

Brief Report

FASTING HYPOKETOTIC COMA IN A
CHILD WITH DEFICIENCY OF
MITOCHONDRIAL 3-HYDROXY-3-
METHYLGLUTARYL-COA SYNTHASE

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FASTING is accompanied by a decrease in the availability of glucose for energy use in peripheral tissues and, consequently, an increased reliance of these tissues on the availability of ketone bodies and fatty acids for energy. The availability of ketone bodies depends almost exclusively on hepatic ketogenesis. Failure of ketogenesis may occur in patients with any defect of the enzymes associated with the mitochondrial oxidation of fatty acids.¹ These defects are typically manifested by hypoglycemia, which results from the inadequate supply of alternative substrate (ketones). Other clinical features are more variable and may include myopathy, cardiomyopathy, hepatocellular damage, and neuropathies. Studies in rats have indicated a pivotal role for mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase in the control of ketogenesis.²⁻⁴

HMG-CoA synthase has cytosolic and mitochondrial forms that, although structurally similar, are controlled by different genes.⁵ Both forms catalyze the combination of acetoacetyl-CoA and acetyl-CoA to form β -hydroxy- β -methylglutaryl-CoA, which in the cytosol is a precursor of sterols and in the mitochondria is converted to acetoacetate (Fig. 1). During fasting, acetyl-CoA produced by mitochondrial β -oxidation of fatty acids in the liver is largely directed toward the production of ketones, with minimal use of the tricarboxylic acid cycle,⁶ so that the vast majority of the two-carbon units produced by fatty-acid oxidation are directed through HMG-CoA synthase to the production of ketone bodies.

In this report, we describe an 11-year-old boy with deficiency of mitochondrial HMG-CoA synthase.

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This case report underlines the importance of the enzyme as a control point in ketogenesis and confirms the functional difference between the cytosolic and mitochondrial forms of HMG-CoA synthase.

CASE REPORT

A boy of Chinese descent first presented at six years of age after mild gastroenteritis with poor oral intake for two to three days that culminated in a brief generalized seizure, which left him semicomatose. His blood glucose concentration was 9 mg per deciliter (0.5 mmol per liter), and a urine dipstick test was negative for ketones. He had previously been well and tolerated minor illnesses without difficulty. Physical examination was normal. The child responded within five minutes to intravenous dextrose, with further improvement over the next hour. Blood lactate and plasma ammonia, aminotransferase, and carnitine concentrations were normal, as was urinary excretion of organic acids when measured two days later.

The patient was given a normal diet, and the parents were advised not to allow the boy to go without food for prolonged periods. Subsequently, he continued to tolerate minor illnesses with no difficulty, and on one occasion strenuous exercise for one hour did not provoke increases in serum creatine kinase or cholesterol concentrations. His physical and mental development during the next five years were normal, as were plasma carnitine, creatine kinase, creatinine, cholesterol, and aminotransferase concentrations on several occasions. Plasma alanine and lactate concentrations were normal even during periods of stress, suggesting that gluconeogenesis was normal. There have been no further seizures; the seizure on presentation was attributed to hypoglycemia. The boy's parents are not related, and he has one sister, who is healthy and has never had any symptoms such as his.

METHODS

Provocative Tests

At the age of seven the patient fasted for 22 hours. Blood was collected every two to four hours from hour 14 to hour 22 for measurements of blood glucose and plasma free fatty acid and β -hydroxybutyrate concentrations. Urine was collected for organic-acid analysis at the end of the fast. After a 15-hour fast, the boy was given 1.5 g of medium-chain triglycerides per kilogram of body weight orally, and then plasma β -hydroxybutyrate was measured at hourly intervals for 3 hours, blood glucose was measured every 15 to 30 minutes for 3 hours, and urine and plasma were collected for organic-acid analysis at 3 hours. A similar protocol was followed after the child had fasted for 12 hours and was then given long-chain triglycerides in the form of safflower oil (1.5 g per kilogram). The boy's parents gave informed consent for all the diagnostic investigations.

Analyses of Enzymes and Metabolites

HMG-CoA lyase activity in cultured transformed lymphoblasts, plasma and tissue carnitine, plasma nonesterified fatty acids and 3-hydroxybutyrate, and plasma and urine acylcarnitines and organic acids were measured as previously described.⁷⁻¹² Assays of long-chain, medium-chain, and short-chain acyl-CoA dehydrogenases in skin fibroblasts were kindly performed by Dr. A. Adams (Melbourne, Australia); assays of long-chain and short-chain hydroxyacyl-CoA dehydrogenases, enoyl-CoA hydratase, and thiolases were performed by Dr. R. Wanders (Amsterdam); assays of carnitine palmitoyltransferases I and II were performed by Dr. F. Demaugre (Paris); and a screening test for defective fatty-acid oxidation based on palmitate and myristate oxidation was performed by Drs. B. Wilcken and J. Hammond (Sydney, Australia).¹³

Liver Biopsy

The boy underwent wedge biopsy of the liver at the age of nine years, with the written informed consent of his parents, for histo-

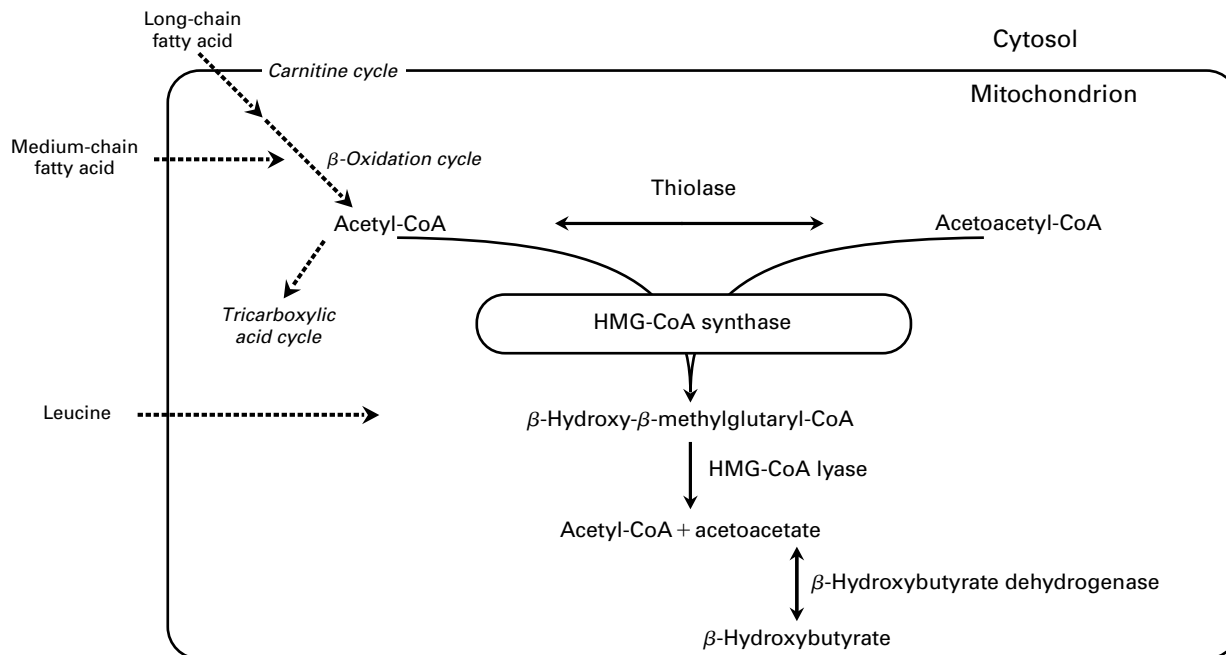


Figure 1. Role of 3-Hydroxy-3-Methylglutaryl-CoA (HMG-CoA) Synthase in Hepatic Mitochondrial Ketogenesis.

The three enzymes of the HMG-CoA cycle — thiolase, synthase, and lyase — convert acetyl-CoA, the end product of mitochondrial fatty-acid β -oxidation, to acetoacetate and β -hydroxybutyrate, which are released from the liver for use as fuels by the brain and other tissues. Small amounts of ketones can be made from the branched-chain amino acid leucine without the need for HMG-CoA synthase.

logic examination and measurement of HMG-CoA synthase activity. Control specimens of normal liver were obtained from two persons who had died accidentally (the interval between death and collection was less than two hours) and from one person who underwent open liver biopsy during an investigation for suspected metabolic disease, which was subsequently ruled out.

HMG-CoA Synthase Activity

HMG-CoA synthase activity was measured in whole homogenates of liver tissue and in cultured skin fibroblasts by radioisotopic modification¹⁴ of a coupled enzyme assay.¹⁵ The assay measures the conversion of acetyl-CoA to acetoacetate by means of HMG-CoA and therefore depends on the activities of acetoacetyl-CoA thiolase, HMG-CoA synthase, and HMG-CoA lyase. In the liver, 90 percent of acetoacetate is produced in mitochondria.¹⁴ Citrate synthase activity was measured in the liver homogenates as a control mitochondrial enzyme to assess sample preservation.¹⁶

RESULTS

After fasting for 22 hours, the patient had a blood glucose concentration of 41 mg per deciliter (2.3 mmol per liter) and a plasma β -hydroxybutyrate concentration of 0.2 mmol per liter (normal, >2.0). He rapidly became comatose, waking about 30 minutes after receiving a bolus intravenous injection of dextrose (500 mg per kilogram) followed by a continuous infusion at a rate of 10 mg per kilogram per minute. This response is slower than that expected in children with brief episodes of hypoglycemia, in whom coma usually resolves within minutes.

After the ingestion of long-chain triglycerides, the

patient's plasma free fatty-acid concentrations increased appropriately, but plasma β -hydroxybutyrate concentrations remained below 0.15 mmol per liter (normal response, >0.5). The patient's plasma β -hydroxybutyrate concentrations (Table 1) also remained low after the ingestion of medium-chain triglycerides, indicating that enzyme function after the oxidation of long-chain fatty acids, the first step in ketone-body formation, was abnormal. Four hours after the ingestion of medium-chain triglycerides the boy became comatose, with a blood glucose concentration of 50 mg per deciliter (2.8 mmol per liter). He was then given intravenous dextrose (in the same dose as before) and responded in about one hour.

Urinary excretion of organic acids was normal on several occasions during minor illnesses and after fasting and the ingestion of long-chain triglycerides. After the ingestion of medium-chain triglycerides plasma concentrations of hydroxy fatty acids, including 3-hydroxyhexanoate and 3-hydroxyoctanoate, increased markedly (Table 1). Urinary excretion of ethyl malonate was slightly elevated (38 μ mol per millimole of creatinine), and traces of 3-ketohexanoate were also present. Hydroxy fatty acids were not detected in plasma during fasting, and their presence after the ingestion of medium-chain triglycerides therefore most likely reflects defective metabolism of

TABLE 1. PLASMA CONCENTRATIONS OF 3-HYDROXYCARBOXYLIC ACIDS AFTER THE INGESTION OF MEDIUM-CHAIN TRIGLYCERIDES.*

VARIABLE	3-HYDROXY- HEXANOATE	3-HYDROXY- OCTANOATE	3-HYDROXY- DECANOATE	β -HYDROXY- BUTYRATE
	$\mu\text{mol/liter}$			
Patient				
At base line	6	3	ND	—
After ingestion of medium-chain triglycerides	510	2400	25	ND
Normal subject†				
After ingestion of medium-chain triglycerides	20	20	ND	—
Normal range after ingestion of medium-chain triglycerides‡	—	—	—	500–3000

*ND denotes not detected.

†The normal subject was a man who was given a similar dose of medium-chain triglycerides on the basis of weight under similar conditions.

‡The normal range was established by measuring levels in 16 age-matched children who were undergoing tests for defects in fat oxidation, all of whom were subsequently proved not to have such defects.

TABLE 2. ACTIVITIES OF HMG-CoA SYNTHASE AND CITRATE SYNTHASE IN LIVER SPECIMENS FROM A PATIENT WITH HMG-CoA SYNTHASE DEFICIENCY AND THREE NORMAL SUBJECTS.*

SUBJECT	HMG-CoA SYNTHASE	CITRATE SYNTHASE
	$\mu\text{mol/min/g}$ of wet weight of whole liver	
Patient	0.02, 0.05	4.3
Normal subjects†		
1	0.32, 0.30, 0.32	3.5
2	0.20, 0.23	2.6
3	0.31	3.9
Both patient and normal subjects (mixing experiments)‡	0.37, 0.30	

*Results from duplicate or triplicate assays performed on separate days are shown.

†Normal liver was obtained from two persons who died accidentally and at open liver biopsy from one subject who was being assessed for a metabolic disease. Metabolic disease was subsequently ruled out.

‡The results shown are those of two experiments in which 0.86 and 0.98 mg of the patient's liver and 0.98 and 0.94 mg of liver from normal subjects, respectively, were mixed. The two mixing experiments show that activity in the combined samples approximates the sum of that in the samples from the patient and normal subjects, indicating that an inhibitor could not be responsible for the low level of activity in the samples from the patient.

medium-chain triglycerides, raising the possibility that fatty-acid toxicity contributed to the patient's coma. After the ingestion of long-chain triglycerides, plasma 3-hydroxyhexanoate and 3-hydroxyoctanoate concentrations were 6 and 2 μmol per liter, respectively, as compared with respective base-line values of 4 and less than 2 μmol per liter.

Histologic examination of the liver showed mild fatty infiltration, mainly in the periportal regions, but no other abnormalities. Electron microscopy revealed moderate variation in the size of the mitochondria, and many contained nonspecific crystalline inclusions. The total carnitine content of the liver was 1278 nmol per gram of wet weight (normal, 900 to 1800), and the free carnitine content was 698 nmol per gram of wet weight (normal, approximately 70 percent of the total content). Analysis of blood spots on filter paper by tandem mass spectrometry revealed a normal acylcarnitine profile.

The HMG-CoA synthase activity in the patient's liver was 5 to 20 percent of that in samples of normal liver (Table 2). In mixing experiments, HMG-CoA synthase activity in combined samples was equal to the sum of the values in samples from the patient and the normal subjects. This finding rules out the possibility that an inhibitor was responsible for the reduced activity in the patient's liver and confirms that the defect was in the rate-limiting step (i.e., at the level of HMG-CoA synthase). Hepatic citrate synthase activity was similar in the patient and the normal subjects. The activities of other enzymes involved in fatty-acid oxidation in fibroblasts were normal.

DISCUSSION

The occurrence of episodes of hypoketotic, hypoglycemic coma during fasting in our patient is typical of deficiencies of enzymes involved in mitochondrial fatty-acid oxidation.¹ The studies performed while the patient was fasting confirmed that ketogenesis was defective, and the fat-loading studies indicated that the defect was not in the metabolism of long-chain fatty acids. Plasma carnitine concentrations are low in many patients with defects of fatty-acid β -oxidation, with normal values previously having been associated with carnitine palmitoyltransferase I deficiency and multiple mild defects in dehydrogenation. The normal plasma and liver values in our patient, together with the results of the fat-loading tests and the finding of normal urinary excretion of organic acids, raised the suspicion of a fault in the final stages of ketogenesis. Our patient had no evidence of HMG-CoA lyase deficiency, and acetoacetyl-CoA thiolase deficiency typically presents with hyperketosis. Thus, a deficiency of HMG-CoA synthase appeared likely. The patient's hepatic HMG-CoA synthase activity was approximately 10 percent of that in normal liver. This residual activity almost certainly re-

flected cytosolic HMG-CoA synthase activity, which contributes about 10 percent of the overall HMG-CoA synthase activity in normal liver.¹⁷ The results in our patient therefore support the diagnosis of a deficiency of hepatic mitochondrial HMG-CoA synthase activity.

Control of hepatic ketogenesis is exerted by two major regulatory enzymes, carnitine palmitoyltransferase I and mitochondrial HMG-CoA synthase.¹⁸ Carnitine palmitoyltransferase I has a clear role in controlling the initiation of β -oxidation and, hence, ketogenesis, but appears to have little influence on later control (down-regulation) of the process.¹⁹ HMG-CoA synthase is active in both the initiation and the down-regulation of ketogenesis,²⁻⁴ as well as having a specific role in the control of β -oxidation of medium-chain triglycerides. This specific role would explain the sensitivity of our patient to medium-chain triglycerides and the appearance of abnormal metabolites after the ingestion of medium-chain triglycerides. Although the mitochondrial HMG-CoA synthase gene has been sequenced in rats²⁰ and birds,²¹ the location of the gene in humans is not known.

The diagnosis of mitochondrial HMG-CoA synthase deficiency is not straightforward. Like other defects of fatty-acid oxidation, this condition should be considered in any patient with coma induced by fasting or a life-threatening event from the newborn period through infancy to middle childhood. In particular, this deficiency may possibly contribute to the sudden infant death syndrome and Reye's syndrome. Low levels of HMG-CoA synthase have been identified in lymphocytes, intestine, kidney, testis, and ovary,^{22,23} but our patient had no symptoms to suggest the involvement of these tissues. HMG-CoA synthase therefore appears to cause symptoms only in relation to its hepatic location, and these symptoms are similar to those that occur in patients with most other defects in fatty-acid oxidation. However, some of these defects involve multiple organs, and the absence of such involvement in the presence of normal plasma carnitine concentrations and normal urinary excretion of organic acids should raise the suspicion of HMG-CoA synthase deficiency. However, the results of diagnostic tests of value in other disorders of fatty-acid oxidation were normal in our patient, and specific diagnosis currently relies on liver-enzyme assay.

The clinical course in our patient confirms the importance of hepatic synthesis of ketones to the maintenance of the energy supply during fasting. The absence of any demonstrable disturbance of cholesterol metabolism underlines the functional distinction between the cytosolic and mitochondrial forms of HMG-CoA synthase. Our patient was asymptomatic except during prolonged fasting. In contrast, patients with deficiency of HMG-CoA lyase, the fi-

nal enzyme of the ketogenic pathway, typically have more prominent symptoms. This difference could be due to the fact that metabolic stress on pathways of protein metabolism, as well as on those of fatty-acid metabolism, can induce symptoms in the latter condition²⁴ or, possibly, to the variation in acyl-CoA accumulation between the two disorders, the latter being reflected in the relative derangements in carnitine metabolism. Although ketones are thought to be important for the developing brain,²⁵ the normal neurologic development in both our patient and many with HMG-CoA lyase deficiency indicates that ketone synthesis is not essential to brain development as long as prolonged fasting is avoided.

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REFERENCES

- Roe CR, Coates PM. Mitochondrial fatty acid oxidation disorders. In: Scriver CR, Beaudet AL, Sly WS, Valle D. The metabolic and molecular bases of inherited disease. 7th ed. Vol. 1. New York: McGraw-Hill, 1995:1501-33.
- Casals N, Roca N, Guerrero M, et al. Regulation of the expression of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene: its role in the control of ketogenesis. *Biochem J* 1992;283:261-4.
- Ayte J, Gil-Gomez G, Hegardt FG. Methylation of the regulatory region of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene leads to its transcriptional inactivation. *Biochem J* 1993;295:807-12.
- Serra D, Casals N, Asins G, Royo T, Ciudad CJ, Hegardt FG. Regulation of mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme A synthase protein by starvation, fat feeding, and diabetes. *Arch Biochem Biophys* 1993;307:40-5.
- Ayte J, Gil-Gomez G, Haro D, Marrero PF, Hegardt FG. Rat mitochondrial and cytosolic 3-hydroxy-3-methylglutaryl-CoA synthases are encoded by two different genes. *Proc Natl Acad Sci U S A* 1990;87:3874-8.
- Des Rosiers C, David F, Garneau M, Brunengraber H. Nonhomogeneous labeling of liver mitochondrial acetyl-CoA. *J Biol Chem* 1991;266:1574-8.
- Wysocki SJ, Hahnel R. 3-Hydroxy-3-methylglutaric aciduria: 3-hydroxy-3-methylglutaryl-coenzyme A lyase levels in leucocytes. *Clin Chim Acta* 1976;73:373-5.
- Cederblad G, Lindstedt S. A method for the determination of carnitine in the picomole range. *Clin Chim Acta* 1972;37:235-43.
- Fouéré C, Rota M, Vassault A, Bonnefont JP, Nicolas A, Bailly M. Miniaturisation et automatization du dosage du glucose, du pyruvate, du lactate, de l'acetoacetate, du β -hydroxybutyrate, des acides gras nonesterifiés sanguins. *Acta Pharm Biol Clin* 1987;4:420-3.
- Millington DS, Kodo N, Terada N, Roe D, Chace DH. The analysis of diagnostic markers of genetic disorders in human blood and urine using tandem mass spectrometry with liquid SIMS. *Int J Mass Spectrom* 1991;111:211-6.
- Duran M, Bruinvis L, Ketting D, de Klerk JBC, Wadman SK. Cis-4-decenoic acid in plasma: a characteristic metabolite in medium-chain acyl-CoA dehydrogenase deficiency. *Clin Chem* 1988;34:548-51.
- Tanaka K, West-Dull A, Hine DG, Lynn TB, Lowe T. Gas-chromatographic method of analysis for urinary organic acids. II. Description of the procedure, and its application to diagnosis of patients with organic acidurias. *Clin Chem* 1980;26:1847-53.
- Manning NJ, Olpin SE, Pollitt RJ, Webley J. A comparison of [9,10-³H]palmitic and [9,10-³H]myristic acids for the detection of fatty acid oxidation defects in intact cultured fibroblasts. *J Inher Metab Dis* 1990;13:58-68.

14. Stanley CA, Gonzales E, Baker L. Development of hepatic fatty acid oxidation and ketogenesis in the newborn guinea pig. *Pediatr Res* 1983;17:224-9.
15. Williamson DH, Bates MW, Krebs HA. Activity and intracellular distribution of enzymes of ketone-body metabolism in rat liver. *Biochem J* 1968;108:353-61.
16. Stanley CA, Hale DE, Berry GT, Deleeuw S, Boxer J, Bonnefont J-P. A deficiency of carnitine-acylcarnitine translocase in the inner mitochondrial membrane. *N Engl J Med* 1992;327:19-23.
17. Quant PA, Tubbs PK, Brand MD. Treatment of rats with glucagon or mannoheptulose increases mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase activity and decreases succinyl-CoA content in liver. *Biochem J* 1989;262:159-64.
18. Guzman M, Geelen MJ. Regulation of fatty acid oxidation in mammalian liver. *Biochim Biophys Acta* 1993;1167:227-41.
19. Grantham BD, Zammit VA. Role of carnitine palmitoyltransferase I in the regulation of hepatic ketogenesis during the onset and reversal of chronic diabetes. *Biochem J* 1988;249:409-14.
20. Gil-Gomez G, Ayte J, Hegardt FG. The rat mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme-A-synthase gene contains elements that mediate its multihormonal regulation and tissue specificity. *Eur J Biochem* 1993;213:773-9.
21. Kattar-Cooley PA, Wang HH, Mende-Mueller LM, Miziorko HM. Avian liver 3-hydroxy-3-methylglutaryl-CoA synthase: distinct genes encode the cholesterologenic and ketogenic isozymes. *Arch Biochem Biophys* 1990;283:523-9.
22. Curi R, Williams JF, Newsholme EA. Formation of ketone bodies by resting lymphocytes. *Int J Biochem* 1989;21:1133-6.
23. Royo T, Pedragosa MJ, Ayte J, Gil-Gomez G, Vilaro S, Hegardt FG. Testis and ovary express the gene for the ketogenic mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase. *J Lipid Res* 1993;34:1636.
24. Thompson GN, Chalmers RA, Halliday D. The contribution of protein catabolism to metabolic decompensation in 3-hydroxy-3-methylglutaric aciduria. *Eur J Pediatr* 1990;149:346-50.
25. Williamson DH. Ketone body metabolism during development. *Fed Proc* 1985;44:2342-6.

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