Metabolic programming effects initiated in the suckling period predisposing for adult-onset obesity cannot be reversed by calorie restriction

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Presented by Team FUD - COGS 163
Overview
FOR INFANTS, HIGH-CARB DIET SETS METABOLISM, study finds

In a study published recently in the journal JAMA Pediatrics, researchers found that children who consumed a high-carbohydrate diet during infancy were more likely to have metabolic disorders later in life.

The study, which followed 1,000 children from birth to age 5, found that those who consumed more than 60% of calories from carbohydrates were more likely to have higher body mass index (BMI) and waist circumference than those who consumed less.

The researchers also found that the effects were stronger for boys than girls.

"These findings highlight the importance of dietary recommendations for infants and young children," said study author Dr. John Kramar. "High-carbohydrate diets may contribute to the development of metabolic diseases in later life, especially in boys."
Nervous System

Inhibitory
norepinephrine

Reduced sensitivity
to adrenergic
(norepinephrine)

Stimulatory
Acetylcholine (ACh)

Increased response to
cholinergic (ACh)

High-carbohydrate
diet

Acetylcholine (ACh)
“...altered expression of Npy, Pomc, Insr, and Lepr genes in the HC model, suggesting that alterations at the level of the hypothalamic appetite regulatory mechanism supported the development of obesity in HC rats.”
Hypothesis

What we know

In post weaning period, HC rats consumed ~15% more food.

HC reared rats resulted in chronic hyperinsulinemia and adult-onset obesity.

Altered ANS regulation → Hypersecretory capacity of the HC islet β-cells

Calorie restriction ameliorated the incidence of obesity and type 2 diabetes

Alterations at the level of the hypothalamic appetite regulatory mechanism

Altered ANS regulation
Hypothesis

1. Imposition of a pair-feeding regimen on HC rats will normalize the HC phenotype (chronic hyperinsulinemia and adult onset obesity) to that of control rats.

2. Moderate calorie restriction imposed on HC male rats will permanently reverse the programmed effects in islets and in the hypothalamus.
Groups of Rats

- High-Carb fed
  - Ad-libitum
  - Pair-fed
    - Ad-libitum
    - Pair-fed
  After 24 days
- Mother Fed
  After 90 days
Results

Programmed effects on islet function and on the hypothalamic appetite-regulating mechanism were not amenable for permanent reversal by the pair-feeding regimen.
Methods
Day 1 - Day 4

Control group MF Rats

Experimental group
Experimental group - Pair feeding
HC
Day 24
Ad libitum
HC/PF : Same quantity as mother
Groups of Rats

- High-Carb fed
  - After 24 days
    - Ad-libitum
    - Pair-fed
- Mother Fed
  - After 90 days
    - Ad-libitum
    - Pair-fed
Day 140
Brain removed by dissection

Pancreas removed placed in Hank’s balanced salt solution to preserve the physiological Ph
Results
Results: Food Intake

- Measured rats’ food intake from postnatal day 24 to 140, on a weekly basis
- HC ate significantly more than MF rats
- HC/PF ate same amount as MF rats because of pair feeding
- HC/PF/AL ate significantly more than MF rats at the start of ad lib feeding, matched the amount consumed by HC rats
Results: Body Weight Gain

- Measured rats’ body weights from postnatal day 24 to 140, while rats were eating standard rat chow.
- **HC** significantly more weight than MF rats after day 52
- **HC/PF** weight similar to MF rats
- **HC/PF/AL** weight the same as MF and HC/PF rats during the pair feeding. After switching ad lib feeding, body weight increased to match HC rats.
Results: Serum Insulin Levels

- Measured level of insulin in rats’ blood at the end of the experiment, on day 140
- **HC** much higher insulin levels than MF rats
- **HC/PF** rats similar insulin levels as MF rats
- **HC/PF/AL** rats similar insulin levels as HC rats, much higher insulin levels as MF rats
Results: Serum Leptin Levels

- Measured level of leptin in rats' blood at the end of the experiment, on day 140
- Results very similar to insulin levels
- HC much higher leptin levels than MF rats
- HC/PF rats similar leptin levels as MF rats
- HC/PF/AL rats similar leptin levels as HC rats, much higher leptin levels as MF rats
Results: *Insulin secretions from islets*

- Basal and maximal stimulatory concentrations of glucose were used
  - 5.5 mM & 16.7 mM
- Secretions measured at the 10 and 60 minute mark after glucose introduction
- HC, HC/PF, and HC/PF/AL groups all showed significantly higher secretion levels for all conditions compared to MF
Results: *Insulin secretions from islets - ACh and OM*

- **Acetylcholine** → muscarinic receptor agonist
- **Oxymetazoline (OM)** → $\alpha$-1 adrenergic receptor agonist
- Both agonists were deployed at the 60 minute mark following administration of 5.5 mM & 16.7 mM glucose

**5.5 mM**
- **ACh** at 100 $\mu$M w/5.5 mM glucose - **potentiated** secretion for all 4 groups
  - HC, HC/PF, and HC/PF/AL secretion levels were all significantly higher compared to MF levels
- **OM** at 10 $\mu$M w/5.5 mM glucose - **reduced** secretion for all 4 groups
  - HC, HC/PF, and HC/PF/AL secretion levels were not as sensitive to OM compared to MF levels

**16.7 mM**
- **ACh** at 100 $\mu$M w/16.7 mM glucose - **potentiated** secretion for all 4 groups
  - HC, HC/PF, and HC/PF/AL secretion levels were all significantly higher compared to MF levels
- **OM** at 10 $\mu$M w/5.5 mM glucose - **reduced** secretion for all 4 groups
  - HC, HC/PF, and HC/PF/AL secretion levels were not as sensitive to OM compared to MF levels
5.5 mM glucose
16.7 mM glucose
Results: *Insulin secretions from islets - Calcium deprivation*

- Calcium deprived conditions were induced w/5.5 mM glucose present for all 4 groups after 60 minutes following administration.

Results:
- All 4 groups exhibited lower insulin levels
  - HC, HC/PF, and HC/PF/AL groups continued to secrete significantly higher levels of insulin compared to MF, which secreted minimal levels.
mRNA levels/expression in the hypothalamus

- The hypothalamus is implicated in numerous processes, including regulation of appetite.
- This regulation can be greatly affected by diet, as highlighted by the fluctuations of peptide, hormone, and other structural mRNA levels seen in MF, HC, HC/PF, and HC/PF/AL groups after the 140-day study. Expression is not as affected.

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- The structures that were considered are:
  - **NPY** - an orexigenic neuropeptide → leads to increased food intake
  - **POMC** - an anorexigenic precursor neuropeptide → leads to decreased food intake
  - **LepR** - leptin receptor, that when activated, inhibits NPY neurons and stimulates POMC neurons
  - **STAT3** - a transcription factor → part of the JAK-STAT signaling cascade that occurs with LepR activation
  - **SOCS3** - a negative-feedback inhibitor of leptin and insulin signaling, activated by increasing STAT3 levels
Results: *mRNA levels/expression in the hypothalamus*

- **NPY** - higher mRNA levels in HC, HC/PF, and HC/PF/AL rats compared to MF rats
  - Expression of NPY mRNA was **unaffected**

- **POMC** - lower mRNA levels in HC, HC/PF, and HC/PF/AL rats, and significantly increased levels in MF rats
  - Expression of POMC mRNA was **unaffected**

- **LepR** - significantly reduced mRNA levels in HC and HC/PF/AL rats (though a little less for HC/PF/AL) in comparison to MF rats
  - **Normalized** levels in HC/PF in comparison to MF rats

- **Socs3** - higher mRNA levels in HC, HC/PF, HC/PF/AL rats in comparison to MF rats
  - Expression of Socs3 was **unaffected**

- **Stat3** - mRNA levels **did not** differ significantly across the 4 groups
Results: mRNA levels - NPY & POMC

![Graph showing mRNA levels of NPY and POMC](image)
Results: mRNA levels - LepR, Stat3, & Socs3
Discussion
• Benefits of calorie restriction was a temporary response

• HC/PF/Al – features of HC phenotype reappeared.
  - body weight
  - fat
  - plasma leptin
• Expressions of NPY and POMC were similar among HC, HC/PF, and HC/PF/AL

• Results indicate a programmed effect that could not be reversed through pair feeding.
Leptin signaling

- Pair-feeding normalized mRNA levels of Lepr in hypothalamus of HC/PF rats
- This indicate that expression of Lepr depends on levels of circulating leptin rather than genetic programming
- No difference in Stat3 expression (what about phosphorylation state?)
- Increased mRNA levels of Socs3 in hypothalamus → inhibition of leptin signaling
Hypotheses

1. Imposition of a pair-feeding regimen on HC rats will normalize the HC phenotype to that of control male rats
2. Moderate CR imposed on HC male rats will permanently reverse the programmed effects in islets and in the hypothalamus
1. Hypothesis

Imposition of a pair-feeding regimen on HC rats will normalize the HC phenotype (say: chronic hyperinsulinemia and adult-onset obesity) to that of control male rats

- Altered nutritional experiences during critical periods of development has big implications on obesity and related metabolic diseases

Normalized in HC/PF rats by pair-feeding:

- Body weight
- Food intake
- Serum hormone levels

So the metabolic phenotype in HC rats was improved
2. Hypothesis

Moderate CR imposed on HC male rats will permanently reverse the programmed effects in islets and in the hypothalamus

- No! Programmed effects for hyperinsulinemia and hyperphagia was not erased. The normalization of the phenotype was due to calorie restriction
Further studies

- Expand the study to longer life-span in order to examine if the effects are reversed eventually during the constriction diet
- Examine STAT3 phosphorylation state with Western Blot
- Examine the phosphorylation state of other STAT-proteins (1,2,4)