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Long-Chain Acyl-Coenzyme A Dehydrogenase Deficiency: Biochemical Studies in Fibroblasts from Three Patients[1]

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ABSTRACT. We studied fibroblasts from three patients with long-chain acyl-coenzyme A dehydrogenase (LCADH) deficiency; siblings H.C. and J.C. had milder clinical phenotypes than unrelated patient R-1. In H.C., J.C., and R-1 oxidation of [9,10(n)-^{3}H]palmitate was 50, 48, and 28% of control, respectively, with R-1 having significantly less activity than H.C. and J.C. ($p < 0.05$). Assays of mitochondrial short-chain and medium-chain acyl-coenzyme A dehydrogenases were normal in H.C. and J.C. However, mitochondrial LCADH activities in all three ranged from 17 to 21% of control. Flavin adenine dinucleotide addition increased LCADH activities in all three to 27–36% of control. In the presence of monospecific medium-chain acyl-coenzyme A dehydrogenase antiseria, LCADH activity decreased 17% in controls, and fell to ≤11% of control in J.C. and R-1. The heterogeneity observed in the [^{3}H]palmitate oxidation studies was not explained by differences in LCADH activities under any assay condition. (*Pediatr Res* 23: 603–605, 1988)

Abbreviations

CoA, coenzyme A
LCADH, long-chain acyl-CoA dehydrogenase
LCD, long-chain acyl-CoA dehydrogenase deficiency
SCADH, short-chain acyl-CoA dehydrogenase
MCADH, medium-chain acyl-CoA dehydrogenase
FAD, flavin adenine dinucleotide
ETF, electron transfer flavoprotein

LCD has been identified in one of two siblings with nonketotic hypoglycemia and dicarboxylic aciduria (1) and in three patients with nonketotic hypoglycemia, carnitine deficiency, cardiomyopathy, and cardiorespiratory arrests associated with fasting (2, 3). LCD differs somewhat from other inborn errors of fatty acid metabolism (4–7) in that accumulated long-chain acyl-CoA produced a medium and long-chain (C_6–C_{14}) dicarboxylic aciduria in one patient (8). The inability of skeletal and cardiac muscle to oxidize long-chain fatty acyl-CoA presumably produces muscle weakness and cardiomyopathy in some patients (3). We report herein studies in fibroblasts from two siblings with LCD (H.C. and J.C.) (8) and compare them to a previously reported LCD patient (R-1) (3). The clinical phenotypes of H.C. and J.C. in infancy and childhood have been well documented (8). The LCADH deficiency of H.C. has been reported briefly (3); ADH studies have never been reported for J.C. At 20 yr of age, H.C. has a mild skeletal myopathy and profound carnitine deficiency without cardiomyopathy. She has repeated episodes of myalgias, myoglobinuria, and greatly elevated serum creatine phosphokinase levels provoked by fasting, exercise, fat ingestion, or emotional stress. She has elevated circulating levels of long-chain acyl-carnitines. J.C., her 18-yr-old sister, avoids fatty foods, takes frequent small meals, and has had few episodes of hypoglycemia or muscle weakness since childhood (Roe C, personal communication). Patient R-1 presented with cardiorespiratory arrest, lethargy, hypertrophic cardiomyopathy, and carnitine deficiency (3). To explore possible explanations for this marked clinical heterogeneity, we performed [9,10(n)-^{3}H]palmitate cell oxidation studies, and assayed LCADH in the presence and absence of added FAD and MCADH antiserum.

METHODS

[9,10(n)-^{3}H]palmitate (380 mCi/mmol) was obtained from Research Products International, Arlington Heights, IL. FAD and N-ethylmaleimide were purchased from Sigma Chemical Co., St. Louis, MO. Pure pig liver MCADH and ETF were gifts from Dr. Carole L. Hall, Georgia Institute of Technology, Atlanta, GA. The pure pig kidney MCADH used for antisera preparation was a gift from Dr. C. Thorpe, University of Delaware, Newark, DE. Skin fibroblasts were obtained from patients H.C., J.C., and R-1 (courtesy of P. Coates, Children's Hospital of Philadelphia, Philadelphia, PA) and six normal male infants. Fibroblasts were cultured in Eagle's minimal essential medium, supplemented with 10% fetal bovine serum, 2 mM glutamine, 140 μM penicillin, and 86 μM streptomycin. Fibroblasts were subcultured 1:4 every 2 wk and the media changed weekly. Cells of passage 6–19 were used in all experiments. ^{14}C-labeled substrate oxidation to $^{14}CO_2$ by intact fibroblasts in suspension was performed as described earlier (9). Tritium release from [9,10(n)-^{3}H]palmitate (380 mCi/mmol, 22 μM) from fibroblast monolayers was performed as described previously (10). Preparation of fibroblast mitochondrial sonic supernatants and dye reduction ADH and ETF assays are described elsewhere (7, 9). FAD and monospecific MCADH antisera were preincubated with mitochondrial supernatants at 30° C for 5 min before ADH assay. Rabbit antiserum was raised to pure pig kidney MCADH as described earlier (4). This antiserum preparation completely inhibited MCADH activity towards octanoyl-CoA in human fibroblast and mitochondrial sonicates, and pure pig liver and kidney MCADH (4) (other data not shown). The estimate of variance used is the SEM. Means were compared with the Student's t test; all p values given are two-tailed and compare the patients' data to the respective control values.

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RESULTS

In preliminary experiments, fibroblasts from H.C. oxidized [1-^{14}C]butyrate, L-[2-^{14}C]leucine, and [1,4-^{14}C]succinate normally (77–141% of control levels; data available on request). However, [1-^{14}C]-palmitate oxidation was significantly decreased (43% of control, $p < 0.05$; data available on request), comparable to the results obtained by Hale *et al.* (3) with the cells of R-1. H.C., J.C., and R-1 oxidized [9,10(n)-^{3}H]palmitate at 50, 48, and 28% of control levels, respectively. Using this substrate, the metabolic defect of R-1 appeared more severe than that in J.C. and H.C. ($n = 15$; $p < 0.05$) (Table 1).

Mitochondrial LCADH activities in patients H.C., J.C., and R-1 were low and identical at 21, 17, and 20% of control values, respectively ($p < 0.01$), whereas SCADH and MCADH activities were normal (Table 2). Inasmuch as low intramitochondrial FAD pools *in vivo* could result in deficient ADH activities, we added 20 μM FAD to mitochondrial supernatants. LCADH activities increased significantly to 27–36% of control in all three patients (one tail $p = 0.03$; Table 2). We also assayed mitochondrial MCADH and LCADH activities after incubation with monospecific rabbit antisera to pig kidney MCADH, which completely inhibits human fibroblast MCADH activity (4) and permits accurate assay of LCADH activity alone (Table 2). Under our assay conditions, 17% of palmityl-CoA dehydrogenation in normal fibroblasts appeared due to MCADH activity. LCADH activities in patients J.C. and R-1 fell to 6 and 11% of control, respectively.

In all three patients, ETF activities were normal (≥74% of control, $p > 0.5$), making it unlikely that any represent an ETF-deficient multiple acyl-CoA dehydrogenation disorder variant (Table 3).

DISCUSSION

We describe herein detailed studies in fibroblasts from three patients with LCD. The radiolabeled substrate oxidation studies revealed defective long-chain fatty acid oxidation in all three patients. Preliminary [^{14}C]substrate oxidation studies revealed no significant differences between H.C. and R-1, two patients with widely varying clinical phenotypes. However, patient R-1 catabolized [^{3}H]palmitate less well than either H.C. or J.C. ($p < 0.05$), consistent with their differing clinical courses.

ADH assays in mitochondrial sonicates revealed low LCADH activites in all three patients (≤21% of control), whereas MCADH and SCADH activities were normal. In our assay system, the residual activities with palmityl-CoA were somewhat higher in R-1 than those reported by Hale *et al.* (3) and higher than anticipated for isolated LCADH deficiency. This observation may result from overlapping substrate specificities of human fibroblast ADH, as reported for the bovine enzymes (11). In many species, three different enzymes dehydrogenate straight-chain fatty acids; LCADH is active with acyl-CoA of 8–22 carbons, MCADH, 4–12 carbons, and SCADH, 4–6 carbons (11, 12). Whereas the chain length specificities of human ADH are not known, human fibroblast MCADH can dehydrogenate a broad range of acyl-CoA. In two patients with SCADH deficiency, apparent SCADH activities were ≤53% of controls, but fell to ≤11% of control after addition of MCADH monospecific antisera (4). The apparent SCADH activities resulted from MCADH acting on butyryl-CoA, the optimal substrate for SCADH. MCADH-deficient patients also have low SCADH activities (42 to 73% of control) (5, 13) reflecting the loss of MCADH activity toward butyryl-CoA. MCADH-deficient patients also have LCADH activities 79–96% of control, suggesting MCADH also dehydrogenates palmityl-CoA. Herein addition of

Table 1. *[9,10(n)-^{3}H]Palmitate oxidation by intact fibroblasts (nmol/mg protein/h ± SEM)**

Cell line	
Normal controls:	
1	0.37 ± 0.05
2	0.43 ± 0.04
H.C.	0.20 ± 0.04†
J.C.	0.19 ± 0.02†
R-1	0.11 ± 0.03†

* Cells from H.C., J.C., and R-1 and two controls were assayed simultaneously under identical conditions in 15 experiments as described previously (10). *p* values refer to comparisons between patient and control data.

† $p < 0.01$.

Table 3. *ETF activities in fibroblasts [pmol/min/mg protein ± SEM (n)]**

Cell line	Cell Preparation: Mitochondrial supernatants	Cell Preparation: Fibroblast supernatants
Normal controls	460 ± 54 (2)	80 ± 20 (6)
H.C.	ND†	59 ± 11 (3)
J.C.	514 (1)	ND
R-1	353 (1)	ND

* Activities were determined as previously described (7). The number of determinations totaled two to six using four control lines and one to three for each patient line.

† Not determined.

Table 2. *ADH activities in fibroblast mitochondrial sonic supernatants [pmol/min/mg protein ± SEM (n)]**

Cell line	Enzyme assayed: / Substrate: / Additions:	MCADH Octanoyl-CoA: SCADH Butyryl-CoA None	MCADH Octanoyl-CoA: None	MCADH Octanoyl-CoA: MCADH antiserum	LCADH Palmityl-CoA: None	LCADH Palmityl-CoA: FAD (20 μM)	LCADH Palmityl-CoA: MCADH antiserum
Normal controls		830 ± 96 (10)	1580 ± 115 (10)	0.0 ± 0.0 (10)	790 ± 102 (10)	805 ± 95 (10)	656 ± 70 (9)
H.C.		639 ± 52 (6)	1469 ± 120 (8)	ND†	166 ± 20 (14)‡	290 ± 32 (6)‡	ND
J.C.		672 ± 41 (2)	1185 ± 40 (2)	ND	134 ± 31 (3)‡	217 ± 36 (3)‡	39 ± 15 (3)§
R-1		ND	ND	ND	158 ± 45 (3)‡	242 ± 41 (3)‡	72 ± 18 (4)§

* ADH activities were assayed by the dye reduction methods described earlier (4, 5). The number of determinations totaled nine to 10 using six control lines and two to 11 for each patient line; in all experiments, at least two control lines were assayed simultaneously with patient lines. *p* values refer to comparisons between patient and control data.

† Not determined.

‡ $p < 0.01$.

§ $p < 0.001$.

monospecific MCADH antisera revealed LCADH activities to be ≤11% of control in J.C. and R-1.

All three straight chain ADH bind FAD ionically (12). As purified rat liver LCADH apoenzyme is converted to its holenzyme after incubation with FAD (12), we preincubated control and patient mitochondrial sonicates with this cofactor to maximize holoenzyme formation. Control LCADH activity increased little, whereas LCADH activities increased in all patients. This *in vitro* phenomenon may not reflect a physiologically significant FAD response, however, riboflavin supplementation might merit a therapeutic trial in LCD patients.

Whereas patient R-1 had a more severe clinical course than H.C., who had more episodes than her sibling, J.C., all three have a similar degree of enzymatic deficiency *in vitro*, both in the presence and absence of FAD and MCADH antiserum. However, our [^{3}H]palmitate oxidation results parallel the clinical severities in these three patients and suggest that other biochemical mechanisms improve β-oxidation efficiency in H.C. and J.C. Relevant possibilities include an altered LCADH with significantly greater activity *in vivo* than in disrupted cells or mitochondria. However, inasmuch as our [^{3}H]palmitate oxidation assay does not distinguish between mitochondrial and peroxisomal β-oxidation, the differences in [^{3}H]palmitate oxidation seen in these patients could be due to increased activity of peroxisomal β-oxidation in the siblings H.C. and J.C. We did not perform complementation analyses (10) between these three patients because all biochemical analyses suggest that all have the same enzymatic defect. Further study of these and other LCD patients should improve our understanding of molecular and biochemical variations of β-oxidation in man.

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