Purines and Neurodegenerative Disorders

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Fructose

- Hexose naturally found in fruits and honey
- Mammals have developed the ability to use fructose as a metabolic substrate over time
  - There may be some evolutionary advantage to fructose consumption
- Twentieth century sparked increase in fructose consume through the technological development of chocolate bars, ice cream, and sodas
Sugar Consumption

- In North America, South America, Oceania, and Europe, sugar consumption has increased drastically
  - 20-30 g/day in early twentieth century
  - 140-150 g/person/day now
  - Sugar has become 20% of total daily energy intake
Does HFCS play a role in obesity?

There’s some controversy....

Increase in HFCS consumption and obesity prevalence between 1970-2000

- Suggested that HFCS provided way more fructose than sucrose - but they’re actually pretty similar quantities
- Same proportions of fructose and glucose, but HCFS has free forms of hexoses
- There’s no data that they exert different metabolic effects than sucrose
In the human body...

- Starch broken down to maltose and oligosaccharides by pancreatic and salivary amylases
- Then split into glucose by maltase and isomaltase
- Starch released in circulation as glucose - 1 of 2 energy substrates used in the body (other is ketone bodies from Fatty Acids)
- BUT, fructose, galactose, and alcohol cannot be directly used by cells in our bodies
  - They are digested and absorbed by enzymes (sucrase-isomaltase for sugar, lactase for lactose) and transporters (GLUT5 for fructose)
  - Then released into portal circulation so they reach the liver first where they are converted to glucose to allow extra-hepatic cells to use them as energy substrates
Liver as a metabolic plant

- TGs are repackaged with apoproteins to become chylomicrons-TG & reach systemic circulation
  - Can be hydrolyzed to lipoprotein lipase to convert to TG for storage
- Non-esterified Fatty Acids (NEFA) released by adipocytes and can be used for energy by most cells (except neuronal)
  - Released when glucose availability is low
Important Transporters

**Sodium-Glucose Co-Transporter (SGLT-1)** - found on apical side of enterocyte and transports glucose into the enterocyte
- Energy-dependent process mediated by Na-K ATPase on basolateral membrane of enterocytes
- Constant Na coming out of the cell

**GLUT-5** - found on apical membrane of enterocytes and allows for fructose absorption
- Expressed in gut, liver, and other cell types (ie. brain)
- “Specific” glucose transporter

**GLUT-2** - facilitative hexose transporter found in basolateral membrane that releases glucose and fructose into the bloodstream
- Also in apical membrane to allow transport of glucose and fructose into lumen of intestine

**GLUT9, SLC2A9** - fructose transporters that are expressed in extrahepatic tissues
Fructose Metabolism in Enterocytes

- Enterocytes in the proximal small bowel express enzymes required for de novo fatty acid synthesis
  - may convert part of fructose into triglycerides
  - package newly formed triglycerides into chylomicrons
  - contribute to dyslipidemia

- Demonstrated in rodents (with high-fructose diet) but has not been directly assessed in humans
**Fructose Metabolism in Hepatocytes**

- Fructokinase and aldolase B are not regulated by insulin
  - Because they are not inhibited by ATP or citrate, there is no feedback inhibition of the initial steps of fructose metabolism
  - Hepatic glycogen synthesis is also stimulated, but this pathway is quantitatively limited

- Hepatic glycogen, lactate and glucose may become saturated, and hepatic DNL is turned on
  - Results in an increase of intrahepatic TG and an enhanced secretion of VLDL
Addition of sucrose to rats' diet or drinking water

- Causes excess body weight gain
- Insulin resistance
- Increased total and visceral (epididymal/perirenal) fat mass
- Increased energy intake
- Multifactorial mechanisms
- Impaired glucose tolerance, or overt diabetes mellitus
- Dyslipidemia
- Hepatic steatosis
- Hyperuricemia
- High blood pressure
Insulin Resistance in Rodents with HFD

- HFD has been used as a model in rodents for diet-induced obesity and DM
- Most rodent studies compare obese, HF fed animals to chow-fed, control rats
  - One recent study, in which rats were given a slightly hypocaloric, high-fructose diet, suggests that many of these effects may be secondary to exposure to fructose independently of excess energy intake
- Overfeeding and acute intravenous fructose administration impairs the suppression of hepatic glucose production by insulin
  - Effects are observed with high amounts of fructose (approximately 20–30% total energy intake) & not associated with clinically significant increases in fasting glycemia
  - Few studies included direct measurements of whole body insulin sensitivity with the gold standard method, the hyperinsulinemic euglycemic clamp
Fructose & Blood Lipids

- High Daily Fructose Intake → Hypertriglyceridemia
  - Cardiovascular Risk Factor

- Mechanisms
  - De Novo Lipogenesis in Liver
    - Build up of Triose Phosphates → Conversion to Fatty Acids
      - Secreted as VLDL Triglycerides
      - Stored as Intrahepatic Fat (NAFLD)
  - Low Postprandial Blood Triglyceride Clearance with Fructose vs. Glucose
    - Fructose Less Insulinogenic than Glucose
      - Lesser Adipocytic Lipoprotein Lipase Response?
Fructose and Non-Alcoholic Fatty Liver Disease (NAFLD)

- High Fructose Diet \textbf{MAY} Cause NAFLD
  - High Fructose Doubled Intrahepatic Fat after 3 Days in Healthy Subjects
    - **Counterpoint**
      - Below NAFLD Threshold
      - May not be Fructose Specific
      - No Effect without PNPLA3
    - **Point**
      - Higher Risk with Obesity
      - Longer Studies Lacking
  - Fructose Metabolism $\rightarrow$ Inflammation & Tissue Fibrosis
    - Hepatic Oxidative Stress
    - Methyglyoxal
Effects of Fructose by Gender

- Female Sex Hormones Mitigate Effects of Fructose
  - Healthy Pre-Menopausal Women vs. Men:
    - Glucose Homeostasis Less Affected
    - Attenuated Hypertriglyceridemia
    - No Increase in Visceral Fat
Uric Acid & Neurodegenerative Diseases
Neurodegenerative Diseases

- Parkinson’s Disease (PD)
  - Resting Tremor
  - Bradykinesia
  - Rigidity

- Huntington’s Disease (HD)
  - Chorea
  - Cognitive Impairment
  - Psychiatric Symptoms

- Multiple Sclerosis (MS)
  - Pain & Weakness
  - Loss of Muscle Control
  - Impaired Vision & Speech

- Alzheimer’s Disease (AD)
Uric Acid ($\text{C}_5\text{H}_4\text{N}_4\text{O}_3$)

- Byproduct of Purine Metabolism
  - Fructose metabolism requires ATP
    - ATP $\rightarrow$ AMP
      - AMP $\rightarrow$ Uric Acid

- Enzymatic Deficiencies Boost Uric Acid
  - HGPRT & Glucose-6-Phosphatase

- Kidney Issues Boost Uric Acid
  - Excreted in urine
Uric Acid Sweet Spot

● Too much (Hyperuricemia)
  ○ Lesch-Nyhan Disease
  ○ Cardiovascular Issues
  ○ Gout
  ○ CNS Inflammation

● Too little (Hypouricemia)
  ○ Oxidation
  ○ Neuronal Cytoskeleton Breakdown
  ○ Rampant Glutamate Neurotoxicity following Neuronal Injury
Hyperuricemia

- Over-Formation of Monosodium Urate (MSU)
  - Gout
    - MSU deposits in Joints, Tendons, Kidneys, etc.
      - Activates Caspase-1/Inflammasome Complex
        - Proinflammatory Cytokines IL-1β & IL-18
  - CNS Inflammation
    - MSU activates intracellular NLRP3’s
      - Activates NALP3 inflammasome complex
        - NLRP3
        - ACS
        - Caspase-1
          - Activates Proinflammatory Caspase-1
            - Pre-caspase-1 precursor → p20-p10 heterodimer form
              - Proinflammatory Cytokines IL-1β & IL-18
CNS Inflammation & Neurodegenerative Diseases

- Inflammation ➔ Neuronal Dysfunction & Death
- Aβ Activates NLRP3s
- High Uric Acid in MS
Hypouricemia

- **Increased Oxidation**
  - Decreases SOD1 & SOD3
    - Allows formation of Superoxide & Peroxynitrite
      - Peroxynitrite deactivates cellular enzymes supporting cytoskeleton
    - Too much free iron
      - Increased iron-dependent ascorbate oxidation

- **Under-expression of astroglial glutamate transporter**
  - Glutamate buildup following neuronal injury
    - Increased neuron death
Hypouricemia & Neurodegenerative Diseases

- Higher Uric Acid \(\rightarrow\) Slower HD

- Gout \(\rightarrow\) PD Risk Down 30%
  - Decreased Neuron Death in Animal Models

- Low Uric Acid in AD & MS
  - Uric Acid Halts MS Progression
    - Decreases Symptoms in Some
Huntington’s Disease & Brain Urea

Handley et al. (2017)
Huntington’s Disease (HD)

- Neurodegenerative disorder
- Most common **inherited** neurodegenerative disorder
  - Prevalent in ~1 in 10,000
  - No environmental causes
- **Autosomal dominant**
  - Having 1 HD parent = a 50% chance of inheriting
- Average age of onset = **40** years old or earlier
- Programmed neuronal death in the **Basal Ganglia**
  - AKA **Huntington's Chorea**
    - **Weight loss**
      - Energy expenditure far exceeds that utilized in movement, despite high calorie intake
    - **Psychosis**
Huntington’s Disease - Cause

**Mutation in the Huntingtin (HTT) gene**

**CAG sequence repeat**

- a trinucleotide that codes for glutamine amino acid
- in the IT15 gene on Chromosome 4
- Average 20-35 CAG repeats ⇒ OK
- >35 or 40 ⇒ glutamate excitotoxicity ⇒ MUTATION
  - More repeats = more severe
Huntington’s Disease Progression

1. Early Stage:
   a. Fully functional
   b. **No impaired motor symptoms**
   c. Mild cognitive symptoms and psychiatric changes - early degeneration

2. Early Intermediate Stage:
   a. May not still be employed
   b. **Weakness and Fatigue** - carry out daily activities despite some difficulties
   c. **Chorea**

3. Late Intermediate Stage:
   a. Non-functional
   b. Worsening in cognitive, motor, psychiatric and behavioral symptoms

4. Early Advanced Stage
   a. Super non functional
   b. Require major assistance - extended care facility

5. Advanced Stage:
   a. Less chorea, more Parkinson-like symptoms
   b. Inability to swallow, extreme fluctuations in blood pressure and temperature
   c. Death
Urea Cycle

- Protein catabolism $\rightarrow$ Ammonia $\rightarrow$ Urea

- Hyperammonemia $\rightarrow$ **Urea Cycle Disorder (UCDs)**
- **Urea Cycle Disorder** symptoms:
  - Fatigue
  - Abnormal circadian rhythm
  - Acute psychosis, weight loss, weakness, seizure, and coma
Experiment on Prodromal Sheeps

- Prodromal sheep model - **OVT73**
  - Pre stage 1 symptoms
    - Circadian rhythm
    - Metabolites in brain and liver
- Performed **RNA-sequencing** analysis
  - **mRNA Gene expression** in **anterior striatal tissue** of OVT73 vs. control sheep
    - 24 → 10 differentially expressed at the nominal significance level
<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene function (GO terms)</th>
<th>Control (count ± SEM)</th>
<th>OVT73 (count ± SEM)</th>
<th>Fold difference (OVT73/Control)</th>
<th>P-value</th>
<th>Adjusted P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC14A1</td>
<td>Urea channel (GO:0015265), water transporter (GO:0005372)</td>
<td>483.6 ± 82.1</td>
<td>1061.5 ± 149.9</td>
<td>2.20</td>
<td>0.01</td>
<td>0.06</td>
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<td>OXTR</td>
<td>Oxytocin receptor (GO:0004990), Vasopressin receptor (GO:0005000)</td>
<td>50.5 ± 13.5</td>
<td>110.3 ± 14.6</td>
<td>2.18</td>
<td>0.01</td>
<td>0.06</td>
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<tr>
<td>SMO2C2</td>
<td>Calcium ion binding (GO:0005509), Glycosaminoglycan binding (GO:0005539)</td>
<td>62.2 ± 13.3</td>
<td>113.4 ± 14.8</td>
<td>1.82</td>
<td>0.03</td>
<td>0.08</td>
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<tr>
<td>SLC5A7</td>
<td>Choline: sodium symporter (GO:0005307)</td>
<td>300.4 ± 29.7</td>
<td>458.5 ± 37.9</td>
<td>1.53</td>
<td>0.01</td>
<td>0.06</td>
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<tr>
<td>ETV5</td>
<td>Transcriptional activator activity (GO:0001228)</td>
<td>1780.4 ± 192.6</td>
<td>2651.7 ± 252.6</td>
<td>1.49</td>
<td>0.02</td>
<td>0.07</td>
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<tr>
<td>RHCG</td>
<td>Ammonium transmembrane transporter (GO:0008519)</td>
<td>80.7 ± 11.4</td>
<td>118.6 ± 12.3</td>
<td>1.47</td>
<td>0.05</td>
<td>0.11</td>
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<tr>
<td>SIAH3</td>
<td>Ubiquitin protein ligase (GO:0061630)</td>
<td>167.2 ± 10.6</td>
<td>230.5 ± 9.3</td>
<td>1.38</td>
<td>0.0001</td>
<td>0.03*</td>
</tr>
<tr>
<td>CBS</td>
<td>Cystathionine-beta synthase activity (GO:0004122), Protein-binding (GO:0005515)</td>
<td>456.0 ± 18.5</td>
<td>546.0 ± 24.7</td>
<td>1.20</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>ITGB4</td>
<td>G-protein coupled receptor binding (GO:0001664)</td>
<td>94.2 ± 8.3</td>
<td>60.2 ± 8.1</td>
<td>0.64</td>
<td>0.02</td>
<td>0.06</td>
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<tr>
<td>CPAMD8</td>
<td>Serine-type endopeptidase inhibitor (GO:0004867)</td>
<td>42.4 ± 8.1</td>
<td>22.0 ± 4.0</td>
<td>0.52</td>
<td>0.05</td>
<td>0.11</td>
</tr>
</tbody>
</table>
SLC14A1 transcripts are elevated in OVT73 striatum

SLC14A1 = Urea transporter

Upregulation response to high level of urea
Urea and SLC14A1 are elevated in the OVT73 sheep brain by 1.5-fold. Did not differ significantly overall in the other brain regions examined: cerebellum, motor cortex, and hippocampus.
There’s a positive correlation between RHCG and SLC14A1

RHCG = Ammonia Transporter
Brain urea is elevated in a large postmortem HD case cohort
Take away

PRE early stage (Stage 0) → Early Stage (Stage 1)
Sheep model → Human

Starts @ Striatum → spreads → Cerebellum and Superior Frontal Gyrus

High levels of urea were present before any symptoms of dementia began

Urea spreads with later progression!
Why is Urea increasing in the first place?

Possibility #1: Neuronal Death
Dead neurons must be processed, which requires catabolism of their protein components

Possibility #2: High Metabolism
Energy expenditure far exceeds that utilized in movement (chorea) → high protein catabolism
Possibility #1: Neuronal Death

Highly unlikely

High Urea in prodromal sheep

Pre early stage of HD

Low cell death
Possibility #2: High Metabolic

Probably

High Metabolism

→ high protein catabolism
→ higher ammonia and urea
→ both transporters increase
Why is this important?

“Together with its precursor ammonia, urea is neurotoxic in excess and could certainly contribute to HD pathogenesis,”

Could lowering brain levels of urea and/or ammonia be a worthwhile therapeutic target in HD?

Further research is required:

- To identify the source of the elevated urea in HD
- To verify that ammonia level actually went up
Mitochondrial Dysfunction in Neurodegenerative Disease
The Mitochondria

- Key energy producing organelle in the cell
- Mitochondrial fission, fusion, and movement maintain cellular homeostasis
Mitochondria in Neurons

- Nervous system is 2% body mass and uses 20% of all energy
- Mitochondria function is critical for maintaining proper synaptic activity
- Mitochondria dysfunction is seen in Alzheimer’s, Huntington’s and Parkinson’s disease
Huntington’s Disease
Huntingtin

- Pleiotropic protein that performs many functions in the body
  - Required for normal mitochondrial motility
  - Mediates vesicle trafficking
  - Transcriptional regulator
- Mutation involves adding multiple CAG repeats
Mitochondrial Import

- 99% of proteins in the mitochondria are encoded by nuclear DNA
- These proteins must be imported into mitochondria
Mutant Huntingtin and TIM23

- Blocks mitochondrial protein import by associating with TIM23
- GST - glutathione S-transferase
- Httex1 - mutated Htt exon 1 with varying number of CAG repeats
- pOTC - radio labeled precursor matrix protein
Mutant Huntingtin in Neurons

- Mutant Htt-expressing neurons have reduced protein import
- Deficient protein import contributes to neuronal death
Parkinson’s Disease
Mitophagy

- Controlled degradation of mitochondria
- Necessary to remove damaged or dysfunctional mitochondria
Free Radicals and Reactive Oxygen Species

- Free radical - molecule that needs one more electron to have a complete valence shell
- Reactive oxygen species are produced by mitochondria
Parkin and Pink1

- Pink1 - mitochondria targeted serine/threonine kinase
- Parkin - E3 ubiquitin ligase
- Pink1 phosphorylates Parkin, causing Parkin to ubiquitinate other proteins
Parkin and Pink1 Pathway

- Key enzymes in mitochondrial degradation pathway
- Pink1 accumulates on depolarized mitochondria
- Recruits Parkin to mitochondria
- Ubiquitination identifies mitochondria for mitophagy
Parkinson’s Disease

- Caused by loss of dopaminergic neurons in the substantia nigra
- Familial link in ~10% of cases
- Loss-of-function mutations in Parkin or Pink1 lead to Parkinson’s disease
Enhancing Autophagy

- mTOR suppresses autophagy under nutrient rich conditions
- Rapamycin inhibits mTOR activity
- Rapamycin protects against neuronal death in Parkinson’s