Neonatal Insulin Action Impairs Hypothalamic Neurocircuit Formation in Response to Maternal High-Fat Feeding

Team Neurocontributors

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First things first…Mother’s Day was on Sunday!

Conception, pregnancy, and childbirth

Pregnant women need nutrients to stay alive and to nurture the growing fetus in their womb

- Proteins
- Carbohydrates
- Vitamins
- Minerals
A New Theory of Pregnancy

Mother and fetus enter a harmonious and “Silent Struggle” for nutrients
What Makes Up the Baby Weight, Anyway?

- Uterus growth: 2 to 5 lbs. (0.9 to 2.3 kilograms)
- Baby: 8 lbs. (3.6 kg)
- Fat stores: 5 to 9 lbs. (2.3 to 4 kg)
- Placenta: 2 to 3 lbs. (0.9 to 1.4 kg)
- Amniotic fluid: 2 to 3 lbs. (0.9 to 1.4 kg)
- Breast tissue: 2 to 3 lbs. (0.9 to 1.4 kg)
- Blood supply: 4 lbs. (1.8 kg)

The weight from the blood supply, fat stores and breast tissue is not localized to the belly.

SOURCES: NATIONAL INSTITUTES OF HEALTH, SHUTTERSTOCK  KARL TATE / © LiveScience.com
<table>
<thead>
<tr>
<th>Prepregnancy BMI</th>
<th>BMI* ($\text{kg/m}^2$) (WHO)</th>
<th>Total Weight Gain Range (lbs)</th>
<th>Rates of Weight Gain* 2nd and 3rd Trimester (Mean Range in lbs/wk)</th>
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<td>Underweight</td>
<td>&lt;18.5</td>
<td>28–40</td>
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<td></td>
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<td>(1–1.3)</td>
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<tr>
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<td>25–35</td>
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<td></td>
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<td>(0.8–1)</td>
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<tr>
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<td>15–25</td>
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<td></td>
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<td>11–20</td>
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<tr>
<td></td>
<td></td>
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<td>(0.4–0.6)</td>
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* To calculate BMI go to www.nhlbischolar.com/bmi/

* Calculations assume a 0.5–2 kg (1.1–4.4 lbs) weight gain in the first trimester (based on Siega-Riz et al., 1994; Abrams et al., 1995; Carmichael et al., 1997)
Obesity is on the rise for pregnant women

15-40% of pregnancies are complicated by maternal obesity (obesity rates for pregnant women in the U.S. are 18.5-38.3%).

Many are aware of certain teratogens, but not aware that high fat diets can also cause health problems - for mom and fetus.

Factors:

“Eating for two” mentality
Hypertension = high blood pressure

Preeclampsia = increased blood pressure, edema, proteinuria

Increase risk of fetal death, increases as BMI increases

Eclampsia = seizures, mental changes, can go into a coma and cause maternal death
Breastfeeding

Pituitary gland releases prolactin and oxytocin

Colostrum is released during the first few days

Gives temporary immunity to infections

True lactation begins 2-3 days after delivery

Pros of breastfeeding for mothers
The Breastfed Baby

Immune system.
Responds better to vaccinations. Human milk helps to mature immune system. Decreased risk of childhood cancer.

Skin.
Less allergic eczema in breastfed infants.

Joints and muscles.
Juvenile rheumatoid arthritis is less common in children who were breastfed.

Throat.
Children who are breastfed are less likely to require tonsillectomies.

Eyes.
Visual acuity is higher in babies fed human milk.

Higher IQ.
Cholesterol and other types of fat in human milk support the growth of nerve tissue.

Mouth.
Less need for orthodontics in children breastfed more than a year. Improved muscle development of face from suckling at the breast. Subtle changes in the taste of human milk prepare babies to accept a variety of solid foods.

Bowels.
Less constipation.

Appendix.
Children with acute appendicitis are less likely to have been breastfed.

Kidneys.
With less salt and less protein, human milk is easier on a baby’s kidneys.

Respiratory system.
Breastfed babies have fewer and less severe upper respiratory infections, less wheezing, less pneumonia and less influenza.

Digestive system.
Less diarrhea, fewer gastrointestinal infections in babies who are breastfeeding. Six months or more of exclusive breastfeeding reduces risk of food allergies. Also, less risk of Crohn’s disease and ulcerative colitis in adulthood.

Heart and circulatory system.
Breastfed children have lower cholesterol as adults. Heart rates are lower in breastfed infants.
How diets in mothers affect breastfeeding

Alcohol inhibits milk production and caffeine affects baby’s sleep.

Diets devoid of cow’s milk, fish, and eggs in the first 3 months of breastfeeding decreased incidence and severity of skin allergy for infants (link)

Trans-fat foods can cause higher levels of body fat in infants.

So already, we know that diets of mothers can have different effects on breast milk and its production.

Can have lifelong effects.
What happens during pregnancy?

Many physiological, anatomical, metabolic, and hormonal changes occur to mobilize fuel stores for fetal growth and prepare the mother for delivery.

Demand for nutrients grows as fetus grows.

Causes necessary changes in maternal metabolism.
Pregnancy & Insulin Resistance

It is actually normal/healthy for insulin resistance to progressively increase during pregnancy!

Allows increase in circulating glucose, increasing availability to fetus

Expanding fetal brain demands more glucose

Maternal obesity

More insulin resistance

IR

More Hyperlipidemia

Inc availability of fuel to fetus

Fat storage

Interferes w/ normal development!

All of this can lead to metabolic dysfunction, insulin resistance & inflammation!
Maternal obesity: why should we care?

Sustained global rise in prevalence of obesity and Type 2 Diabetes Mellitus over last few decades

“Heavy mothers have heavy babies”

Need to start investigating from birth or even before

We know that maternal obesity/maturity diabetes predispose offspring to developing metabolic disorders

This has long-term implications

Metabolic programming
Maternal obesity: It’s a big deal

Asthma

Studies link maternal obesity to higher risk of Asthma

Autism

Human children study found that obese mothers are 67% more likely to have a child with ASD

ADHD

Children born to obese mothers are more likely to show symptoms of ADHD
Maternal obesity: A significant healthcare issue!

Clearly, maternal obesity has many significant health implications for the offspring (physically and cognitively)

What causes this, and when exactly does it occur?
Metabolic programming

Multicellular organisms have normal programmed development

Organism also has ability to respond to abnormal situations/environments during early critical periods of life

These early adaptations permanently change physiology and metabolism of organism

Continue to be expressed even in absence of stimulus/stress that initiated them

This is “metabolic programming”
Nutritional experiences in the immediate postnatal life

- Undernourishment
  - Hyperinsulinemia
  - Hyperleptinemia
  - Hypothalamic alterations
  - Increased growth
  - Obesity
  - Growth retardation
  - Hypoinsulinemia

- Overnourishment
  - Hyperinsulinemia
  - Hypothalamic alterations
  - Increased growth
  - Obesity

- Calorie-redistribution
  - Hyperinsulinemia
  - Hypothalamic alterations
  - Hyperphagia
  - Increased growth
  - Obesity

- Lactation by diabetic mother
  - Hyperphagia
  - Hypothalamic alterations
  - Obesity

Metabolic Syndrome and Associated Diseases
What about the brain?

HYPOTHALAMUS, of course!

Integrates hormonal/nutritional signals from periphery and conveys them into neuroendocrine/autonomic responses

Hypothalamic neurocircuits

Includes brain regions, neurons & axonal projections of:

ARH: NPY/AgRP, POMC
PVH: TRH
DMH
LH
Hypothalamus

ARH [arcuate nucleus of hypothalamus]

Mediobasal collection of POMC and AgRP neurons which project to other sites within hypothalamus

Modulated primarily by leptin and insulin

PVH [paraventricular nucleus of hypothalamus]

Receives projections from ARH including POMC and AgRP/NPY

Expression of TRH, anorexigenic hormone

Projects to and receives inputs from the brainstem (Nucleus of the Solitary Tract)

DMH [dorsomedial nucleus of hypothalamus]

Role in feeding, drinking, and body weight regulation, as well as regulation of circadian rhythm

19
adipose tissue $\rightarrow$ ↑LEPTIN

hypothalamus
What about the brain?

Abnormal changes during development $\rightarrow$ persistent changes in function of hypothalamic neurocircuits

Hypothalamic neurocircuits physiologically regulate energy and glucose metabolism

In humans: develops at birth

In rodents: develops until 3rd week of postnatal life

Includes formation of neuronal networks, axonal projections, and synaptic connections occurring during lactation.
Hypothalamic neurocircuits

As a result of impaired maternal health, exposure to an altered developmental environment during pregnancy/lactation results in gross changes of hypothalamic neurocircuits

- Includes differential neuropeptide gene expression
- Altered hypothalamic neuronal cell numbers
- Impaired formation of hypothalamic axonal projections
Findings

Maternal high-fat feeding during lactation offspring programed to have impaired energy and glucose homeostasis throughout their lifetime.

Impaired insulin signaling in the offspring interferes with the formation of the hypothalamic neural circuits that contribute to metabolic status (our study).

HFD During 3rd Trimester = Greatest Risk - Metabolic Factors
Connections to other topics/Lit Review

Newer studies show the importance of the Hypothalamic-Pituitary-Adrenal (HPA) axis in fetal development

Suggest a molecular and endocrine connection between mother and fetus

Importance of mother diet and stress levels in determining fetal...
Maternal and Fetal Connection

Molecular Connection:

Maternal and Fetal blood separated by just a few layers of cells of placenta.

Fetal Development and growth depend on Maternal nutrient supply via trans-placental passage.

Example of Endocrine Connection:

Influence of Maternal adrenal cortical hormones on fetus.

Maternal Stress may affect fetal development.
Recent Research Cont

Importance of Hypothalamic Pituitary Adrenal Axis (HPA)

2016- found that overfeeding during a critical postnatal period exacerbates HPA axis responses to immune challenges

Results show that neonatal diet influence how rats combat bacterial infection

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4751608/

HPA- importance

Interactions major part of the neuroendocrine system

Controls of stress

Digestion
Other Effects of HFD

**Leptin:**

Study found obese phenotype from mothers can be passed onto next generation, possibly associated with hypothalamic leptin resistance (2015)

**Grehlin:**

Early postnatal overnutrition results in central resistance to peripheral ghrelin during periods of hypothalamic development

May contribute to metabolic defects observed in postnatally overnourished mice

**Neonatal Obesity:**

Predispose individuals to obesity throughout life
Neonatal Overfeeding Cont.

Effects on Microglial Cells

Microglia- glial cell throughout CNS= brain and spinal cord

Functions: Immune regulation, synaptic and neuronal pruning and extracellular signaling

Neonatal overfeeding sensitizes microglia of the hypothalamus, contributing to central pro-inflammatory profile and an altered response to neuroimmune challenge throughout life.
Sources

Aims of this study

Establish mouse model of metabolic programming to identify the most sensitive period of hypothalamic neurocircuit development in response to maternal HFD.

Use this model to understand the specific role of neuronal-insulin signaling in mediating the lifelong predisposition for metabolic disorders in offspring of obese mothers.
DNA (deoxyribonucleic acid) is made up of ~20k genes

Strands of this double stranded molecule are found inside the nucleus of each cell

The normal human genome has 23 pairs of chromosomes

Each chromosome is made up of many genes

Each gene stores the information needed to synthesize proteins.

Wildtype mice contain 99% of all of their DNA in the nucleus of each cell.
Knockout Mice (CRE - LOX System)

https://www.youtube.com/watch?v=ZRWMxMhrzQo
Measures

**Homeostatic Model Assessment (HOMA-IR)** - a method used to quantify insulin resistance - compares fasting blood glucose and insulin levels using a radioimmunoassay.

**ITT** - insulin is injected into a fasting animal and their blood glucose response is measured.

**GTT** - fasting animal has basal glucose levels measured & then are administered glucose (orally or IV). Blood samples taken in subsequent intervals.

**Immunofluorescence** - after perfusion, tissue samples are mounted and stained
Neonatal Hypothalamic Mystery

Big Question: When is the most sensitive time period of hypothalamic neurocircuit development in response to maternal HFD feeding?

Challenging Points:

Exact timing requirements to affect metabolic fate of offspring (when does it have long term implications)
Methods of the paper’s experiment

Fed C57Bl/6 mice - Normal Chow Diet or High Fat Diet 8 weeks prior to and during gestation

Day of Birth:

Half of NCD Mothers switched to a HFD and other half remain on a NCD fed diet

Half of HFD Mothers switched to a NCD and the other half remain on a HFD
More About the Study

Ex: NCD/HFD (4 different Maternal groups)

The first half (NCD) - refers to **pre DOB diet (prior and during gestation)**

The second half (HFD) - refers to **Lactation diet**

Was critical timing period due to gestation or lactation?

NCD = 53.5% carbs 18.5% protein 5.5% fat

HFD = 32.7% carbs 20% protein 35.5% fat

Animal Care - 3-4 animals per cage; 12 hour light/dark cycle; Free-Roam in cages
Findings at 7 weeks (prior to gestation)
Mothers at 7 weeks on a HFD resulted in:

- Moderately increased body weight (1A)
- Elevated fasting blood glucose concentration (1B)
- 7 fold increase in homeostatic model assessment of insulin resistance (HOMA-IR)

What does this mean?

Indicates pre-gestational HFD feeding causes insulin resistance

Increased weight
Maternal HFD during lactation/Post Weaning

HFD exposure during lactation resulted in

Slight elevation of serum insulin concentrations in the Mothers (D)

Increased serum insulin levels in offspring at 3 weeks of age

Post weaning:

Offspring were fed a NCD until 8 weeks of age

All fed NCD to account for confounds

Offspring - kept at 6-7 pups

Control for nutritional availability
Maternal High Fat Feeding
Offspring: Post Weaning

After 8 weeks on a NCD each group of offspring divided into different groups for the following 12 weeks

Exposed to either NCD or HFD

Results in 8 different groups
Maternal HFD exclusively during lactation

NCD/HFD = half of NCD-fed mothers exposed to HFD

NCD/NCD = half of NCD-fed mothers exposed to NCD

HFD/NCD = half of HFD-fed mothers exposed to NCD

HFD/HFD = half of HFD-fed mothers exposed to HFD

Assessed how maternal HFD at this time affected offspring health

Monitored offspring from each of the 4 groups and had them fed either a NCD or HFD after 8 weeks (8 groups total)
Exclusively during lactation - at 20 weeks of age

One aim of the study:
“The exact timing requirements of metabolic insults to affect the metabolic fate of the offspring”
Exclusively during lactation - at 20 weeks of age

So why should humans care?

Sex!!!

Leptin damages sperm cells

“Don’t cook your balls”
Exclusively during lactation - at 15 weeks of age

(E) HFD during lactation = increased glucose and insulin in milk = hyperinsulinemia of offspring at 3 weeks of age
Exclusively during lactation - at 15 weeks of age

Critical period of development where the diet of the mother will have strongest effect of metabolic health in offspring is lactation.
Maternal HFD during lactation & hypothalamic neurocircuitry

Comparison of NCD/NCD with NCD/HFD mice

Want to define molecular mechanism underlying obese NCD/HFD mice

Determined mRNA expression of hypothalamic neuropeptide genes critically involved in regulation of energy and glucose homeostasis:

- POMC (anorexigenic)
- AgRP (orexigenic)
- NPY (orexigenic)
- TRH (anorexigenic)
Results

1. No difference in expression of ARH neuropeptide genes

Significantly lower expression of TRH [anorexigenic downstream target in PVH]
2. No change in ARH neuronal cell number in NCD/HFD
3. POMC processing impaired in NCD/HFD mice?

No!
4. Effect of NCD/HFD on electrophysiological properties of POMC neurons in offspring

No difference in spontaneous firing freq of POMC neurons

No difference in POMC neuron resting membrane potential

No difference in relative synaptic input onto POMC neurons
What does this mean??

We know that maternal HFD exclusively during lactation (NCD/HFD) decreases anorexigenic TRH expression: a target of POMC and AgRP neurons in ARH

BUT WITHOUT ALTERING

1. ARH neuropeptide gene expression
2. ARH neuronal cell number
3. POMC processing
4. Electrophysiological properties of POMC neurons
Lactation is the phase of hypothalamic neurocircuit development in which axonal projections are formed (in rodents)

Strongest impact on metabolic fate of offspring

Want to see if axonal projections of ARH neurons to target sites within the hypothalamus are impaired during this time (for NCD/HFD mice)

Looked at fiber densities of α-MSH and AgRP in 3 main ARH downstream hypothalamic projection areas

(alphaMSH & AgRP)
Results

Significant reduction in alpha-MSH and AgRP fiber densities in NCD/HFD offspring compared to NCD/NCD offspring.
What does this mean?

Formation of POMC and AgRP projections to hypothalamic target sites is severely impaired. This is likely due to impaired axon formation in offspring.

Could this have something to do with the *impaired pathway* leading to the decrease in TRH in the PVH (which we just saw)?

**Impaired axon formation** → **less fiber density** → **less excitatory signal projected to PVH** → **less excitation of TRH** → **less anorexigenic signaling** → **eat more**
Neuronal Insulin Mechanisms in the Hypothalamus

1. Neuronal insulin signaling & metabolic disorders

2. POMC-specific IR deficiency & altered projections
Maternal HFD → increased glucose and insulin levels in milk → hyperinsulinemia in offspring → ???

Goal: Uncover mechanisms of action between hyperinsulinemia and “downstream” dysfunction.

Experiment

Transgenic mice with selectively-inactivated POMC insulin receptors.

4 groups of animals:

(1) NCD/NCD ctrl (2) NCD/HFD ctrl (3) NCD/NCD -POMCIR (4) NCD/HFD -POMCIR

Each group was given normal chow from weaning until week 8 and HFD from week 8 - end of testing.
Maternal HFD during lactation made no difference in body weight (A)

NCD/HFD offspring developed:

Greater % adiposity (B)

Increase perigonadal fat pad weight (C)
Measures of insulin sensitivity revealed impairments in both NCD/HFD genotypes.

HOMA-IR (E)

Insulin Tolerance Test (F)
Animals fed HFD while lactating tend to produce offspring which have increased adiposity and impaired insulin sensitivity regardless of the presence of working insulin receptors on POMC neurons.

Therefore neither insulin sensitivity nor increased adiposity in offspring is caused by insulin receptor activity in hypothalamic POMC neurons.
Glucose Tolerance Test (GTT) revealed:

NCD/HFD ctrl mice (nontransgenic) displayed glucose intolerance
Conclusions:

Increased neuronal insulin signaling from maternal HFD during lactation predisposes offspring to impaired glucose tolerance throughout lifetime.

Animals which would have otherwise developed an impaired glucose tolerance (NCD/HFD -POMCIR) because of maternal HFD diet were unable to.

Therefore, the mechanism which drives glucose tolerance relies on the presence of working insulin receptors.
Maternal HFD during Lactation

Persistent hyperinsulinemia in offspring

Increased Insulin activity @ HT POMC

Impaired glucose tolerance

How is increased insulin activity impairing glucose regulation and increasing blood glucose?
(2) POMC - Specific IR Deficiency & Altered Projections

Goal: to better understand how POMC-specific IR deficiency protected NCD/HFD-POMCIR mice from developing a dysfunctional glucose response.

Experiment

Analyzed fiber densities of ARH neurons projecting to the distinct subregions of PVH

Animals analyzed using immunofluorescence procedures:

@ 8 weeks (PVH)

@ 20 weeks (PVH, DMH, LH)
Loss of POMC IR did not affect alpha-MSH or AgRP fiber densities to any hypothalamic target site in NCD/NCD offspring at any age.

What, then, is the effect of the hyperinsulinemia in the hypothalamus?
NCD/HFD showed a decrease in fiber density in Anterior PVH (alpha-MSH and AgRP) at both 8 weeks and 20 weeks.

Maternal HFD $\rightarrow$ ↓↓ neuronal fiber density regardless of genotype.
NCD/HFD ctrl mice experienced a pronounced reduction in alpha-MSH fiber density.

NCD/HFD -POMCIR mice did not show a decrease in alpha-MSH fiber density in the Posterior PVH.

Levels ~ NCD/NCD
Maternal HFD during Lactation

Persistent hyperinsulinemia in offspring

Increased Insulin activity @ HT POMC

Decreased alpha-MSH fiber density

Impaired glucose tolerance
(3) POMC - Specific IR Deficiency & Pancreatic β cells

Reciprocal neural connections exist between the hypothalamus and the periphery.
Goal: identify the possible changes in peripheral organs that result from excessive POMC activity.

Stained pancreatic islets for vesicular acetylcholine transporter (vAChT)
NCD/HFD ctrl offspring significantly reduced number of vAChT sites per islet area.

NCD/HFD -POMCIR seemed to be protected from the effects of hyperinsulinemia.
Found that C-peptide levels were substantially smaller @ 5 min in ctrl group.

-POMCIR ~ NCD/NCD

There was no change in beta cell mass or islets size to account for differences between genotypes.

Serum insulin levels were unaffected by L-arginine
Maternal HFD during Lactation

Persistent hyperinsulinemia in offspring

Increased Insulin activity @ HT POMC

Impaired glucose tolerance

Decreased alpha-MSH fiber density (posterior PVH projections)

Decreased parasympathetic innervation

Decreased glucose-stimulated insulin secretion
(1 + 2 + 3)
Summary of Results

Maternal HFD-feeding during lactation impairs metabolic health of the offspring

Maternal HFD during lactation impairs formation of melanocortin projection

Abnormal insulin action in POMC- neurons impairs POMC projection to preautonomic PVH

Abrogating POMC insulin action improves glucose metabolism despite maternal HFD

Overall: Maternal HFD during lactation predisposes offspring for obesity, impaired glucose homeostasis in mice and impairment of hypothalamic melanocortin circuitry
Desi, Shuying, Lauren, Scotty Out.