grove 2010
Grove/Smith Group
Dr. M. Susan Smith
Dr. Bernadette Grayson
Dr. Maria Glavas
Dr. Elinor Sullivan (U of P)
Dr. Paul Kievit
Dr. Li Fan (Beijing)
Dr. Antonio Frias (Ob/Gyn)
Dr. Jonathan Stoehr
Dr. Saurabh Verma
Dr. Lynley Pound
Dr. Arain Baquero
Sarah Williams
Cadence True
Shin Draper

Dr. Jed Friedman and Lab (UC Denver)
Dr. Carrie McCurdy – fatty liver studies
Dr. Daniel Marks (OHSU) – inflammatory system
Dr. Melanie Gillingham (OHSU) – Lipid Analysis

Epigenetic Modification
Dr. Robert Lane (Univ. Utah)
Dr. Kjersti Tillery-Aagaard (Baylor)

Placental/Cardiovascular Function
Dr. Kent Thornburg (OHSU)

Respiratory & Immune Dysfunction
Dr. Eliot Spindel (ONPRC)
Dr. Ilhem Messaoudi (ONPRC)

Melissa Kirigiti  Amanda Vedder
Diana Takahashi  Ashely Kostbra
Lindsay Pranger  Rene Lindsley
Anne Evans  Jeanette Valleau
Karalee Baquero  Elizabeth Hoyt
Leigh Ann Bauman  Peter Blundell

DAR staff, Hormone Core, Imaging Core
NIDDK and NICHD ONPRC Pilot Project
Summary: Effects of Diet reversal

• Maternal diet reversal
  – Chronic HFD moms switched to a chow diet just prior to pregnancy
  – Reversed most of the abnormalities in the fetal offspring (including brain, liver and pancreas)

• Diet reversal of juvenile offspring at weaning
  – Increased AgRP/MSH ratio and orexigenic drive for palatable diets
  – No improvement in serotonin system in females
  – Partial improvement in CV endpoints
  – Partial improvement in pancreas islet
Fetal Origins of Disease
Hales and Barker

• Thrifty phenotype hypothesis
  – Based on maternal under- (mal-) nutrition
  – Placental insufficiency
  – Maternal protein restriction

• Why does maternal over-nutrition or postnatal over-feeding result in a familiar phenotype?

• Common links with gestational diabetes and/or maternal obesity.
Food Preference Test - control
5-HT<sub>1A</sub>R & SERT mRNA Expression

**5-HT<sub>1A</sub>R mRNA Expression Increases with Exposure To HFD (both male & female) → 5-HT tone**

**No Impact of HFD on SERT Expression**
Maternal HFD causes a small decrease in fetal weight (early 3rd trimester)

McCurdy et al, JCI, Feb 2009

G130 fetus < 0.1% body fat

Cardiovascular complications?