thematic review

Thematic Review Series: Calorie Restriction and Ketogenic Diets

Ketone ester effects on metabolism and transcription

Richard L. Veech[1]

Laboratory of Metabolic Control, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892

Abstract Ketosis induced by starvation or feeding a ketogenic diet has widespread and often contradictory effects due to the simultaneous elevation of both ketone bodies and free fatty acids. The elevation of ketone bodies increases the energy of ATP hydrolysis by reducing the mitochondrial NAD couple and oxidizing the coenzyme Q couple, thus increasing the redox span between site I and site II. In contrast, metabolism of fatty acids leads to a reduction of both mitochondrial NAD and mitochondrial coenzyme Q causing a decrease in the ΔG of ATP hydrolysis. In contrast, feeding ketone body esters leads to pure ketosis, unaccompanied by elevation of free fatty acids, producing a physiological state not previously seen in nature. The effects of pure ketosis on transcription and upon certain neurodegenerative diseases make approach not only interesting, but of potential therapeutic value.—Veech, R. L. **Ketone ester effects on metabolism and transcription. *J. Lipid Res.* 2014.** 55: **2004–2006.**

Supplementary key words D-beta-hydroxybutyrate - R-1,3 butanediol monoester • elevated blood ketones • Alzheimer's disease • antioxidants • diabetes • fatty acid • deacetylase • ketosis

PRODUCTION OF KETONE BODIES

Ketone bodies are formed in the liver from free fatty acids released from adipose tissue. As the blood concentration of free fatty acids increases, concentration of blood ketone bodies is correspondingly increased (1, 2). Ketone bodies serve as a physiological respiratory substrate and are the physiological response to prolonged starvation in man (3, 4), where the blood level of ketones reaches 5–7 mM (5). If the release of free fatty acids from adipose tissue exceeds the capacity of tissue to metabolize them, as occurs during insulin deficiency of type I diabetes or less commonly in the insulin resistance of type II diabetes, severe and potentially fatal diabetic ketoacidosis can occur, where blood ketone body levels can reach 20 mM or higher (2) resulting in a decrease in blood bicarbonate to almost 0 mM and blood pH to 6.9. Diabetic ketoacidosis, which is a pathological state, differs from the ketosis of prolonged starvation, which is a normal physiological state without known medical consequences. Elevation of blood ketones can however lead to elevation of blood uric acid, which can produce a gouty crisis. This can be prevented by feeding oral potassium citrate (6)

Ketosis occurs during starvation in man. It occurs only with difficulty in species other than lactating cows where ketosis can occur due to the large requirement for lactose production in milk production. This form of ketosis can be treated by the administration of glucose. In this situation, ketosis occurs because of the competition for oxaloacetate, required for gluconeogenesis, where its shortage prevents acetyl-CoA in the liver from entering the Krebs cycle with a resultant increase in ketone body production (1).

In vitro, ketone body production has been observed during the metabolism of leucine in glial cells (7). However, the quantitative importance of this pathway has not been established in vivo.

While ketone bodies are usually produced in the liver by free fatty acids released by adipose tissue, effects of this elevation of free fatty acids result in the elevation of the PPAR transcription factors with many undesirable effects which include diabetes and atherosclerosis (8). Feeding a high fat ketogenic diet, of the Mayo Clinic type, results in large increases in blood cholesterol and LDL cholesterol predisposing the atherosclerosis (9). Accordingly, to obtain the desirable effect of ketosis in treating Alzheimer's or Parkinson's disease (10), increasing the $\Delta G'$ of ATP hydrolysis (11), decreasing and overcoming the insulin resistance present in many acute injuries (12), or combating free radical toxicity (13, 14); an orally (15) absorbable ketone body ester was synthesized, which was comprised of the mono-ester of D-β-hydroxybutyrate and (R)-1,3-butanediol (16). An ester was required, because feeding a mole of ketones as the salt or acid would result in undesirable consequences of cation overload or acidosis. (R)-1,3-butanediol was chosen because this alcohol is readily converted in the liver to ketone bodies (17). This ester is

Manuscript received 10 December 2013 and in revised form 28 February 2014.

Published, JLR Papers in Press, April 8, 2014
DOI 10.1194/jlr.R046292

[1]To whom correspondence should be addressed.
e-mail: rveech@mail.nih.gov

readily hydrolyzed in the gut to ketone bodies, which can reach blood levels equivalent to those seen in prolonged fasting and equivalent to the K_m of transport into the brain (18), without observable toxic effects (15, 19).

FEEDING KETONE BODY ESTERS TO A RAT LOWERS THE BLOOD GLUCOSE, WHILE AT THE SAME TIME LOWERING BLOOD INSULIN, DEMONSTRATING THAT KETOSIS INCREASES INSULIN SENSITIVITY

Blood glucose decreased by about 50% from 5 to 2.8 mM while insulin decreased from 0.54 to 0.26 ng/ml in rats fed a diet where 30% of the calories from starch were replaced with ketone esters (20). Plasma leptin was also decreased from 3.12 to 1.83 ng/ml in rats, where ketone esters were substituted equi-calorically for starch. The ability of the metabolism of ketone bodies to increase insulin sensitivity has been demonstrated previously in the working perfused rat heart (11, 13).

A major effect of insulin is to increase the activity of the pyruvate dehydrogenase multienzyme complex (21, 22), thus increasing the provision of acetyl-CoA for use in the Krebs cycle.

Addition of insulin or ketone bodies to the working perfused heart increased acetyl-CoA 9- and 15-fold, respectively (11), demonstrating that ketone body metabolism could mimic the metabolic effects of insulin. The decrease of insulin by the metabolism of ketone bodies, in addition to its effects on metabolism, also has effects on transcription.

A DECREASE IN INSULIN DECREASED THE TRANSCRIPTION FACTOR, SREBP, RESPONSIBLE FOR THE TRANSCRIPTION OF THE GENES OF FATTY ACID AND CHOLESTEROL SYNTHESIS

Insulin selectively increases sterol regulatory binding protein (SREBP) in the liver, which enhances transcription of the genes encoding cholesterol and fatty acid synthesis (23).

Intracellular cholesterol is synthesized by a pathway involving HMG-CoA reductase, an enzymatic step inhibited by statins. Intracellular cholesterol can also be increased by the uptake of LDLs from blood; the number of LDL receptors is also variable depending upon the intracellular levels of cholesterol (24). Because the feeding of ketone esters decreases insulin levels, it would be expected that the level of blood cholesterol would go down on feeding ketone esters.

A SECOND TRANSCRIPTION FACTOR RESPONSIVE TO ELEVATED BLOOD GLUCOSE, ChREBP, ALSO CONTROLS THE TRANSCRIPTION OF THE ENZYMES OF FATTY ACID SYNTHESIS

Elevation of blood ketones prevents the carbohydrate response element binding protein (ChREBP) from entering the nucleus, thus preventing the increased transcription of the enzymes of fatty acid synthesis (25). Ketone bodies activate the interaction between cytoplasmic ChREBP and 14-3-3 proteins, inhibiting the ChREBP-importin interaction, which prevents ChREBP from entering the nucleus and increasing the transcription of lipogenic enzymes.

THE KETONE BODY, D-β-HYDROXYBUTYRATE, IS A NATURAL HISTONE DEACETYLASE INHIBITOR

Treatment of cells with D-β-hydroxybutyrate increases histone acetylation at the FOX3A and MT2 promoters, thus increasing the transcription of antioxidant enzymes, including superoxide dismutase, catalase, and metallothionein (14), thus adding to the removal of reactive O_2 species brought about by the reduction of the NADP system induced by ketone body metabolism (13).

It has earlier been reported that treatment of implanted astrocytoma by caloric restriction resulted in a marked shrinkage of tumor size (26). Although there are metabolic factors such as the ability of normal brain, but not brain cancers, to utilize ketone bodies as an energy substrate, other factors may also play a role. Cancer cells are often associated with global hypoacetylation of chromatin, the major substrate for which is N-acetyl aspartate in the brain (27). An increase in brain acetate, by feeding glycerol triacetate, inhibited the growth of certain glioma cells. It has now emerged that deleted breast cancer 1 (DBC1) acts as a master regulator of transcriptional processes through its inhibition of the NAD-linked histone deacetylase Sirt 1, leading to the hyperacetylation of tumor suppressor protein, P53, leading to activation of the apoptotic pathway (28). DBC1 also inhibits histone methyltransferase, which is involved in heterochromatin formation.

In conclusion, the metabolism of ketone bodies not only alters cellular metabolism by increasing the $\Delta G'$ of ATP and increasing the reducing power of mitochondrial NAD and cytoplasm NADP couples, but also alters transcription of a number of important pathways involved in protection against free radical damage, but ketosis protects against apoptosis through P53.

REFERENCES

1. Krebs, H. A. 1966. The regulation of the release of ketone bodies by the liver. *Adv. Enzyme Regul.* **4:** 339–354.
2. Cahill, G. F., Jr., and R. L. Veech. 2003. Ketoacids? Good medicine? *Trans. Am. Clin. Climatol. Assoc.* **114:** 149–161; discussion 162–163.
3. Cahill, G. F., Jr. 1970. Starvation in man. *N. Engl. J. Med.* **282:** 668–675.
4. Cahill, G. F., Jr. 2006. Fuel metabolism in starvation. *Annu. Rev. Nutr.* **26:** 1–22.
5. Reichard, G. A., O. E. Owen, A. C. Haff, P. Paul, and W. M. Bortz. 1974. Ketone-body production and oxidation in fasting obese humans. *J. Clin. Invest.* **53:** 508–515.
6. Sampath, A., E. H. Kossoff, S. L. Furth, P. L. Pyzik, and E. P. Vining. 2007. Kidney stones and the ketogenic diet: risk factors and prevention. *J. Child Neurol.* **22:** 375–378.
7. Murín, R., and B. Hamprecht. 2008. Metabolic and regulatory roles of leucine in neural cells. *Neurochem. Res.* **33:** 279–284.

8. Kersten, S., B. Desvergne, and W. Wahli. 2000. Roles of PPARs in health and disease. *Nature.* **405:** 421–424.
9. Kwiterovich, P. O., Jr., E. P. Vining, P. Pyzik, R. Skolasky, Jr., and J. M. Freeman. 2003. Effect of a high-fat ketogenic diet on plasma levels of lipids, lipoproteins, and apolipoproteins in children. *JAMA.* **290:** 912–920.
10. Kashiwaya, Y., T. Takeshima, N. Mori, K. Nakashima, K. Clarke, and R. L. Veech. 2000. D-beta-hydroxybutyrate protects neurons in models of Alzheimer's and Parkinson's disease. *Proc. Natl. Acad. Sci. USA.* **97:** 5440–5444.
11. Sato, K., Y. Kashiwaya, C. A. Keon, N. Tsuchiya, M. T. King, G. K. Radda, B. Chance, K. Clarke, and R. L. Veech. 1995. Insulin, ketone bodies, and mitochondrial energy transduction. *FASEB J.* **9:** 651–658.
12. Li, L., and J. L. Messina. 2009. Acute insulin resistance following injury. *Trends Endocrinol. Metab.* **20:** 429–435.
13. Kashiwaya, Y., M. T. King, and R. L. Veech. 1997. Substrate signaling by insulin: a ketone bodies ratio mimics insulin action in heart. *Am. J. Cardiol.* **80:** 50A–64A.
14. Shimazu, T., M. D. Hirschey, J. Newman, W. He, K. Shirakawa, N. Le Moan, C. A. Grueter, H. Lim, L. R. Saunders, R. D. Stevens, et al. 2013. Suppression of oxidative stress by β-hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science.* **339:** 211–214.
15. Clarke, K., K. Tchabanenko, R. Pawlosky, E. Carter, M. Todd King, K. Musa-Velosa, M. Ho, A. Roberts, J. Robertson, T. B. Vanitallie, et al. 2012. Kinetics, safety and tolerability of (R)-3-hyroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects. *Regul. Toxicol. Pharmacol.* **63:** 401–408.
16. Veech, R. L. 2004. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot. Essent. Fatty Acids.* **70:** 309–319.
17. Veech, R. L., and M. A. Mehlman. 1972. Liver metabolite content, redox and phosphorylation states in rats fed diets containing 1,3-butandiol and ethanol. *In* Energy Metabolism and the Regulation of Metabolic Processes in Mitochondria. M. A. Mehlman and R. W. Hanson, editors. Academic Press, New York. 171–183.
18. Vannucci, S. J., and I. A. Simpson. 2003. Developmental switch in brain nutrient transporter expression in the rat. *Am. J. Physiol. Endocrinol. Metab.* **285:** E1127–E1134.
19. Clarke, K., K. Tchabanenko, R. Pawlosky, E. Carter, N. S. Knight, A. J. Murray, L. E. Cochlin, M. T. King, A. W. Wong, A. Roberts, et al. 2012. Oral 28-day and developmental toxicity studies of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate. *Regul. Toxicol. Pharmacol.* **63:** 196–208.
20. Kashiwaya, Y., R. Pawlosky, W. Markis, M. T. King, C. Bergman, S. Srivastava, A. Murray, K. Clarke, and R. L. Veech. 2010. A ketone ester diet increased brain malonyl CoA and uncoupling protein 4 and 5 while decreasing food intake in the normal Wistar rat. *J. Biol. Chem.* **285:** 25950–25956.
21. Mukherjee, C., and R. L. Jungas. 1975. Activation of pyruvate dehydrogenase in adipose tissue by insulin. Evidence for an effect of insulin on pyruvate dehydrogenase phosphate phosphatase. *Biochem. J.* **148:** 229–235.
22. Denton, R. M., P. J. Randle, B. J. Bridges, R. H. Cooper, A. L. Kerbey, H. T. Pask, D. L. Severson, D. Stansbie, and S. Whitehouse. 1975. Regulation of mammalian pyruvate dehydrogenase. *Mol. Cell. Biochem.* **9:** 27–53.
23. Shimomura, I., Y. Bashmakov, S. Ikemoto, J. D. Horton, M. S. Brown, and J. L. Goldstein. 1999. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. *Proc. Natl. Acad. Sci. USA.* **96:** 13656–13661.
24. Goldstein, J. L., R. A. DeBose-Boyd, and M. S. Brown. 2006. Protein sensors for membrane sterols. *Cell.* **124:** 35–46.
25. Nakagawa, T., Q. Ge, R. Pawlosky, R. M. Wynn, R. L. Veech, and K. Uyeda. 2013. Metabolite regulation of nucleo-cytosolic trafficking of carbohydrate response element-binding protein (ChREBP): role of ketone bodies. *J. Biol. Chem.* **288:** 28358–28367.
26. Seyfried, T. N., T. M. Sanderson, M. M. El Abbadi, R. McGowan, and P. Mukherjee. 2003. Role of glucose and ketone bodies in the metabolic control of experimental brain cancer. *Br. J. Cancer.* **89:** 1375–1382.
27. Long, P. M., S. W. Tighe, H. E. Driscoll, J. R. Moffett, A. M. Namboodiri, M. S. Viapiano, S. E. Lawler, and D. M. Jaworski. 2013. Acetate supplementation induces growth arrest of NG2/PDGFRα-positive oligodendroglioma-derived tumor-initiating cells. *PLoS ONE.* **8:** e80714.
28. Joshi, P., O. I. Quach, S. Giguere, and I. Cristea. 2013. A functional proteomics perspective of DBC1 as a regulator of transcription. *J. Proteomics Bioinform.* **Suppl 2:** 002.