Ketones Inhibit Mitochondrial Production of Reactive Oxygen Species Production Following Glutamate Excitotoxicity by Increasing NADH Oxidation

M. MAALOUF, P. G. SULLIVAN, L. DAVIS, D. Y. KIM AND J. M. RHO (2007)
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Presentation by Joey Miller

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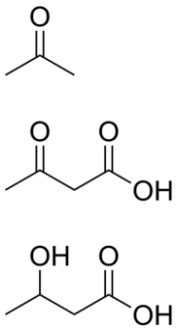
FASTING AS EPILEPSY CURE.

Osteopaths Hear That 22 Days on Water Usually End Fits.

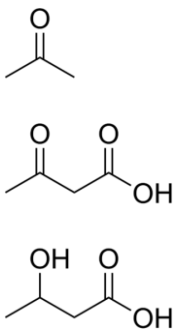
LOS ANGELES, July 5.—Epilepsy may be cured by fasting, Dr. Hugh Conklin told the twenty-sixth annual convention of the American Osteopathic Association, now in session here. Epilepsy, according to Dr. Conklin, is caused by the improper functioning of certain glands in the bowels. By fasting for twenty-two days, taking only water, a cure may be effected, he said.

"Many people," added Dr. Conklin, "fast thirty days and are never afflicted by fits again. The longest fast which any patient ever took under my direction lasted sixty days. Out of thirty-seven tests in which children were used as patients, only two still are affected by the disease. The children all were under the age of 11 years, but we effect cures in older patients in from 50 to 60 per cent. of the cases we undertake."

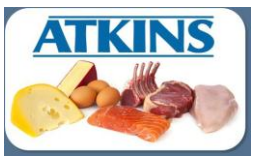


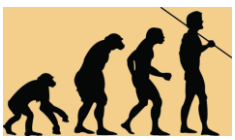
Ketosis

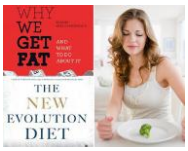
- Metabolic state of elevated levels of 'ketone bodies'
- Produced as a result of fatty acid breakdown
- Can cross the blood-brain barrier to fuel the brain during starvation



Controversial Applications of the Ketogenic Diet







Neuroprotection

What is glutamate excitotoxicity?

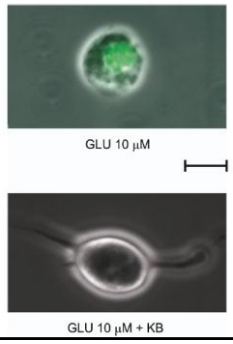
- Implicated in stroke, epilepsy, trauma, Alzheimer's disease
- Associated with higher cellular levels of Reactive Oxygen Species (ROS)

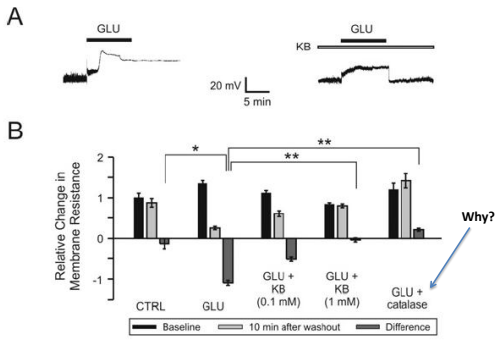
Maalouf et. al (2007)

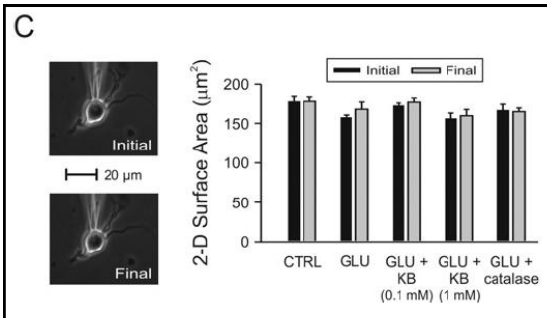
- Studied effects of two ketone bodies on excitotoxicity
  - Beta-hydroxybutyrate (BHB)
    - Prevents death of hippocampal neurons exposed to amyloid plaque
    - Reduces brain injury due to ischemia
  - Acetoacetate (ACA)
    - Protects hippocampal neurons against glycolysis inhibition
  - Acetone
    - Not physiologically relevant, excluded from study

Ketones Reduce Neuronal Swelling and Death

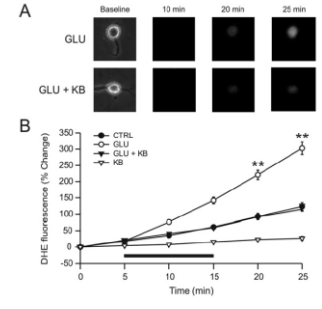
- Neurons incubated with PI, exposed to 10 uM glutamate:
  - 6/12 displayed PI
- BHB and ACA applied, 1 mM each:
  - 0/10 displayed PI



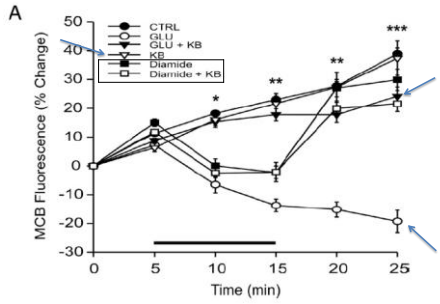




No observed morphological changes; no significant measurable changes in surface area

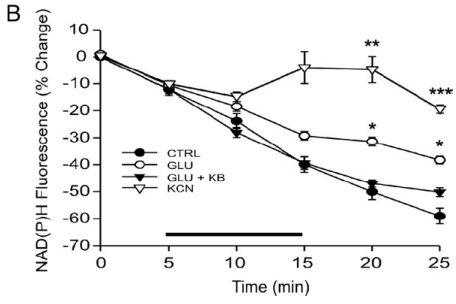


DHE fluorescence reflects superoxide radical levels. 1 mM each of BHB and ACA.
Conclusion: Radical count is reduced. What is the mechanism?

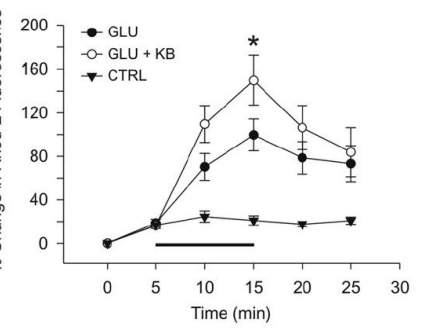


MCB fluorescence reflects levels of active glutathione, an endogenous antioxidant.

Conclusion: Activity is consistent with KB acting independently of glutathione to prevent ROS buildup

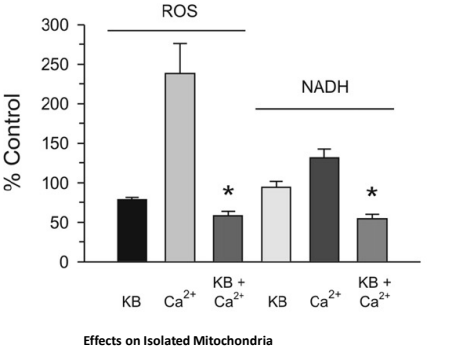


Study of NAD(P)H autofluorescence. Higher levels of NADH are associated with increases in free radical production; oxidation of NADH results in reduction of ROS



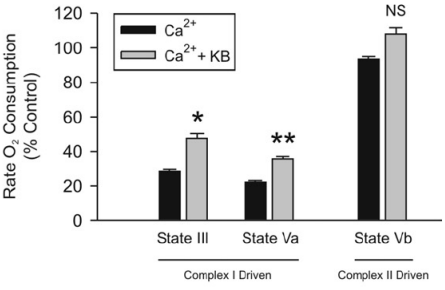
Effects on Isolated Mitochondria

Recall: Glutamate excitotoxicity causes Ca2+ to leak into mitochondria which increases [ROS]. Rhod-2 is an indicator of mitochondrial Ca2+



Effects on Isolated Mitochondria

KB reduces effects of Ca2+ (0.5 mM) on both ROS and NADH back to baseline.



Effects on Isolated Mitochondria

State III: Addition of ADP
State Va: Addition of FCCP
State Vb: Addution of succinate
Ca2+ (0.5 mM) impairs Complex 1, which consumes NADH; KB reverses this

Summary

- Oxidative stress occurs during glutamate excitotoxicity and damages biomolecules, contributes to energy failure, and can cause apoptosis
- Ketones prevent glutamate excitotoxicity by reducing ROS without altering glutathione levels
- This occurs through enhanced NADH oxidation and mitochondrial respiration

Other research has implicated ketones as causing the effects of calorie restriction…

Proc Natl Acad Sci U S A. 2000 May 9;97(10):5440-4.

D-beta-hydroxybutyrate protects neurons in models of Alzheimer's and Parkinson's disease.

Kashiwaya Y, Takeshima T, Mori N, Nakashima K, Clarke K, Veech RL.
Division of Neurology, Tottori University Faculty of Medicine, Yonago, 683-8503 Tottori, Japan.

Abstract
The heroin analogue 1-methyl-4-phenylpyridinium, MPP(+), both in vitro and in vivo, produces death of dopaminergic substantia nigral cells by inhibiting the mitochondrial NADH dehydrogenase multienzyme

Role of glucose and ketone bodies in the metabolic control of experimental brain cancer

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