

## SUPPLEMENT - KETOGENIC DIET AND TREATMENTS

# Assessing ketosis: Approaches and pitfalls

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### SUMMARY

Although the mechanism of action of the ketogenic diet (KD) remains unknown, ketones have been used as a marker of efficacy. Acetoacetate, acetone, and beta-hydroxybutyrate can be measured in urine, blood, and breath. The value of

these measures is reviewed in this paper. Future brain measures hold promise as markers of efficacy, but research focused on the mechanisms of the KD should help to develop more reliable markers of efficacy.

**KEY WORDS:** Ketogenic diet, Acetoacetate, Acetone, Beta-hydroxybutyrate, Brain imaging.

The first reference supporting the efficacy of the ketogenic diet (KD) for the treatment of epilepsy comes from the Bible. In modern times, Guelpa and Marie were the first to publish the effect of dietary manipulation for epilepsy (Guelpa & Marie, 1911). These authors recommended a regimen of fasting, followed by a restrictive and vegetarian diet in the treatment of epilepsy. A decade later, Geyelin published a report confirming its efficacy in suppressing seizures (Geyelin, 1921). Managing the KD requires an understanding of the mechanisms by which the human metabolism switches its main source of energy from carbohydrate to fatty acids and ketones. The goal of this paper is to review the mechanisms through which this is done and to discuss the techniques used to monitor these changes and assess their clinical value.

### THE FORMATION OF KETONE BODIES

When one begins to fast, carbohydrate intake decreases and adipocytes start breaking down triglycerides into glycerol and fatty acids. Subsequently, free fatty acids are released into the circulation. The liver and muscles use fatty acids as an energy source. The main process that transforms fatty acids into energy is mitochondrial beta-oxidation. The fatty acids are oxidized into ketone bodies, which are then used as an energy substrate by the brain and other tissues, including muscle. Much knowledge about the

function of hepatocytes has been gained in recent years in efforts to understand the role of the adipocytes in obesity (Cunnane, 2004a; Fukao et al., 2004).

#### Beta-oxidation

By this process, fatty acids repetitively lose 2-carbon fragments at the carboxy-terminal end. It is essential as fatty acids greater than 12 carbons in size can only penetrate the outer mitochondrial membrane after being transformed into their acyl-coA esters. The larger fatty acids are initially cleaved by their size-specific enzymatic system, and subsequently use the smaller size systems. There is some overlap in the size specificity of these systems. There are also differences in the rate of oxidation between fatty acids, depending on their structure. For example, the oxidation of alpha-linolenic acid is twice that of linoleic acid or oleic acid. Therefore, alpha-linolenic acid and linoleic acid are the most ketogenic fatty acids and enriching a high-fat diet with those compounds increases its ketogenic potential. This is the reason that a diet high in flaxseed oil (which contains 60% of alpha-linolenic acid) is more ketogenic and offers more seizure protection than those using other dietary sources of ketones, at least in animal models (Likhodii et al., 2000).

In the next step, acyl-coA esters interact with the carnitine-palmitoyl transferase-1, which attaches carnitine and removes the coA, producing acylcarnitine derivatives. This complex is then carried through the inner mitochondrial membrane by the carnitine or acylcarnitine transporter into the mitochondrial matrix. The carnitine-palmitoyl transferase-2 enzyme, which is located at the internal surface of the inner mitochondrial membrane, removes the carnitine and reattaches the coA residue,

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forming an acyl-coA ester again. Carnitine-palmitoyl transferase-1 is the rate-limiting step in the beta-oxidation of fatty acids, and it is the site of action of the lipolysis inhibitor malonyl-coA. Medium-chain fatty acids cross the inner mitochondrial membrane freely. In fact, a medium-chain fatty acid infusion in fed animals promptly induces ketosis, an effect that is not seen with long-chain fatty acid infusions (Weissman et al., 1988).

### Ketone formation

Ketone bodies include beta-hydroxybutyrate, acetone, and acetoacetate. Ketone formation takes place mostly in the liver, and to a lesser extent, in the kidneys, using plasma free fatty acids as the substrate. This process takes place inside the mitochondria. Most hepatically-produced ketone bodies are released into the circulation to serve as energy substrate to other tissues, mainly muscle and brain. Lipolysis produces acetyl-coA. Two molecules of acetyl-coA combine to form acetoacetyl-coA. The latter undergoes enzymatic conversion to acetoacetate. The enzyme 3-hydroxy-3-methylglutaryl-coA synthase, which helps in this conversion, is upregulated in animals on the KD. The direction of the interconversion of beta-hydroxybutyrate and acetoacetate depends on the mitochondrial redox state. Acetoacetate can also be decarboxylated into acetone by a slow, nonenzymatic spontaneous reaction. These interactions demonstrate the importance of measuring more than one of the ketone bodies to gain a complete picture of the patient's metabolic state (Cullingford, 2004).

## THE EFFECTS OF KETONE BODIES

Ketone bodies are one of the main sources of energy during starvation greater than 48 h and during the neonatal period (Morris, 2005). During fasting, when acetoacetate reaches the brain, it is combined with succinyl-coA to form acetoacetyl-coA and succinate; it then enters the Krebs cycle to generate energy through the formation of adenosine triphosphate (ATP). The same is true in virtually every other tissue in the body except the liver, since the enzyme acetoacetate-succinyl-coA transferase is not present in the hepatocytes. Acetoacetyl-coA may also be converted by beta-thiolase into acetyl-coA, which enters the Krebs cycle for the production of energy. In the target tissues, such as the CNS, beta-hydroxybutyrate is converted into acetoacetate as the concentration of acetoacetate is decreased by its utilization in the energy metabolism. The brain also locally produces ketones via astrocytes, which support the energy metabolism of adjacent neurons (Cullingford, 2004).

Children have a greater capability to extract and oxidize ketones (Persson et al., 1972). This effect is even more pronounced during the state of chronic ketosis, when children seem to have an adaptive mechanism that facilitates even more ketone extraction from the blood into the brain. An increase in blood-brain barrier permeabil-

ity to ketones during starvation has also been reported. The blood-brain barrier appears to be the rate-limiting step in metabolizing ketones from the blood. This rate-limiting step is probably due to a saturable monocarboxylic acid carrier mechanism, which facilitates the transport of beta-hydroxybutyrate through the blood-brain barrier. Human data have confirmed that the brain utilization of beta-hydroxybutyrate is directly proportional to the serum level (Pan et al., 2000). Clinical observations suggest that ketosis tends to be more difficult to induce in patients younger than 1-year old and older than 10-year olds. However, our center and others have been able to induce ketosis and show benefit of the KD in infants (Nordli et al., 2001).

## METHODS OF KETONE MEASUREMENT

### Urinary dipstick ketone test

This is the most widely available test and has the advantage that it can be used at home. The urinary dipstick test measures acetoacetone, as it reacts with the nitroprusside reagent present on the dipstick, leading to a change of color varying from pink to maroon. The color matches the acetoacetone levels on an exponential scale. However, the dipstick has a tendency to overestimate ketosis when urinary ketone bodies are measured enzymatically. More importantly, the urinary dipstick ketone test does not necessarily correlate with blood ketosis (Gilbert et al., 2000). Differences between urinary and blood ketone bodies vary based on hydration/urine volume, acid-base balance, renal hemodynamics, and excretion (Marliss et al., 1970). Another disadvantage of this test is that it does not measure other ketone bodies.

### Plasma ketone measures

This is the most accurate method. However, unlike the urinary test, there is a delay before the results can be obtained. Beta-hydroxybutyrate can be measured by a commercially available assay. Acetone and acetoacetate require HPLC or gas chromatography to obtain accurate measures. Acetoacetate is unstable—as it decarboxylates spontaneously—and thus needs to be assessed immediately.

### Breath measures

As it is volatile, acetone can be measured in the breath. Breath acetone levels have been shown to be a significant predictor of blood ketones (Musa-Veloso et al., 2006). Actually, plasma ketones can be predicted based on an equation derived from measures obtained on breath acetone. This can be done by chromatography or with a recently developed breathalyzer. However, the machine is insensitive to levels below 500 nmol/L and values obtained in patients on the KD range from 200 to 10,000 nmol/L. The patient needs to be able to cooperate and calibration is required

once a month. Finally, this method does not discriminate between acetone and its metabolites.

## CONCLUSION

It would be optimal to be able to measure all three ketones and compare the levels obtained with seizure control. However, serum ketone levels have an uncertain correlation with brain ketone levels. Acetone diffuses into the brain but acetoacetone and beta-hydroxybutyrate are transported by a monocarboxylate transporter. A study comparing cerebrospinal fluid (CSF) versus plasma levels showed higher acetoacetone levels in CSF than in blood but the opposite for beta-hydroxybutyrate and free fatty acids (Klepper et al., 2004). Future studies using positron emission tomography (PET) and magnetic resonance spectroscopy (MRS) may be used to perform such correlation, now that some of these metabolites can be measured using modern imaging techniques (Authier et al., 2008). Some studies that failed to show a clear correlation between seizure control and ketosis found a significant relationship with plasma lipid profile and the role of these molecules need to be evaluated further (long-chain polyunsaturated fatty acids in particular) (Cunnane, 2004b). A better understanding of the mechanisms of action of the KD remains the key to developing reliable markers of its efficacy.

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I confirm that I have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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