Abnormal urinary excretion of unsaturated dicarboxylic acids in patients with medium-chain acyl-CoA dehydrogenase deficiency

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Abstract Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is the most frequently described metabolic disorder of fatty acid oxidation in humans. Acute episodes are usually characterized biochemically by the appearance of nonketotic dicarboxylic aciduria. In addition, other abnormal metabolites, such as suberylglycine, n-hexanoylglycine, 3-phenylpropionylglycine, and octanoylcarnitine, are excreted in the urine. Urinary organic acids were determined using dual capillary column gas–liquid chromatography and gas–liquid chromatography/mass spectrometry. In three cases of MCAD deficiency we observed a disproportionate increase in the excretion of unsaturated dicarboxylic acids compared to either fasting control children with expected ketotic dicarboxylic aciduria or patients with nonketotic dicarboxylic aciduria not associated with MCAD deficiency. The most significant increase was in the urinary excretion of *cis*-4-decenedioic acid. Additionally, the urinary excretions of *cis*-3-octenedioic and *cis*-5-decenedioic acids were slightly decreased whereas the excretion of *cis*-5-dodecenedioic acid was increased. These data are consistent with the notion that as a result of MCAD deficiency the metabolic oxidation of unsaturated fatty acids such as linoleate and oleate is inhibited more than saturated fatty acids. **—Tserng, K-Y., S-J. Jin, D. S. Kerr, and C. L. Hoppel.** Abnormal urinary excretion of unsaturated dicarboxylic acids in patients with medium-chain acyl-CoA dehydrogenase deficiency. *J. Lipid Res.* 1990. **31:** 763–771.

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Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is a commonly described metabolic disorder (1–3). Screening for MCAD deficiency is based, in large part, on the determination of urinary organic acids. Massively increased urinary excretion of medium-chain (C_6–C_{10}) dicarboxylic acids in the absence of ketosis, i.e., nonketotic dicarboxylic aciduria, is a common feature among patients with MCAD deficiency during acute episodes. However, nonketotic dicarboxylic aciduria is not specific for MCAD deficiency, as other defects of fatty acid oxidation also show increased urinary excretion of medium-chain dicarboxylic acids (3, 4). Attempts to enhance the selectivity for the differential diagnosis based on urinary organic acid analysis have led to the identification of urinary suberylglycine (5), hexanoylglycine (6), 3-phenylpropionylglycine (7), octanoic acid (8), and octanoylcarnitine (8, 9) as possible specific marker compounds for MCAD deficiency (1).

In three cases of MCAD deficiency, we have found additional unusual features of the dicarboxylic aciduria, which potentially may help differentiate this disorder from other defects of fatty acid oxidation. In MCAD deficiency, the urinary excretion of specific unsaturated medium-chain. (C_{10}–C_{12}) dicarboxylic acids is disproportionately increased. This feature is similar to that observed in rats treated with hypoglycin, an inhibitor of short-chain and medium-chain acyl-CoA dehydrogenases (10–12).

MATERIALS AND METHODS

Urinary organic acid analysis

Urine samples were acidified and extracted with a solvent mixture (ethyl acetate–diethyl ether) as previously described (13). Trimethylsilyl derivatives were prepared

Abbreviations: DC6, adipic acid; DC8, suberic acid; DC10, sebacic acid; DC12, dodecanedioic acid; t2DC6, *trans*-2-hexenedioic acid; c3DC8, *cis*-3-octenedioic acid; c4DC8, *cis*-4-octenedioic acid; t3DC8, *trans*-3-octenedioic acid; c5DC10, *cis*-5-decenedioic acid; c4DC10, *cis*-4-decenedioic acid; c5DC12, *cis*-5-dodecenedioic acid; GLC, gas–liquid chromatography; MS, mass spectrometry; MCAD, medium-chain acyl-CoA dehydrogenase; OH-B, 3-hydroxybutyric acid.

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and analyzed with a dual-capillary column gas chromatograph, using a 30-m SPB-1 (bonded dimethylpolysiloxane phase) and a 30-m SPB-35 (bonded 35% diphenyl: 65% dimethylpolysiloxane phase) fused silica capillary columns (0.25 mm id, 0.25 μm film thickness, both from Supelco, Bellefonte, PA) in a Hewlett-Packard 5890A gas chromatograph equipped with a split/splitless capillary injector, two flame ionization detectors, and HP 3354 Laboratory Automation System interfaced through a HP 18652A analog-digital converter (Hewlett-Packard, Avondale, PA). The column temperature was programmed to increase at 4°C/min from 60°C to 250°C and a 50 to 1 split injection ratio was used.

Quantitation of dicarboxylic acids and related compounds with dual-capillary column gas chromatograph

Adipic, suberic, and octenedioic acids were eluted from SPB-1 and SPB-35 without any known interfering peak. Data from the SPB-1 column were used for quantification. Their methylene units (MU) calculated relative to hydrocarbon standards have been reported (14).

Cis-5-decenedioic acid (c5DC10), *cis*-4-decenedioic acid (c4DC10), sebacic acid (DC10), dodecanedioic acid (DC12), and *cis*-5-dodecenedioic acid (c5DC12) were determined from the peak areas in SPB-1 column. Occasionally, an interfering peak occurred around DC10. In these cases, the peak area of the interfering compound determined from SPB-35 was subtracted from the peak area determined from SPB-1. The determinations of these dicarboxylic acids in the present investigation were all confirmed with GLC–MS analysis.

Gas–liquid chromatography–mass spectrometry

A Hewlett-Packard (Palo Alto, CA) 5985B gas chromatograph/mass spectrometer was used as described before (13). Electron impact (70 eV) ionization and repetitive scanning (300 AMU/s) from *m/z* 49 to 550 were used (15). For compound identification, a specific ion (usually the M–15 ion) expected for the compound of interest was retrieved from the repetitive scanned mass spectra file and displayed (mass chromatogram technique). The complete mass spectrum (from *m/z* 49 to 550) was then obtained and compared with authentic standards. The criteria for identification of a compound were that both the retention times on the two columns and the mass fragmentation spectrum were identical to those obtained from authentic standards.

Urine samples from patients with MCAD deficiency

Urine samples obtained from three patients with MCAD deficiency were investigated. A total of nine urine samples from these patients during episodes of hypoglycemia accompanied by nonketotic dicarboxylic aciduria were included in the analysis.

Patient 1 had recurrent respiratory infections as an infant and at 7 mo of age had a cardiac arrest after a prolonged episode of hypoglycemia. He had moderate enlargement of his heart and liver. Plasma and urinary carnitine levels were very low. A combined biopsy of rectus abdominus skeletal muscle and liver was performed and mitochondria were isolated. The activity of muscle MCAD was 6% of controls, and liver MCAD was 18% of controls. Activities of other enzymes of fatty acid oxidation and electron transport were normal in mitochondria from both tissues. Hexanoylglycine, phenylpropionylglycine, and suberylglycine in the concentration ranges described by Rinaldo et al. (1) for MCAD-deficient patients were consistently found in urine samples obtained when in acute hypoglycemic episode or during fasting. *Cis*-4-decenoic acid was detected in all plasma samples taken during hypoglycemic episodes.

Patient 2 was a child who was referred as a sudden infant death syndrome. A urine sample was obtained at autopsy. The diagnosis of MCAD deficiency was established after urine organic acid analysis. Patient 3 was similar to patient 1 with hypoglycemic episodes and low plasma carnitine. The liver was not enlarged and cardiac and neuromuscular examination appeared normal. Because no biopsy sample was available for the evaluation of enzyme activity, the diagnosis of MCAD deficiency in both patients was based on urinary organic acid analysis. Characteristic urinary organic acids, n-hexanoylglycine, phenylpropionylglycine, and suberylglycine in the concentration ranges (1) reported for MCAD-deficient patients were consistently found. Octanoylcarnitine was also found in the urine of all patients. The normal excretions of urinary glutaric and ethylmalonic acids, as well as branched-chain amino acid metabolites, further ruled out the possibility of glutaric aciduria type II and ethylmalonic-adipic aciduria.

Urine samples from fasting control children

Six children with matching ages ranging from 2 to 8 years underwent fasting for up to 36 h for diagnostic purposes, because of a suspected hypoglycemic disorder (16). These six children were classified retrospectively as normal reference subjects, because of lack of evidence for hypoglycemia. Urine was collected from these subjects whenever voided during the fasting test. These urine samples were grouped into several time intervals.

Urine samples from other patients with nonketotic dicarboxylic aciduria

Nonketotic dicarboxylic aciduria due to defects other than MCAD deficiency was defined according to the following criteria: *1*) a urinary 3-hydroxybutyrate/adipic ratio less than 1; *2*) a urinary adipic acid concentration higher than 150 mg/g creatinine; and *3*) absence of urinary suberylglycine, phenylpropionylglycine, or n-hexanoyl-

glycine during acute episodes or fasting. Biopsy specimens from some of these patients were also analyzed and showed normal activities of MCAD. Nine urine samples from a total of seven patients were used for comparison with the three MCAD-deficient patients described above. Of these seven patients, three had a suspected defect in 3-hydroxyacyl-CoA dehydrogenase (3-hydroxydicarboxylic aciduria) (4); one had systemic carnitine deficiency due to a defect in carnitine transport similar to that described by Treem et al. (17); one had a defect in the electron transport complex (18); and the other two had unexplained nonketotic dicarboxylic aciduria.

Urine samples of patients with ketotic dicarboxylic aciduria

Urine samples submitted for routine organic acid screening were selected according to the following criteria: *1*) urinary OH-B/DC6 ratio above 1; *2*) urinary adipic acid concentration higher than 150 mg/g creatinine; and *3*) absence of urinary n-hexanoylglycine, phenylpropionylglycine, and suberylglycine. A total of seven samples from seven subjects was included in this group for comparison.

Statistical analysis

Student's *t*-test was used for the comparison among different groups.

RESULTS

Identification of urinary *cis*-4-decenedioic, *cis*-5-decenedioic, and *cis*-5-dodecenedioic acids

Using dual capillary column gas–liquid chromatography, gas–liquid chromatography–mass spectrometry, and synthetic authentic samples, we previously identified the major hexenedioic and octenedioic acids in human urine as being *trans*-2-hexendioic, *cis*-3-octenedioic, *cis*-4-octenedioic, and *trans*-3-octenedioic acids (14). In urine samples obtained from fasting normal subjects and children with dicarboxylic aciduria, three isomeric decenedioic acids were detected using the mass chromatogram technique displaying *m/z* 329 (the M–15 ion expected for decenedioic acids) from repetitive scanning mass chromatograms (**Fig. 1**). By comparison with urinary organic acids obtained from hypoglycin-treated rats, we identified the middle of the three decenedioic acids as *cis*-4-decenedioic acid (**Fig. 2**). The gas chromatographic retention of the first peak suggested a *cis*-5-decenedioic acid structure since this is the only unsaturation that could have shorter retention than a *cis*-4 structure (14). The structure of peak 1 as *cis*-5-decenedioic acid was later confirmed by comparison with synthetic authentic sample of *cis*-5-decenedioic acid (19).

In urine samples from fasting normal children and patients with dicarboxylic aciduria not associated with MCAD deficiency, the amount of dodecenedioic acids was almost negligible. However, in urine samples from

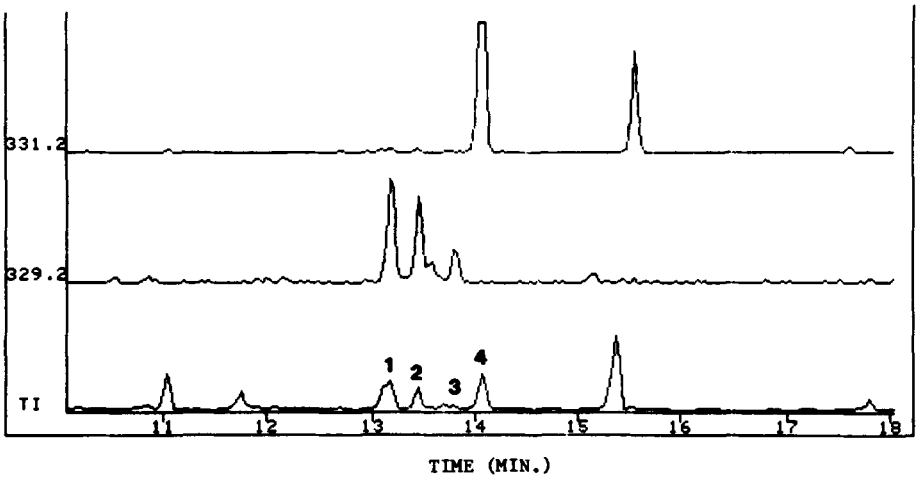

Fig. 1. The mass chromatogram of a urine extract from a patient with undiagnosed nonketotic dicarboxylic aciduria shows the existence of three isomeric decenedioic acids eluted before sebacic acid on an SPB-1 capillary column. The sample was analyzed using a gas chromatograph-mass spectrometer with repetitive scanning from *m/z* 49 to 550. The M–15 ions expected for decenedioic acid (*m/z* 329) and sebacic acid (*m/z* 331) are shown. Through comparison of mass spectra and retention times on dual capillary columns, the peaks are identified as: 1, *cis*-5-decenedioic acid; 2, *cis*-4-decenedioic acid; 3, *trans*-3-decenedioic acid (?); and, 4, sebacic acid. The gas chromatograph was programmed from 110°C to 250°C with a 4°C/min rate increase. A 20 to 1 split ratio was used for analysis.

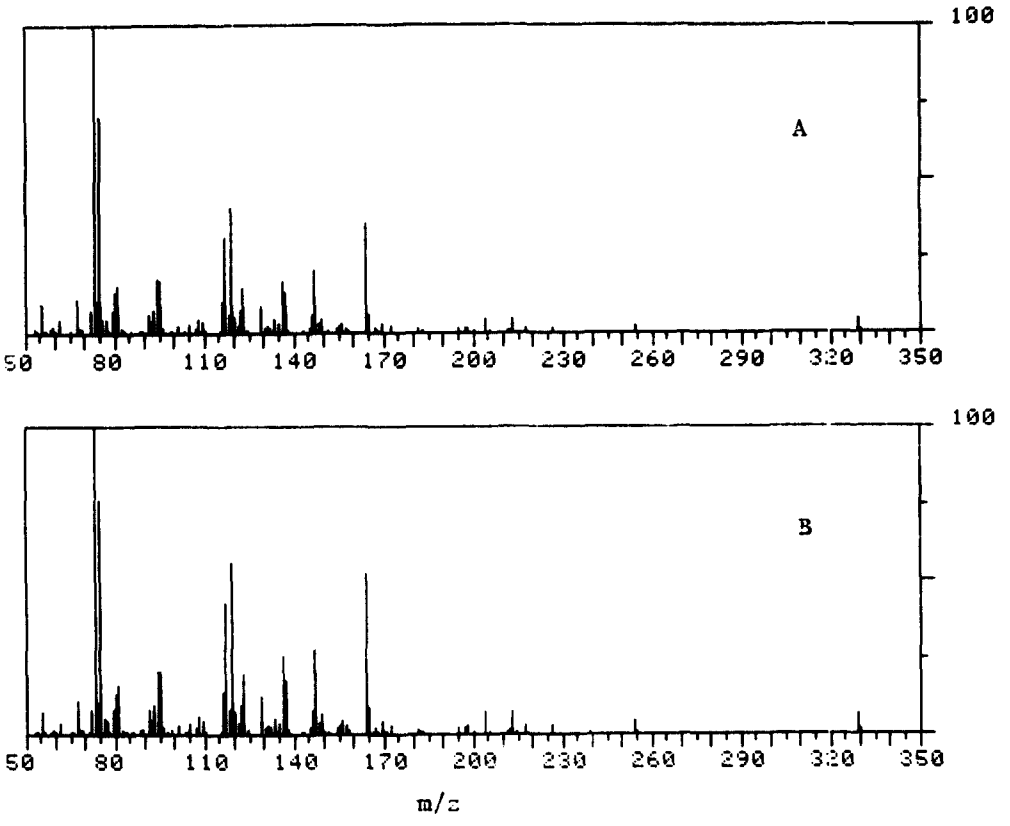

Fig. 2. Mass spectra of *cis*-4-decenedioic acid bis-trimethylsilyl derivative: A: obtained from the urine of patient 1 with medium-chain acyl-CoA dehydrogenase deficiency; B: obtained from the urine of a rat treated with hypoglycin.

MCAD-deficient children, the amount of dodecenedioic acids was significantly increased. Using the mass chromatogram technique) (**Fig. 3**), the three peaks that eluted before dodecanedioic acid have *m/z* 357 mass fragments. Mass spectra of these three peaks show that they are isomeric dodecenedioic acids. The first eluted peak was always the major one and was assigned as *cis*-5-dodecenedioic acid, which was also confirmed by comparison with a synthetic authentic sample (19).

Urinary excretion of dicarboxylic acids in patients with MCAD deficiency

For comparison of the amounts of various organic acids excreted in different disorders associated with dicarboxylic aciduria, we found it helpful to use the ratio of each acid to adipic acid since adipic acid is usually the most abundant end product of omega-oxidation. For fasting control values, the data collected with adipic acid above 150 mg/g creatinine are used for comparison because these values are more compatible with the samples obtained from groups with nonketotic dicarboxylic aciduria. These samples were generally obtained after more than 24 h of fasting (**Table 1**).

The ratios of 3-hydroxybutyric/adipic (OH-B/DC6) for fasting controls with adipic acid above 150 mg/g creatinine ranged from 12 to 32 (mean ± SD, 21 ± 9). In contrast, the ratios ranged between 0.02 and 0.13 (mean ± SD, 0.05 ± 0.03) for patients with MCAD deficiency, and between 0.02 and 0.36 (mean ± SD, 0.13 ± 0.11) for other patients with nonketotic dicarboxylic aciduria not associated with MCAD deficiency. In these samples, the urinary concentrations of adipic acid ranged between 156 and 3884 mg/g creatinine for MCAD-deficient patients and between 177 and 5889 mg/g creatinine for other nonketotic patients, while those of fasting controls were between 223 and 761 mg/g creatinine. Since the control fasting group had less urinary adipic acid excretion than nonketotic dicarboxylic aciduric groups, another group of patients (n = 7) with ketotic dicarboxylic aciduria was also compared. In this group, the ranges of DC6 were 237 to 2605 mg/g creatinine with an OH-B/DC6 ratio of 23.9 ± 33.9 (ranges 2.30–93.4).

The ratios of urinary saturated dicarboxylic acids (DC8, DC10, and DC12) to that of adipic acid are shown in **Fig. 4**. The urinary excretion of suberic acid (DC8) and sebacic acid (DC10), normalized to adipic acid, were

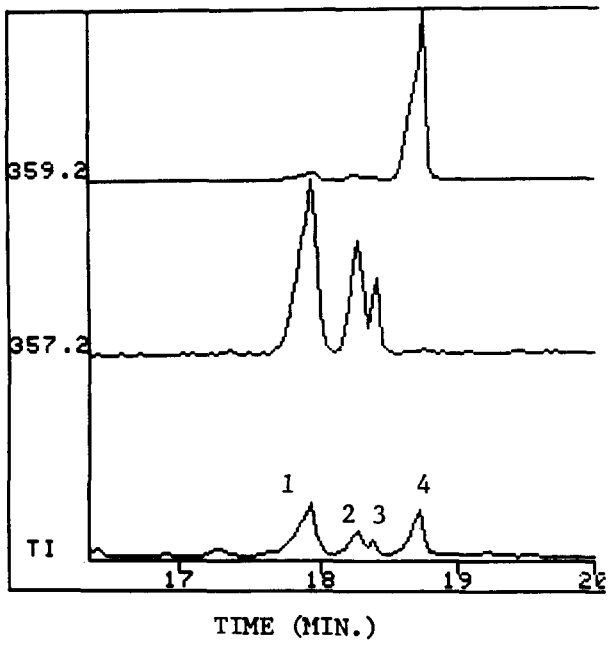

Fig. 3. The mass chromatogram of a urine extract from a patient with MCAD deficiency shows the existence of three isomeric dodecenedioic acids eluted before dodecanedioic acid on an SPB-1 capillary column. The analytical conditions were the same as described for Fig. 1. The M-15 ions expected for dodecenedioic acids (*m/z* 357) and dodecanedioic acid (*m/z* 359) are shown. Mass spectra from these peaks indicated a structure of dodecenedioic acids. The identity of dodecanedioic acid (peak 4) was confirmed; the structure of the peak 1 of dodecenedioic acids was identified as *cis*-5-dodecenedioic acid.

significantly increased ($P < 0.01$) in MCAD-deficient patients. However, some overlap exists between MCAD-deficient patients and other nonketotic dicarboxylic aciduria. This is to be expected since the latter group is a collection of samples with heterogenous etiologies. Even though less than DC8 and DC10, dodecanedioic acid (DC12) in MCAD-deficient patients is also significantly elevated compared to fasting controls ($P < 0.001$) and other nonketotic dicarboxylic aciduria ($P < 0.05$).

The urinary excretion of unsaturated dicarboxylic acids is shown in **Fig. 5**. A highly elevated ($P < 0.001$ for all comparisons) urinary *cis*-4-decenedioic acid (c4DC10) in MCAD-deficient patients was observed. There was no overlap in the ratios of c4DC10/DC6 in this group with the other nonketotic dicarboxylic acidurias or fasting controls. However, prospectively, there is a possibility that some urinary DC6 could be derived from dietary sources even though this contribution is usually small in dicarboxylic aciduria. This potential artifact would lower the true ratio of endogenous acids normalized to DC6. Therefore, the normalization to other urinary dicarboxylic acids is also presented. When the excretion of c4DC10 is normalized to DC10 (c4DC10/DC10 ratio) the group with MCAD deficiency (ranges: 0.39–1.42; median 0.95) is still significantly higher than the group with nonketotic dicarboxylic aciduria not associated with MACD deficiency (ranges: 0.15–0.84; median: 0.18). In this comparison (c4DC10/DC10 ratio), some overlap (three samples from the MCAD-deficient group) occurred between the two groups. The three samples from MCAD-deficient patients with the lowest c4DC10/DC10 ratios (0.39–0.56) were the only three urine samples collected after carnitine treatment. These data imply that some biochemical changes occurred in MCAD-deficient patients that contributed to the disproportionately increased urinary excretion of c4DC10. In addition, this increase in urinary c4DC10 was partially reversed with carnitine treatment.

When the urinary excretion of c4DC10 is normalized to the excretion of c5DC10, the ratios c4DC10/c5DC10 for

TABLE 1. Urinary excretion of dicarboxylic acids and other related acids in normal children (n = 6) during fasting

	Fasting Duration (h)					
Acids[a]	<8	12	18	24	30	36
	mg/g creatinine					
OH-B	166 ± 106[b]	25 ± 13	251 ± 386	586 ± 905	3128 ± 5142	6970 ± 3314
DC6	17 ± 7	10 ± 2	42 ± 33	44 ± 12	110 ± 60	374 ± 286
t2DC6	0	0	1 ± 2	6 ± 1	8 ± 2	13 ± 9
c3DC8	3 ± 2	2 ± 3	4 ± 6	11 ± 3	13 ± 5	25 ± 17
c4DC8	1 ± 1	1 ± 1	3 ± 5	8 ± 3	7 ± 5	17 ± 12
t3DC8	0	0.3 ± 0.6	2 ± 3	7 ± 5	11 ± 3	35 ± 30
DC8	3 ± 6	2 ± 3	3 ± 6	21 ± 12	31 ± 14	84 ± 68
c5DC10	0	0	2 ± 3	12 ± 6	16 ± 7	38 ± 34
c4DC10	0	0	0	0	0	2 ± 4
DC10	0	0	0	2 ± 5	8 ± 7	17 ± 23

[a]Abbreviations: OH-B, 3-hydroxybutyric; DC6, adipic; t2DC6, *trans*-2-hexenedioic; c3DC8, *cis*-3-octenedioic; c4DC8, *cis*-4-octenedioic; t3DC8, *trans*-3-octenedioic; DC8, suberic; c5DC10, *cis*-5-decenedioic; c4DC10, *cis*-4-decenedioic; DC10, sebacic.

[b]Organic acid concentrations are expressed as mg/g creatinine (mean ± SD).

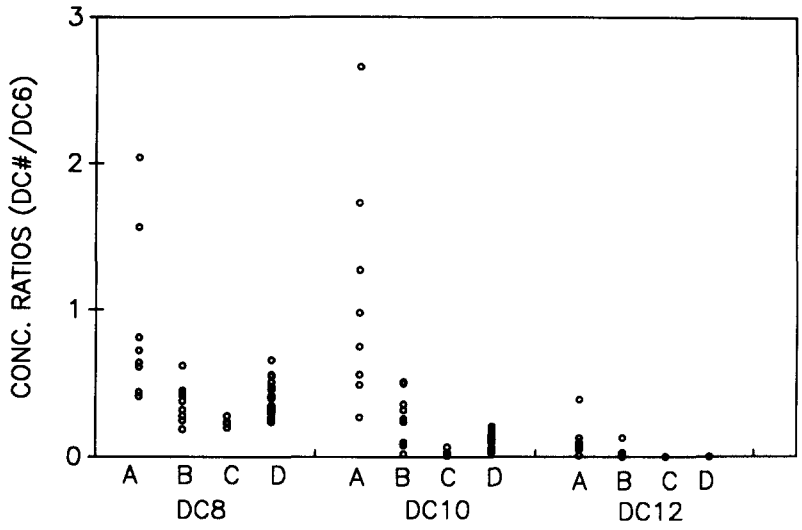

Fig. 4. The comparison of urinary concentration ratios of saturated dicarboxylic acids to adipic acid in four groups: A, patients with MCAD deficiency; B, patients with nonketotic dicarboxylic aciduria other than MCAD deficiency; C, fasting normal controls with urinary adipic acid over 150 mg/g creatinine; and D, fasting normal controls with urinary adipic acid lower than 150 mg/g creatinine. The abbreviations are: DC6, adipic acid; DC8, suberic acid; DC10, sebacic acid; and DC12, dodecanedioic acid.

MCAD-deficient patients (ranges 1.69–10.70, mean ± SD, 5.58 ± 3.01) are even more profoundly different than those of fasting controls (ranges, 0.20–0.34, mean ± SD, 0.27 ± 0.05) and nonketotic dicarboxylic aciduria other than MACD deficiency (ranges 0.22–1.36, mean ± SD, 0.75 ± 0.40). This difference resulted because, in MCAD-deficient patients, the urinary excretion of c5DC10 is slightly depressed as shown below, while, at the same time, the excretion of c4DC10 is elevated.

The group with ketotic dicarboxylic aciduria not associated with controlled fasting was not different from the fasting group or nonketotic dicarboxylic aciduria not associated with MCAD deficiency in c4DC10/c5DC10 ratios (mean ± SD; 0.50 ± 0.21; ranges: 0.14 ± 0.72) and c4DC10/DC6 (mean ± SD: 0.04 ± 0.03; ranges: 0.01–0.09). It appears that nonspecific ketotic dicarboxylic aciduria is not different from controlled fasting in terms of urinary excretion of dicarboxylic acids. Therefore, this group was dropped from further comparison.

Other unsaturated dicarboxylic acids (c4DC8, c3DC8, and c5DC10) did not show significant differences (when normalized to DC6 as shown in Fig. 5) from either fasting controls or other nonketotic dicarboxylic aciduria. When c3DC8 and c5DC10 are normalized to their individual saturated counterparts, i.e., DC8 and DC10, both show slightly lower ratios ($P < 0.05$) in MCAD-deficient patients than the other nonketotic group and significantly lower ratios ($P < 0.001$) than fasting controls.

Besides the increase in the urinary excretion of DC12 in MCAD-deficient patients shown in Fig. 4, the unsaturated dodecenedioic acids were also increased. In MCAD-deficient patients, the ratios c5DC12/DC12 were 1.68 ± 1.00 (ranges, 0.47–2.99). For patients with nonketotic dicarboxylic aciduria of other etiologies and with uri-

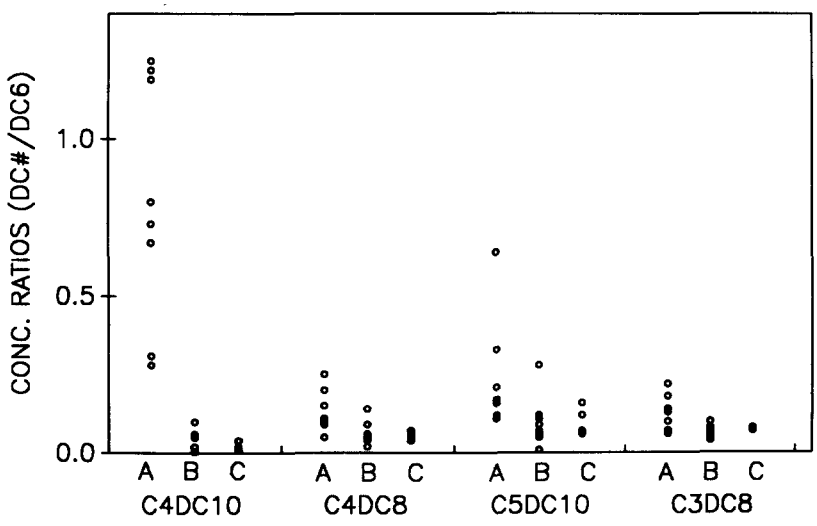

Fig. 5. The comparison of urinary concentration ratios of unsaturated dicarboxylic acids to adipic acid in three groups. Designations for groups are the same as in Fig. 4. Abbreviations: DC6, adipic acid; c3DC8, *cis*-3-octenedioic acid; c4DC8, *cis*-4-octenedioic acid; c5DC10, *cis*-5-decenedioic acid; and c4DC10, *cis*-4-decenedioic acid.

nary dodecanedioic and dodecenedioic acids sufficiently high for reliable measurement, the ratios of c5DC12/DC12 were 0.22 ± 0.14 (n = 7, range: 0.08–0.40). For the other decenedioic acids, the amount in urine was not enough for measurement. Therefore, at least the ratio for c5DC12/DC12 is significantly higher ($P < 0.001$) in MCAD-deficient patients than in other nonketotic dicarboxylic aciduria.

Fasting urinary organic acids in a patient with MCAD deficiency

Patient 1 from the MCAD deficiency group was subjected to controlled fasting for diagnostic purposes. The urinary excretion of dicarboxylic acids and acylglycines of diagnostic value (i.e., n-hexanoylglycine, phenylpropionylglycine, and suberylglycine) are listed in **Table 2**. In comparison to normal fasting controls shown in Table 1, these data (Table 2) show that the abnormality in the urinary excretion of dicarboxylic acids, i.e., high DC8/DC6, DC10/DC6, DC12/DC6, c4DC10/DC6, c4DC10/DC10, C4DC10/c5DC10, persisted throughout the fasting period. At the end of 20 h fasting, the blood glucose dropped to 57 mg/dl while the free fatty acid concentrations increased from 0.10 mM to 2.02 mM. The blood 3-hydroxybutyrate increased from 0.14 mM to 0.40 mM at the end of fasting.

DISCUSSION

The organic acids from urine samples with elevated levels of dicarboxylic acids include a number of unsaturated species. These dicarboxylic acids can be grouped into two metabolic series, i.e., the *cis*-4 series as well as the *cis*-3 and *cis*-5 series. The metabolic precursor of *cis*-4 unsaturated dicarboxylic acids is linoleic acid; while that of *cis*-3 and *cis*-5 unsaturated dicarboxylic acids is likely to be oleic acid (14).

TABLE 2. Urinary excretion of dicarboxic acids and glycine conjugates (mg/g creatinine) in a patient with medium-chain acyl-CoA dehydrogenase deficiency

	Fasting Duration (h)				
Acids[a]	5	8	12	16	22
	mg/g creatinine				
OH-B	11	29	15	25	52
DC6	63	35	58	387	1743
t2DC6	0	0	0	12	49
c3DC8	8	8	20	51	181
c4DC8	7	8	20	57	194
t3DC8	0	0	9	33	100
DC8	40	35	88	312	1111
c5DC10	18		21	83	304
c4DC10	46		72	458	1395
DC10	0	0	90	493	944
c5DC12	0	0	8	71	94
DC12	0	0	25	150	92
HG	8	41	91	88	176
PG	7	22	38	33	60
SG	8	15	143	309	721

[a]Abbreviations: same as Table 1. In additon, c5DC12, *cis*-5-dodecenedioic acid; DC12, dodecanedioic acid; HG, hexanolyglycine; PG, phenylpropionylglycine; and SG, suberylglycine.

In this investigation, we found elevated urinary excretions of *cis*-4-decenedioic and *cis*-5-dodecenedioic acids in patients with MCAD deficiency. This finding suggests abnormal handling of unsaturated fatty acids in these patients. The pathway for the oxidation of linoleic acid has been revised recently (20, 21). After stepwise beta-oxidation and an isomerization step, *cis*-4-decenoyl-CoA is produced as a metabolic intermediate (**Fig. 6**). The continued oxidation of *cis*-4-decenoyl-CoA requires several enzymes—MCAD, 2,4-dienoyl-CoA reductase, and *trans*-3-*trans*-2-isomerase. Inhibition or deficiency of any of these enzymes is likely to prevent the further beta-oxidation of *cis*-4-decenoyl-CoA. As a consequence, *cis*-4-decenoyl-CoA will accumulate and may be enzymatically hydrolyzed to *cis*-4-decenoic acid, a substrate for the microsomal omega-hydroxylation system. *Cis*-4-decenedioic acid thus produced in the extramitochondrial compartment is then transported back to mitochondria and/or peroxisomes for further beta-oxidation to *cis*-4-octenedioic acid. In hypoglycin-treated rats (22), the metabolic degradation of linoleic acid is inhibited. The fatty acid metabolic enzymes, medium-chain acyl-CoA dehydrogenase and short-chain acyl-CoA dehydrogenase, are known to be inhibited by hypoglycin metabolites (11, 12). It suggests that the inhibition of MCAD by hypoglycin metabolites seems sufficient to explain the increased excretion of *cis*-4-dicarboxylic acids. Using purified enzymes from bovine liver, Dommes and Kunau (23) showed that the dehydrogenation of *cis*-4-decenoyl-CoA required MCAD. According to their results, long chain acyl-CoA dehydrogenase (LCAD) oxidized *cis*-4-decenoyl-CoA at a rate of only 2.7% of that obtained with decanoyl-CoA as substrate, whereas *cis*-4-decenoyl-CoA was a slightly better substrate than decanoyl-CoA for MCAD. As a consequence, the inhibiton of MCAD will have a lesser effect on the production of saturated dicarboxylic acids than on *cis*-4-dicarboxylic acids inasmuch as the further metabolism of decanoyl-CoA can be partially compensated by LCAD. According to this rationale, MCAD deficiency will produce disproportionately higher urinary excretion of *cis*-4-dicarboxylic acids in humans if the chain specificity of MCAD and LCAD toward *cis*-4-decenoyl-CoA is the same in human as in cows. As seen in Fig. 5, the ratios of c4DC10 to DC6 and to DC10 increased several-fold in MCAD-deficient patients when compared to patients with nonketotic dicarboxylic aciduria due to other etiologies.

Further support to our hypothesis is the detection of *cis*-4-decenoic acid, the alleged intermediate postulated in

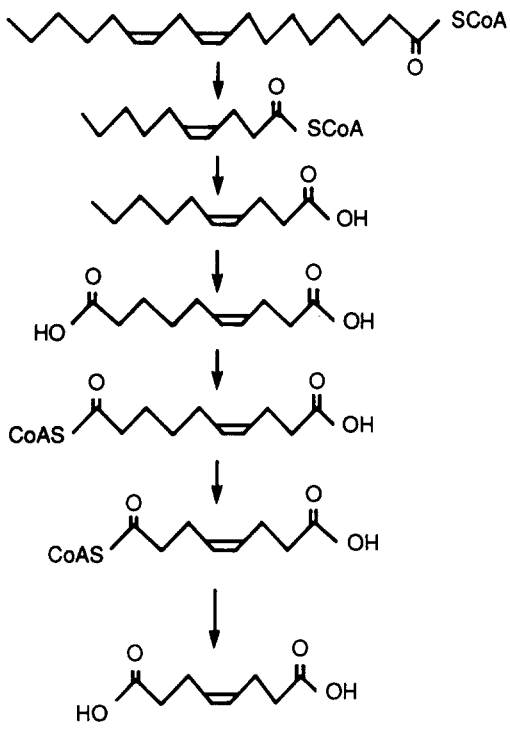

Fig. 6. Postulated metabolic pathway for the production of *cis*-4-octenedioic and *cis*-4-decenedioic acids. Linoleoyl-CoA, after four cycles of beta-oxidation, is oxidized to *cis*-4-decenoyl-CoA. The further beta-oxidation of *cis*-4-decenoyl-CoA requires medium-chain acyl-CoA dehydrogenase (MCAD). A reduced activity of MCAD can cause the accumulation of this acyl-CoA, which may be hydrolyzed to release *cis*-4-deceneoic acid. The omega oxidation of *cis*-4-deceneoic acid produced *cis*-4-decenedioic acid, which can be beta-oxidized from the newly created carboxylic end to yield *cis*-4-octenedioic acid.

Fig. 6, in plasma. The same observation has also been reported by Duran et al. (24). The occurrence of plasma *cis*-4-decenoic acid, which is not detectable in plasma samples from patients with other nonketotic dicarboxylic acidurias, and the increased urinary excretion of *cis*-4-decenedioic acid would be predicted from decreased linoleic acid metabolism as a result of MCAD deficiency. Gregersen et al. (6) did not find any significant urinary excretion of unsaturated dicarboxylic acids in their three cases of MCAD deficiency. However, in the cases described by Duran et al. (25) and Del Valle et al. (26), a significantly higher urinary excretion of unsaturated octenedioic and decenedioic acids is apparent. The reason for this discrepancy is not known.

In MCAD-deficient patients, carnitine treatment appears to reduce some of the abnormal urinary excretion of *cis*-4-decenedioic acid. The mechanism of this reversal is not known. However, if the inhibiton of linoleate metabolism is not totally a consequence of reduced enzyme activity in the dehydrogenation of *cis*-4-decenoyl-CoA, and part of the abnormality in linoleate metabolism is due to the inhibition of 2,4-dienoyl-CoA reductase or isomerase by toxic metabolites produced as a consequence of MCAD deficiency, then the treatment with carnitine could remove part of this abnormality by removing the toxic CoA derivatives as carnitine esters (27, 28).

Cis-3-octenedioic and *cis*-5-decenedioic acids are postulated to derive from oleic acid (14). In MCAD-deficient patients, the ratio of these acids to their saturated counterparts decreased significantly when compared to fasting normal children or other nonketotic dicarboxylic aciduria. In addition, there was a significant difference between MCAD deficiency and other nonketotic dicarboxylic acidurias in the excretion of dodecenedioic acids. The urinary excretion of *cis*-5-dodecenedioic acid was significantly elevated relative to dodecanedioic acid in MCAD-deficient patients, but not in other nonketotic hypoglycemic patients. It appears that MCAD deficiency also affects the metabolism of oleic acid more profoundly than saturated fatty acids. The high excretion of c5DC12 and the lower excretion of c5DC10 as well as c3DC8 suggests a possible metabolic block at the beta-oxidation of c5DC12.

Recently, characteristic abnormal urinary metabolites have been proposed as marker compounds for the diagnosis of MCAD deficiency (1). n-Hexanoylglycine, octanoylcarnitine, and phenylpropionylglycine have been proposed by various investigators (1). In the present investigation, we found several additional abnormalities in the urinary excretion of organic acids. Based on the higher ratios of DC8, DC10, and DC12 to DC6 (Fig. 4), it points to a possible abnormality in the metabolism of medium-chain fatty acids in these patients. However, higher ratios of medium-chain dicarboxylic acids to adipic acid do not necessarily indicate MCAD deficiency since nonketotic dicarboxylic aciduria not associated with MCAD deficiency has been shown to have some overlap in these ratios. In addition, the adminstration of medium-chain triglycerides to children was found to cause the same type of elevated ratios (29). However, the elevated excretion of *cis*-4-decenedioic and *cis*-5-dodecenedioic acids seems to be an interesting feature among patients with MCAD deficiency and this abnormality can be explained by the pathophysiology of the disease. In our patient population, we have three patients with this characteristic feature out of a total of three cases with criteria for the diagnosis of MCAD deficiency. However, whether this specific feature is generally applicable to all MCAD deficiency cases has to be confirmed by the analysis of more patient samples. Nevertheless, the biochemical basis of this disease (MCAD deficiency) predicts such an outcome of urinary unsaturated dicarboxylic acid excretion.

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