Mechanisms of Ketogenic Diet action

COGS 163: FLAMING DINOS
What is the keto diet?

- High fat, low carb, adequate protein
- Force body to burn fats not carbs
- NOT Atkins Diet
- Used to treat epilepsy
HOW A NORMAL BODY PROCESSES GLUCOSE
GLYCOLYSIS

GLUCOSE → PYRUVIC ACID

2 ADP + 2 (P) → 2 ATP
2 NAD+ → 2 NADH + 2H+
KREB’S CYCLE (SIMPLIFIED)
ELECTRON TRANSPORT CHAIN

GLYCOLYSIS
Glucose → Pyruvate

KREBS CYCLE

ELECTRON TRANSPORT CHAIN AND OXIDATIVE PHOSPHORYLATION

ELECTRON TRANSPORT CHAIN

Electrons carried via NADH and FADH$_2$

Mitochondrion

Substrate-level phosphorylation

ATP synthase

Oxidative phosphorylation

Substrate-level phosphorylation

ATP

ATP

Cytosol

ATP

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WHAT HAPPENS IN KETOSIS?

- Reduced blood glucose levels
- Fatty acid oxidation in liver
- Production of ketone bodies

=> alternative energy source to glucose in brain
OVERVIEW OF GLUCOSE REGULATION

AMPLE CARBS

- Glucose => Co2, acetylco-A is intermediate goes into krebs -> ATP

NO CARBS

- Fatty acids=> acetylco-A (not recycled in Krebs) because OAA all used to make more glucose
  - ACCUMULATION OF ACETYLCO-A ACTIVATES KETOGENESIS
Historical and Clinical Perspectives

- Mild dehydration necessary
- Decreased pH (acidosis)

Approved Dietary Therapies

- Ketogenic Diet
- Modified Atkin’s Diet
- Low-Glycemic Index Treatment
Animal models: A Cautionary tail

- In some studies KD works, sometimes it doesn’t
- You can’t do a cross-comparison because of highly variable methodologies
- Acute Models vs. Chronic Models
  - Inducing seizures vs. epileptic mice
### Acute Animal Models (the cross-comparison that wasn’t meant to be...)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Type</th>
<th>% Fat</th>
<th>Who?</th>
<th>Length</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corneal electroshock</td>
<td>electric</td>
<td>55%</td>
<td>JM mice</td>
<td>short</td>
<td>KD: protective effects</td>
</tr>
<tr>
<td>Hydration electroshock</td>
<td>electric</td>
<td>55%</td>
<td>JM mice</td>
<td>short</td>
<td><strong>KD: less protective than control</strong></td>
</tr>
<tr>
<td>Maximal electroshock</td>
<td>electric</td>
<td>78%</td>
<td>AM rats</td>
<td>long</td>
<td><strong>KD: ^ severity, less WG</strong></td>
</tr>
<tr>
<td>Pentyletetrazol</td>
<td>chemical</td>
<td>78%</td>
<td>AM rats</td>
<td>long</td>
<td>KD: elevated threshold, Less WG</td>
</tr>
<tr>
<td>Bicuculline</td>
<td>chemical</td>
<td>78%</td>
<td>JM rats</td>
<td>long</td>
<td>KD: Fewer threshold seizures, longer seizure onset time</td>
</tr>
<tr>
<td>Semicarbazide</td>
<td>chemical</td>
<td>55%</td>
<td>JM mice</td>
<td>short</td>
<td>KD: more protective than control</td>
</tr>
<tr>
<td>Kainate</td>
<td>chemical</td>
<td>81%</td>
<td>AM mice</td>
<td>short</td>
<td>KD: Delay seizure onset, decrease Hippocampal loss</td>
</tr>
<tr>
<td>Fluorothyl</td>
<td>chemical</td>
<td>91%</td>
<td>JM/AM mice</td>
<td>short</td>
<td>KD: less WG+ ^ thrshld in JM</td>
</tr>
<tr>
<td>Maximal electroshock</td>
<td>electric</td>
<td>76%</td>
<td>JM/AM mice</td>
<td>short</td>
<td>KD: increased threshold to shock</td>
</tr>
<tr>
<td>Treatment</td>
<td>Type</td>
<td>% Fat</td>
<td>Who?</td>
<td>Length</td>
<td>Effect</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>---------------</td>
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<td>-------------------------------------------------</td>
</tr>
<tr>
<td>Kainate-induced status epilepticus</td>
<td>chemical</td>
<td>78.8%</td>
<td>JM Rats</td>
<td>short</td>
<td>KD: less WG, worse water maze performance</td>
</tr>
<tr>
<td>Succinic semialdehyde dehydrogenase</td>
<td>chemical</td>
<td>80%</td>
<td>J Mice</td>
<td>Long</td>
<td>KD: WG, fewer seizures</td>
</tr>
<tr>
<td>EL (seizure-susceptible inbred)</td>
<td>genetic</td>
<td>Injected BHB</td>
<td>JF Mice</td>
<td>short</td>
<td>ACA, acetone: protective BHB: depends + mildly toxic DBA: dose dependent protection</td>
</tr>
<tr>
<td><em>Kcnal</em>-null mice</td>
<td>genetic</td>
<td>86%</td>
<td>J Mice</td>
<td>short</td>
<td>KD: fewer seizures</td>
</tr>
</tbody>
</table>
**Ketone bodies themselves?**

- Seizure control lost when ketosis breaks
- Ketone body plasma levels correlate with seizure protection
- Mechanisms?
  - Interconversion of ACA to Acetone and BHB
  - BHB and ACA open $K_{ATP}$ channels of GABAergic neurons in SNr
  - ACA inhibits vesicular glutamate transporters
- Not sure though!

\[
\text{CH}_3\text{CCH}_2\text{CO}_2^- \quad \xleftrightarrow{\text{NAD}^+ \text{ NADH} + \text{H}^+} \quad \text{CH}_3\text{CCH}_2\text{CO}_2^- \quad \xrightarrow{\text{H}^+} \quad \text{CH}_3\text{CCH}_2\text{O}^- \\
\beta\text{-hydroxybutyrate} \quad \text{Acetoacetate} \quad \text{Acetone}
\]
\( K_{\text{ATP}} \) channels open

Resting state

\[ \text{Voltage-gated Ca}^{2+} \text{ channel closed} \]

No Insulin

Low glucose

\( K^+ \)

\( K_{\text{ATP}} \) channel open

ATP↓

ADP↑

GLUT2

ACA inhibits Glu transport

A

Glutamate

Ketone bodies

\( \text{Cl}^- \)

B

Signal transmission

Fasting

Ketogenic diet
Age dependence?

- KD more effective in children?
- Higher fat content in breast milk → need efficient mechanisms
- Higher expression of MCT1 and MCT2 in infants/children
- Who knows though?
Age dependence?

Ketone Bodies Inhibit

Development

A

B

Depolarization & Excitation of immature neurons

Hyperpolarization & Inhibition of adult neurons
GABAergic Mediated Inhibition

Decrease seizures?
Noradrenergic system, Leptin, and KD

- NE reuptake inhibitors prevent seizures (rat model)
- NE lacking mice have no response to KD
- KD increases NE levels in hippocampus by two-fold
- Leptin modulates neuronal excitability and suppresses seizures
  - KD increases leptin
Polyunsaturated Fatty Acids

- PUFAs decrease neuronal excitability
- PUFA levels elevated during KD from clinical evidence
- PUFAs and seizure control?
  - Not sure!
  - PUFA load, treatment duration, degree of ketosis as important variables
- PUFAs activate PPAR → could be anticonvulsant
Metabolic Mechanisms

“Consistent with increased energy reserves, ketogenic diet fed animals were highly resistant to the metabolic stress induced by low glucose conditions”

Ketogenic Diet:

- ↑ ATP, bioenergetic substrates, cerebral energy reserve, brain adenine
- ↑ metabolic enzymes and mitochondrial proteins
- ↓ Seizures and Convulsions
Effects on Mitochondrial Function

- 1) ACA/BHB oxidizes NADH
- 2) KB reduces ROS generation
- 3) protects against MRC inhibitors, elevates seizure threshold in patients with impaired MRC function
- 4) KD/KB elevates ATP production
- 5) Fatty acids activate mitochondrial uncoupling proteins
- 6) KB elevates the threshold for mitochondrial permeability transition activation
  - MPTp is a pore on the inner layer of the membrane
How does ATP prevent seizures?

- Maintain ionic gradients through Na/K ATP pumps?
  - No evidence for or against
- Lowered blood glucose levels
  - Causes ATP release from cells
  - Which is broken down into adenosine extracellularly
  - Activates a1 receptors
  - Open $K_{\text{ATP}}$ Channels and hyperpolarizes cell
  - Neuron is less excitable
**Effects of Decreased Glucose Metabolism**

- 2DG, a glucose analog that inhibits glycolysis
  - Anticonvulsant and antiepileptic
  - Decreases synaptic transmission via adenosine

- Confounding findings about 2DG:
  - ↓ GABA\(_A\)R function
  - Decreases BDNF and TrkB
Anaplerosis

**Definition:** process of replenishing Kreb’s Cycle intermediates

- Seizures have been correlated to cause deficiencies in oxaloacetate and α-ketoglutarate
- Has been shown to oppose seizure generation
Ketogenic Diet and Neuroprotection

KD is not just helpful for epilepsy...
Ketogenic Diet and Neuroprotection
KD is not just helpful for epilepsy...

“...a prominent neuroprotective mechanism of KD action involves a reduction in mitochondrial free radical production, which would decrease oxidative stress, and potentially neuronal injury.”

Research in:
- Alzheimer’s, Parkinson’s, TBI, stroke, obesity, ALS, cancer!!
- Thought to improve: lifespan, general mood, age-related deficits in learning, motor coordination, weight
Ketogenic Diet and Neuroprotection

KD is not just helpful for epilepsy...

Ketone Bodies/PUFAs and Mitochondrial Function:
- Raise ATP levels in hippocampus
- Diminish ROS production (↑NADH oxidation)
- Inhibit mitochondrial permeability

More about ROSs:
- PUFAs → UCP production → reduced ROS production (and ↓ATP synthesis, ↓calcium influx into mitochondria, ↓heat)
- UCP protects against kainate-induced excitotoxicity (probably by reducing ROS production)
Discussion...

- It’s becoming very apparent that the mechanisms are not simple.
  - There have to be many mechanisms working in a coordinated fashion
- What is evident, however, is the increasing importance of studying neurometabolism.
- This research is important for epilepsy, but also for an expanding set of neurodegenerative diseases
Conclusions...

How Does the Ketogenic Diet Work?
Conclusions