Early Life Origins of Obesity: Role of Hypothalamic Programming

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A Striking Example of Environmental Control Phenotypic Polymorphism

Honey bee larvae with identical DNA

Worker Food

Royal Jelly

Worker

Queen

↑ weight (x2)

↑ longevity (x20)

↑ fertility
Early Growth and Later Risk of Diabesity in Humans

Perinatal Food Restriction

↑ Obesity
↑ Diabetes
↑ Cardiovascular Risks

Perinatal Overfeeding
Catch-up Growth
Key Role for the Brain in Body Weight Regulation

Hetherington and Ranson, 1940
Reeves, 1969
Hypothalamic Circuits Involved in Appetite Regulation

Bouret, JPGN, 2009
Critical Periods for Hypothalamic Development

from Bouret, Forum Nutr, 2010
“Bedside to Bench”: Animal Models of Postnatal Over- and Under-nutrition

- Mechanistic studies can easily be done in animal models
- Brain feeding pathways are highly conserved during evolution
- Studies in the human hypothalamus have been limited in part by instrument resolution
Mouse Model of Postnatal Over- and Under-nutrition: Divergent Litter Size

Small Litter (3) overnourished

Normal Litter (7)

Large Litter (14) undernourished

![Graph showing body weight over postnatal days for Small Litter, Normal Litter, and Large Litter groups.](image)
Adult Growth of Neonatally Under- and Over-Nourished Animals

- Normal Litter (7)
- Large Litter (14) undernourished
- Small Litter (3) overnourished

Body Weight (g)

Catch-Up Growth

Postnatal Day

P4-P22
Method to Study Development of Hypothalamic Feeding Circuits

adapted from, www.hhmi.org “Science of Fat”
Impact of Early Postnatal Nutrition on Formation of Hypothalamic Neural Projections

- Normal Fed (P12)
- Overfed (SL) (P12)
- Underfed (LL) (P12)

![Images showing the hypothalamic neural projections in different feeding conditions.](Image)

![Bar graph showing the density of arcuate Dil-labeled fibers in the PVH.](Image)

- Density of arcuate Dil-labeled fibers in the PVH:
  - SL: b
  - NL: a
  - LL: b

Note: The bar graph indicates significant differences among the groups, with different letters representing significant differences at the p < 0.05 level.
Secretion Pattern of Peripheral Signals in Postnatally Under- and Over-Nourished Animals

**Cytokines**

- IL-6 (P4, P8, P12)
- TNF-α (P4, P8, P12)

**Chemokines**

- MCP-1 (P4, P8, P12)

**Metabolic Hormones**

- Insulin (P4, P8, P12, P18)
- Leptin (P4, P6, P8, P10, P12, P16, P18, P20, P22)
- Resistin (P4, P8, P12)
Is Leptin a Signal for Hypothalamic Development?

Leptin Relative Levels

Postnatal Age (weeks)

Weaning

Body Weight (g)

Postnatal Age (days)

0 7 8 9 10 11

Leptin

Vehicle

Caron et al., J Comp Neurol, 2010

Leptin-induced pSTAT3

Leptin Receptor mRNA

V3

ARH

me

me

V3

ARH

Caron et al., J Comp Neurol, 2010
Leptin Deficiency and Hypothalamic Neural Projections
Leptin Acts during a Neonatal Critical Period to Induce its Neurodevelopmental Effects

**Neonatal Treatment**

- $+/+$
- $ob/ob$
- $ob/ob +$ leptin

**PVH**

**Adult Treatment**

- $+/+$
- $ob/ob$
- $ob/ob +$ leptin

**PVH**

Diagrams showing Dil Fiber Density and AgRP Fiber Density with statistical comparisons.

Bouret et al., Science, 2004
Postnatal Secretion of Metabolic Hormones

Ghrelin
Leptin

Relative Levels

Postnatal Age (weeks)

Weaning
Puberty

?adapted from Grove and Cowley, 2005
The answer to this latter problem was solved by the discovery and development of Spiegelmers (Klussmann et al., 1996; Vater & Klussmann, 2003). These molecules, which are biostable aptamers, have all of the diversity characteristics of aptamers but possess a structure that prevents enzymatic degradation.

2) Identification of Spiegelmer Products

While aptamers are created from the natural D-nucleotides, which are recognized by the nucleic acid degrading enzymes, a molecule synthesized as the mirror image L-oligonucleotide will not be degraded by any nucleases since there are no such enzymes in the body capable of interacting with these unnatural molecules. As such, the amplification methods used to create large aptamer libraries cannot be employed to synthesize the L-nucleic acid-containing molecules. However, NOXXON has developed the methods to form high affinity L-oligonucleotide aptamers that possess outstanding binding affinities and functionality. These molecules are termed “Spiegelm” from the German “Spiegel” or mirror.
Programming Actions of Ghrelin on Metabolism

Pre-Weaning Growth Curve

Post-Weaning Growth Curve

Adult Food Intake

Adult Glycemia
Long-Term Effects of Postnatal Ghrelin Blockade on Adiposity

Control

Anti-Ghrelin

perirenal

visceral

subcutaneous
Postnatal Ghrelin Blockade Increases ARH Neural Projections

Control P12

Anti-Ghrelin P12

PVH

PVH

Density of ARH D1 Fibers in PVH

Control Anti-Ghrelin
Disturbances in Metabolic Hormones’ Action (leptin, insulin, ghrelin, etc.)

Adverse Effects on Fetal and Postnatal Hypothalamic Development (neurogenesis, circuit formation)

Obesity
Type 2 diabetes
Hypertension

Metabolic syndrome
Conclusion II:
Adverse Perinatal Nutritional and Hormonal Environments Increase the Susceptibility to Adult Metabolic Disease
Conclusion III:
Hormonal Programming: Timing is Important!

**Postnatal Exposure**

**Adult Exposure**

It is important to determine when key developmental events are particularly sensitive to hormonal signals during restricted ontogenetic periods.

Steculorum and Bouret, unpublished

Adapted from Shearman et al., 2006