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In humans, beta-hydroxybutyrate is synthesized in the liver from acetyl-CoA in the fasting state. The biosynthesis is catalyzed by the enzyme beta-hydroxybutyrate dehydrogenase.

Although not a ketone itself, the concentration of beta-hydroxybutyrate, like that of other ketone bodies, is raised in ketosis. The compound can be used as an energy source by the brain when blood glucose is low.

Diabetic patients can have their ketone levels tested via urine or blood to indicate diabetic ketoacidosis. In alcoholic ketoacidosis, this ketone body is produced in greatest concentration. Both types of ketoacidosis result in an increase beta-hydroxybutyrate to oxaloacetate ratio, resulting in TCA cycle stalling and shifting of glucose towards ketone body production.

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Last update: 2025/05/13 02:16

