Ketogenic Diet: Update of proposed mechanisms of action

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Introduction
• High-fat, low-carbohydrate, adequate-protein
• Mimic beneficial effects of fasting on epilepsy
• Well-documented clinical efficacy
• Little knowledge about mechanism(s)
Basic Concepts
Ketogenic Diet

- **Ketogenic:**
  - Generating ketone bodies
  - Producing ketosis

- **Ketosis:**
  - Systemic elevation of ketone bodies

- **Ketone body:**
  - Acetoacetate (ACA)
  - β-Hydroxybutyrate (BHB)
  - Acetone
Acetone

Spontaneous

ACA

NADH + H^+ → BHB dehydrogenase

NAD^+

BHB
KETONE BODY PRODUCTION

LONG-CHAIN FATTY ACIDS

- Major precursors to ketone bodies
- LCFAs released from adipose tissue triacylglycerol stores in response to a decrease in blood glucose (e.g., in starvation, fat feeding) & concomitant decrease in plasma insulin
- transported in the plasma bound to albumin
- extracted dependent upon their plasma concentrations
- cross liver cell memb & bind to cytosolic-binding proteins
• Within the liver,
  1. re-esterified to form TG & phospholipids
  2. enter mitochondria via the carnitine acyltransferase system to undergo β-fatty acid oxidation.

• The resultant acetyl CoA
  1. converted to ketone bodies (Acac & β-OHB) via the hydroxymethylglutaryl-CoA pathway
  2. undergo complete oxidation in the TCA cycle.
MEDIUM-CHAIN FATTY ACIDS (≤ 12 carbons),

- another important source of precursors for ketogenesis
- relatively high conc in maternal milk and MCT oil
- readily absorbed from stomach into portal venous system
- cross directly into the inner mitochondrial memb of liver, thus bypassing the carnitine acyltransferase system

**Within liver mitochondrial matrix,**

1. MCFAs converted to acylCoA derivatives
2. undergo **β-oxidation** in a manner similar to LCFAs
KETONE BODY METABOLISM

- Large increase during fasting, after exercise, with high-fat diet, late in pregnancy, & during suckling period in most mammals
- When elevated, serve as metabolic fuel and substrates for critical physiologic processes such as lipogenesis and myelinogenesis
- While prolonged fasting, 65% ↑ total brain energy requirements
- Dramatic increase around the time of delivery,
  particularly when mother is fasted during a prolonged labor,
  → important alternate source of energy to fetus just before birth
• **Free FAs** as alternate energy substrate in most tissues in body not readily cross **BBB**
  converted to **KBs** in **liver** before used by the **brain**.

• Once converted, very good respiratory fuel:
  - 100 g of **glucose** $\rightarrow$ 10.7 kg of ATP
  - 100 g of $\beta$-**OHB** $\rightarrow$ 12.7 kg of ATP
  - 100 g of **aetoacetate** $\rightarrow$ 11.4 kg of ATP

• Immature animals, infants, and children have a greater capacity, as compared with adults, to produce, extract, & use KBs as primary energy source in brain under conditions of fasting, low-CHO intake, high-fat diet.
KETOGENIC POTENTIAL OF FOOD

- KD developed originally by Wilder & Woodyatt (1921)
- based on the concept that some foods are more likely to increase body's production of ketone bodies - “ketogenic”, while others are “anti-ketogenic.”

- Any glucose anti-ketogenic because completely burned in body
- 10% fat, 54% protein, & all carbohydrates broken to glucose and are anti-ketogenic.

"K / AK" = (0.9 F +0.46 P) / (1.0 C + 0.1 F +0.54 P) [in gram scale]
"ketogenic/anti-ketogenic ratio" of food in a diet

- ↑ 1.5:1 for noticeably elev levels of KBs in blood 
  & urine
- ↑ 3:1 for best seizure control
- Calories must be limited to maintain ketosis.

However, most clinicians currently prefer to use the term "ketogenic ratio" to "ketogenic/anti-ketogenic ratio" because it is more simple.

"Ketogenic ratio" = F / (C + P) [in gram scale]
Suggested Mechanisms
HOW DOES THE KETOGENIC DIET WORK?

- The exact mechanism of action is currently not known, but we know that in order for it to be successful, the child must remain in ketosis and generate ketone bodies.

- Ketosis
- Acidosis
- Dehydration
- Lipid effect
- Cerebral energy metabolism
- Direct effect on neuronal excitability
A: phosphofructokinase
B: pyruvate dehydrogenase
C: α-ketoglutarate dehydrogenase
D: 3-oxoacid-CoA transferase
Basic Research Findings
• The protective effect of a ketogenic diet on kainic acid-induced hippocampal cell death in the male ICR mice.

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Representative photomicrographs of the Cresyl violet-stained hippocampus of the normal ICR mouse brain (A–C); an ND-fed mouse brain (D–F); and a KD-fed mouse (G–I) 2 days after KA (25 mg/kg, i.p.) administration. (A), (D), and (G) represent low-power photomicrographs of cresyl violet-stained hippocampal (CA1, CA2, and CA3) and (C, F, I) are higher magnification views (boxed areas in A, D, and G) of CA1, and CA2. Two days after KA injection, most of the pyramidal layer of the CA1 (E) and CA3 (F) of ND-fed mice was disrupted and filled with pyknotic cells (arrows). On the contrary, well-preserved pyramidal cell layers of the CA1 (H) and CA3 (I) were observed in the KD-fed mice.
Figure 4: Semi-quantitative RT-PCR analysis of mRNA expression of caspase-3 in the hippocampus. M: g/kb DNA standard ladder, lanes 1, 2: KD-fed mice, lane 3: ND-fed mice 2 days after KA administration; lane 4: ND-fed mice 21 days after KA administration. RT-PCR was performed with hippocampal total RNA from each pool of five animals. The values were normalized to the β-actin values. Expression of caspase-3 mRNA increased in the ND- and KD-fed mice 2 days after KA administration compared with that of both ND- and KD-fed mice. In contrast, the KD-fed mice, 2 days after KA administration, had a decreased caspase-3 mRNA expression compared to the KA-treated ND-fed mice.
Ketogenic Diet: Mechanism

Fig. 3. Caspase-3 immunoreactivity in the hippocampi of KD-fed mice. (A-D) 2 days after KA administration. Arrows indicate caspase-3-positive cells. (E) Immunoreactivity was decreased in the CA1 and CA3 (P < 0.01 vs. ND-fed).
• Ketogenic diet increases calbindin-D28k in the hippocampi of male ICR mice with kainic acid seizures.

• Ketogenic diet prevents clusterin accumulation induced by kainic acid in the hippocampus of male ICR mice.

Fig. 1: Immunohistochemistry of clusterin in the hippocampus of ND-fed (A) and KD-fed mice (B) and 2 days after KA (25 mg/kg i.p.) administration in the ND (C,E,G) and KD (D,F,H)-fed mice. No clusterin-IR was detected in the hippocampus of ND-fed (A) and KD-fed mice (B) but 2 days after KA administration, strong clusterin-IR was showed in the nucleus of pyramidial neurons in the CA1 (C,E,G) and CA3 (C,G) in the ND-fed mice. Note the low level of clusterin-IR in the KD-fed mice (D,F,H) administration in the CA1 (D,F) and CA3 (D,H). Arrows indicate clusterin-positive cells. Representative photomicrography of TUNEL-stained hippocampi of ND-fed mice (I) and KD-fed mice (J) 2 days after KA (25 mg/kg i.p.) administration. Panels (I) and (J) represent the low-power photomicrography. Scale bars in panels A–D = 125 μm; panels E–H = 50 μm; panels I–J = 125 μm.
Fig. 2. Western blotting detection of nuclear clusterin (nCLU) 2 days after KA administration. The 55-kDa form of nCLU was detected by Western blot analysis using anti-clusterin antibody in the hippocampal nuclear protein of ND- and KD-fed mice 2 days after KA administration (we represented that ND + KA or KD + KA). 20 μg of nuclear protein was loaded in each lane. Ponceau S dye staining was used as the control to verify identical protein loading. No 55-kDa nCLU was detected in the hippocampal nuclear protein of ND-fed or KD-fed mice (these data represented in the graph and α-tubulin was used as the control to verify identical protein loading). Data were analyzed by the Mann–Whitney U test; *P < 0.01; results are mean ± SD.
• Increased nitric oxide caused by the ketogenic diet reduces the onset time of kainic acid-induced seizures in ICR mice.

Fig. 2—Effects of KD on the immunoreactivity of nNOS (A–G) and levels of NOx (H) in the hippocampus of ND- and KD-fed mice. (A–F) Photomicrographs of immunoreactive nNOS in the hippocampus of ND- (A, C, and E) and KD-fed mice (B, D, and F). nNOS positive cells were found in the stratum oriens (so), stratum pyramidale (sp) and stratum radiatum layer (sr) of the CA1 (A and B), in the CA3 (C and D), and in the granule cell layer (G) and hilus (h) of dentate gyrus (E and F) in the ND- and KD-fed mice; M, molecular cell layer of dentate gyrus. Arrows indicate nNOS positive cells that had strong immunoreactivity within soma and arrowhead indicates nNOS positive cells that had strong immunoreactivity within dendrites. A significant increase in nNOS immunoreactivity in KD-fed mice was observed in the sp and sr in the CA1/3, and in the h of the dentate gyrus compared with the ND-fed mice. Scale bar = 100 μm. (G) Quantitative analysis of changes in nNOS positivity in the hippocampus of ND- and KD-fed mice. Data represent the means ± SEM of 30 sections/10 animals/group. *P < 0.005 and **P < 0.0005 (statistically significant differences compared with ND-fed mice). (H) Levels of NOx in hippocampus at day 28 of the respective diet treatments. The content of NOx in KD-fed mice was higher than that of the ND-fed mice. There was a significant difference between the two diet groups (*P < 0.05) according to the Student’s t test. Each data point represents the mean ± SEM of 15 mice.
• Ketogenic diet decreases the level of proenkephalin mRNA induced by kainic acid in the mouse hippocampus.

Fig. 1. Effects of the KD on the KA-mediated induction of PENK mRNA expression in the hippocampus. (A) Representative autoradiograms of PENK mRNA expression after KA-induced seizures. The basal expressions of PENK mRNA were detected by in situ hybridization in the striatum (arrow head), but not in the hippocampus (a). 3 h after KA injection, PENK mRNA was dramatically induced by KA in the dentate gyrus (arrow) (b). However, the induction of PENK mRNA by KA administration was suppressed by pretreatment with the KD (c). Part (d) shows the absence of labeling with the sense probe. (B) Northern blot analysis of PENK mRNA levels (n = 5/group). Total RNA (10 μg) extracted from the pooled mice hippocampal samples (n = 5/group) was used for determination of the PENK mRNA levels. PENK mRNA was autoradiographed, with an exposure time of 5 days. Levels of a 1.4-kb transcript corresponding to PENK mRNA were increased 3 h after KA treatment. Note that the PENK mRNA level induced by KA was decreased in the KD-fed mice.
• Effects of the ketogenic diet on neurogenesis after kainic acid-induced seizures in mice.

Fig. 2 Blood β-hydroxybutyrate (BHB) levels in male ICR mice treated with the ketogenic diet (KD). Bars represent mean ± S.D. The asterisk (*) indicates a significant difference ($P < 0.001$, Student’s $t$-test) between the normal diet (ND)-fed mice and the KD-fed mice (each $n = 25$).
Fig. 3  Influence of the ketogenic diet (KD) on the kainic acid (KA)-induced seizure onset time in male ICR mice. In the KD-fed mice \((n=14)\), the latency to a seizure onset was delayed compared with the normal diet (ND)-fed mice \((n=14)\). Bars represent mean \(\pm\) S.D. The asterisk (*) indicates a significant difference \((P<0.01\), Student’s t-test\) between the ND-fed and the KD-fed mice.
Fig. 4  BrdU-positive cells in the hippocampal dentate gyrus of male ICR mice. Mice were divided into four groups: (1) seizure-free normal diet (ND), (2) seizure-free ketogenic diet (KD), (3) KA-seizure ND, and (4) KA-seizure KD groups. (A) Baseline mitotic activity in the hippocampal dentate gyrus of group (1). (B) No remarkable difference in BrdU-positive cells of group (2) compared with (1). (C) Increased BrdU-positive cells of group (3) compared with (1) and (2). (D) Remarkable increase in BrdU-positive cells of group (4) compared with (1), (2), and (3). Scale bars: 200 μm.
Fig. 5  The number of BrdU-positive cells in the male ICR mice. Mice were divided into four groups: (1) seizure-free normal diet (ND) group \((n=5)\), (2) seizure-free ketogenic diet (KD) group \((n=5)\), (3) KA-seizure ND group \((n=6)\), and (4) KA-seizure KD group \((n=7)\). Bars represent mean. Although measurement of BrdU-positive cells showed no significant differences between the seizure-free ND and KD groups, BrdU-positive cells after KA-induced seizures significantly increased in the KD-fed mice compared with the ND-fed ones \((P<0.05\), ANOVA with interaction\).
Fig. 6  The neuronal phenotype of proliferating cells in the hippocampal dentate gyrus of a ketogenic diet (KD)-fed mouse after kainic acid (KA)-induced seizure. (A) and (B) BrdU-positive cells (green) in the hippocampal dentate gyrus. (C) Cells immunostained by neuronal marker NeuN (red). (D) Cells immunostained with astrocytic marker GFAP (red). (E) Colocalization of BrdU with NeuN shown by yellow nuclei. (F) BrdU rarely colocalized with GFAP. (G) High-power photograph of (E). (H) High-power photograph of (F). Scale bars: (A–F) 200 μm; (G) 25 μm; (H) 20 μm.
Fig. 7  Alterations in the percentages of BrdU-positive cells that were neurons (NeuN) or "other" cells in the male ICR mice. Mice were divided into four groups: (1) seizure-free normal diet (ND), (2) seizure-free ketogenic diet (KD), (3) KA-seizure ND, and (4) KA-seizure KD groups (each n = 6). Cells that were BrdU-positive, but lacked a co-localization of NeuN were classified as "other". There were no significant differences among respective groups in the cellular phenotype (percentages) of BrdU-positive cells (F = 1.35, P = 0.28, ANOVA). Bars represent mean ± S.D.
Conclusion
KETOCGENIC DIET

ACUTE/DIRECT EFFECTS:
- Ketones
- Lipids

MODULATORS:
- Genetic Factors
- Age
- Neuromodulators, hormones
- Epilepsy syndrome
- Other anticonvulsants

SUPPRESSION OF EXCITABILITY/ANTICONVULSANT EFFECT
- seizure threshold
- seizure spread
- seizure termination
- interictal excitability

LONG-TERM EFFECTS:
- Metabolic adaptation/energy charge
- Synaptic reorganization
- Membrane lipid alterations
KD is a legitimate antiepileptic therapy.

If mechanism(s) of KD action were known,
1. to modify or simplify existing KD protocols, in terms of formulation, administration, or monitoring,
2. to devise new AEDs.
Many Thanks for Your Attention !!!