Maternal Obesity and the Developmental Programming of Offspring Appetite and obesity: A role for leptin?

Paul D Taylor, Anne-Maj Samuelsson, Clive W Coen and Lucilla Poston.
Maternal Obesity

Glucose, insulin, leptin, lipids, inflammatory response

Fetal macrosomia, increased adiposity Persistently altered energy balance

Childhood and Adulthood Obesity

Transgenerational ‘Acceleration ‘ of Obesity?
Maternal pre-pregnancy BMI and gestational weight gain
Risk of offspring obesity: Hochner 2012 Jerusalem study

- Adjusted means of offspring obesity outcomes at age 32 by quartiles of maternal pp BMI and GWG
- Greater mppBMI, independently of GWG, was significantly associated with higher offspring BMI, and WC.
- The observed associations were independent of characteristics reflecting the pre- peri- and post-natal environment, including current measures of SES and lifestyle.
- A similar associations of mppBMI and GWG for DBP and SBP was observed, but this disappeared after controlling for offspring current BMI.
Why use animal models to study mechanisms of Developmental Programming?

- Mice, rats, and humans share all but 1% of each other's genes
- Reduce possible genetic influence
- Relatively quick life cycle
- Environment can be tightly controlled
- Enable different diets to be tested
- Investigate critical periods in development

“Future studies that explore mechanisms underlying the intergenerational cycle of obesity are warranted to identify potentially novel targets for cardiometabolic risk-reduction interventions”. Hochner et al 2012
Breeding Offspring of Obese Rats and Mice (OffOb)

Control Diet
Highly Palatable Obesogenic Diet: Pellets with 20% FAT + Sweetened Condensed Milk

Mating

Weaning

6 week Gestation & Lactation

6 week Gestation & Lactation

DAMS

OFFSPRING

Day 30 Characterization of pre-Obese Phenotype

Day 90 Characterization of Obese Phenotype

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Maternal Characteristics

A

Body weight (g)

-5 -4 -3 -2 -1 0 2 4 6 8 10 12 14 16 18 20

Time (weeks) Time (Gestational days)

Calorific intake (kcal animal⁻¹ day⁻¹)

-5 -4 -3 -2 -1 0 2 4 6 8 10 12 14 16 18 20

Time (weeks) Time (Gestational days)

Control Dam Obese Dam

Fat mass (g)

34.4 14.8

Lean mass (g)

18.8 20.5

Control Obese

BAT mass (g)

0 0.1 0.2 0.3

Control Obese

WAT mass (g)

0 1 2 3 4 5 6 7

Control Obese

***

Lean mass (g)

20.5 18.8

Fat mass (g)

3.6 9.8

% Fat

14.8 34.4

Control Dam Obese Dam

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Maternal Characteristics

Late gestation

Weaning
Offspring Phenotype: hyperphagia and adult obesity

A

MALE

FEMALE

Calorific intake
(kcal animal-1 week-1)

Time (weeks)

B

body weight (g)

Time (weeks)

C

Concentrations

Time (weeks)
Summary (1): Maternal Obesity in rodents

Maternal Phenotype

- High-fat high-sugar diet leads to hypercalorific intake & maternal obesity.
- Hyperleptinaemia, hyperinsulinaemia in pregnancy
- Further hyperglycaemia, dyslipidaemia during suckling.

Offspring Phenotype

- Increased birthweight & weight at weaning (milk content)
- Hyperphagic and hypertensive from weaning
- Abdominal obesity, hyperleptinaemia, dyslipidaemia, hyperinsulinaemia and reduced muscle mass by 3 months.
- Hyperglycaemia (type 2 DM) and fatty liver disease (NAFLD) by 6 months.
Neonatal serum leptin and insulin in OffOb

Figure 1. Neonatal serum leptin and insulin concentrations in offspring of control and obese dams. Serum leptin (A) and insulin (B) were measured in offspring of control dams (open bars) and obese dams (closed bars) over the suckling period. * p<0.05, ** p<0.01 and *** p<0.001 versus offspring of control dams for the same period (n = 3-6). For longitudinal comparisons, a significant difference (p<0.05) from the preceding period is indicated by # for offspring of control dams and by † for offspring of obese dams.

Fig 2. Leptin mRNA expression in adipose tissue from in offspring from control (open bars) and obese dams (closed bars). Day 2-8 (n=5-10), 9-11 (n=3-4), 13-18 (n=4-8) mRNA expressed as copy number divided by the geometric mean of 2 reference genes (28S, Actin beta), multiplied by 1000. Days 13-18, **P<0.005 vs control.
Leptin in Developmental Programming

- Postnatal leptin surge in rodents (Ahima 1998) – a key developmental signal for the hypothalamus.
- Leptin in early life modulates the formation of neural energy-regulation circuits in the hypothalamus (Bouret et al. 2004).
- Maternal undernutrition can influence the timing and amplitude of the leptin surge (Delahaye et al. 2008; Yura et al. 2005; Stocker et al. 2007; Attig et al. 2008).
- Genetic models of obesity?
Leptin in the neuro-development of the brain

Exogenous leptin rescues arcuate nucleus development in neonatal but not adult Lep$^{ob}$/Lep$^{ob}$ mice (Bouret et al, Science 2004)
Leptin Challenge in Day 30 Offspring

24 hr Food Intake

Males
- Saline
- Leptin

Females
- Saline
- Leptin

Δ Body Weight

Males
- Saline
- Leptin

Females
- Saline
- Leptin

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A role for the melanocortin system in programming of appetite?

From Ozanne et al, Clinical Science 2005
### Leptin Signalling in Day 30 Offspring

#### ARC pSTAT3-ir Cells

- **~3.30 mm Caudal to Bregma**

- **OffCon**
- **OffOb**

#### VMH pSTAT3-ir Cells

<table>
<thead>
<tr>
<th>Bregma</th>
<th>Group</th>
<th>Mean</th>
<th>SEM</th>
<th>n</th>
<th>p</th>
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<tr>
<td>VMH</td>
<td>-2.30 mm</td>
<td>OffCon</td>
<td>339</td>
<td>36</td>
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<td>&quot;</td>
<td></td>
<td>OffOb</td>
<td>304</td>
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<td>NS</td>
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<td>VMHdI</td>
<td>-3.30 mm</td>
<td>OffCon</td>
<td>1146</td>
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<td>OffOb</td>
<td>1152</td>
<td>288</td>
<td>NS</td>
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<tr>
<td>VMHvI</td>
<td>-3.30 mm</td>
<td>OffCon</td>
<td>765</td>
<td>50</td>
<td>8</td>
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<td>&quot;</td>
<td></td>
<td>OffOb</td>
<td>789</td>
<td>185</td>
<td>5</td>
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</table>
AgRP Immunoreactivity in Female PVH at Day 30

AgRP Fluorescence (Bregma -1.80 mm)

OffCon  OffOb

AgRP Fluorescence

AgRP Fluorescence (Bregma -1.80 mm)

AgRP Fluorescence

Bregma -1.80 mm

AgRP-Immunoreactivity (% Area PVH)

OffObOffCon  OffObOffCon

AgRP-Immunoreactivity (% Area PVH)
• Maternal obesity has adverse consequences for offspring energy balance including **leptin resistance and hyperphagia**.

• We hypothesise that leptin resistance in the ARC is **acquired** during a critical period due to an amplified and prolonged neonatal leptin surge.

• **Deficits in leptin-signalling** may impair leptin’s neurotrophic effects on AgRP projections to the PVH.

• Abnormal neuronal development in the appetite regulatory centres of the brain may permanently programme hyperphagia and therefore obesity in adult life.
Leptin Treatment in naïve rat pups Postnatal Day 9-15

Neonatal serum leptin concentrations achieved leptin (3mg/kg ip) and saline treated pups (male and female combined) ***P<0.001, **P<0.01 versus saline using t-test, n=4-7.
Figure S2. Weight gain and food intake (24hr) following a bolus leptin challenge (10 mg/kg i.p.) in fasted saline-treated (S-Tx) or leptin-treated (L-Tx) male and female rats at (A) 30 days and (B) 5 months of age. ***P<0.001, **P<0.01, *P<0.05 versus saline injection using t-test, n=8. Abbreviation S-Tx-saline treated; L-Tx-leptin treated.
Table S1. Body weight, inguinal WAT, heart –, and liver weight in 5- and 12-month-old male and female rats treated with saline or leptin in neonatal period.
### Table S1. Body weight, inguinal WAT, heart –, and liver weight in 5- and 12-month-old male and female rats treated with saline or leptin in neonatal period.

<table>
<thead>
<tr>
<th>Weight</th>
<th>5 months male</th>
<th>5 months female</th>
<th>12 months male</th>
<th>12 months female</th>
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<tr>
<td></td>
<td>S-Tx</td>
<td>L-Tx</td>
<td>S-Tx</td>
<td>L-Tx</td>
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<tr>
<td>Bodyweight (g)</td>
<td>222.9±6.9</td>
<td>238.2±12.1</td>
<td>188.5±10.9</td>
<td>194.9±9.1</td>
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<tr>
<td>Inguinal WAT (mg)</td>
<td>1.32±0.15</td>
<td>2.03±0.35*</td>
<td>1.01±0.15</td>
<td>1.86±0.05†</td>
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<tr>
<td>Heart (mg)</td>
<td>0.87±0.02</td>
<td>1.12±0.09*</td>
<td>0.71±0.02</td>
<td>0.82±0.03*</td>
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<tr>
<td>Liver (mg)</td>
<td>9.08±0.29</td>
<td>8.82±0.29</td>
<td>7.35±0.79</td>
<td>7.57±0.79</td>
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<tr>
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<td>600.0±29.6</td>
<td>631.3±23.7</td>
<td>346.8±7.2</td>
<td>402.2±17.2†</td>
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<tr>
<td></td>
<td>5.31±0.41</td>
<td>9.25±1.21†</td>
<td>5.23±0.76</td>
<td>12.3±1.35‡</td>
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<tr>
<td></td>
<td>1.61±0.03</td>
<td>1.77±0.03*</td>
<td>1.19±0.03</td>
<td>1.56±0.10†</td>
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<tr>
<td></td>
<td>16.8±0.42</td>
<td>19.1±1.00</td>
<td>9.16±0.60</td>
<td>10.77±0.05</td>
</tr>
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</table>
Sugar Preference Tests Performed at Day 30

Sucrose Preference (2%) at Day 30

Rats with experimental neonatal hyperleptinaemia have increased sucrose preference at Day 30.
Leptin’s Involvement in the Central Control of Mesolimbic Dopamine Pathway

Leinninger & Myers, 2008
D2 receptor binding

**D2 Binding Males**

- **Brain Region**
  - NAcc Sh
  - NAcc Core
  - CPu Med
  - CPu Lat
  - Olf Tub

- **Specific [3H]-Raclopride binding nCi/mg**

- **Legend**
  - Vehicle treated
  - Leptin-treated

**D2 Binding Females**

- **Brain Region**
  - NAcc Sh
  - NAcc Core
  - CPu Med
  - CPu Lat
  - Olf Tub

- **Specific [3H]-Raclopride binding nCi/mg**

**Total binding:** [3H]-Raclopride

**Non specific binding:** [3H]-Raclopride + Butaclamol

**Nissl Stain**

- CPu Lat
- CPu Med
- NAcc Core
- NAcc Sh
- Olf Tub

**D2 Binding**

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**Mu Opioid Receptor Binding**

**Mu Opioid Binding Males**

![Graph showing mu opioid binding in males with Vehicle-treated and leptin-treated groups.]

**Mu Opioid Binding Females**

![Graph showing mu opioid binding in females with Vehicle-treated and leptin-treated groups.]

*Total Binding: [3H]-DAMGO
*Non Specific Binding: [3H]-DAMGO + Naloxone
Leptin receptors on dopaminergic neurons in the VTA and also on neurotensin neurons in the lateral hypothalamus influence dopamine VTA neurons (Leinninger & Myers, 2008).

Leptin resistance at these sites may increase dopaminergic activity in the VTA, thereby leading to down-regulation of receptors in the nucleus accumbens.

The extent to which these receptor changes underlie the increased sucrose preference remains to be determined.
Overall Summary

• High leptin levels during a critical period for hypothalamic neurodevelopment can permanently influence the regulation of appetite and energy balance resulting in hyperphagia and obesity in the offspring of obese rodents.

• Experimental Hyperleptinaemia in the immediate postnatal period mimics the leptin resistant and obese phenotype we have previously described in offspring of obese rodents.

• Neonatal leptin exposure programmes a ‘selective leptin resistance’ in which the anorectic action of leptin is lost (the cardiovascular response to leptin is preserved).

• Hyperleptinaemia in the immediate postnatal period affects sucrose preference and reward-related receptor density in the mesolimbic dopamine pathway.

• Increased perinatal leptin exposure secondary to maternal obesity may predispose to increased appetite, altered food preference and risk of obesity.
Maternal Obesity

- Glucose, insulin, leptin, lipids, inflammatory response

Increased neonatal adiposity
Persistently altered energy balance

Offspring Obesity

Transgenerational ‘Acceleration’ of Obesity?
Developmental origin of leptin resistance

Fetal/neonatal Hyperleptinaemia & Hyperinsulinaemia?

Excessive Weight Gain & Adiposity

Selective leptin resistance

↓ Metabolic action

Hyperphagia & Obesity

Hyperinsulinaemia?

↑ SNS activity

Hypertension

Pregnancy & Lactation

Offspring Phenotype

Schematic: mechanisms in fetal programming of obesity and hypertension.
Relevance to human obesity?

- Rodents are *altricial species*: The relative maturity between the human brain and that of the rodent post-partum requires consideration.
- Non-human primates and sheep support translation to precocial species and provide evidence for postnatal development of neurite projections.
- Nothing is known about the sensitivity of the developing human hypothalamic circuitry to leptin in late pregnancy/early infancy.
- Susceptibility to the *neurotrophic* influences of leptin could also occur in both ante-natal and post-partum periods.
- Cord blood leptin concentration is elevated in neonates, falls rapidly post-partum, - developmental signal?.
- Maternal obesity associated with raised cord blood leptin (Catalano et al 2009).
- On-going RCT intervention studies in obese pregnant women offer a unique opportunity to study effects on childhood appetitive behaviour and obesity risk.
Thanks to:

Developmental Programming Research Group

Principle Investigators
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Professor Clive Coen (Professor of Neuroscience)
Dr Paul Taylor (Senior Lecturer in Developmental Programming, HEFCE)

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Dr Olena Rudyk (Research Fellow, BHF)
Dr Shona Kirk (Research Fellow, BHF)
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Dr Laura Cox (Texas) Dr Caroline Relton (UK)
Dr Susan Ozanne (UK) Dr Eugene Jensen (BE)
Dr Claude Remacle (BE), Dr Jude Oben (UCL).
Leptin impacts the peptidergic innervation of preautonomic divisions in adult Ob/Ob mouse PVH

Maternal HFD Feeding Exclusively during Lactation Predisposes the Offspring for Metabolic Disorders

Maternal HFD Feeding Exclusively during Lactation Predisposes the Offspring for Metabolic Disorders and Impairs Axonal Projections of ARH Neurons to Intrahypothalamic Target Sites

Fiber Density $\text{PVH}_{\text{ant}}$

Fiber Density $\text{PVH}_{\text{post}}$

POMC-specific IR Deficiency in NCD/HFD offspring Rescues POMC Axonal Projections to Preautonomic Regions in the PVH
Milk Contents (Pre-weaning Stomach Contents)

A. Leptin (pg/ml)

B. Cholesterol (umol/g)

C. Free Fatty Acids (FFA)

D. Triglycerides (umol/g)

E. Glucose (umol/g)

Postnatal Day
Milk Contents (Pre-weaning Stomach Contents)

A. Leptin (pg/ml)
- Days: 2, 7-8, 9-11, 13-14, 15-18
- Significance: **, #

B. Cholesterol (umol/g)
- Days: 2, 7-8, 9-11, 13-14, 15-18
- Significance: *, **, †

C. Free Fatty Acids (FFA) (umol/g)
- Days: 2, 7-8, 9-11, 13-14, 15-18
- Significance: *, #

D. Triglycerides (umol/g)
- Days: 2, 7-8, 9-11, 13-14, 15-18
- Significance: †

E. Glucose (umol/g)
- Days: 2, 7-8, 9-11, 13-14, 15-18
- Significance: **, #, †
# 30 day Body weights and Organ weights in L-Tx rats

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<tr>
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<td>0.18±0.03</td>
<td>0.20±0.03</td>
</tr>
<tr>
<td>Heart weight (mg)</td>
<td>0.35±0.02</td>
<td>0.41±0.01*</td>
</tr>
<tr>
<td>Liver weight (mg)</td>
<td>2.53±0.13</td>
<td>2.58±0.10</td>
</tr>
<tr>
<td>Heart weight (mg/g BW)</td>
<td>5.53±0.17</td>
<td>6.34±0.28*</td>
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<tr>
<td>Liver weight (mg/g BW)</td>
<td>40.2±2.9</td>
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**Table 1.** Body weight, inguinal WAT, and heart weight in 30 day-old male and female rats treated with saline (S-Tx) or leptin (L-Tx) in neonatal period. Values given as mean± SEM. *P<0.05 versus saline using t-test, n=4-8.
30 day Body weights and Organ weights in L-Tx rats

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<tr>
<td>Heart weight (mg)</td>
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<td>0.41±0.01*</td>
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<td>0.42±0.01*</td>
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<td>Liver weight (mg)</td>
<td>2.53±0.13</td>
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<td>39.1±1.7</td>
<td>38.7±2.0</td>
<td>42.3±2.6</td>
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