Effects of Ketogenic Diet on the Microbiome and Epilepsy

ATPeeps

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Agenda

● Background
  ○ Ketone bodies
  ○ Ketogenic diet
  ○ Insulin resistance
  ○ Gut Microbiota
  ○ Epilepsy
    ■ Treatment methods
  ○ Refractory epilepsy

● Studies previously done
  ○ Mice & Human
    ■ Methods & Results
  ○ Findings

● Conclusion
Ketone Bodies (KB)

Recap: What are ketone bodies?
- Three water-soluble molecules containing the ketone group that are produced by the liver from fatty acids during periods of low food intake, carbohydrate restricted diets, starvation, prolonged intense exercise, alcoholism, or untreated type 1 diabetes.
- Not enough insulin = unable to use glucose for energy = breakdown of fat and protein
- Raised levels of ketone bodies in the body tissues = ketosis

3 main ketone bodies:
- β-hydroxybutyrate (BHB)
- Acetoacetate (AcAc)
- Acetone
Acetoacetate (AcAc), Acetone and β-hydroxybutyrate (BHB)

- **Acetoacetate**
  - principle ketone body
  - produced and utilized during intermediary metabolism
  - other ketone bodies are derived from AcAc

- **Acetone**
  - simplest ketone
  - produced by the spontaneous decarboxylation of acetoacetate
  - least abundant ketone body

- **β-hydroxybutyrate (BHB)**
  - In the liver, the BHB-dehydrogenase enzyme reduces AcAc to form BHB
  - BHB is the most prevalent circulating ketone body as cells adapt to carbohydrate restriction
  - BHB is not a technically a ketone due it structure
  - Still considered a ketone because BHB is closely related to AcAc and acetone and affect the body similarly
Ketone Bodies Mediate Anti-Seizure Efficacy

- New data from in vivo and in vitro studies support ketone bodies as having anti-seizure actions.
- Ketones also influence neuronal excitability directly through molecular targets:
  - The mitochondria
  - Antioxidant systems, inflammatory mediators, and
  - Histone deacetylation (HDAC) status
- The anti-seizure efficacy is due to the effects of......
Ketogenic Diet (KD)

- Low carbohydrate, adequate protein, and high-fat diet
- Body burn fats rather than carbohydrates
- Diet promotes weight loss though high caloric foods are consumed
- Ketones → fatty acid production → decreased glucose uptake
Examples of Ketogenic Diet (KD) Foods
Ketogenic Diet Used as Treatment

- Treatment for:
  - Alzheimer’s disease
  - Type II Diabetes
  - Polycystic Ovarian Syndrome
  - ASD
  - Epilepsy

- Insulin resistance mediated by KD is a key player in how the diet can be used as a treatment for some of these diseases
Insulin Resistance

- KD diet has been found to result in **insulin resistance** which increases hepatic lipid content
  - Increased diacylglycerol (DAG) content → increased protein kinase C (PKC\(\varepsilon\)) activation → inhibition of insulin signalling
    - In muscle → failure to promote glycogen synthesis: rather promote fatty acid (FA) prod.
    - Increased lipid prod. → NAFLD (Non-alcoholic fatty liver disease)
  - CAN be fixed with exercise!
Recent studies suggest KD may improve insulin sensitivity

**Insulin resistance** leads to a decrease in insulin-stimulated heart muscle and brown adipose tissue (BAT) glucose uptake
  - In the heart, it reduces its ketolytic capacity → fatty acid oxidation

Additionally, 23% reduction of glucagon → increase FGF21 (regulates energy)
  - Contributes to reduction in fasting plasma glucose and insulin concentrations
  - Inhibits glucagon secretion from rat pancreatic α-cells

Overall, FGF21 has been suggested to play a crucial role in ketogenesis, increases energy expenditure and thermogenic activation of BAT

Hepatic expression of FGF21 is implicated in long-term regulation of energy balance and metabolism
- The microbe (microorganism) population living in our intestine
- Gut flora or gastrointestinal microbiota
- ⅓ of our gut microbiota is common to most people and ⅔ are specific to one person
  - Each person/animal has a unique microbiota
- Even with unique microbiotas they all fulfill physiological functions which impact our health:
  - Helps with digestion
  - Production of some vitamins like B and K
  - Helps with host metabolism and behavior
  - etc.
- Intermediary in diet and host physiology
  - Composition and function is shaped by diet → changing
  - Nutrients available depend on microbial metabolism
• The microbiota plays an important role in providing materials needed for ketone body synthesis and can affect **ketone body synthesis** during caloric restriction.

• Changes are reproducible and persistent → lasting impact on host
  
  ○ **Diet-induced pathologies** due to changes in the gut microbiota in mouse models
    ■ Atherosclerosis = carnitine-rich diet
    ■ Undernutrition = Malawian diet
    ■ Abnormal social behavior = maternal high-fat diet
  
  ○ KD alters the composition of gut microbiota in mice
  
  ○ **Ketosis** is associated with alterations of gut microbiota

• ↑ association with changes in factors relevant to neurotransmission (neurotransmitter signaling, synaptic protein expression, long-term potentiation, myelination, etc)
  
  ○ Changes in host behavior like stress-induced, social, and cognitive behaviors

*ketosis = presence of ketone bodies*
Epilepsy

- Disorder where nerve cell activity in the brain is disturbed, causing seizures.
- Symptoms:
  - Fainting, fatigue, muscle spasms, amnesia, anxiety, depression, headaches, temporary paralysis after a seizure, loss of consciousness, etc.
- 4th most common neurological disorder
- Affects all ages
- Also known as "seizure disorder"
Epilepsy is a spectrum condition with a wide range of seizure types:

- **Focal** - specific place in the brain
  - Without loss of consciousness and with impaired awareness
- **Generalized** - all over the brain
  - Absence, tonic, atonic, clonic, myoclonic, tonic-clonic seizures
  - **Tonic-clonic seizures**: can cause abrupt loss of consciousness, body stiffening and shaking, and sometimes loss of bladder control or biting of the tongue.

**Causes:**

- Genetic influences, head trauma, brain conditions, infectious disease, prenatal injury, developmental disorders
Current Treatments for Epilepsy

- **Medication**
  - Depends on what type of seizures patient has, frequency, age, sex, other medications, and pregnancy
  - Anti-seizure or anticonvulsants
    - Change the way the brain cells work and communicate with each other

- **Nerve stimulation**
  - **Vagus nerve stimulation**
    - Device sends small bursts of electricity through the nerve in chest/abdomen to your brain
    - Medication still may be needed
  - **Responsive neurostimulation**
    - Device under the scalp, looks for patterns in brain activity that lead to seizure, when pattern detected sends pulse to interrupt it

- **Surgery**
  - **Resective surgery**
    - Part of brain that causes seizures is removed, mostly done when the part is very small with small boundaries, along with controlling important parts like speech, movement, sight, or hearing.
  - **Disconnective surgery**
    - Instead of removing part of brain, path of nerves involved in the seizures are cut

- **Diet**
  - **Ketogenic diet**
Refractory Epilepsy

- **Refractory epilepsy:**
  - Condition affecting > ⅓ epileptic individuals, defined by failure to respond to existing anticonvulsant medications
  - A disorder in which nerve cell activity in the brain is disturbed, causing seizures
- Also known as intractable, or drug-resistant epilepsy.
- Medicine is **NOT** bringing seizures under control
Several studies have shown with antibiotic treatment increases the risk of status epilepticus and symptomatic seizures

- **Status epilepticus**: repeated seizures one after another without recovery of consciousness between them
- **Acute symptomatic seizures**: Clinical seizures occurring at the time of a systemic insult (such as a stroke, traumatic brain injury, subdural hematoma, active CNS infection, active phase of MS, other autoimmune disease, etc.)

**Hypothesis** that gut microbiota (GM) impacts the anti-seizure effects of the KD
Mice Microbiota Experiments with Ketogenic Diet
The Ketogenic Diet Alters the Gut Microbiota

- Tested using 6-Hz induced seizure model of refractory epilepsy
- Mice fed KD exhibit elevated seizure thresholds in response to 6-Hz stimulation
- KD alters composition of gut microbiota by 4 days post-dietary treatment
  - Decreased alpha diversity observed at each time point → KD-induced losses of particular bacterial taxa
    - KD increase relative abundance of Akkermansia (Akk)
    - Parabacteroides, Sutterella, and Erysipelotrichaceae increase in KD-fed mice
    - Allobaculum, Bifidobacterium, Desulfovibrio increased in CD (control diet)–fed mice
- Composition of gut microbiota is rapidly and substantially altered in response to KD
The GM is Necessary & Sufficient for Anti-Seizure Effects of KD

- 6-Hz Psychomotor Seizure Assay
- Experimental groups:
  - Germ free or antibiotic treated mice fed a ketogenic diet
  - Postnatally conventionalized germ free mice fed a ketogenic diet
- SPF mice fed KD for 14 days exhibited increased seizure thresholds and altered microbiota
- Gut microbiota is required for KD-mediated increases in seizure protection
  - Actively mediates seizure protection through pathways that are independent of pre-weaning developmental processes
  - Microbial effects on seizure resistance do not correlate with changes in serum BHB or glucose levels
  - Gut microbes modulate seizure susceptibility through mechanisms that do not involve alterations in BHB levels
The Gut Microbiota Confers Seizure Protection to Mice Fed the Control Diet

- Study to determine if ketogenic diet-associated gut microbes confer anti-seizure effects in mice fed a control diet:
  - Mice treated with antibiotics were transplanted with the microbiota from specific pathogen free mice and fed either a control diet or ketogenic diet
  - Mice were then evaluated on their susceptibility to 6-Hz seizures after 4 days of dietary treatment
  - Mice transplanted with microbiota from the control diet specimen and fed a ketogenic diet demonstrated an increased seizure threshold
  - Mice fed a control diet demonstrated an increased seizure threshold when transplanted with the microbiota from the ketogenic diet specimen
    - The ketogenic diet microbiota confers seizure protection even in mice fed a control diet
The Gut Microbiota Confers Seizure Protection to Mice Fed the Control Diet

- A confound to these anti-seizure effects arises when specific pathogen free mice and germ free mice treated with antibiotics and fed a control diet demonstrate an increase in seizure threshold
- To combat this confound:
  - Exogenous bacterial treatment administered to mice fed a control diet
  - Specific pathogen free mice gavaged bi-daily for 28 days with $10^9$ colony forming units A. muciniphila and Parabacteroides or with vehicle
    - Treatment increased seizure thresholds in comparison to vehicle gavaged controls
    - Seizure protection not observed in mice treated with A. muciniphila alone
    - Treatment with heat-killed bacteria decreases seizure threshold
  - Persistent exposure to A. muciniphila and Parabacteroides is necessary
  - Fecal transplant of the ketogenic diet microbiota paired with long term bacterial treatment with the ketogenic diet associated taxa confer protection against 6-Hz seizures in mice fed the control diet
Fecal Microbiome Transplant (CD/KD fecal transplant, CD/KD diet):

Antibiotic Depletion & Bacterial Enrichment (SPF Abx + Pb / Akk / AkkPb / Bif):

AkkPb 28-Day Probiotic Treatment (SPF CD + veh / AkkPb / Akk / heat-killed AkkPb):
KD-Associated Bacteria Mediating Anti-Seizure Effects of the KD

- That increase in seizure threshold in SPD CD mice treated with Abx (antibiotics) alone and GF Cd mice relative to SPF (Specific pathogen-free) CD controls caused uncertain results.

- Bacterial treatment increased seizure threshold in relation to vehicle-gavaged controls.

- The Co-administration of A Muciniphilia and Parabacteroides is required for seizure protection.
**Antibiotic Depletion & Bacterial Enrichment (SPF Abx + AkkPb):**

- **Antibiotic (AVN):** Water, Gavage
- **Bacterial:** Ad libitum, Dietary Treatment, EEG Transmitter Implantation & Recovery, EEG Recording

**SPF Kcnat1**:
- Days 1-7
- Day 9
- Days 9-31
- Days 23-28
- Days 29-31

**Seizures**
- Per Day
- Duration Per Day (min)

- **SPF CD**: Kcnat1+/+ vs Kcnat1−/−
- **SPF KD**: Kcnat1+/+ vs Kcnat1−/−
- **Abx AkkPb**: Kcnat1+/+ vs Kcnat1−/−

**EEG Monitoring**
- 2s (200 uV)
- 250 ms
- 1200 uV

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**Graphs and Data**

- Relative abundance of bacterial species:
  - Akkermansia muciniphila
  - Parabacteroides spp.

- Seizure frequency and duration analysis:
  - Comparison between control and experimental groups.
Temporal Lobe Epilepsy and SUDE

- KD increases A Muciniphilia and parabacteroides in KCNA mice
- Decrease in the occurrence of seizures and duration in both KD and CD fed mice.
- A. muciniphila and Parabacteroides had a reduced seizure frequency and duration vs vehicle control.
- Microbial dietary metabolism regulates secondary metabolites
- An impact on the likelihood of a seizure happening
The Microbiota Modulates Gut, Serum, and Brain Metabolomes

- Hypothesis: microbial dietary metabolism regulates secondary metabolites that impact seizure susceptibility.
- Widespread decreases in subsets of ketogenic gamma-glutamylated amino acids
  - Gamma-glutamated forms of the amino acids were particularly affected
  - Gut microbiota modulates gamma-glutamylation itself or selective metabolism of ketogenic GG-amino acids
  - Increased ketogenic GG-amino acids are associated with seizure susceptibility
- Gut microbiota modulates intestinal and systematic metabolomic responses to KD
- Association between KD-induced seizure protection and microbiota-dependent alterations in levels of ketogenic GG-amino acids
What About HUMANS?
Ketogenic Diet Treatment in Adults with Refractory Epilepsy

- KD is used to treat refractory epilepsy mainly for children.
- Not many studies.
- Untested assumption that adults would not comply with the unpalatable diet.
Treatment Compliance

35 eligible TOTAL

- 23 fully compliant
- 6 mild
- 5 moderate

12 subjects left:
- 8 women
- 4 men

ages (24-65)

reluctant to give up regular diet OR thought KD was too complex.
Side Effects Observed in Children

- Constipation
- Diarrhea
- Nausea
- Vomiting
- Nephrolithiasis (kidney stones)
- Metabolic acidosis (excessive quantities of acid)
- Hyperuricemia (high uric acid)
- Hypocalcemia (electrolyte imbalance)
- Hypomagnesemia (low magnesium)
- Weight loss
- Hyperlipidemia (too many lipids)
- Bruising
- Osteopenia (bone loss)

But KD is still safe!
METHODS

SUBJECTS

- Men and women aged 25-65 with
  - Refractory primary generalized epilepsy (PGE)
  - Localization-related epilepsy (LRE)
  - Failed treatment with ≥ 3 antiepileptic drugs & seizure frequency of ≥ 1 every 2 months

- Fast for first 24-48 hours
- KD initiated during 4-5 day long hospitalization → daily caloric increase (33, 66, 100%)
- Used 3:1 \([\text{fat}] : [\text{protein + carbohydrate}]\) ratio by weight, 90% calories derived from fat
  - 4:1 ratio usually used in children
RESULTS

- No subjects discontinued treatment because of adverse effects → SAFE
- 10/12 subjects improved
  - 6/12 had 50% seizure reduction during the whole treatment
  - 4/12 had 20-50% seizure reduction during the whole treatment
  - 1 did not change
  - 1 worsened
- Efficacy data in study similar to those from pediatric studies → KD may be very effective in proportion of adults

(Chart showing adverse events:
- Mild Intermittent Hunger: 3
- Diarrhea: 2
- Constipation: 1
- Nausea: 2
- Abdominal Cramps: 4
- Transient Nausea: 1
- Isolated Vomiting: 2)
<table>
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<th>Subject No.</th>
<th>Treatment duration (months)</th>
<th>Seizure frequency/month</th>
<th>% Change</th>
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a Compliance grading: 0 = none; 1 = mild, 2 = moderate, 3 = complete.
b Subject dropped out after 4 days and is not included in data analysis.
c \( P = 0.05 \).
d \( P = 0.04 \).
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Monthly Frequency of Only Generalized Tonic-Clonic & Complex Partial Seizures
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Mean (SD) 2.07 (2.36)
Seizure frequency did not improve.
Comparing graphs together:

Monthly Frequency of Only Generalized Tonic-Clonic & Complex Partial Seizures

- **Seizure Frequency Per Month**
  - **Subject Number**
  - **Baseline**
  - **Whole Treatment**

Percentage of Change After KD Treatment of Tonic-Clonic & Complex Partial Seizures

- **% of Change**
  - **Subject Number**
Seizure-free months for 4-month baseline, KD months 1–4, and whole treatment duration.

<table>
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<th>Subject No.</th>
<th>Treatment duration (months)</th>
<th>Seizure-free months (Baseline)</th>
<th>KD months 1–4</th>
<th>Compliance(^b)</th>
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N/A, not applicable.

\(^a\) Treatment months 1–4 are 4 months except for subjects who discontinued treatment early, for whom number of seizure-free months/number of months on treatment is given.

\(^b\) Compliance grading: 0 = none; 1 = mild, 2 = moderate, 3 = complete.

\(^c\) Subject dropped out after 4 days and is not included in data analysis.

\(^d\) \(P = 0.04\).
Adults VS. Children

- In pediatric studies, antiepileptic effect may persist after KD discontinuation.
- Previously, adult studies have not evaluated effect of KD on epilepsy after treatment discontinuation.

- In this study, seizure improvement did NOT outlast KD treatment → difference between adult and pediatric responses to KD
Conclusion

- Ketone bodies mediate anti-seizure efficacy of the KD
- Insulin resistance mediated by KD is a key player in reducing anti-seizure effects in epilepsy
- Effects of a ketogenic diet on epileptic seizures are also mediated by the gut microbiome through their modulation of hippocampal GABA/glutamate ratios
- Changes in the gut microbiota are required for the anti-seizure effects of the KD
- Due to previous adaptations in adult gut microbiota, the ketogenic diet affects adult patients ONLY during treatment → diet must be continued to have anti-seizure effects
References

- [https://www.mayoclinic.org/diseases-conditions/epilepsy/symptoms-causes/syc-20350093](https://www.mayoclinic.org/diseases-conditions/epilepsy/symptoms-causes/syc-20350093)
- [https://www.webmd.com/epilepsy/guide/treating-epilepsy#1](https://www.webmd.com/epilepsy/guide/treating-epilepsy#1)
Bing Word Bank

- **BHB**: B-hydroxybutyrate
- **AcAc**: Acetoacetate
- **KB**
- **KD**
- **ETC**: Electron transport chain
- **HDAC**: Histone deacetylase
- **mPT**: Mitochondrial permeability transition
- **Ac-CoA**: Acetyl coenzyme A
- **GABA**: gamma-aminobutyric acid
- **Akk**: Akkermansia muciniphila
- **FISH**: Fluorescence in Situ Hybridization
- **Abx**: Antibiotics
- **CFU**: colony forming units
- **SPF**: Specific pathogen-free
- **GF**: Germ free
- **CD**: Control diet
- **BF**: Bifidobacterium longum
- **BHI**: Brain heart infusion
- **OTUs**: Operational taxonomic units
- **KO**: Knock out
- **GGT**: gamma-glutamyltranspeptidase
- **AU**: Absorbance units
- **EEG**
- **ROS**: reactive oxygen species