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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

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Abstract

We studied the effects of L-carnitine supplementation at a small dose on the profiles of acylcarnitines in serum and urine, as well as the renal handling of acylcarnitines, in a patient with multiple acyl-coenzyme A dehydrogenation defect. After supplementation with L-carnitine at a dose of 20 mg/kg/day, the concentration of each acylcarnitine measured both in the serum and in the urine had increased significantly, with the exception of that of an acylcarnitine with a carbon chain length (C) of 8 (C8 acylcarnitine). The magnitude of increase in the concentrations of the acylcarnitines in the serum was not associated with chain length, whereas in the urine, the magnitude tended to be greater in proportion to the shortness of the chain length. The fractional excretions of C2–C5 acylcarnitines exceeded 100%, indicating that they were produced in, or transported across, renal tubular epithelial cells and secreted into the urine. These results indicate that supplementation with a relatively small amount of L-carnitine can enhance the renal excretion of accumulated short-chain-length acylcarnitines through tubular excretion, in addition to basic glomerular filtration.

Keywords: Multiple acyl-coenzyme A dehydrogenation defect; Carnitine; Acylcarnitine

1. Introduction

Multiple acyl-coenzyme A (CoA) dehydrogenation defect (MADD), or glutaric aciduria type II [1], is a syndrome caused by an inherited defect either in electron transfer flavoprotein (ETF) [2,3] or an enzyme, ETF:CoQ oxidoreductase (ETF:QO) [4–6]. This enzyme reoxidizes the reduced ETF and transfers the resulting hydrogen to coenzyme Q in the mitochondrial respiratory chain. ETF serves as the

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hydrogen acceptor in the dehydrogenation reactions of both the fatty acyl-CoA in the mitochondrial β-oxidation spiral as well as those of individual acyl-CoAs derived from lysine, tryptophan, hydroxytryptophan, and the three branched-chain amino acids; leucine, isoleucine, and valine. Therefore, a defect in ETF or ETF:QO causes a combined disorder in both fatty acid oxidation and amino acid catabolism.

This disease has been classified into three clinical forms [4]: neonatal forms with or without malformations of the internal organs, and a late-onset form in which onset is delayed until early infancy or late adolescence. The former type is refractory to any therapeutic approach and is almost inevitably fatal, whereas the latter type occasionally responds to therapy. Some patients with the late-onset disease respond to a pharmacological dose of riboflavin and the course of disease remains uneventful on such a regimen [7,8]. Supplementation with L-carnitine or D,L-carnitine has also been used [9–12]. Previously reported chemical responses to L-carnitine supplementation in patients with this disorder were moderate increases in acylcarnitines derived from amino acids, minimal increases in acylcarnitines derived from straight-chain fatty acids, and increases in acetylcarnitine in the urine [9]. Changes in individual acylcarnitine concentrations in blood and their renal handling after introduction of L-carnitine supplementation have not been previously determined. In addition, a rationale for a particular adequate dosage of L-carnitine in patients with this disease has not yet been established. We report here the sequential changes observed among concentrations of individual acylcarnitines in the blood and urine, as well as their renal handling before and after supplementation with L-carnitine was administered to a patient with MADD.

2. Case report

A boy, RK, was referred to us at the age of 85 days due to a positive family history suggestive of a mitochondrial fatty acid oxidation defect. His elder brother, TK, had undergone an uneventful course, with the exception of mild motor retardation until 8 months of age, at which time he was brought to a hospital due to staring and screaming after a brief febrile illness. He was found to have cardiorespiratory arrest upon arrival. Autopsy revealed fatty changes in the liver. The mother of the boys was a strict vegetarian and had refused to ingest any animal protein while she breast-fed her children.

Physical examination of the present patient, RK, revealed a slight case of jaundice with minimum hypotonia of the trunk and extremities, stridor, and blue sclerae. The liver was palpated 2 cm below the right costal margin. Because his elder brother's history suggested a defect in mitochondrial fatty acid transport or in the β-oxidation spiral, determinations of carnitine concentrations, and analyses of acylcarnitines in the serum and of organic acids in the urine were performed. The results of these analyses, as will be described in the Results section, suggested a diagnosis of MAAD.

Although the determination revealed a marked decrease in the concentration of carnitine (3.76 μmol/l) and an increase in acylcarnitine (47.3 μmol/l) in the serum obtained at that time, L-carnitine supplementation was withheld by the mother's request until 132 days of age, when oral L-carnitine supplementation was begun at a dose of 20 mg/kg/day. The patient was placed on a dietary intake of 1.2–1.5 g/kg of protein per day and energy of 80–100 kcal/kg/day (1 cal=4.1868 J). The patient's health has been stable since then and he has tolerated several episodes of febrile illnesses without symptomatic worsening. He was able to walk unaided at 19 months of age, and was capable of some speech at 22 months of age. After he gained the ability to walk, he had three episodes of pathological fracture, which led to a diagnosis of osteogenesis imperfecta. The ETF protein, as assessed by immunoblotting using anti-rat liver ETF raised in rabbits as described elsewhere [13], revealed no significant difference in molecular mass and in band intensity in comparison with the control.

3. Experimental

3.1. Analysis of acylcarnitines in the serum and urine

[methyl-$^2H_3$]$_3$ Iodomethane (Campro Scientific, Veenendaal, The Netherlands) and [methyl-$^2H_3$]$_3$car-

nitine (Cambridge Isotope Labs., MD, USA) were purchased from respective manufacturers. 4-Amino-3-hydroxybutyric acid, butanolic HCl (10%), HPLC-grade acetonitrile, methanol, and distilled water were products of Nacalai Tesque (Kyoto, Japan). [methyl-$^2H_3$]$_3$Carnitine was synthesized with 4-amino-3-hydroxybutyric acid and [methyl-$^2H_3$]$_3$ iodomethane, as previously described [14]. Stable isotope-labeled acylcarnitines were synthesized by treating [methyl-$^2H_3$]carnitine or [methyl-$^2H_3$]$_3$carnitine with a corresponding acid hydrochloride in trifluoroacetic acid, and the resulting acylcarnitine was purified by repeated crystallization, as previously described [15].

Analysis of acylcarnitines was performed by electrospray ionization tandem mass spectrometry (ESI-MS–MS), as described elsewhere [16]. A 100-μl portion of serum or a 20-μl portion of urine was added with 1.0 nmol of [$^2H_3$]acetylcarnitine, 0.2 nmol of [$^2H_3$]butyrylcarnitine, 0.4 nmol of [$^2H_9$]isovalerylcarnitine, 0.2 nmol of [$^2H_3$]hexanoylcarnitine, 0.2 nmol of [$^2H_9$]octanoylcarnitine, and 0.2 nmol of [$^2H_3$]decanoylcarnitine as internal standards, and was deprotenized with 800 μl of ethanol. The supernatant was dried under a nitrogen stream, then the dried residue was derivatized with butanolic HCl, dried, and dissolved in 100 μl of 50% aqueous acetonitrile. ESI-MS–MS analysis was performed using a Model TSQ7000 triple stage mass spectrometer (ThermoQuest, Tokyo, Japan), equipped with a Model LC10 high-performance liquid chromatography (HPLC) system and a Model SIL-10ADVP autoinjector (Shimadzu, Kyoto, Japan). Each 20-μl portion of the sample was automatically injected into a HPLC system employing 50% aqueous acetonitrile as the mobile phase at a flow-rate of 20 μl/min. The resolution was set at 0.7 on both mass spectrometers. Scan was performed with precursor ions of m/z 85, ranging from 210 to 505 m/z, at a collision energy of −30 eV, and scan time was 2.0 s.

3.2. Determination of L-carnitine in the serum and urine

The concentration of L-carnitine was determined by a colorimetric method employing the L-carnitine dehydrogenase from *Alcaligenes* sp., as described elsewhere [17].

3.3. Analysis of organic acids in the urine

Urease (Sigma–Aldrich, St. Louis, MO, USA), margarate and hydroxylamine HCl (Wako, Tokyo, Japan), tetracosane (Seikagaku-Kogyo, Tokyo, Japan), and tropate, *N*,*O*-bis(trimethylchlorosilyl)-fluoroacetamide (BSTFA), and trimethyl-chlorosilane (TMCS) (Nacalai Tesque, Kyoto, Japan) were purchased from respective manufacturers.

The general profile of metabolites in the urine was analyzed by gas chromatography–mass spectrometry (GC–MS), as previously described [18]. In brief, to a portion of urine specimen containing 0.2 mg of creatinine, 20 μg each of margarate and tetracosane and 40 μg of tropate were added as internal standards. After oximation of 2-ketoacids with hydroxylamine HCl, urine was acidified with 6 *M* HCl, saturated with 1 g of NaCl, and extracted twice with 6 ml of ethyl acetate and once with 6 ml of diethylether. The extracts were combined, dehydrated with 5 g of anhydrous sodium, and dried under nitrogen stream. The residue was then trimethylsilylated with 100 μl of BSTFA and 10% TMCS at 80 °C for 30 min. The derivatized specimens were injected into a GC–MS system (Shimadzu QP 5050, Shimadzu) equipped with a fused-silica DB-5 capillary column (J & W Scientific, Folsom, CA, USA). Mass spectra were obtained in the electron impact ionization mode, scanning every 0.4 s from 50 to 650 m/z. The temperature program was started with initial holding at 100 °C for 1 min, followed by increase at a rate of 4 °C/min up to 300 °C, and a final holding at 300 °C for 15 min.

3.4. Analysis of acylglycines in the urine

[$^{13}C_2$]Dodecanedioate (MSD Isotopes, Montreal, Canada) and [$^2H_4$]glutarate (C/D/N Isotopes, Quebec, Canada) were purchased from respective manufacturers, and the remaining labeled compounds used were synthesized as previously described [19]. *N* - (*tert*.-Butyldimethylsilyl)-*N*-methyltrifluoroacetamide (BDMMTFA) (Aldrich, Milwaukee, WI, USA), dimethylformamide and ethylmalonate (Nacalai Tesque), and isovalerylglycine (Tokyo-Kasei Kogyo, Tokyo, Japan) were supplied by respective sources. Other acylglycine standards were

the gift of Dr. Piero Rinaldo (Yale University, New Haven, CT, USA).

Quantification of the metabolites of interest was carried out with deuterated [^{2}H]- or [^{13}C]-labeled compounds as internal standards [19]. A portion of urine containing 0.4 mg of creatinine was treated with 20 units of urease at 37 °C for 30 min; spiked with 20 nmol each of [$^2H_3$]ethylmalonate, [$^2H_4$]glutarate, [$^2H_4$]isovalerylglycine, [$^{13}C_2$]hexanoylglycine, and [$^{13}C_2$] suberylglycine and 10 nmol of [$^{13}C_2$]dodecanedioate as internal standards; filled up to 4.0 ml with water; acidified to pH 1.0 with 6 *M* HCl, to which was added 1 g of NaCl; and extracted with a 6 ml portion of ethyl acetate three times. The extracts were combined, dehydrated with 5 g of anhydrous sodium sulfate, and dried under a nitrogen stream. The residue was derivatized with BDMMTFA and dimethylformamide at 80 °C for 30 min to form *tert*.-butyldimethylsilyl (tBDMS) esters. The three carboxylic acids and the three glycine conjugates were quantified by selected ion monitoring on a gas chromatograph–mass spectrometer (Shimadzu QP 5050) equipped with a fused-silica DB-5 capillary column (J & W Scientific). Mass spectra were obtained by electron impact ionization-selective ion monitoring (EI-SIM) at the scan rate of 0.4 s/cycle. The temperature program was started at 100 °C with an initial holding for 4 min and increased at the rate of 4 °C/min to 290°C, with a final holding for 10 min. Each compound was quantified by comparing the peak area of a quantitative ion of the compound ($[M-57]^+$) with that of the corresponding internal standard.

3.5. Calculation of fractional excretion and statistical analyses

Fractional excretion (Fe) of carnitines was calculated by the following equation:

$$\mathrm{Fe} = \frac{[\text{carnitine}]_u}{[\text{creatinine}]_u} \cdot \frac{[\text{creatinine}]_s}{[\text{carnitine}]_s} \cdot 100\,(\%)$$

where $[\text{carnitine}]_u$ and $[\text{carnitine}]_s$ represent the concentrations of non-esterified or acyl-carnitine in urine and serum, and $[\text{creatinine}]_u$ and $[\text{creatinine}]_s$ denote the concentrations of creatinine in the urine and serum, respectively.

A comparison of the means of two sets of values was performed by Student's paired *t*-test. The strength of the relationship between fractional excretions of carnitines was evaluated by Pearson's correlation. The relation between fractional excretions of acylcarnitines and their chain length was determined by regression analysis.

The intra-assay and inter-assay variations of acylcarnitine determinations, as defined as relative standard deviation (RSD), were 5–11% and 7–15%, respectively.

4. Results

4.1. Acylcarnitine profile in the serum and organic acids and glycine conjugates in the urine

Analysis of acylcarnitine in the patient's serum at the age of 85 days revealed an abnormally high signal intensity of acylcarnitines with a chain length of even number (C4–C12), as well as one C5 (Fig. 1).

The qualitative analysis of metabolites in the urine obtained at the age of 85 days disclosed increases in ethylmalonate, glutarate, suberate, and sebacicate, as well as nonspecific mild increases in oxalate and 3-hydroxyphenylacetate (Fig. 2). The quantitative analysis of the same urine specimen demonstrated that concentrations of ethylmalonate, glutarate, hexcanoylglycine, and suberylglycine were abnormally high; taken together, these results suggested a diagnosis of MADD, although the concentrations of isovalerylglycine and dodecanedioate were within the respective normal control ranges (Table 1).

4.2. Effects of L-carnitine on acylcarnitine profiles in the serum and excretion into the urine

After the introduction of oral L-carnitine supplementation at a dose of 20 mg/kg/day, non-esterified carnitine and every acylcarnitines in the serum were measured; significant increases were observed, namely, 1.4- (C12) to 4.3-fold (C4), with the exception of C8 acylcarnitine (Table 2), in comparison with those measured before L-carnitine supplementation. The excretion of all of the C2 to C12 acylcarnitines, barring C8 acylcarnitine, had in-

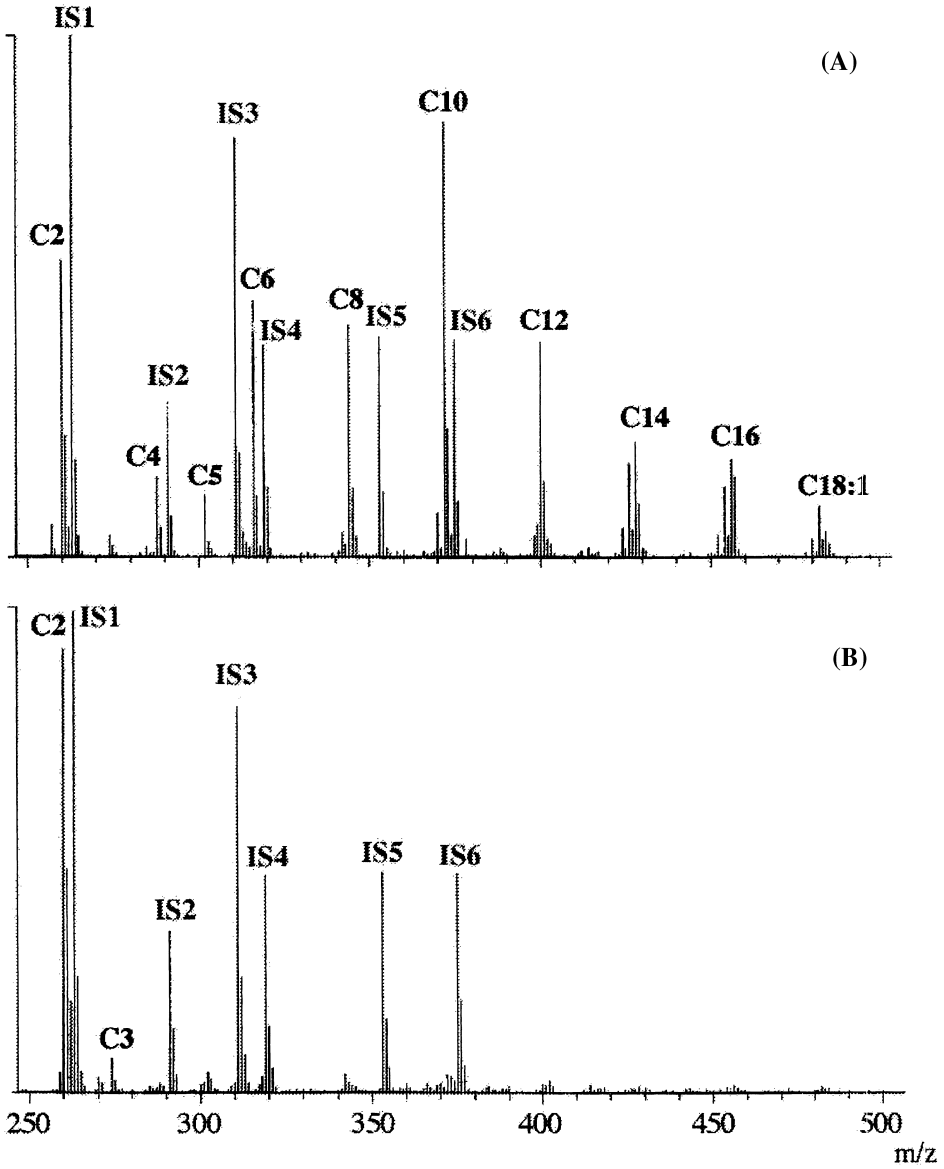

Fig. 1. Serum acylcarnitine profiles obtained by ESI-MS–MS analysis with precursor ion scanning of m/z 85. The ion intensities for C4–C18:1 acylcarnitines were increased in the MADD patient (A) in comparison with those in a control (B). The ion peaks indicate the molecular ions of the butyrated acylcarnitines, and those highlighted with I.S. numbers are the stable isotope-labeled internal standards (1: [$^2H_3$]acetylcarnitine, 2: [$^2H_3$]butyrylcarnitine, 3: [$^2H_9$]isovalerylcarnitine, 4: [$^2H_3$]hexanoylcarnitine, 5: [$^2H_9$]octanoylcarnitine and 6: [$^2H_3$]decanoylcarnitine).

creased significantly after the introduction of L-carnitine (Table 2), and the increments tended to be greater in proportion to the shortness of the chain length of acylcarnitine. Fractional excretions of acylcarnitines with a chain length of C5 or less exceeded 100% after L-carnitine supplementation (Table 3), indicating that they were actively excreted from the renal tubules.

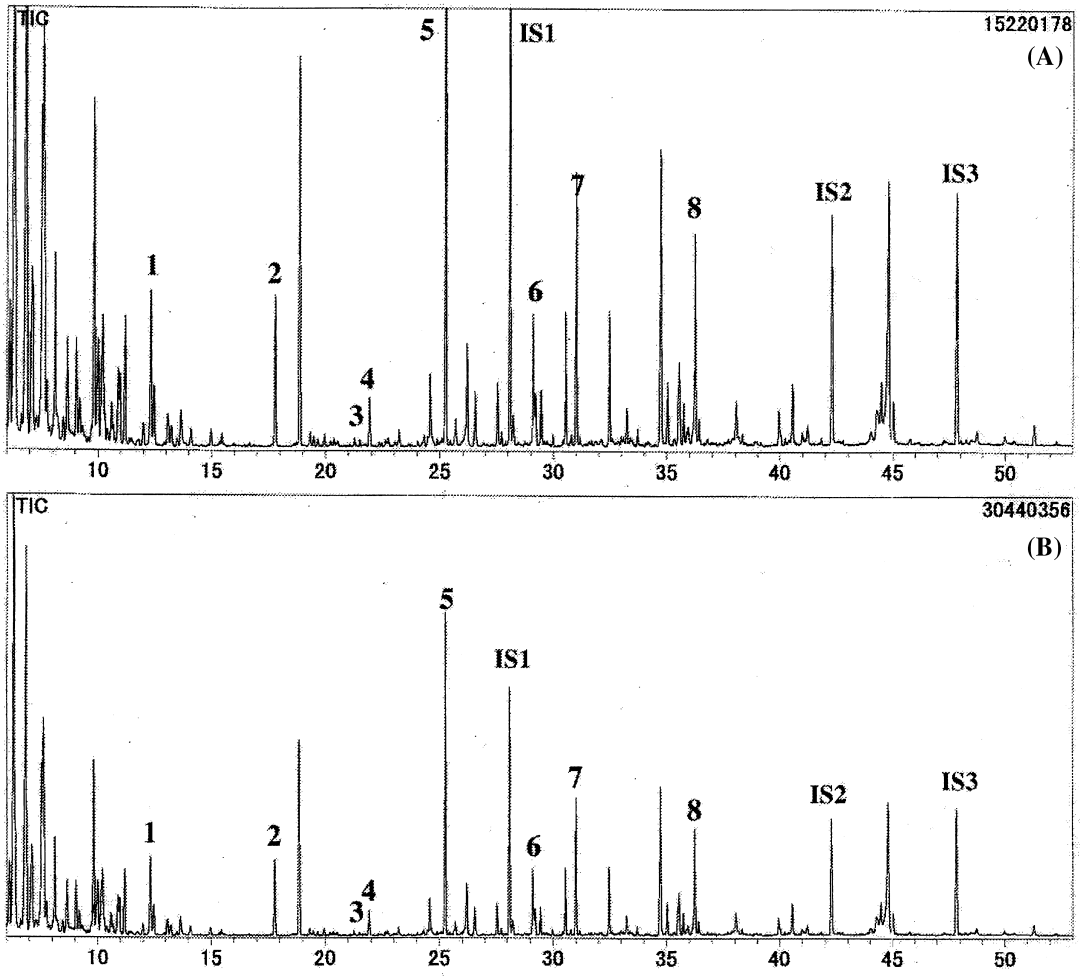

Fig. 2. Total ion chromatogram of organic acids and related compounds in urine of patient (A) and control (B). Peaks: 1 = oxalate, 2 = ethylmalonate, 3 = isobutyrylglycine, 4 = glutarate, 5 = isovalerylglycine + adipate, 6 = 3-hydroxylphenylacetate, 7 = octenedioate + suberate, 8 = sebacicate, and I.S. (internal standard) 1 = margarate, 2 = tetracosane, 3 = tropate.

4.3. Fractional excretions of C2–C12 acylcarnitines by their chain length, and correlation between fractional excretions of non-esterified carnitine and acylcarnitines

There was a significant inverse correlation between log-transformed fractional excretions of acylcarnitines and their chain lengths ($r^2 = 0.74$, $P = 0.0129$) (Fig. 3). The fractional excretion of each of the acylcarnitines correlated strongly with that of the non-esterified carnitine ($P > 0.700$, C2–C10) or, in one case, weakly ($0.700 > P > 0.400$, C12); (Table 3).

5. Discussion

Supplementation with L-carnitine in patients with a defect in the mitochondrial β-oxidation spiral has long been a matter of controversy. In the present patient, supplementation with L-carnitine was chosen because the patient exhibited marked hypocarnitinemia. This initial hypocarnitinemia may have been linked to the fact that, in addition to the disease, the mother of the patient was a strict vegetarian and she adhered to this regimen when breast-feeding the patient, thus rendering the patient carnitine-deficient. In the present patient, after introduction of 20 mg/

Table 1
Concentrations of carboxylic acids and glycine conjugates in the urine[a] (patient age: 85 days)

Compound	Patient	Same batch control			Control ($n=10$)
		1	2	3	
Ethylmalonic acid	108.9	8.5	5.3	3.7	1.4–6.9
Glutaric acid	22.0	2.8	8.4	2.8	1.5–10.5
Dodecanedioic acid	nd[b]	nd[b]	nd[b]	nd[b]	0.0–0.2
Isovalerylglycine	1.0	0.3	0.3	0.3	0.2–1.9
Hexanoylglycine	33.8	nd[b]	nd[b]	nd[b]	0.0–1.4
Suberylglycine	5.9	nd[b]	nd[b]	nd[b]	0.0–0.1

[a] μmol/mmol creatinine.
[b] Not detectable.

kg/day of L-carnitine, every acylcarnitine species in the serum that was measured, with the exception of C8 acylcarnitine, had increased. The increases in serum acylcarnitines with shorter chain lengths (<C6) were more pronounced than those with longer chain lengths, as was also observed in the urine. It seems likely that the preceding deficiency of L-carnitine was countered by the administration of the supplement and that the replenishment with L-carnitine restored the transport of long-chain fatty acids into the mitochondrial matrix space, thus allowing them easier access to the mitochondrial β-oxidation spiral, yielding shorter chain length acyl-CoAs, which were then esterified by L-carnitine. This hypothesis is consistent with a previous observation that in a patient with MADD, the ratio of 3-hydroxybutyric acid to that of non-esterified fatty acids in the blood increased after supplementation, indicating an improvement in the β-oxidation of long-chain fatty acids in the mitochondria; in that study, L-carnitine supplementation was administered at a dosage of 100 mg/kg/day [12]. However, deleterious effect of L-carnitine supplementation at a smaller dosage was reported as likely in a patient with MADD [20]. In patients with a defect in the mitochondrial β-oxidation spiral, when a preceding L-carnitine deficiency is normalized, and transport into the mitochondria of long chain length fatty acids is also normalized, acyl-CoAs would accumulate instead of being oxidized by the defective reaction, and consequently, in such cases, free CoA would be depleted in the mitochondria. Therefore, excessive supplementation with L-carnitine may have some drawbacks in patients with disorders of the mitochondrial β-oxidation spiral. In contrast, in disorders of amino acid degradation pathways, excessive L-carnitine would not stimulate entry of precursors into the mitochondrial matrix, but it would enhance the removal of accumulated acyl-CoAs out of the mitochondria as acylcarnitines and regeneration of free coenzyme-A, as is likely the case in patients with MADD. Challenges with a larger amount of L-carnitine were not attempted in the present patient due to parental refusal.

Table 2
Concentrations of non-esterified carnitine and acylcarnitines in the serum and urine[a] before and after supplementation with oral L-carnitine

Chain length	Before	After	P
Serum		μmol/l	
	($n=5$)	($n=9$)	
0[b]	39.7±7.3[c]	56.0±10.4[d]	0.0213*
2	6.3±1.9	11.8±3.5	0.0094**
4	0.6±0.1	2.6±1.3	0.0188*
5	0.4±0.2	0.6±0.3	0.0077**
6	1.0±0.6	2.7±0.9	0.0087**
8	1.3±0.7	2.5±0.8	0.0914
10	1.6±0.9	3.5±1.3	0.0159*
12	1.0±0.6	1.4±0.5	0.0094**
Urine		μmol/mmol creatinine	
	($n=5$)	($n=10$)	
0[b]	4.8±2.5[c]	89.6±96.5[d]	0.0636[e]
2	13.6±6.1	1089±1535.7	0.0292*[e]
4	20.7±22.5	642.2±1043.3	0.0078**[e]
5	5.5±4.3	82.9±128.6	0.0182*[e]
6	2.0±1.3	45.7±76.1	0.0174*[e]
8	3.1±1.4	32.2±106.6	0.1644[e]
10	2.9±1.2	10.5±12.2	0.0092**[e]
12	1.8±1.0	3.2±2.9	0.0058**[e]

[a] Values are given as the mean±SD.
[b] A chain length of ''0'' denotes non-esterified carnitine.
[c] $n=4$.
[d] $n=8$. * $P<0.05$, ** $P<0.001$.
[e] A comparison of carnitine concentrations in the urine was made after raw data were log-transformed.

Little information is available on the concentrations of acylcarnitine species in the bodily fluids in MADD patients with or without supplementation with L-carnitine. The quantitatively major acylcarnitines in the urine before L- or D,L-carnitine supplementation have been determined to be acetylcarnitine, propionylcarnitine, butyrylcarnitine, 2-

Table 3
Fractional excretions of acylcarnitinies by their chain lengths and correlation between fractional excretions of non-esterified carnitine and acylcarnitines after L-carnitine supplementation

Chain length	Fractional excretion (%)[a] ($n=8$)	Correlation between fractional excretions of non-esterified (C0) carnitine and acylcarnitines (r[b])
0[c]	3.0±3.3	–
2	112.9±196.3	0.759
4	276.3±443.1	0.770
5	179.3±294.3	0.762
6	20.7±38.8	0.756
8	52.4±100.1	0.772
10	7.1±9.9	0.756
12	5.5±5.7	0.459

[a] Values are given as the mean±SD.
[b] r is the correlation coefficient.
[c] A chain length of ''0'' denotes non-esterified carnitine.

methylbutyrylcarnitine, isovalerylcarnitine, hexanoylcarnitine, and octanoylcarnitine [1,9,12], with a prominent increase in isobutyrylcarnitine and isovalerylcarnitine after supplementation with L-carnitine [12]. In the blood, acylcarnitines with longer chain lengths (C8–C12) are also increased [21–23] in the absence of L-carnitine supplementation. As determined by the profile of acylcarnitines in the serum of the present patient, the buildup of acylcarnitines derived from fatty acids was more marked than the corresponding buildup from amino acids (C5, isovalerylcarnitine plus 2-methylbutyrylcarnitine). This observation suggests that acyl-CoAs that are produced in the β-oxidation of fatty acids are major contributors to the acylcarnitine fraction and probably behave as major aggravating factors in a metabolic decompensation, whereas those derived from amino acids would play a secondary role in quantitative terms, at least in the infantile form of MADD.

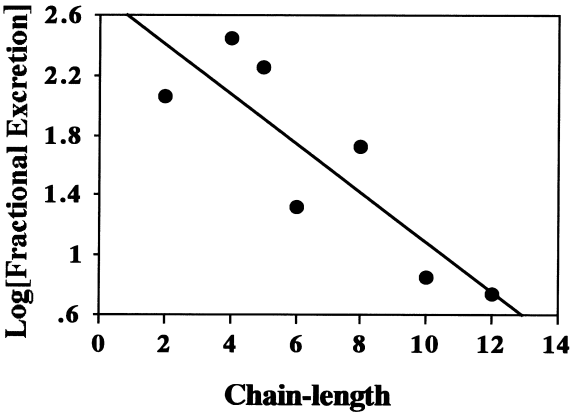

Fig. 3. Correlation between fractional excretions of acylcarnitines and their chain lengths. There is a significant inverse correlation between log-transformed fractional excretions of acylcarnitines and their chain lengths ($r^2=0.74$, $P=0.0129$).

L-Carnitine is transported in a sodium gradient-dependent manner across the renal brush border in the rat kidney [24]. The cDNA encoding the human high-affinity L-carnitine transporter, OCTN2, was cloned, and expressed in vitro; its functional characteristics are well understood [25]. It was later confirmed that OTCN2 is the plasma membrane L-carnitine transporter of primary physiological significance; this was made clear because mutations of the gene SLC22A5, which encodes OTCN2, cause human systemic carnitine deficiency [26]. The existence of another organic cation transporter, OCTN1, is also recognized [27]. Both OCTN1 and OTCN2 are localized at the luminal side of the renal tubule in humans and both of these transporters exert unidirectional transport from the luminal side into the cellular matrix [27,28].

The fractional excretion of acylcarnitine in normal individuals does not exceed 25% [29–31]. In the present patient, fractional excretions of C2–C5 acylcarnitines increased logarithmically and ex-

ceeded 100% after supplementation with L-carnitine was introduced. In normal subjects and in patients with disorders not associated with the buildup of specific acyl-CoAs, the tubular reabsorption of non-esterified carnitine and short-chain acylcarnitine may compete with each other [29,32], suggesting the existence of an absorptive mechanism(s) common to L-carnitine and short-chain acylcarnitines, including OTCN2. Such a mechanism may have been responsible for the results in the present patient when L-carnitine was administered. However, this mechanism was probably not the major cause of the enhanced acylcarnitine excretion after L-carnitine supplementation, because the fractional excretions of acylcarnitines positively correlated with those of non-esterified carnitine (Table 3). Many organic cations undergo bidirectional active resorption and secretion within nephrons [33]. Therefore, the transport of acylcarnitines from the vascular side to the luminal side across the tubular epithelial cells, and the secretion of acylcarnitines produced in such cells into the urine are both possible, although such specific carrier systems have not been identified. OCTN1 and OCTN2 are candidates for such carriers, but there is as of yet no evidence that they exert bidirectional transport or that they can handle any of the acylcarnitines other than acetylcarnitine [25,27].

An inverse correlation was observed between the log-transformed mean values of fractional excretions of acylcarnitines and their chain lengths. This result may simply imply that the shorter the chain length of an acylcarnitine, the more water-soluble and hence more easily diffusible that acylcarnitine will be. However, it is also possible to speculate that the movement of acylcarnitines across the biomembrane is mediated by an active transport system(s) with relaxed substrate specificity; such a transport system might exhibit a higher affinity toward acylcarnitines with shorter chain lengths. Additionally, in the present case, a positive correlation was found between the fractional excretion of L-carnitine and the fractional excretion of acylcarnitines with chain lengths of up to 8. This observation implies that if the excretion of acylcarnitine is indeed mediated by a transport system or systems, then such a system would be independent of that for L-carnitine.

The present observations pose an interesting therapeutic implication. Supplementation with a relatively small amount of L-carnitine can induce the excretion of acylcarnitines into the urine at efficiency greater than the glomerular filtration rate without overt adverse effects. It remains to be determined whether such a tubular secretion is a physiological response encountered in normal individuals when a marked buildup of acylcarnitine occurs, or if it reflects a pathological response that is associated with MADD. There is growing evidence that supports the presence of tubulopathy in the mild form of MADD, as well as in the severe form [34].

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