Nutritional Programming of Hypothalamic Development: Critical Periods and Windows of Opportunity

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Team FENAPS (Finesse)
Overview

- Introduction
- Influence of Hypothalamus on Energy Control
- Critical Periods of Hypothalamus Development
- Prenatal Influences
- Postnatal Influences
- Role of Leptin in Hypothalamic Development
- Windows of Opportunity (It’s not too late!)
Reviewing the Obesity Epidemic

- From 2015-2016, 38.9% of the US population was reported to suffer from obesity.
- Obesity is a complex condition
  - Factors are:
    - Biological
    - Genetic
    - Social
    - Environmental
- Effect on children concerning
  - Obese children more likely to be obese as adults

Image Credit: https://www.cdc.gov/obesity/data/prevalence-maps.html
Reviewing the Obesity Epidemic (Cont’d)

● Evidence shows that perinatal changes in the hormonal and nutritional environments affect risk of developing obesity later in life
  ○ Critical periods of development
● Possibility of developmental changes as an evolutionary advantage
  ○ Maximize energy storage, minimize energy losses
    ■ Not as necessary in Western world!
  ○ Is obesity really a disease?

Scope: Consider the long-term effects of pre- and post-natal nutrition on body circuitry

Metabolic imprinting: critical impact of the perinatal environment on the regulation of energy homeostasis Barry E. Levin
Review of the Hypothalamus

- Energy balance is controlled centrally by the hypothalamus.
  - Contains neurons directly tied to metabolic regulation
  - Responds to hormonal and nutritional signals
- The body as a feedback control system for energy expenditure

Image Credit: https://www.researchgate.net/figure/Hypothalamic-leptin-pathways-and-body-weight-regulation-Increased-fat-mass-due-to_fig1_51774467

Not so different after all...
Hypothalamus

- **Key areas:**
  - Arcuate nucleus (ARH or ARC)
  - Ventromedial nucleus (VMH)
  - Dorsomedial nucleus (DMH)
  - Paraventricular nucleus (PVH)
  - Lateral hypothalamic area (LHA)

- **Circuits within ARH most responsive to blood-borne signals**
  - ARH contains orexigenic and anorexigenic neurons that counteract each other
  - Controls energy intake, bodyweight
Hypothalamus Specifics

- More specifically, the ARH contains:
  - Neurons that produce Neuropeptide Y (NPY)
  - Neurons that produce Agouti-related peptide (AgRP)
    - Inhibited by Leptin, activated by Ghrelin
  - Proopiomelanocortin (POMC) neurons - regulation of bodyweight
    - Synthesis of POMC and cocaine and amphetamine regulated transcript peptides (CART)
- Response to peripheral hormonal signals like Leptin
- NPY/AgRP- and POMC-containing neurons project to the PVH, DMH and LHA
  - These nuclei contain neurons that regulate energy balance and produce anorexigenic peptides
  - Namely Galanin, Enkephalin and Dynorphin in the PVH
  - Also contains orexigenic neuropeptides in LHA

http://www.cellbiol.net/ste/alpobesity4.php

Takeaway: Hypothalamus is a complex system containing many interconnected neural pathways
Energy Balance

• POMC and AgRP/NPY neurons project from ARH to PVN
  ○ Controls food intake
• Signalling can be influenced by:
  ○ GCs (glucocorticoids)
  ○ Leptin
  ○ Insulin
Leptin Returns

- **Recall:** Leptin is made by adipocytes (cells specializing in fat storage)
  - Reduces food intake and body weight by regulating synthesis of orexigenic and anorectic peptides (Shown right)
- **Leptin can activate the IRS-phosphatidylinositol 3-kinase (PI3K) cascade**
  - Leads to restriction of food intake through modulation of extracellular related kinases
  - Hypothalamic AMP-activated protein kinase (AMPK, increases food intake) is inhibited
- **Studies on administration of leptin to counteract obesity**
Leptin in the Hypothalamus

● Leptin influences transcriptional activity
  ○ Binds to ARH and other areas in hypothalamus

● Leptin is crucial for the brain
  ○ Fiber density
  ○ Accurate projections from ARH throughout Hypothalamus
  ○ Nervous system development
  ○ Altered levels lead to increase in food intake!

● Energy regulation pathway is vulnerable to changes in leptin levels
  ○ Pathways can be affected by malnutrition
  ○ These changes can affect leptin sensitivity

● Exact mechanisms for which changes arise are unknown

Takeaway: In addition to energy regulation, Leptin has a role in developmental responses to nutritional and metabolic changes.
Insulin - Energy Balance

- Insulin can act in the ARH to produce orexigenic effect
  - Reduce food intake, reduce body weight.
- Insulin receptors co-localize with POMC and AgRP neurons
  - Activation of insulin signaling pathway decreases NPY and AgRP expression
  - Simultaneous increase in POMC to produce anorexigenic effect
- Like with leptin, development of insulin resistance can occur
  - Insulin not as effective at reducing food intake in obese patients with insulin resistance

Takeaway: Insulin plays a role in the ARH for energy balance and regulation of food intake
Ghrelin’s back

- Ghrelin is produced mostly in the stomach
- Acts through growth hormone secretagogue receptor (GHS-R) in hypothalamus
- Ghrelin-induced increase in food intake is mediated by NPY/AgRP pathway
  - Genetic removal or pharmacological inactivation of AgRP and NPY blocks orexigenic effects of Ghrelin
Factors In Hypothalamus Development

● Maternal Overnutrition
  ○ Development of obesity in the offspring accompanied by impaired insulin and glucose homeostasis, as well as many other outcomes such as cardiovascular diseases
  ○ Conflicting results - Neuropeptide expressions vary

● Undernutrition
  ○ Rodent offspring exhibited hyperphagia in postnatal life and develop obesity, insulin resistance in adulthood

● Effect depends on:
  ○ Extent of overnutrition or malnutrition
  ○ Timing - critical periods of development, post-natal vs. pre-natal
    ■ Rats born to calorie-restricted mothers but nourished during post-natal period display catch-up growth
    ■ Early catch-up growth reduces improper hypothalamic neural projections and is beneficial for brain development
Critical Periods for Hypothalamic Development

Shayna Kaler
Human Developmental Critical Periods

- Brain development begins in embryonic period GW3-8 (gestational weeks 3 - 8)
  - Basic structures of brain, CNS, and PNS defined

- Fetal development GW8-38
  - Rapid growth of cortical and subcortical structures
  - Cell migration, synaptogenesis, neural network formation
    - Major fiber pathways, e.g. Thalamocortical pathway
    - Hypothalamic neurogenesis GW9-10

- Early postnatal period
  - Synaptic exuberance pruned by experience dependent processes
  - Differentiation and maturation of cells continues
  - Neurogenesis: olfactory bulb and dentate gyrus
Hypothalamic Development

- Determination of cell numbers
  - Neurogenesis: proliferative zone of 3rd ventricle
    - Rats/mice: majority of hypothalamic neurons born between E12 and E14
      - DMH and LHA: E12-E14
      - ARH and VMH: E12-E16
      - PVH: E12
    - NHP: first quarter of gestation
    - Humans: GW 9-10
  - Neuron migration
  - Cell death
Hypothalamic Development cont’d.

- **Formation of functional circuits**
  - Axon growth and synaptogenesis
  - Rats/mice: hypothalamus is relatively immature at birth and continues to develop during first two weeks postnatally
    - ARH axons reach target nuclei between P6 and P16
    - Efferent projections DMH → PVH and LHA fully established by P6
    - Projections containing AgRP/NPY develop in similar temporal pattern
      - Unknown if anorexigenic ARH neurons develop at the same time
    - Suggests pathways other than ARH projections regulate weight and feeding during early postnatal life
      - DMH and VMH?
Critical Periods

- Rodent hypothalamus develops in two distinct environments
  - Intra-uterine: cell numbers are determined
  - Extra-uterine: neuronal connectivity established
  - Synapses form after projections, mature into adulthood
- Disruption → severe structural/functional abnormalities
- Humans/NHPs
  - Hypothalamus develops prenatally
  - Rodents are good model
Leptin’s contradictory role

- **Endogenous leptin surge during postnatal period**
- **Anorectic role during early postnatal development?**
  - Administration of exogenous leptin before weaning did **NOT** increase milk intake or metabolic rate - after weaning it does
  - Crosses BBB, hypothalamic receptors are developed and functioning
  - Why?
- **Neonatal surge in leptin during critical period for circuitry development**
  - Leptin deficient (ob/ob) mice: ARH projections severely reduced
  - Delay in ARH projection formation
    - P12: significantly reduced amount of ARH axons innervating PVH
    - P60: lower density of ARH projections to nuclei - disruption appears to be permanent
Leptin Disruption

● Restricted critical period: ~P1-14
● Molecular factors?
  ○ Kurrasch et al.: microarray analysis studying genes enriched in VMH
    ■ Satb2: VMH gene regulated exclusively by leptin during postnatal development
● Neonatal leptin treatment → long term reduction in food intake for ob/ob mice
● Inhibit leptin surge → inc susceptibility to DIO
● Premature leptin surge → changes in weight gain, glucose homeostasis, leptin sensitivity
Ghrelin

- Inhibitory role?
- Block ghrelin → enhanced ARH projections
  - Long term metabolic effects
  - No effect on DMH
- Chronic administration of ghrelin postnatally
  - Impaired development of ARH axons
- Direct ghrelin exposure ARH
  - Blunted axon growth
  - Blocked neurotrophic effect of Leptin
- Chronic exposure neonatally
  - Reduced leptin STAT3 signaling in ARH
Obesity and Famine Exposure

- Dutch famine of 1944-45
- 300,000 men exposed to famine prenatally and during early infancy
- 3rd trimester and early infancy
  - Significantly lower obesity rates ($P < 0.005$)
- First half of pregnancy: GW1-20
  - Significantly HIGHER obesity rates ($P < 0.0005$)
- Timing of nutritional deprivation predicts long term metabolic changes
Prenatal Influences

By: Fernando Beltran Jr
Prenatal Influences

- Genetic basis for a subpopulation of obese patients

- 1 out of 5 women are obese when they give birth
Studying Consequences of Obesity

- Most common approach of obesity in both rodents and NHPs:
  - High fat diet (HFD)
    - 30-60% of total calories from fat
  - Offspring of HFD-induced obese females:
    - Progressively overweight regardless if fed during gestation OR gestation & lactation
  - Temporary HFD leading to metabolic syndrome while pregnant:
    - Predisposes offspring to obesity
      - Increase in food intake, body weight, & fat mass
OWP (Obese While Pregnant)

- Do mothers then **have to be obese** for the length of the pregnancy to induce changes in the offspring’s hypothalamus and predispose them to metabolic effects?
  - **Appears not according to this study!**
  - Transient exposures of nutritional and hormonal imbalances during gestation are sufficient to produce long term metabolic effects!
Levin’s Diet Induced Obesity (DIO) Model in Rats

- Good model since effects observed in rats are analogous to humans
  - Share several features with human obesity, including polygenic inheritance
- Mothers genetically predisposed to DIO and fed HFD:
  - Offspring are obese, hyperphagic, & glucose intolerant
- Offspring born to diet-resistant (DR) rats:
  - Did not show any effects related to DIO with HFD rats!
Consequences of An HFD while pregnant

- Impaired organization of hypothalamic circuits in Maternal HFD:
  - Increase in hypothalamic cell proliferation resulting in higher numbers of orexigenic neurons in the PVH & LHA, including galanin, enkephalin, dynorphin, melanin-concentrating hormone & orexin neurons in offspring.
    - **More orexigen neurons = more hunger induced**
  - Common structural abnormalities observed across subjects:
    - Decrease in density of ARH AgRP containing fibers innervating the PVH
      - Impaired metabolic responses during adult life
      - Impaired hypothalamic activation of STAT3 pathway after leptin administration
  - Significant remodeling of synapses in hypothalamus in DIO rats:
    - Increase in inhibitory inputs to POMC neurons in ARH compared with DR rats
Similar Effects Observed in Hypothalamic Neurons in Cross Fostered Rodents

- HFD offspring cross-fostered with control mothers during lactation:
  - Displayed similar effects in orexigenic cell numbers & metabolic outcomes to pups raised by HFD mothers during pregnancy & lactation
What’s the Cause of All This?

- Many animal models of maternal obesity display hyperglycemia & insulin resistance
  - Changes due to diet or consequences of maternal obesity?
  - Test manipulation of glucose levels without alterations in the diet.
- Test by:
  - Manipulating glucose levels using **Streptozotocin**
    - Pancreatic beta cell toxin
  - Pups born to non-obese diabetic moms
    - Experience a reduction in both the density of AgRP & anorexigenic containing fibers that innervate the PVH
Prenatal Malnutrition/Altered Growth

- Perinatal rodents born & raised by caloric restricted (CR) or protein restricted (PR) rats during pregnancy & lactation:
  - Experience growth retardation
  - Low birth weight
  - Slow growth during pre-weaning period

- Same CR/PR pups raised by control lactating mothers fed ad lib:
  - Display rapid catch-up growth during first postnatal week
  - Adult animals born to malnourished mothers display normal body weight
    - however, are hyperphagic, glucose intolerant, & increased sensitivity to DIO
Maternal Malnutrition

- Associated with reduction in the number of orexigenic neurons in offspring’s hypothalamus
  - Including reduction in NPY-IR neurons in ARH
    - Remains to be investigated, however
  - Thickness of cortical plate is reduced in fetuses of PR dams
    - Supports the idea that maternal malnutrition reduces cell proliferation during embryonic life
    - Various genes involved in brain development are down-regulated
    - Reduced number of astrocytes & reduction of glia-to-neuron ratio
Neonate Exposed to a reduced nutrient Environment

- Density of AgRP-IR & POMC-IR nerve fibers in PVH reduced in rodents
- Timing of catch-up growth in intrauterine growth retarded animals is critical!
  - Early catch-up growth ameliorates abnormal organization of hypothalamic neural projections
    - Highly beneficial for markers of brain development, including cell adhesion & axon elongation
  - Late catch up growth causes detrimental neurodegenerative effects in the hypothalamus
Maternal Overnutrition, Postnatal Influences & Epigenetics

Alexus Jones
Quick Rundown of what I am Talking About

- Maternal Overnutrition
  - Hypothalamic Energy Regulating pathway
- Postnatal Influences
  - Overnutrition
  - Malnutrition

- Epigenetics
  - Mechanisms
  - DNA Methylation
  - Histone Modifications
  - Epigenetic Programming of Obesity
Maternal Overnutrition

- Why do we care?
  - Rise in Obesity, laziness, increased intake high calorie diets
  - Study in 2013 - Bariatric Surgery
    - Gotta be a link
Animal Models - Maternal/Postnatal Overnutrition

- Standard Animal Model
  - Problem: lactation or gestation

- Programming
  - Persistent change in structure and function via some stimuli or insult

- Conflicting Evidence until 2013

- Comparing Some Studies
  - Langly-Evans Review
    - Steculum and Bouret 2011
    - Plagemann 1999
  - Vogt et al. 2013
Maternal Gestational Diabetes
Steculorum -- AgRP and POMC neurons

- Studied by injecting Mom’s with a toxin -- Streptozotocin STZ
  - Structural changes in
    - AgRP
    - α-MSH

- Kind of reversible
- AgRP, POMC, α-MSH, Galanin and NPY -- altered programming

Plagemann -- NPY and Galanin

- Increased number of galanin and NPY neurons
Postnatal Influences
Images from Vogt et al. 2013
So, Postnatal Overfeeding Causes

- Maternal HFD during lactation leading to obese offspring
- Formation of POMC and AgRP projections that are being affected
Quick Catch Up

- HFD lactating moms will cause - decrease in of ARH neuronal fiber densities in the hypothalamus
- HFD - Obese- Diabetic moms will cause - neuropeptide expression in orexigenic neuropeptide galanin to increase.
  - See decreased electrophysical response changes of PVH
    - NPY, AgRP, α-MSH, and CART
Postnatal Overnutrition/Malnutrition

- How its Studied
  - Changing the Litter Size
    - SL -- Overnutrition
      - Decreasing litter size will decrease competition
      - Side note: only keep males to avoid estrogen interference
      - Good model
        - Show more growth than normal litter size during pre-weaning period
        - Have higher body weight
        - Stay fat
    - LL -- Malnutrition
      - Decreases Milk availability
      - Statistically causes decrease in pre-weaning weight
Postnatal Influence - Malnutrition

- Decrease in α-MSH and NPY/AgRP fibers in hypothalamus
- Rats show an increase in NPY neurons with increased NPY in ACH at weaning
- Neurons aren’t missing they just are not extending into the hypothalamus as much as they should be
Malnutrition

- Neuropeptide Kisspeptin
  - Disrupted Normal Development
  - Altered puberty onset/fertility problems
We didn’t forget Daddio

- Altered Drosophila protein and sugar diets
- It only takes 2 days of this to create an effect in the offspring
- Results:
  - Protein diet had no change
  - Sugar diet had a 3x increase in triglyceride storage
  - Alters Offspring Heterochromatin
Mechanisms for Hypothalamic Programming

- Cool Immunostaining images from earlier
  - Nutritional insults cause structural and functional changes
- They forgot something
  - Epigenetic Modifications
    - Regulation of gene expression
      - Spatially
      - Time
Epigenetic Mechanisms

- Not changing the innate DNA Sequence
- Methylation
  - Most Prominent
  - Highly stable
  - Works Via two mechanisms
- Histone modification
DNA Methylation

- Two Mechanisms
  - Methyl group directly stops transcription factors from binding to their sequence
  - Silences Gene expression through co-repressor complexes
    - Histone Deacetylases
    - Histone methyltransferase
Histone Methylation

- Histones Package DNA
- Two important players
  - Lyine
    - activation/repression depending on the reside
  - Arginine
    - Transcriptional activity
Why do we care?

- Overfeeding pups leads to hypermethylation in the promoter of the insulin receptor
- A second study
  - Promoter hypomethylation of the hypothalamic dopamine and opioid related genes
  - Increased gene expression and they also behaviorally preferred food that was higher in sugar and fat
Leptin’s back

Question to ask:

- Why??
- What is Leptin’s role in hypothalamic development?
Neonatal Leptin Treatment..., Vickers MH et al. 2005

- To investigate the effects of neonatal leptin treatment.
- Using different variables to measure effectiveness of treatment.
Subjects used:

- Virgin Wistar rats used (aged ~100)
- 25C room, 12h dark and light cycles
- 2 nutritional groups: 1) undernutrition of a standard diet (UN) & 2) standard diet (AD)
- Postnatal weigh-in & litters reorganized to match 8 pups per litter for standardized nutrition before weaning
- Cross-fostered rats with UN & AD moms
- Post-natal day 3, UN & AD pups randomized to receive saline or leptin by sc injection
- Weaning, pups were weight matched and placed on standard rat chow or a high fat diet
Measurements/Tools used

- Whole body DEXA scan for bone density
- RIA for plasma leptin
- ELISA used for fasting plasma insulin
- YSI glucose analyzer for fasting glucose
- Optimax behavioral testing apparatus for locomotion
- Stat analysis: SigmaStat & StatView packages
- 3 way ANOVA for differences between groups (prenatal nutrition, treatment, postnatal diet)
- Bonferonni corrections (multiple comparisons)
Results
Results continued...

Groups

AD & UN = Nutritional Groups

Treatment

S & L = Saline and Leptin

Food intake

C & HF = Chow and High Fat
Leptin as a potential hormonal mechanism

- Maternal obesity/diabetes and overnutrition increase leptin levels
  - Increased leptin levels
  - Leptin resistance
  - Reduced STAT3 in the arcuate nucleus (ARH)
- Leptin levels decrease due to malnutrition during pregnancy and lactation by hindering the naturally occurring postnatal leptin surge
  - Disrupted development of ARH axonal projections vs wild-type mice
- Leptin treatment in early postnatal life can normalize abnormalities, but treatment in adulthood has no effect
Windows of “opportunity”

- So what can be done about all these drastic changes due to over/malnutrition?
- Is it reversible?
Conclusion

- Nutrition in hypothalamic development is crucial to proper development
- There is a small window to reverse course, but...
- Eat right regardless!
- And exercise...
References


