Tohoku J. Exp. Med., 2010, **221**, 191-195

Carnitine Palmitoyltransferase 2 Deficiency: The Time-Course of Blood and Urinary Acylcarnitine Levels during Initial L-Carnitine Supplementation

Tomohiro Hori,[1] Toshiyuki Fukao,[1,2,3] Hironori Kobayashi,[4] Takahide Teramoto,[1] Masaki Takayanagi,[5] Yuki Hasegawa,[4] Tetsuhiko Yasuno,[6] Seiji Yamaguchi[4] and Naomi Kondo[1]

[1]Department of Pediatrics, Gifu University Graduate School of Medicine, Gifu, Japan
[2]Medical Information Science Division, United Graduate School of Drug Discovery and Medical Information, Gifu University, Gifu, Japan
[3]Clinical Research Division, Nagara Medical Center, Gifu, Japan
[4]Department of Pediatrics, Shimane University School of Medicine, Shimane, Japan
[5]Department of Metabolism, Chiba Children's Hospital, Chiba, Japan
[6]Division of Nephrology and Rheumatology, Department of Internal Medicine, Fukuoka University School of Medicine, Fukuoka, Japan

Carnitine palmitoyltransferase 2 (CPT2) deficiency is one of the most common mitochondrial beta-oxidation defects. A female patient with an infantile form of CPT2 deficiency first presented as having a Reye-like syndrome with hypoglycemic convulsions. Oral L-carnitine supplementation was administered since serum free carnitine level was very low (less than 10 μmol/L), indicating secondary carnitine deficiency. Her serum and urinary acylcarnitine profiles were analyzed successively to evaluate time-course effects of L-carnitine supplementation. After the first two days of L-carnitine supplementation, the serum level of free carnitine was elevated; however, the serum levels of acylcarnitines and the urinary excretion of both free carnitine and acylcarnitines remained low. A peak of the serum free carnitine level was detected on day 5, followed by a peak of acetylcarnitine on day 7, and peaks of long-chain acylcarnitines, such as C16, C18, C18:1 and C18:2 carnitines, on day 9. Thereafter free carnitine became predominant again. These peaks of the serum levels corresponded to urinary excretion peaks of free carnitine, acetylcarnitine, and medium-chain dicarboxylic carnitines, respectively. It took several days for oral L-carnitine administration to increase the serum carnitine levels, probably because the intracellular stores were depleted. Thereafter, the administration increased the excretion of abnormal acylcarnitines, some of which had accumulated within the tissues. The excretion of medium-chain dicarboxylic carnitines dramatically decreased on day 13, suggesting improvement of tissue acylcarnitine accumulation. These time-course changes in blood and urinary acylcarnitine levels after L-carnitine supplementation support the effectiveness of L-carnitine supplementation to CPT2-deficient patients.

Keywords: carnitine palmitoyltransferase 2; CPT2; L-carnitine; acylcarnitine profile; carnitine administration
Tohoku J. Exp. Med., 2010, **221** (3), 191-195.

Carnitine palmitoyltransferase 2 (CPT2) deficiency (EC 2.3.1.21, OMIM 600650) is one of the most common disorders of mitochondrial fatty acid oxidation. CPT2 deficiency has several clinical presentations (Bonnefont et al. 1999). The adult form is characterized by episodes of rhabdomyolysis triggered by prolonged exercise. The infantile form presents as severe attacks of hypoketotic hypoglycemia, occasionally associated with sudden infant death or a Reye-like syndrome (Demaugre et al. 1991; Hug et al. 1991). The most severe kind, the neonatal form, is almost always lethal during the first month of life.

Secondary carnitine deficiency, characterized by low levels of total and free carnitines associated with an increase in the long-chain acylcarnitine fraction, is observed in the infantile form of CPT2-deficient patients (Bonnefont et al. 2004; Longo et al. 2006). Hence, L-carnitine supply might be useful in severe CPT2 deficiencies (Bonnefont et al. 2004), although supplementation with L-carnitine in patients with beta-oxidation defects of long-chain acyl-CoA has long been a matter of controversy (Costa et al. 1998;

Received March 23, 2010; revision accepted for publication May 17, 2010. doi:10.1620/tjem.221.191
Correspondence; Toshiyuki Fukao, M.D., Ph.D., Department of Pediatrics, Gifu University Graduate School of Medicine, Yanagido 1-1, Gifu 501-1194, Japan.
e-mail:toshi-gif@umin.net

Liebig et al. 2006; Primassin et al. 2008).

In this report, we describe a CPT2-deficient patient who presented as having a Reye-like syndrome with secondary carnitine deficiency. We focused on time-dependent changes in the serum and urinary acylcarnitine profiles after intial L-carnitine supplementation.

Clinical Report

The patient, a female, was born to nonconsanguineous Japanese parents. She had been well until 15 months of age when she suddenly had tonic-clonic convulsions at 3:00 a.m. for about 30 minutes and became unconscious. Ten days before the convulsions, she had a cold and was given Cefteram pivoxil (CFTM-PI) for four days. When she arrived at another hospital, she had hypoglycemia (blood glucose 1.1 mmol/L), hepatic dysfunction (AST 85 IU/L, ALT 55 IU/L, LDH 402 IU/L), and mild hyperammoniemia (NH3 84 μmol/L). Urinary ketones were not detected. Brain MRI and cerebrospinal fluid were normal. She was suspected of being affected by a Reye-like syndrome and transferred to Gifu University Hospital.

On admission, her height was 72 cm (−1.5S.D.) and her weight was 10 kg (+0.73S.D.). She had a fever (38.3°C) and exhibited lethargy. Physical examination revealed mild hepatomegaly. A laboratory test showed AST 382 IU/L, ALT 441 IU/L, LDH 557 IU/L, PT 31%, NH3 84 μmol/L, and blood glucose 4.7 mmol/L.

We tentatively diagnosed her as having a Reye-like syndrome and treated her with intravenous glucose. Her consciousness level became clear on the 4th hospital day and she started oral intake of food. An abdominal CT scan still showed hepatomegaly and a fatty liver (20HU) on the 6th hospital day. The finding of cardiac ultrasonography was normal. Urinary organic acid analysis during the hypoglycemic condition showed hypoketotic dicarboxylic aciduria. The initial measurements of serum free carnitine and acyl-

Table 1. Time-course of serum and urinary acylcarnitine levels measured by tandem MS.

	Day	- 1	3	5	7	9	13
Serum (μmol/L)	range						
C0	10 - 55	2.98	12.70	40.75	24.31	18.49	58.22
C2	4 - 60	2.25	3.85	14.87	20.15	8.37	14.8
C8	- 1.0	0.035	0.024	0.088	0.058	0.073	0.10
C8DC	- 0.25	0.035	0.046	0.12	0.89	0.97	0.063
C10	- 0.8	0.055	0.062	0.25	0.12	0.17	0.21
C10DC	- 0.1	0.063	0.12	0.24	0.33	0.53	0.19
C12:1	- 0.2	0.038	0.038	0.18	0.15	0.15	0.091
C12DC	- 0.05	0.053	0.064	0.19	0.14	0.27	0.054
C14:1	- 0.1	0.075	0.16	0.47	0.58	0.68	0.18
C16	- 0.5	1.01	1.29	2.99	4.45	8.07	2.56
C18	- 0.3	0.49	0.65	1.46	1.67	3.07	0.99
C18:1	- 0.46	1.50	1.84	4.21	6.09	10.03	3.62
C18:2	- 0.3	0.46	0.67	1.47	1.43	2.05	0.98
(C16+C18:1)/C2	- 0.36	1.12	0.81	0.48	0.52	2.16	0.42
C total		12.35	26.74	86.07	84.99	67.46	85.52
Urine (μmol/mmol Cr)	range*						
C0	5.67 - 56.09	0.61	1.31	82.33	37.85	45.95	329.15
C2	6.87 - 60.48	0.56	0.02	25.44	128.00	41.83	53.58
C4	0.07 - 0.74	0.31	0.47	0.92	0.47	1.38	2.32
C6	0.04 - 0.48	0.18	0.09	0.21	0.22	0.61	0.23
C6DC		1.25	1.34	1.63	15.69	83.33	2.93
C8	0.05 - 0.39	0.00	0.02	0.33	0.98	1.33	0.62
C8DC		0.25	0.52	0.83	23.90	122.99	1.11
C10	0.03 - 0.36	0.05	0.06	0.11	2.66	1.76	0.12
C10DC		0.11	0.02	0.10	0.75	4.03	0.08
C12DC		0.00	0.02	0.01	0.23	1.52	0.01
C16	0.05 - 1.55	0.04	0.02	0.02	0.18	0.63	0.08
C total		4.75	6.86	122.16	226.51	344.91	408.34

* Reference values for urine acylcarnitines were obtained from data reported by Mueller et al. (2003) (10th - 90th percentile)

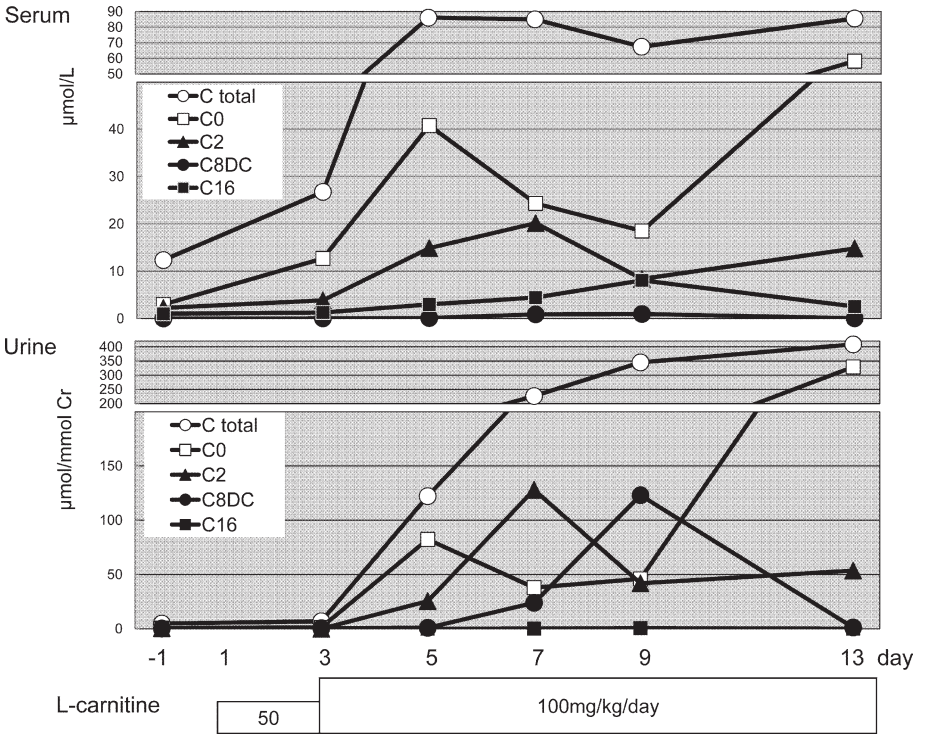

Fig. 1. Time-course of serum and urinary acylcarnitine levels measured by tandem MS. The levels of representative acylcarnitines are shown. The first day of L-carnitine supplementation is designated as day 1. Urinary carnitines were assayed using the first urine in the morning.

carnitine fractions by the enzymatic cycling method were 9.5 and 5.9 μmol/L, respectively. The initial serum acylcarnitine profile (Table 1) showed a very low free carnitine level and relatively high long-chain acylcarnitine levels. This profile was compatible with the secondary carnitine deficiency due to CPT2 or translocase deficiency.

After confirmation of the carnitine deficiency, we supplied her with L-carnitine orally from the 15th hospital day (day 1 in the Table 1 and Fig. 1) at a dose of 50 mg/kg/day for the first two days and 100 mg/kg/day from day 3. Blood and urinary samples were obtained before plus 3, 5, 7, 9 and 13 days after L-carnitine supplementation. During carnitine supplementation, the patient had continuous intravenous glucose infusion of 2.5 mg/kg/min until day 11. We analyzed the serum and urinary acylcarnitines by tandem mass analysis, as previously reported (Mueller et al. 2003; Kobayashi et al. 2007a,b). Table 1 shows details of the analyses. Fig. 1 shows the changing patterns of free carnitine (C0), acetyl-carnitine (C2), C8DC representing medium-chain dicarboxylic acylcarnitines, and C16 representing long-chain acylcarnitines in the serum and urine. Urinary excretion of C0 and acylcarnitines remained at very low levels on day 3. Sequential peaks of free carnitine (day 5), acetylcarnitine (day 7), and long-chain acylcarnitines (day 9) were found in the serum, which corresponded to peaks of free carnitine, acetylcarnitine, and dicarboxylic medium-chain acylcarnitines in the urine.

The fatty liver and hepatomegaly improved as judged by an abdominal CT scan on the 26th hospital day (day 13). Informed consent for a skin biopsy, enzyme assay, and DNA was obtained from the parents. CPT2 activity in the patient's fibroblasts was 0.18 nmol/min/mg of protein (3 controls; 0.82, 1.27, and 1.26 nmol/min/mg of protein), confirming the diagnosis of CPT2 deficiency.

Now the patient is 4 years of age. After carnitine supplementation, she did not experience hypoglycemia at all. She is being treated with 1,000 mg L-carnitine/day (current body weight 19.8 kg). Her growth and development are within normal ranges. She had some rhabdomyolysis attacks (the highest CK recorded was 16,769 IU/L) during a febrile illness even after L-carnitine supplementation.

Discussion

The diagnosis of CPT2 deficiency was first suspected by the data on urinary organic acid analysis and acylcarnitine analysis and was confirmed by enzyme assay using fibroblasts. Our patient is a compound heterozygote of a previously reported E174K mutation from the father and an unknown mutation from the mother which was not detected by exon sequencing. According to an in vitro expression analysis of mutant CPT2 cDNAs carrying E174K, the mutant E174K protein was present as much as a wild type protein and retained 10% residual CPT2 activity (Wataya et al. 1998). This "mild" mutation from the father, together with possible null mutation from the mother, may result in an infantile form of CPT2 deficiency.

Initially, she developed secondary carnitine deficiency. Chronic administration of pivalate-conjugated antibiotics is

a major cause of secondary carnitine deficiency even in healthy children (Stanley 2004). Ten days before the onset of the Reye-like syndrome, she had a cold and was given Cefteram pivoxil (CFTM-PI) for four days. The initial serum acylcarnitine profile showed no elevation of hydroxy-C5 carnitine, nor of pivaloylcarnitine. While the antibiotic might have contributed to secondary carnitine deficiency in part, the acute attack with fasting was more likely the course of the low carnitine in the patient at presentation.

The time-course changes in the serum and urinary acylcarnitine levels after L-carnitine supplementation were studied. These changing profiles suggest that accumulated and potentially toxic long-chain acylcarnitines in the mitochondria were eliminated from the body by day 13. The majority of accumulated long-chain acylcarnitines in the mitochondria may be eliminated by the following steps: 1) a large amount of accumulated long-chain acylcarnitines should be transferred from the mitochondrial matrix by carnitine acylcarnitine translocase if there is a sufficient amount of free carnitine outside of the mitochondrial matrix; 2) then peroxisomal beta-oxidation reduces the chain length of such accumulated fatty acids; 3) the resultant medium-chain fatty acids can be catalyzed in the mitochondria, or further ω-oxidized into dicarboxylic acids in the microsomes; 4) these medium-chain DC and their carnitine conjugates can be excreted into the urine efficiently. It took several days for oral L-carnitine administration to increase the serum carnitine levels, probably because the intracellular stores were depleted and it took several days for them to be replenished. Thereafter, the administration increased the excretion of abnormal acylcarnitines, some of which had probably accumulated within the tissues.

It is noteworthy that the acetylcarnitine in both the serum and the urine was a predominant acylcarnitine on day 7 (Fig. 1). Elevation of acetylcarnitine in the serum and urine indicates the presence of enough acetyl-CoA in the mitochondria and the availability of acetyl-CoA for carnitine acyltransferase reactions in the cells, and might account for the increased beta-oxidation rates upon L-carnitine therapy (Fontaine et al. 1996). In general, acetylcarnitine is a major acylcarnitine in healthy controls and is regarded as a marker of undisturbed beta-oxidation (Costa et al. 1998). Since CPT2-deficient patients have beta-oxidation restrictions of long-chain acyl-CoA, L-carnitine supplementation may increase beta-oxidation of medium-chain acyl-CoAs, which could be supplied via peroxisomal beta-oxidation of long-chain acyl-CoA.

Carnitine supplementation in the treatment of long-chain beta-oxidation defects is still controversial. In patients with a defect in the mitochondrial beta-oxidation spiral, when a preceding L-carnitine deficiency is normalized, and transport into the mitochondria of long-chain fatty acids is also normalized, acyl-CoAs accumulate instead of being oxidized by the defective reaction and, consequently, in such cases, free CoA is depleted in the mitochondria (Yoshino et al. 2003). This may be true in beta-oxidation defects such as very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency and trifunctional protein deficiency. Studies on VLCAD-deficient mice suggested carnitine supplementation results in the induction of acylcarnitine production in various tissues and significant accumulation of potentially toxic intermediate acylcarnitines in tissues (Liebig et al. 2006; Primassin et al. 2008). However, blockage of the CPT2 step causes the accumulation of long-chain acylcarnitines but does not primarily cause the accumulation of intermediate CoA esters in beta-oxidation.

Acknowledgment

This study was supported in part by Health and Labour Science Research Grants for Research on Children and Families from The Ministry of Health, Labour and Welfare of Japan.

References

Bonnefont, J.P., Demaugre, F., Prip-Buus, C., Saudubray, J.M., Brivet, M., Abadi, N. & Thuillier, L. (1999) Carnitine palmitoyltransferase deficiencies. *Mol. Genet. Metab.*, **68**, 424-440.

Bonnefont, J.P., Djouadi, F., Prip-Buus, C., Gobin, S., Munnich, A. & Bastin, J. (2004) Carnitine palmitoyltransferases 1 and 2: biochemical, molecular and medical aspects. *Mol. Aspects Med.*, **25**, 495-520.

Costa, C.G., Dorland, L., de Almeida, I.T., Jakobs, C., Duran, M. & Poll-The, B.T. (1998) The effect of fasting, long-chain triglyceride load and carnitine load on plasma long-chain acylcarnitine levels in mitochondrial very long-chain acyl-CoA dehydrogenase deficiency. *J. Inherit. Metab. Dis.*, **21**, 391-399.

Demaugre, F., Bonnefont, J.P., Colonna, M., Cepanec, C., Leroux, J.P. & Saudubray, J.M. (1991) Infantile form of carnitine palmitoyltransferase II deficiency with hepatomuscular symptoms and sudden death. Physiopathological approach to carnitine palmitoyltransferase II deficiencies. *J. Clin. Invest.*, **87**, 859-864.

Fontaine, M., Briand, G., Vallée, L., Ricart, G., Degand, P., Divry, P., Vianey-Saban, C. & Vamecq, J. (1996) Acylcarnitine removal in a patient with acyl-CoA beta-oxidation deficiency disorder: effect of L-carnitine therapy and starvation. *Clin. Chim. Acta*, **252**, 109-122.

Hug, G., Bove, K.E. & Soukup, S. (1991) Lethal neonatal multiorgan deficiency of carnitine palmitoyltransferase II. *N. Engl. J. Med.*, **325**, 1862-1864.

Kobayashi, H., Hasegawa, Y., Endo, M., Purevsuren, J. & Yamaguchi, S. (2007a) A retrospective ESI-MS/MS analysis of newborn blood spots from 18 symptomatic patients with organic acid and fatty acid oxidation disorders diagnosed either in infancy or in childhood. *J. Inherit. Metab. Dis.*, **30**, 606.

Kobayashi, H., Hasegawa, Y., Endo, M., Purevsuren, J. & Yamaguchi, S. (2007b) ESI-MS/MS study of acylcarnitine profiles in urine from patients with organic acidemias and fatty acid oxidation disorders. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **855**, 80-87.

Liebig, M., Gyenes, M., Brauers, G., Ruiter, J.P., Wendel, U., Mayatepek, E., Strauss, A.W., Wanders, R.J. & Spiekerkoetter, U. (2006) Carnitine supplementation induces long-chain acylcarnitine production—studies in the VLCAD-deficient mouse. *J. Inherit. Metab. Dis.*, **29**, 343-344.

Longo, N., Amat di San Filippo, C. & Pasquali, M. (2006) Disorders of carnitine transport and the carnitine cycle. *Am. J. Med. Genet. C. Semin. Med. Genet.*, **142C**, 77-85.

Mueller, P., Schulze, A., Schindler, I., Ethofer, T., Buehrdel, P. & Ceglarek, U. (2003) Validation of an ESI-MS/MS screening

method for acylcarnitine profiling in urine specimens of neonates, children, adolescents and adults. *Clin. Chim. Acta*, **327**, 47-57.

Primassin, S., Ter Veld, F., Mayatepek, E. & Spiekerkoetter, U. (2008) Carnitine supplementation induces acylcarnitine production in tissues of very long-chain acyl-CoA dehydrogenase-deficient mice, without replenishing low free carnitine. *Pediatr. Res.*, **63**, 632-637.

Stanley, C.A. (2004) Carnitine deficiency disorders in children. *Ann. N.Y. Acad. Sci.*, **1033**, 42-51.

Wataya, K., Akanuma, J., Cavadini, P., Aoki, Y., Kure, S., Invernizzi, F., Yoshida, I., Kira, J., Taroni, F., Matsubara, Y. & Narisawa, K. (1998) Two CPT2 mutations in three Japanese patients with carnitine palmitoyltransferase II deficiency: functional analysis and association with polymorphic haplotypes and two clinical phenotypes. *Hum. Mutat.*, **11**, 377-386.

Yoshino, M., Tokunaga, Y., Watanabe, Y., Yoshida, I., Sakaguchi, M., Hata, I., Shigematsu, Y., Kimura, M. & Yamaguchi, S. (2003) Effect of supplementation with L-carnitine at a small dose on acylcarnitine profiles in serum and urine and the renal handling of acylcarnitines in a patient with multiple acyl-coenzyme A dehydrogenation defect. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **792**, 73-82.