GC Labs

To Detect Inborn Errors of Metabolism (IEM)

Carnitine and Acylcarnitine Quantitative Analysis

Carnitine

Carnitine is an ammonium compound produced in the liver and kidneys from the basic amino acids, like lysine and methionine as needed. It is present in cells and mostly concentrated in skeletal and heart muscles where fatty acids are used as an energy source.

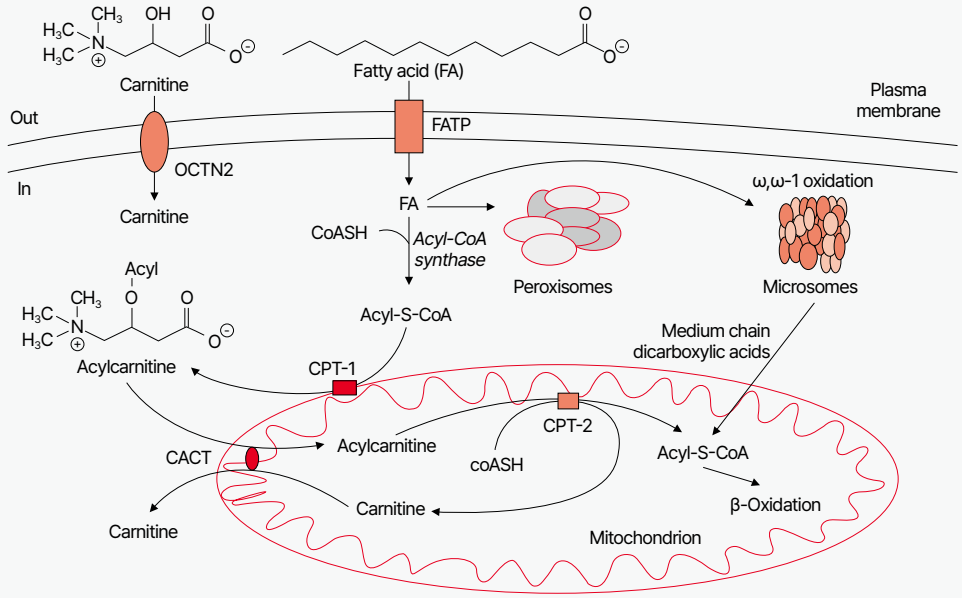
Its main function is to transfer long-chain fatty acids to mitochondria to generate energy. It also transports toxic compounds generated in cell organelles to prevent their accumulation in cells.

Since carnitine plays the role of an enzyme, it was called vitamin B_T in the past. Most people can produce sufficient carnitine in their bodies, but some people (e.g., premature infants) cannot make a sufficient amount, so carnitine can be considered a conditionally essential nutrient.

Acylcarnitine

Carnitine without substituted acyl groups is called free carnitine, while those with substituted acyl groups are all called acylcarnitine, which varies from C2 to C20 or higher.

While carnitine acts as a regulator during β-oxidation, which is the process of fatty acid decomposition, acylcarnitine, a bound form of free carnitine and long-chain fatty acyl CoA, is produced by carnitine palmitoyltransferase I (CPT I). Then it enters a mitochondrion to allow fatty acyl CoA to be transferred again to the β-oxidation step by CPT II. Afterward, it is converted to free carnitine and exits the mitochondrion to be recycled.



Ref) Tietz Textbook of Laboratory Medicine. 7 th edition

Fig 1. The carnitine cycle in fatty acid oxidation. The carnitine cycle is responsible for delivering long-chain fatty acids to the mitochondrial matrix for subsequent beta oxidation. CACT, Carnitine acyl carnitine translocase; CPT-1, carnitine palmitoyl transferase-1; CPT-2, carnitine palmitoyl transferase-2; FA, fatty acid; FATP, fatty acid transporter protein.

Quantitative Analysis of Carnitine and Acylcarnitine and Detection of IEM

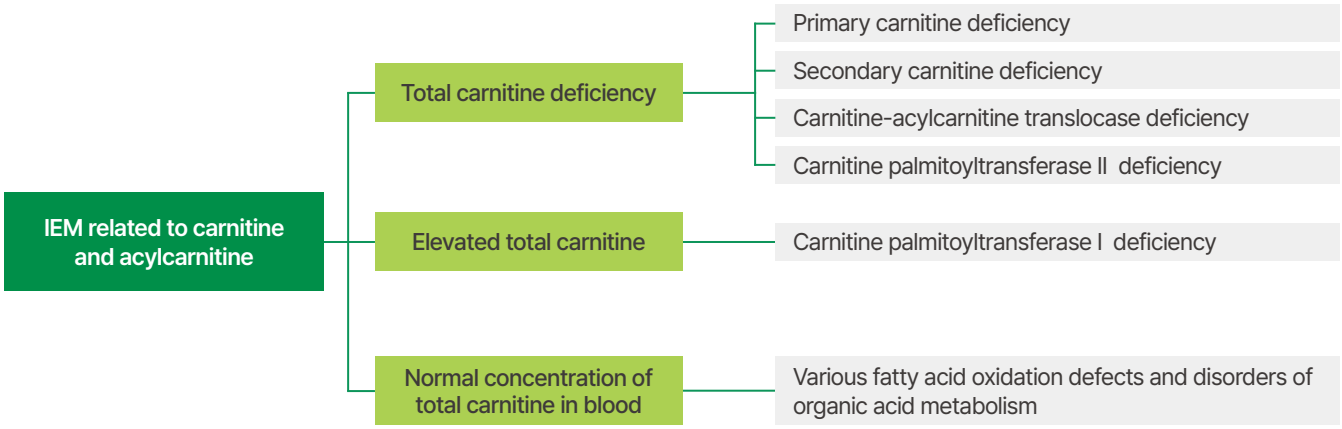


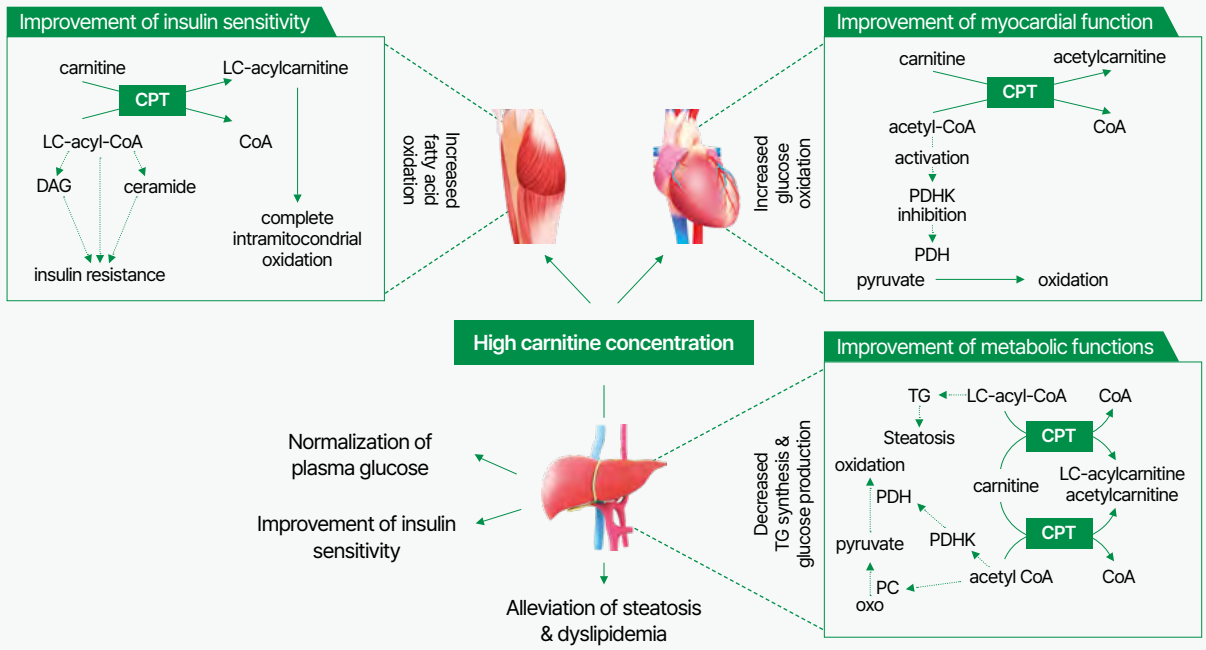
Table 1. Basic acylcarnitine increase and decrease patterns associated with various disease states

Item	Name	Disorder
CO↓	Free carnitine	· Carnitine uptake defect · Secondary carnitine deficiencies
CO↑	Free carnitine	· CPT I deficiency (with C16, C18↓)
C3↑	Propionyl	· Propionic acidemia · Methylmalonic acidemias · Succinyl-CoA synthetase deficiency
C4↑	Butyryl	· SCAD deficiency · Formiminoglutamic aciduria · Ethylmalonic encephalopathy (with C5) · Multiple acyl-CoA dehydrogenase (MAD) deficiency
	Isobutyryl	· Isobutyryl-CoA dehydrogenase (IBD) deficiency
C5↑	Isovaleryl Methylbutyryl	· Isovaleric acidemia · Methylbutyryl-CoA dehydrogenase (SBCAD) deficiency
C4-OH↑	3-Hydroxybutyryl	· SCHAD deficiency
C5-OH↑	3-Hydroxyisovaleryl	· 3-Methylcrotonyl-CoA carboxylase (3-MCC) deficiency · Holocarboxylase synthetase deficiency · HMG-CoA lyase deficiency (with C6DC) · Biotinidase deficiency · 3-Methylglutaconyl-CoA hydratase deficiency
	3-Hydroxy-2-methylbutyryl	· 3-Oxothioase deficiency (with C5:1)
C8↑	Octanoyl	· MCAD deficiency (with C6, C10, C10:1)
C3DC↑	Malonyl	· Malonyl-CoA carboxylase deficiency
C4DC↑	Succinyl/methylmalonyl	· Succinyl-CoA synthetase (SUCLA2) deficiency
C5DC↑	Glutaryl	· Glutaric acidemia type I
C10-OH↑	3-Hydroxy decanoyl	· M/SCHAD deficiency, MCLAT deficiency
C14:1↑	Tetradecenoyl	· VLCAD deficiency (with C14, C14:2)
C16↑	Palmitoyl	· CPT II deficiency (with C18:2, C18:1, C18) · Carnitine-acylcarnitine translocase (CACT) deficiency (with C18:2, C18:1, C18)
C16-OH↑	3-Hydroxypalmitoyl	· LCHAD deficiency (with C16:1-OH, C18:1-OH, C18-OH) · Trifunctional protein (TFP) deficiency (with C16:1-OH, C18:1-OH, C18-OH)

Ref) Piero Rinaldo, et al. ACMG Standards and Guidelines, Acylcarnitine profile analysis. Genet Med 2008

Other Physiological Effects Related to Carnitine

Carnitine supplementation is reported to improve insulin sensitivity, cardiac function, and liver metabolism. Studies are ongoing in a variety of diseases and conditions, including cancer, diabetes, end-stage renal disease, and dialysis.

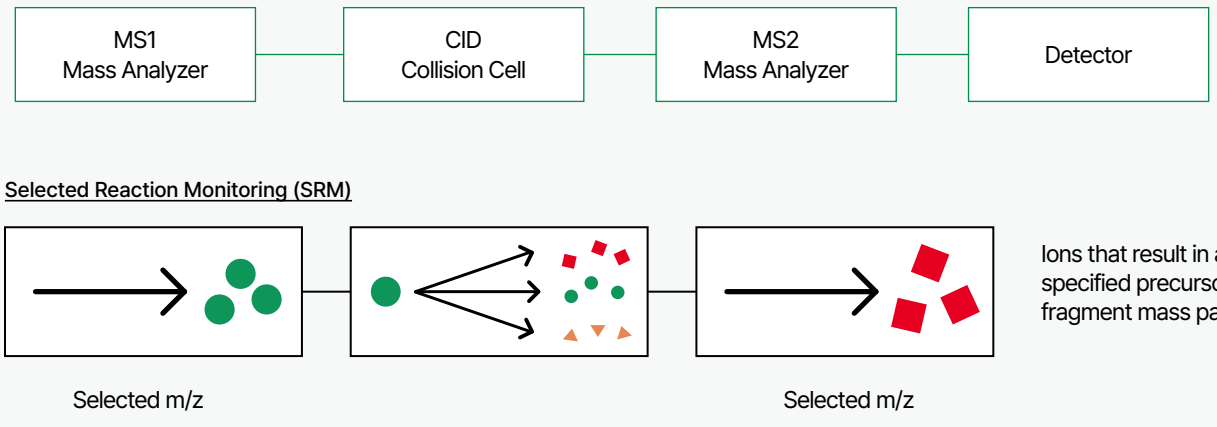


Ref) Arduino Arduini et al. Carnitine in metabolic disease: Potential for pharmacological intervention. Pharmacol Ther. 2008

Fig 2. Potential metabolic effects of high-dose L-carnitine

Carnitine (Vitamin B_T) and Acylcarnitine assay by LC-MS/MS in GC Labs

Tandem MS (or MS/MS) has become the dominant MS-based technique used in clinical laboratories for quantitative analysis of routine samples. The most important features of MS/MS are high selectivity, which in turn often conveys an ability to measure very low concentrations of analytes. The susceptibility of MS/MS to interference is typically very low, especially if it is combined with chromatographic separation. The reason is that a detected compound is separated and characterized by three physical properties: chromatographic retention time, precursor ion m/z, and product ion m/z. Because of its high specificity and potential for high sample throughput, these instruments are widely used for routine analysis in clinical laboratories.



Test Information

Test code	Test item	Specimen(mL)	Test schedule	TAT(day)	Test method
S153	Acylcarnitine [LC-MS/MS]	EDTA plasma 3.0	Mon~Fri	2	LC-MS/MS
C703	Carnitine (Vitamin B_T) [LC-MS/MS]	Serum 1.0 EDTA plasma 1.0	Thu	5	LC-MS/MS

Status of participation in external QC programs

CAP

References

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3. Stanley CA, Bennett MJ. Chapter 80.1. Disorders of Mitochondrial Fatty Acid β-Oxidation. Nelson's Textbook of Pediatrics, 19th Edition.

4. Stephen L Nelson. Free and Total Carnitine. Reference range, Interpretation, Collection and Panels. Medscape. (http://emedicine.Medscape.com/article/2115721-overview)

5. Rinaldo P, Cowan TM, Matern D. Acylcarnitine profile analysis. Genet Med. 2008 Feb;10(2):151-6.

6. Dietzen DJ, Rinaldo P, Whitley RJ, et al. National academy of clinical biochemistry laboratory medicine practice guidelines: follow-up testing for metabolic disease identified by expanded newborn screening using tandem mass spectrometry; exclusive summary. Clin Chem. 2009 Sep;55(9):1615-26

7. Arduini A, Bonomini M, Savica V, Amato A, Zammit V. Carnitine in metabolic disease: potential for pharmacological intervention. Pharmacol Ther. 2008 Nov;120(2):149-56.

8. Flanagan JL, Simmons PA, Vehige J, Willcox MD, Garrett Q. Role of carnitine in disease. Nutr Metab (Lond). 2010 Apr 16;7:30.

9. Tietz Textbook of Laboratory Medicine-7th edition

10. Henry's Clinical Diagnosis and Management by Laboratory Methods – 24th edition