Dietary and pharmacologic approaches to ketosis: potential therapeutic applications to neurological conditions and cancer

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Disclosures

Member of the Moore Family Center for Whole Grain Foods, Nutrition and Preventive Health (Director, Emily Ho, PhD)

No commercial relationships to disclose
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Presentation Outline: Health Implications of Ketosis

- Maintaining energy homeostasis: the role of ketosis
- Dependence of brain, heart and muscle on ketones for energy
- How are ketones made and consumed
- Types of ketosis:
  - Physiological
  - Dietary
  - Pharmacological
- Ketones and neurological function
- Ketones in the treatment of neurological conditions
Production and Consumption of Ketones

• Ketone bodies are synthesized in liver from acetyl-CoA derived primarily from fatty acid oxidation and are transported to extrahepatic tissues for oxidation during physiological states characterized by limited carbohydrate and when fatty acid availability is in excess.

• Ketones can also be produced by astrocytes in the brain to support neural cell function.

• Ketones are water soluble and can be transported across the inner mitochondrial membrane as well as across the blood-brain barrier and cell membranes.

• Tissues that use ketones to produce energy include the brain, heart and muscle.
Ketones Enable Survival in Starvation

- Ketone bodies are metabolized through evolutionarily conserved pathways that support bioenergetic homeostasis, particularly in brain, heart, and skeletal muscle when carbohydrates are in short supply.
- Metabolism of ketone bodies is conserved among eukarya, bacteria, and archaea.

Ketone metabolism: beyond energetics

• Ketone bodies also serve as lipogenic and sterol biosynthetic substrates in many tissues, including the developing brain, lactating mammary gland, and liver.

• Ketone body metabolism can be abnormal in numerous disease states, including types 1 and 2 diabetes, neurological conditions and cancer.
Defining terms in ketosis

- **Ketosis**: a metabolic process that occurs normally and is greatly induced when the body does not have enough glucose for energy. Stored fats are broken down for energy, resulting in production of ketone bodies.

- **Ketoacidosis**: an acute, major, life-threatening complication of type 1 diabetes (but not uncommon in some patients with type 2 diabetes). This condition is a complex disordered metabolic state characterized by hyperglycemia, ketoacidosis, and ketonuria.

<table>
<thead>
<tr>
<th>Blood levels</th>
<th>Normal diet</th>
<th>Ketogenic diet</th>
<th>Diabetic ketoacidosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>80–120</td>
<td>65–80</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>Insulin (μU/l)</td>
<td>6–23</td>
<td>6.6–9.4</td>
<td>≈ 0</td>
</tr>
<tr>
<td>KB conc (mM/l)</td>
<td>0.1</td>
<td>7/8</td>
<td>&gt; 25</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
<td>7.4</td>
<td>&lt; 7.3</td>
</tr>
</tbody>
</table>
Defining Physiologic Ketosis

- Dr. Hans Krebs, pioneering biochemist, defined physiologic ketosis as a *normal metabolic state* that provides an important source of energy in prolonged physical exertion, fasting, and starvation (Krebs HA. The physiological role of the ketone bodies. The Biochemical Journal. 1961;80:225-33).

- Ketosis enables survival in starvation and hibernation, and accounts for as much as 25% of the neonate's basal energy requirements in the first several days of life (Bougneres PF, Lemmel C, Ferre P, Bier DM. Ketone body transport in the human neonate and infant. JCI 1986;77(1):42-8).
The brain cannot store or utilize fatty acids…….

but is adapted to use glucose and secondary metabolite of fatty acids called ketones

Adapted from Melo et al. *Neurochem Int.* 2006 May-Jun;48(6-7):498-507
Ketone Nomenclature
(Historically referred to as ketone bodies)

- [R]-hydroxybutyrate
  - ([R]-HB yields 4.79 kcal/gram)

Acetoacetate
- (AcAc yields 4.24 kcal/gram)

Acetone
Physiological Ketosis: Enabling Survival in Starvation

Ketone Production and Utilization Enable Survival

• In fasting, rates of total ketone body production and oxidation can reach 150 g/day and 129 g/day, respectively (Reichard et al. JCI 1974;53(2):508-15. doi: 10.1172/JCI107584)

• During periods of prolonged fasting or starvation, the human brain is dependent on $\overset{\rightleftharpoons}{\text{HB}}$ and AcAc, which provide up to 60% of the brain’s energy requirement (Owen et al. JCI 1969;48(3):574-83. doi: 10.1172/JCI106016).
A  

**Hepatic Ketogenesis**

1) β-Oxidation

2) ACAT1 → CoA-SH

3) HMGCS2 → CoA-SH

4) HMGCL → Acetyl-CoA

5) Acetone → AcAc → CO2 → βHB

B  

**Ketone Body Oxidation**

1) BDH1 → NAD+ → NADH

2) OXCT1 → Succinyl-CoA → Succinate

3) ACAT1 → CoA-SH

4) Citrate Synthase → Acetyl-CoA → Pyruvate → CoA-SH

1 kcal/g
BDH1 and OXCT1 are constitutively expressed in heart, brain and muscle.
Dependence of the Brain on Ketones

• The brain exerts control over voluntary and involuntary functions via neural and neuroendocrine functions.
• The brain is fueled primarily by glucose in the fed state and ketones and lactate in the fasted state or with ketogenic diets.
• While constituting only 2% of the total body weight, the brain consumes 20% of the oxygen and 25% of the glucose consumed daily.
• The high level of glucose and fatty acid catabolism for energy and sterol metabolism negates the brain’s ability to store energy as fat.

M. D. McCue (ed.), Comparative Physiology of Fasting, Starvation, and Food Limitation, DOI: 10.1007/978-3-642-29056-5_1,
What does the brain use energy for?

1. Signaling through postsynaptic and action potentials, as well as uptake and recycling of neurotransmitters;
2. Maintenance and restoration of ion gradients dissipated by signaling processes listed above, as well as synaptic and action potentials, represent by far the main energetic cost related to maintenance of excitability (Alle et al., 2009). Therefore—it has been estimated that glutamate-mediated neurotransmission is responsible for most (80%) of the energy expended in the gray matter (Sibson et al., 1998; Hyder et al., 2006; Shulman et al., 2004; Attwell and Laughlin, 2001).

Achieving Ketosis by Dietary and Pharmacologic Means
Dietary and pharmacologic methods of achieving ketosis

1. Dietary Means
   • Ketogenic Diet
   • Modified Atkins Diet
   • Low-Glycemic Index Diet

2. Pharmacologic Means
   • Mineral salts of ketones*
   • Ketone mono- and di-esters of $\text{HB}/\text{AcAc}/1,4\text{ butanediol}$*
   • Medium Chain Triglyceride (MCT) Therapy*

3. By a combination of these approaches*

Intellectual property regarding neurological effects of these approaches has been granted to Accela/ Nestle, the inventors of the ketone esters and Dr. Dominic D’Agostino, respectively
<table>
<thead>
<tr>
<th>Methods For Inducing Ketosis</th>
<th>Composition</th>
<th>Guidelines</th>
<th>Side Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Classic” Long-chain Triglyceride KD&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>10-15g/d carbohydrate 1g/d protein Remaining calories from fat</td>
<td>Ketogenic ratio 4:1 or 3:1 (fat: carbohydrate and protein) 90% energy from fat</td>
<td>Gastrointestinal effects (vomiting and constipation) Hunger Taste problems Lack of energy</td>
<td>Eagles, 2008; Neal et al., 2009</td>
</tr>
<tr>
<td><strong>Medium-chain Triglyceride KD&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td></td>
<td>Ketogenic ratio &lt;2.5:1 (fat: carbohydrate and protein) 60% energy from medium-chain triglycerides</td>
<td>Gastrointestinal effects (vomiting, diarrhea, abdominal pain)</td>
<td>Eagles, 2008; Neal et al., 2009</td>
</tr>
<tr>
<td><strong>Modified Medium-chain Triglyceride KD&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td></td>
<td>30% energy from medium-chain triglycerides 30% energy from long-chain triglycerides</td>
<td>Gastrointestinal effects (vomiting, diarrhea, abdominal pain)</td>
<td>Neal et al., 2009</td>
</tr>
<tr>
<td><strong>Modified Atkins Diet&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td>10-15g/d carbohydrate (initially) 15-20g/d carbohydrate increase (based on seizure control) Calories not restricted Fluid not restricted Protein not restricted</td>
<td>Ketogenic ratio 0.9:1 (fat: carbohydrate and protein) 60% energy from fat 30% energy from protein 10% energy from carbohydrates Fiber not counted as carbohydrate Sugar alcohols counted as carbohydrate</td>
<td>Increase total cholesterol Increase blood urea nitrogen (BUN) Possible weight loss</td>
<td>Kang et al., 2007; Kossoff and Dorward, 2008; Kossoff et al., 2008</td>
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<td>Ketone Esters</td>
<td>An esterified ketone compound (commonly β-hydroxybutyrate or acetoacetate esterified to become (R)-3-hydroxybutyl (R)-3-hydroxybutyrate)</td>
<td>Supplementation of between 2 and 40 grams of a ketone ester compound.</td>
<td>Gastrointestinal effects (vomiting, diarrhea, abdominal pain)</td>
<td>Clarke et al., 2012; D’Agostino et al., 2014</td>
</tr>
</tbody>
</table>
Classical and “Liberal” Ketogenic Diets May Be Efficacious As Well
(Michelle Nikolai, Mary Noel, Ken Schwartz)
KetoCal® by Nutricia

• A soybean oil based, nutritionally complete, powdered product, which can be used to administer the classical (4:1) ketogenic diet for children over 1 year of age.

• Rodent studies use paste form water : KetoCal® = 1:2
## Side effects of ketogenic diets

<table>
<thead>
<tr>
<th>Short term</th>
<th>Long term</th>
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<tbody>
<tr>
<td>Acidosis</td>
<td>Bone fractures</td>
</tr>
<tr>
<td>Constipation</td>
<td>Decreased bone mineral density</td>
</tr>
<tr>
<td>Excessive ketosis</td>
<td>Dyslipidemia</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Kidney stones</td>
</tr>
<tr>
<td>Food refusal</td>
<td>Poor linear growth</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>Secondary carnitine deficiency</td>
</tr>
<tr>
<td>Vomiting</td>
<td>Vitamin D deficiency</td>
</tr>
<tr>
<td>Exacerbation of gastro-esophageal reflux disease</td>
<td>Weight loss/insufficient weight gain</td>
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</table>
Efficacy of Ketogenic Diets for Epilepsy

• While the mechanism(s) of action are not known, the ketogenic diet is The effectiveness of ketogenic diets has been shown to produce a 30–40% reduction in seizures compared with comparative controls and considered ‘comparable to modern antiepileptic drugs’.
• The main drawback with the ketogenic diet was difficult tolerability and high dropout rates—given the positive results and the severe side effects common with antiepileptic medications, the development of an easier-to-follow ketogenic diet would be a worthwhile goal.

Proposed mechanisms of action

• Several hypotheses have been put forward trying to explain the mechanism of action of ketogenic diets:
  – (1) a direct anticonvulsant effect of KBs;
  – (2) a reduced neuronal excitability induced by KBs;
  – (3) an effect on the mammalian target of rapamycin pathway.

• These metabolic mechanism(s) activated by ketogenic diets may also influence neurotransmitter activity in neurons.

MCTs and Ketone Esters: Pharmacologic or “Therapeutic” Ketosis

- MCTs consist of mixture of 6-12 carbon medium-chain fatty acids (MCFAs) that are esterified with glycerol (Marten et al. International Dairy Journal. 2006;16(11):1374-82).


- MCFAs are transported to the liver where they undergo β-oxidation in mitochondria to form ketone bodies.

- Modest MCT supplementation at 30 grams per day for 3 weeks can result in elevation of blood ketone concentrations ~290 μmol.

- MCT supplementation has also been shown to have the following therapeutic benefits in animal models: improved athletic performance, treatment of neurodegenerative diseases, and reduction of body fat (Zyl CGV, Lambert EV, Hawley JA, Noakes TD, Dennis SC. Journal of applied physiology. 1996;80(6):2217-25; Tsuji H et al., The Journal of Nutrition. 2001;131(11):2853-9).
FDA-Approved MCT formula treatment of age-associated memory impairment (AAMI) and Alzheimer's disease.


• Accera’s patent consists of oral and/or intravenous MCT administration that results in elevated KB concentrations in a diet where carbohydrate intake is not restricted (Henderson ST, inventor; Accera, Inc., assignee. Use of medium chain triglycerides for the treatment and prevention of alzheimer's disease and other diseases resulting from reduced neuronal metabolism II patent US6835750 B1. 2004 2004/12/28).

• The recommended daily dosage is 40 grams of Axona® powder dissolved in water that contains 20 grams MCTs. The Food and Drug Administration approved Axona® in 2009 for the treatment of age-associated memory impairment (AAMI) and Alzheimer's disease.
Ketone Esters: Ketosis Without Side Effects

• An alternative to producing ketosis pharmacologically, rather than by starvation or feeding a ketogenic diet, has been made possible through the development of ketone salts (KS) and ketone mono- and di-esters (KE). Sodium and potassium salts of $\beta$-HB and AcAc are available commercially.

• KEs serve as an effective vehicle KB for delivery to blood circulation because the KE supplementation eliminates the risk of acidosis observed with supplementation of $\beta$-HB or AcAc in free acid form.

Dr. Dominic D’Agostino
Patent "Methods of Sustaining Dietary Ketosis and its Effects on Lipid Profile"
Experimental Evidence: Ketone Esters

• KE supplementation can raise KB levels without fasting or adherence to a ketogenic diet or elevation of plasma free fatty acid elevation------
  *a physiological state unseen in nature.*

• KBs, including $\beta$-HB, AcAc and/or 1,4 butanediol can be esterified as homo- and heteromeric combinations forming ketone esters (KE) that, result in elevation of blood ketone levels to 2-7 mmol/L, which is similar to those seen in prolonged fasting (Cotter DG, et al., The Journal of Biological Chemistry. 2011;286(9):6902-10).

• In animal models, KE supplementation of a normal rodent diet, has been shown to have therapeutic benefits including
  – delay of seizures caused by central nervous system oxygen toxicity,
  – weight loss,
  – treatment of Alzheimer's disease,
  – improved athletic performance,
  – cancer treatment

Why Do Ketones Appear to Be So Beneficial to the Brain?

• Available evidence indicates that \( \alpha \)-HB, may be able to overcome the hypometabolism of glucose observed in certain neurological conditions.

• Many neurological conditions (e.g., ALS, Parkinson’s, Huntington’s, Alzheimer’s and Cockayne Diseases) have been shown, in animal models, to respond favorably to a ketogenic diet. (Bold= caloric restriction exacerbates progression)
Life without lipid: brain energy metabolism

• The brain uses glucose in the fed state via different pathways to produce ATP and shuttle energy substrates between cell types.
  • glycolysis (leading to lactate production or mitochondrial metabolism),
  • the pentose phosphate pathway (PPP),
  • glycogenesis (in astrocytes only)
• Neurons and glial cells contribute equally to the cell populations in the brain but differ by region (cortex ratio: 3.76 to 1; cerebellum 1: 4.3, respectively (Herculano-Houzel et al. J Comp Neurol. 2009 Apr 10;513(5):532-41).
• Ketones can substitute for most (62%) of the oxidative substrates needed by neuronal cells in starvation (Chowdury et al. (2014) J. Cereb. Blood Flow Metab. 34, 1233-1242).
The ketogenic diet as a cancer therapy
Motivation for Pursuing Ketogenic Diets to Treat Brain Cancer

Eastern Michigan University
Miriam Kalamian; EdM, MS, CNS

- December 2004: Initial Diagnostic MRI
- October/November 2006: Debulking Surgeries
- December 2007: Chemotherapy Stopped
- RW - JPA Diagnostic Pathology
- March 2007: Diet Initiated
- June 2007: 15% Tumor Shrinkage
- March 2008: Stable Tumor
Funding Source

American Institute for Cancer Research, “Pilot Study of a Metabolic Therapy for the Management of Primary Brain Tumors”, Kenneth Schwartz, Norman Hord, L. Karl Olson, Mary Noel, Howard Chang, Michele Nikolai, 2012-2016
A

Hepatic Ketogenesis

1) β-Oxidation
   \[ \text{Acyl-CoA} \rightarrow \text{Acetyl-CoA} \]

2) ACAT1
   \[ \text{Acetyl-CoA} \rightarrow \text{CoA-SH} \]

3) HMGCS2
   \[ \text{HMG-CoA} \rightarrow \text{CoA-SH} \]

4) HMGCL
   \[ \text{Acetyl-CoA} \rightarrow \text{CO}_2 \]

5) BDH1
   \[ \beta\text{HB} \rightarrow \text{Acetone} \]

B

Ketone Body Oxidation

1) BNH1
   \[ \beta\text{HB} \] 4.79 kcal/g

2) OXCT1
   \[ \text{AcAc} \] 4.24 kcal/g

3) ACAT1
   \[ \text{Acetyl-CoA} \rightarrow \text{Fatty Acids} \]

4) Citrate
   \[ \text{TCA Cycle} \]
Hypothesis: **Ability to catabolize ketones for energy is compromised with advancing metastatic potential in gliomas**

Astrocytoma Grade

I II III IV

Expression of ketolytic enzymes decreases

Proliferative potential/ Clinical aggressiveness
Energy Metabolism in Advanced Astrocytic Tumors

Hypothesis:

When compared with normal human brain tissue, energy metabolism in brain cancers is more dependent on glucose and demonstrates a relative inability to metabolize ketones.

Rationale:

Current therapy for high grade brain tumors has a reported survival of around 12 to 14 months. One novel therapy that has been shown to delay growth in two different orthotopically transplanted tumors in mice is the energy restricted ketogenic diet.
Overview of Energy-Restricted Ketogenic Diet Protocol in GBM patients

- Initial MRI
- Tumor Pathology
- Standard Therapies
- Therapeutic Failure
  - History
  - Physical
  - Laboratory Studies
    - Baseline $^{18}$FDG-PET
    - Diet instruction
  - Energy-Restricted Ketogenic Diet
    - 6 wks
    - Second $^{18}$FDG-PET
    - 6 wks
    - Third $^{18}$FDG-PET
    - Assessment of blood glucose and ketones
Hypothesis: Ability to catabolize ketones for energy is compromised with advancing metastatic potential in gliomas

Goals of Energy Restricted Ketogenic Therapy:

Kcal intake: 20-25 kcal/kg

Blood glucose: 50-70 mg/dl

Blood $\beta$-hydroxybutyrate: 4-8 mmol
Efficacy of ERKD in Adults with GBM
(Schwartz et al. Cancer & Metabolism (2015) 3:3)

• METHODS: Patients in this reports of different KD diet treatments including 5 detailed case reports, 2 new patients treated with an ERKD protocol and 19 recently reported patients from Germany.

• RESULTS: Prolonged remissions ranging from more than 5 years to 4 months were reported in detailed case reports. Only one of these patients was treated using the ketogenic diet as monotherapy. The best responses reported in more recent patient series were stable disease for approximately 6 weeks. Two patients with progressive disease after 12 weeks of diet therapy had tissue expression of at least one of 2 mitochondrial ketolytic enzymes succinyl CoA: 3-oxoacid CoA transferase (OXCT-1), β-3-hydroxybutyrate dehydrogenase 1 (BDH1). The 19 patients from Germany treated with a ketogenic diet demonstrated that the diet was safe with no major side effects.

• Trial Registration: ClinicalTrials.gov# NCT01535911
ERKD AND GBM CONCLUSIONS:
(Schwartz et al. Cancer & Metabolism (2015) 3:3)

1. Treatment with a ketogenic diet may be effective in controlling some patients with primary brain cancers, but additional protocol studies are needed;
2. Ketosis can be induced using customary foods and;
3. A ketogenic diet is safe without major side effects.
Conclusions

• βHB and AcAc as an alternative energy sources for the brain under normal physiologic conditions.
• The observed anti-inflammatory effects and metabolic efficiencies of neurons and astrocytes in ketosis may partially explain the efficacy of therapeutic ketosis in certain neurological conditions.
• New, innovative dietary and pharmacological approaches to achieve ketosis have promise to improve the treatment of certain neurological disorders and cancer.