EDUCATIONAL COMMENTARY – BIOCHEMISTRY OF KETOSIS

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Learning Outcomes

On completion of this exercise, the participant should be able to

- discuss the metabolism of fatty acids in the human body;
- review the biochemistry of ketone bodies: acetone, acetoacetic acid, and β-hydroxybutyrate; and
- outline the significance of the ketone test.

Ketosis, or ketogenesis, is a normal metabolic process wherein ketone bodies are generated to supply energy to the body when there is insufficient glucose. Adenosine triphosphate (ATP) is the primary chemical compound that provides energy in the cells to sustain life. The body relies on carbohydrates, fats, and proteins to generate ATP. There are many pathways through which the body produces ATP; should one source become scarce or depleted, the body uses another source for energy production. Carbohydrates provide the most readily available source of energy, with the majority in the form of glucose. Other carbohydrates that can generate ATP include fructose and galactose. However, glucose concentration far exceeds the amount of fructose and galactose combined.

The most common pathway for glucose metabolism is the glycolytic pathway, more commonly known as glycolysis. When glucose is exhausted, as stated, the body turns to alternate pathways for needed energy production. One alternative is to metabolize fat for ATP production. Fat can be turned into fatty acids that are metabolized to produce energy. The body routinely uses fatty acids to produce energy, although at a much lower rate than carbohydrates. Fatty acids are primarily stored in three forms: triglycerides, cholesterol, and phospholipids. In the liver, these fatty acids are converted to ATP through degradation by β-oxidation to acetyl coenzyme A (acytely coA), and further degradation by the Krebs cycle (i.e., citric acid cycle) into biochemical byproducts and ATP.

Under normal conditions, the liver is able to regulate fatty acid degradation to ATP. However when the body is stressed, additional energy is needed, and the liver has reached the limit of its ability to metabolize fatty acids, the acetyl coA that is first formed by β-oxidation condenses into acetoacetic acid (acetoacetate). The excess acetoacetic acid is further catalyzed to β-hydroxybutyric acid (β-hydroxybutyrate, 3-hydroxybutyrate, or β-HB) by the enzyme β-hydroxybutyrate dehydrogenase (β-HB
As the acetoacetic acid is catalyzed, there is also decarboxylation wherein carbon dioxide (CO₂) is liberated, producing the byproduct acetone.

Acetoacetic acid, β-HB, and acetone produced from fatty acid breakdown are known as ketone bodies. They are produced primarily in the mitochondria of liver cells but can be produced in other cells as well. The condition where there is little glucose for energy production and the body cannot use the acetyl coA as fast as it is generated from fatty acids, is called ketosis. Ketosis occurs most commonly in type I diabetes, starvation, and cirrhosis or other liver impairment. With type I diabetes (insulin dependent), the body cannot metabolize the readily available glucose. The opposite occurs during starvation or fasting, in which available carbohydrates are used and metabolized, and the body turns to stores of fat for energy production. With cirrhosis, there are not enough viable liver cells to metabolize and degrade the acetyl coA that is being generated. Basic reactions are shown below:¹

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\begin{align*}
\text{Fatty acid chains} & \xrightarrow{(\beta\text{-oxidation})} \text{Acetyl CoA} \\
\text{Acetyl CoA} & \xrightarrow{(\text{condensation})} \text{Acetoacetic acid} \\
\text{Acetoacetic acid} & \xrightarrow{(\text{catalysis} + \text{decarboxylation})} \beta\text{-hydroxybutyrate} + \text{CO}_2 + \text{acetone}
\end{align*}
\]

Excess production of ketone bodies from fatty acid metabolism can lead to a condition known as ketoacidosis. With diabetes, this condition is known as diabetic ketoacidosis (DKA).² Depending on the reference ranges used, there are varying levels of ketoacidosis as the body becomes more acidic. Diabetic ketoacidosis has 3 levels of acuity: mild, moderate, and severe. Reference ranges for mild DKA are as follows:

- Glucose >240 mg/dL
- pH <7.30
- Serum bicarbonate <18 mEq/L
- Urinalysis: positive for ketone
- Anion gap >10

Other than the high glucose level, one can use these laboratory values as an indicator of ketoacidosis; further testing may be needed for appropriate diagnosis.

The ketone bodies produced circulate in the blood and are carried to the cells. At times of excess, the cells do not have the capacity to convert them to ATP. The excess ketone bodies can lead to extreme acidosis, which can be life threatening. Acetoacetic acid and β-HB are primarily harbored and metabolized in the cells. Acetone itself is an organic compound that is volatile and easily turns into a gas.
It is discharged primarily through exhalation, and a small quantity via urine. Acetone has a sweet smell and supports the diagnosis of ketoacidosis. Persons in starvation or during extreme exercise may also have this sweet smell on their breath.

Signs of diabetic ketoacidosis are well known and will not be discussed in this exercise. However, ketoacidosis occurs in other conditions, as stated; even exercise contributes to excess production of ketones. The most common initial diagnostic test for ketones is a urine dipstick. The dipstick ketone test is based on the reaction of sodium nitroprusside with acetoacetic acid, one of the ketone bodies produced.\(^3\) Calling the dipstick test a ketone test is misleading; it actually tests for only one of the three. There are limited testing methods for further analysis of ketone bodies.

Acetone and acetoacetic acid can be measured using gas chromatography–mass spectrometry, a highly specific method that quantitates an analyte based on its chemical structure. \(\beta\)-hydroxybutyrate is quantitated using an enzymatic reaction catalyzed by \(\beta\)-HB dehydrogenase and cofactor nicotinamide adenine dinucleotide (NAD). During the reaction, the NAD is reduced to nicotinamide adenine dinucleotide + hydrogen (NADH), which further reacts with a colorimetric detector and is measured spectrophotometrically. Quantitation is based on the amount of NADH produced.

When the body is not stressed, ketone bodies are regulated and metabolized to low levels that are not detectable. Excretion in urine is low to none when tested by urine dipstick. When in excess, ketones become detectable in both urine and blood. The clinical effect depends on the reason for the excess ketone bodies. In diabetes, the glucose is not being used. Once insulin is used appropriately and glucose is being metabolized, the rate of fatty acid use diminishes. With cirrhosis, the liver impairments vary and medical management of metabolism is more involved based on the severity of the patient’s needs. With starvation or extreme exercise, in which available carbohydrates are suddenly decreased, fatty acid use increases until the condition or activity stops and carbohydrates are replenished.\(^1\)

Toxic effects depend on many factors, including glucose level, pH, and \(\text{CO}_2\), and the quantity of each of the ketone bodies being produced. A glucose concentration greater than 240 mg/dL in itself may not be concerning. However, coupled with a drop in pH below 7.30, the high glucose concentration may cause metabolic acidosis. Should the body be unable to buffer the bicarbonate to a manageable level, these three combined conditions can be life threatening.

**Summary**

Ketone bodies are generated when fatty acids are metabolized for energy. Occasional increases in ketone bodies indicate that fat is being consumed. However, under certain conditions where there is excess fatty acid degradation and ketone bodies are not cleared as quickly as they are generated, ketosis
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becomes a health issue. Depending on the quantity of each of the types of ketone bodies and the patient’s metabolic condition, individually the ketone bodies may not cause unhealthy conditions. However, when combined to toxic levels, the condition becomes ketoacidosis.

References


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