

## CARNITINE DEFICIENCIES

Carnitine ( $\beta$ -hydroxy- $\gamma$ -N-trimethylaminobutyric acid) is an essential factor in the mitochondrial transport of long-chain fatty-acyl-CoA, in the mitochondrial efflux of short-chain acyl-CoA (propionyl-CoA, methylmalonyl-CoA) and in the maintenance of the free CoA-SH level in the mitochondrial matrix. It is derived from the diet and is synthesized in the liver from the essential amino acids lysine and methionine according to the following scheme:

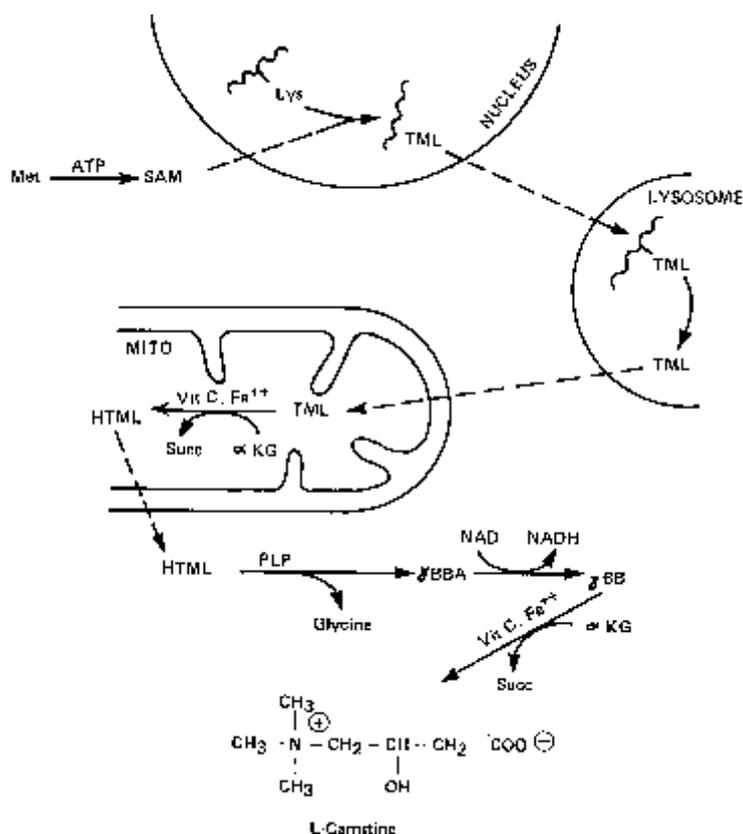


Fig. 1. Biosynthesis of carnitine. Abbreviations: SAM, S-adenosylmethionine; TML, 6-N-trimethyllysine;  $\alpha$ KG,  $\alpha$ -ketoglutarate; Succ, succinate; HTML, 3-hydroxy-6-N-trimethyllysine; PLP, pyridoxal phosphate;  $\gamma$ BBA,  $\gamma$ -butyrobetaine aldehyde;  $\gamma$ BB,  $\gamma$ -butyrobetaine.

While most animal tissues contain the enzymes necessary to convert 6-N-trimethyllysine to  $\gamma$ -butyrobetaine, the formation of carnitine from  $\gamma$ -butyrobetaine occurs only in a few tissues (in humans the  $\gamma$ -butyrobetaine hydroxylase is present in liver, kidney and brain).

Although the individual steps of the overall pathway and the enzymes catalyzing the reactions have been identified, the regulation of carnitine biosynthesis in mammals is still (1998) unresolved. The rate of carnitine biosynthesis is determined by the availability of 6-N-trimethyllysine, which in turn can be produced only in the course of posttranslational modification of protein-bound lysine residues. In this case one can expect that the rate of carnitine biosynthesis could be increased through increased methylation of protein-bound lysine and/or increased protein turnover. Since

neither of these processes is strictly specific for the carnitine biosynthesis, the ability of humans to adapt to changes in the need for carnitine would be severely restricted, consequently increasing dependence on exogenous carnitine in conditions of increased demand.

Humans possess efficient mechanisms to conserve their carnitine pool (Fig. 2.).

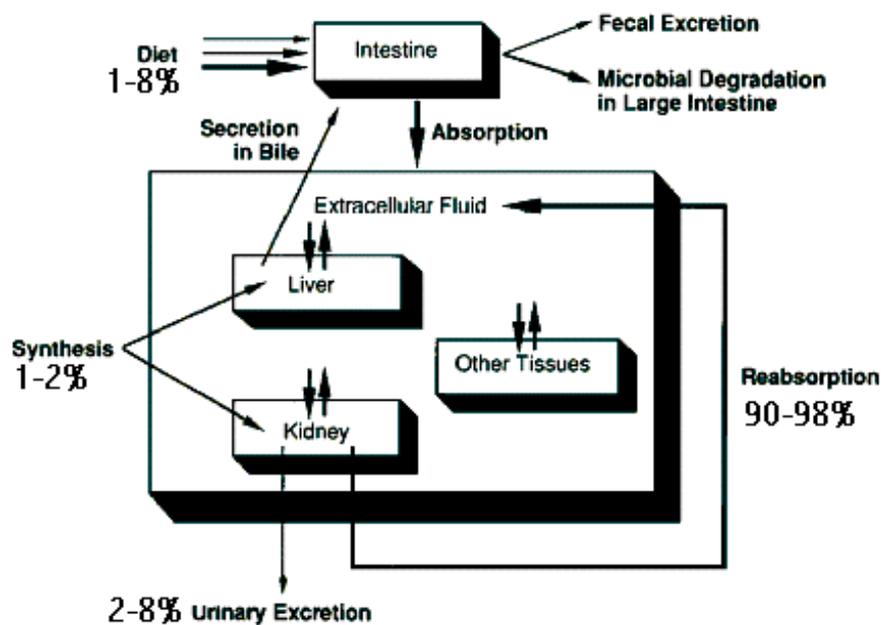


Fig. 2. Carnitine homeostasis. The numbers refer to the relative contribution of different routes to the whole body carnitine turnover.

Humans can not degrade carnitine. The only route to lose carnitine is in the urine in free form or as acyl-carnitine. As shown on Fig.2., kidneys reabsorb carnitine with high efficiency. The lost carnitine is replenished by the dietary carnitine and by the endogenous synthesis; normally the relative contribution of these two sources is approximately equal. The rate of absorption of carnitine in the intestine is regulated, but the mechanism is still unknown; depending on the carnitine demands 54-87% of the dietary carnitine can be absorbed. The rate of reabsorption in the kidneys is also a subject of control. The route with the lowest adaptive capacity is the endogenous synthesis. According to the scheme of Fig. 2. the maximally activated endogenous biosynthesis and kidney reabsorption has sufficient capacity to compensate for deficiency of dietary carnitine (a situation quite possible with vegetarian subjects). Similarly, the carnitine content of mixed-type diet together with the activated kidney reabsorption can completely counterbalance deficiencies of the biosynthesis (e.g. a hypothetical enzyme defect in the synthetic pathway; N.B. no such defect has been described by now, 1998). In contrast, the two sources of carnitine, even if maximally activated, can not meet the carnitine demands in situations when the kidney reabsorption is severely impaired.

Liver is the major site of  $\gamma$ -butyrobetaine hydroxylation and it is the only organ in which net release of carnitine into circulation occurs. The rest of the tissues rely on the uptake of blood carnitine. Due to the unfavourable concentration gradient (40-50  $\mu$ M carnitine in the extracellular space versus 600-700  $\mu$ M intracellular concentration) the uptake requires active transport: one exchange transport system and

two  $\text{Na}^+$ -dependent transporters ( $\text{Na}^+$ -carnitine cotransport) have been described (Table 1.). Recently it has been shown that the high-affinity carnitine transporter is identical to the organic cation transporter OCTN2: a member of the family of organic cation transporters, integral membrane proteins with 12 transmembrane domains and a nucleotide binding fold that are important in detoxification, drug excretion and transplacental solute transfer. The exchange transporter performs two types of transfer: it can exchange intracellular acyl-carnitine for extracellular free carnitine (with no net uptake of carnitine in this case) or it can take up free extracellular carnitine exchanging it for  $\gamma$ -butyrobetaine synthesized within the cell (in this case the energy invested in the  $\gamma$ -butyrobetaine synthesis covers the energy demands of the transport). The separate system for carnitine efflux is saturable and energy independent.

Tissue	$\text{Na}^+$ -dependent high-affinity uptake ( $K_T=2-20 \mu\text{M}$ )	$\text{Na}^+$ -dependent low-affinity uptake ( $K_T=0.2-5 \text{ mM}$ )	Efflux	Exchange
Liver	-	+	+	-
Heart	+	-	-	+
Striated muscle	+	+	+	+
Kidney	+	+	-	-
Fibroblasts	+	+	-	-

Table 1. Tissue distribution of carnitine transporter systems. Abbreviation:  $K_T$ = carnitine concentration at half-maximal transport rate.

Kidneys play a crucial role in whole-body homeostasis: they are responsible for the retention of the whole-body carnitine pool (Figs. 2., 3.).

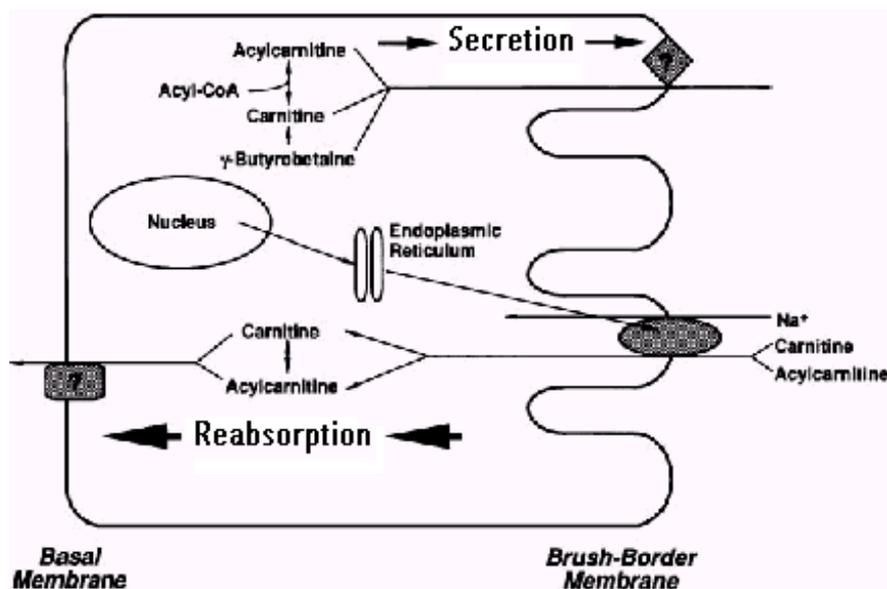


Fig.3. Carnitine metabolism in renal tubular epithelia.

Carnitine deficiency represents a heterogeneous group of diseases with widely varying clinical symptoms. Given the essential role of carnitine in fatty acid oxidation and in the intracellular homeostasis of free and acyl-CoA, the clinical manifestation of the deficiencies (see clinical cases) is not unexpected. Carnitine deficiency is defined as decreased tissue carnitine concentration that is below the requirements for normal metabolism. Based on the etiologies the carnitine deficiencies can be classified as primary and secondary as discussed briefly below.

1. Primary carnitine deficiencies (inherited disorders based on a defect of a component directly involved in the maintenance of carnitine homeostasis)

1.1. Primary systemic carnitine deficiency

Carnitine concentration is severely reduced both in the affected tissues (heart, skeletal muscle, liver) and in the blood plasma. The clinical symptoms are related to progressive cardiomyopathy, myopathy and hypoketotic hypoglycemic encephalopathy coupled with hyperammonemia (see clinical cases). The molecular basis of the disease is a severe renal carnitine leak because of a defect in the OCTN2. Several mutations of the OCTN2 gene have been identified in patients with primary carnitine deficiency: base substitution converting the codon for Arg-282 to a STOP codon, insertion producing a new STOP codon. These mutations decrease the level of mature OCTN2 mRNA and result in nonfunctional transporters. The diagnosis is made by determination of carnitine uptake in cultured fibroblasts, which express the same carrier (Table 1.).

1.2. Primary myopathic carnitine deficiency

The carnitine content is severely reduced in skeletal muscle but is normal in plasma and the liver, with no sign of renal carnitine leak. The clinical symptoms are related to progressive muscle weakness and lipid storage myopathy coupled with elevation of plasma enzymes of muscle origin. Onset may occur early in life or may be delayed until middle age. The molecular basis of the disease is an increased loss of tissue carnitine because of a defect in the carnitine efflux carrier. Direct studies in cultured myoblasts demonstrate normal carnitine uptake and increased carnitine efflux.

2. Secondary carnitine deficiencies (excessive renal loss of carnitine or acyl-carnitine that accompanies inherited or acquired disorders of metabolic pathways not directly involved in carnitine homeostasis)

2.1. Metabolic disorders associated with impaired oxidation of acyl-CoA intermediates in mitochondria (e.g. methylmalonyl-CoA isomerase deficiency, vitamin B<sub>12</sub> deficiency).

2.2. Defects in the mitochondrial respiratory chain (e.g. complex I deficiency in which the shortage of ATP needed to establish the Na<sup>+</sup> gradient could explain the impaired renal reabsorption and tissue uptake of carnitine) .

2.3. Renal tubular Fanconi syndrome (a generalized transport defect affecting tubular reabsorption of small molecules).

## Clinical cases

### CASE I.

This 3-1/2-year-old boy was born to non-consanguineous parents from the state of Chihuahua in Mexico after an uncomplicated pregnancy and delivery. A brother had died with a liver problem after an unexplained coma occurring at three months of age. The parents and three older siblings are well.

At three months of age the patient was admitted in coma to the emergency room of a community hospital, where he had a cardiac arrest. His blood sugar was 15 mg/dl. Hepatomegaly and cardiomegaly were noticed for the first time. Hepatomegaly resolved with appropriate and intensive care, and the patient recovered. At six months of age he was brought to the University of California, Los Angeles, Medical Center in congestive heart failure after an episode of upper-respiratory-tract infection. Hepatomegaly and hypotonia were observed. On day 3, he became lethargic; generalized seizure activity and cardiac arrest developed, but he was successfully revived. Laboratory studies revealed a blood sugar of 15 mg/dl without associated acidosis or ketosis; mild elevation of serum aspartate aminotransferase (SGOT) (337 IU/l; normal, 10 to 45); and hyperammonemia (300  $\mu$ g/dl; normal, < 69). Delayed milestones (developmental quotient 66), proximal-muscle weakness, and growth retardation (weight and height below the third percentile) were noted after recovery. Cardiac catheterization showed a minimal atrial-septal defect and bilateral ventricular hypertrophy. Metabolic studies showed normal glucose, galactose, and fructose tolerance and a normal 10-hour fasting blood sugar with no increase in lactate, pyruvate, or ketone bodies. Cortisol and insulin studies, the blood-sugar response to glucagon stimulation, and urine amino acid and organic acid chromatography also gave normal results. The electroencephalogram, brain scan, and chromosomal studies were unremarkable. Blood sugar and ammonia, SGOT, SGPT, and creatine phosphokinase were all normal between acute episodes. Also normal were the levels of electrolytes, calcium, phosphorus, magnesium, bilirubin, thyroxine, thyroid stimulating hormone, and growth hormone. Results of total protein determination, serum electrophoresis, cerebrospinal-fluid studies, and studies of immune function were also normal.

The patient presented again with cardiorespiratory arrest after upper-respiratory-tract infections at the ages of 20 months, 24 months, and 33 months. He recovered slowly each time. The episodes were associated with liver enlargement, elevation of the transaminases to 2000 or more IU per liter, and elevation of creatine phosphokinase from 1500 to 33,000 IU per liter. Maintenance glucose requirements varied from normal levels (3 mg per kilogram of body weight per minute) to slightly elevated levels (5 to 7 mg per kilogram per minute).

During the 11<sup>th</sup> admission, fatty changes in the liver and the lack of production of ketone bodies after 24 hours of fasting were noted. In addition, carnitine levels in liver, muscle, serum, and urine were determined (Table 2.).

**Therapy:**

The patient was given 4 g of DL-carnitine/day and a low-fat diet. He improved dramatically, hepatomegaly was no longer seen after treatment for two weeks and the SGPT returned to normal. His muscle strength steadily improved. Carnitine administration in such a patient must be life long. Carnitine levels in tissues and serum after treatment are also given in Table 2.

Tissue	Control	Patient before therapy	Patient after 3 months of therapy
Muscle ( $\mu\text{mol/g}$ )	1.22	0.02	0.50
Liver ( $\mu\text{mol/g}$ )	1.24	0.04	4.98
Serum ( $\mu\text{mol/l}$ )	35.5	4.82	18.5
Urine ( $\mu\text{mol/24 h}$ )	231	47	1664

Table 2. Carnitine levels in muscle, liver, serum and urine.

**Questions:**

1. Based on the findings upon admission at six months and prior to recovery, what tentative diagnosis would you propose? What additional lab tests would you order to confirm or rule out your diagnoses?
2. What immediate treatment would you order for the patient at the emergency admission at 6 months of age?
3. What key findings led to the definitive diagnosis?
4. Is the carnitine deficiency in this patient systemic or myopathic?
5. How would you differentiate between the two forms of carnitine deficiency?

6. Would pyruvate oxidation be impaired in this patient?
7. Why did lipid accumulate in the liver (11<sup>th</sup> admission)?
8. Would a pyridoxine deficiency be a possible basis for this disorder?
9. Why was the patient unable to produce ketone bodies after a 24-hour fast?
10. What is the biochemical basis of the marked hypoglycemia and the hyperammonemia that is observed in this patient? How would these conditions contribute to the acute episodes in this patient?
11. How can you interpret the ratio of carnitine content in serum and urine in the patient before and after treatment?

## **CASE II**

The patient, a girl, was the first child of unrelated caucasian parents. After a normal pregnancy, delivery at term was complicated by breech presentation requiring caesarean section. She was breastfed initially, but on day three of life she became tachypneic and hypotonic, and proceeded into coma with profound metabolic acidosis (base deficit, 21 mmol/l). Gasliquid chromatography of her urine and blood plasma followed by mass spectrometry gave a diagnosis of methylmalonic aciduria, and this was confirmed by enzymology on cultured skin fibroblasts. Attempts to treat with vitamin B<sub>12</sub> (1 mg daily IM) failed to improve her clinical and metabolic state. She was subsequently managed by restriction of natural protein intake (up to 1 g/kg body weight daily) with supplementary amino acids (devoid of isoleucine, valine, threonine, and methionine) at a value equivalent to an additional 1 g protein/kg body weight, daily. Supplementary vitamins and minerals were included with additional calories provided by carbohydrate polymer and lipid. Her postnatal course was characterized by multiple hospital admissions for metabolic disturbances, including hypoglycemia, hyperammonemia and hyperglycinemia. The episodes were complicated by anorexia and recurrent vomiting associated with intermittent upper gastrointestinal bleeding. She had generally delayed development with particular delay in speech and poor muscle tone and power. At the time of the study she was 21 months old, with a developmental age of 11 months, height < 3<sup>rd</sup> percentile, weight 3<sup>rd</sup>-10<sup>th</sup> percentile, and head circumference 50<sup>th</sup> percentile.

The patient was tested for a possible secondary deficiency of carnitine. The measured plasma and urinary values for the patient and for normal controls are given in Table 3.

The patient was given an oral dose of 12.4 mmol of D,L-carnitine (200 mg/kg body weight). Urine samples were collected sequentially over a 24 hour period for organic acid analysis and acylcarnitine measurements. During this period 476  $\mu$ mol of carnitine, as free and acylcarnitines, were excreted in the urine. This amount represented 8.1% of the ingested dose. The total carnitines excreted comprised 269  $\mu$ mol free and 207  $\mu$ mol acylcarnitines. Urinary free carnitine excretion paralleled

acylcarnitine excretion throughout the study period. Most of the excretion (77.2%) occurred in the first 12 hours.

<i>Carnitine fraction</i>	<i>Control plasma (<math>\mu\text{M}</math>)</i>	<i>Control urine (nmol/mg creatinine)</i>	<i>Patient plasma (<math>\mu\text{M}</math>)</i>	<i>Patient urine (nmol/mg creatinine)</i>
free carnitine	36.7	51.3	35.8	142
short-chain acylcarnitine	5.7	73.7	17.1	352
long-chain acylcarnitine	3.7		0.3	
Total carnitine	46.1	125	53.2	494

Table 3. Carnitine content of plasma and urine of control subjects and a methylmalonic aciduric patient.

Although there were no notable fluctuations in other metabolites, there were major changes in urinary hippurate, methylmalonate, methylcitrate, and acylcarnitines during the first 12 hours (Fig. 4.). There was a rapid 6-fold increase in hippurate concentration at 30 minutes after the dose, with a peak value attained at 3 hours, and the values decreasing thereafter. In contrast, there was less than a 2-fold increase in acylcarnitine excretion by 30 minutes but then a rapid excretion took place by five hours, representing a 24-fold increase over the baseline value. As the acylcarnitine excretion increased, both methylmalonate and methylcitrate excretion decreased progressively, by 43% and 57% respectively compared with earlier values at 30 minutes. Plasma carnitine analysis at this time showed major changes in short chain acylcarnitine concentrations, from 17.1  $\mu\text{mol/L}$  before carnitine supplementation to 27.4  $\mu\text{mol/L}$  afterwards. Free carnitine increased from 35.8 to 66.1  $\mu\text{mol/L}$ . There was no appreciable change in plasma long chain acylcarnitines. The relation between urinary acylcarnitines and the metabolites, methylmalonate and methylcitrate, persisted for 12 hours, by which time the metabolites had returned to their previous high values, and acylcarnitine excretion had decreased.

The structure of the excreted acylcarnitines was determined by mass spectroscopy. The predominant acylcarnitine was identified as the propionyl derivative. Significant amounts of acetylcarnitine were also present. The patient was given an oral carnitine supplement (25 mg/kg per hr) for 18 months with no apparent complications. During this interval, the patient has not required bicarbonate for correction of metabolic acidosis. Preliminary observations include a marked improvement in the patient's fine and gross motor skills, in her interaction with environment and in her expressive language. These changes were noted within two weeks of carnitine supplementation

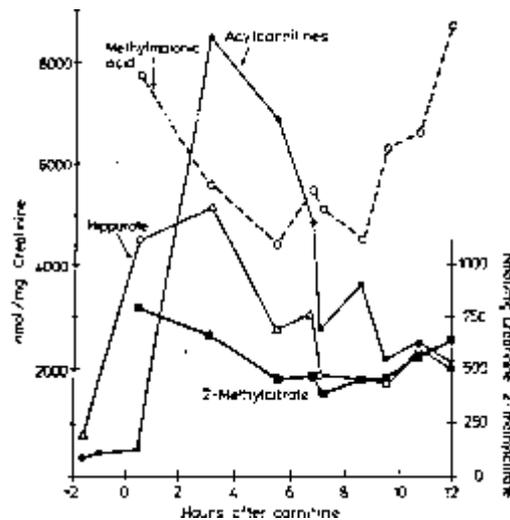


Fig. 4. Metabolic effects in the urine of a patient with methylmalonic aciduria after a single oral dose of D,L-carnitine 200 mg/kg body weight (given at arrow).

Questions:

1. What is meant by a base deficit (i.e., anion gap) of 21 mmol/l? How can this be corrected?
2. What is the rationale for treating the patient with intramuscular injections of vitamin B<sub>12</sub>?
3. What is the biochemical basis for maintaining the patient on a diet that restricted intake of isoleucine, valine, threonine and methionine?
4. What other nutrients are likely to exacerbate the clinical symptoms associated with methylmalonic aciduria? Explain.
5. What reactions would account for the synthesis of propionyl carnitine?
6. Propionyl CoA is an inhibitor of the tricarboxylic acid cycle, the pyruvate dehydrogenase complex, N-acetylglutamate synthetase and the glycine cleavage system. Explain how these effects contribute to the recurrent metabolic disturbances that are associated with methylmalonic aciduria?
7. In what ways would oral carnitine supplementation benefit a patient with methylmalonic or propionic aciduria?

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