0031-3998/92/3106-0552$03.00/0
PEDIATRIC RESEARCH

Vol. 31, No. 6, 1992
Printed in U.S.A.

Short-Chain Acyl-Coenzyme A Dehydrogenase Activity, Antigen, and Biosynthesis Are Absent in the BALB/cByJ Mouse

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ABSTRACT. BALB/cByJ (J) mice have short-chain acyl-CoA dehydrogenase (SCAD) deficiency and an organic aciduria similar to that of human SCAD deficiency. [9,10(n)-^{3}H]- and [15,16(n)-^{3}H]palmitate oxidations in J mouse fibroblasts were 96 and 35% of control, respectively, consistent with an isolated SCAD defect. Acyl-CoA dehydrogenase activities were assayed in muscle and fibroblast mitochondria from BALB/cBy controls (Y) and SCAD-deficient J mice. Medium-chain acyl-CoA dehydrogenase (MCAD) activities were comparable in both J and Y mice from all tissues. In the presence of MCAD antiserum, SCAD activities in J mice were undetectable in both tissues. Apparent Km and V_{max} values in liver mitochondria suggested a somewhat increased affinity of MCAD for butyryl-CoA in J mice, as compared with MCAD from other species. Immunoblot studies using mitochondria revealed identical apparent SCAD molecular weight in liver, muscle, and fibroblasts from Y mice and no detectable SCAD antigen in J mice; MCAD antigen was detected in comparable amounts from both Y and J mice. Radiolabeling and immunoprecipitation studies in J mouse fibroblasts revealed no SCAD synthesis, but normal MCAD synthesis. These data argue against the existence of tissue-specific SCAD isoforms in the mouse and confirm that this mouse strain is a model for the human organic aciduria resulting from this β-oxidation defect. (*Pediatr Res* 31: 552–556, 1992)

Abbreviations

SCAD, short-chain acyl-CoA dehydrogenase
MCAD, medium-chain acyl-CoA dehydrogenase
J, BALB/cByJ
Y, BALB/cBy control

Human SCAD deficiency is an inborn error of fatty acid oxidation reported in three infants (1, 2). Two presented with metabolic acidosis and ethylmalonic aciduria and the third presented with severe skeletal muscle weakness, developmental delay, and muscle carnitine deficiency (1, 2). Detailed enzymatic analyses in fibroblasts revealed an isolated, specific SCAD deficiency (1, 2). SCAD catalyzes the first reaction in the β-oxidation of short-chain fatty acids. It is a 160-kD tetrameric mitochondrial flavoprotein, with subunits of 41 kD (3, 4). SCAD is synthesized in the cytoplasm as a 44-kD precursor and is processed in the mitochondrion to the 41-kD active form (5).

To identify animal models for human metabolic diseases, Wood *et al.* (6) screened mice systematically for inherited metabolic defects. During these studies, they discovered a subline of BALB/c mice (BALB/cByJ) that excreted abnormally high concentrations of ethylmalonate, methylsuccinate, *n*-butyrylglycine, and *n*-butyrylcarnitine (7). These metabolites suggested a defect in short-chain acyl-CoA dehydrogenation, inasmuch as all can be formed intramitochondrially from accumulated butyryl-CoA (1, 2, 7). Other investigators reported previously that this substrain has a null allele for butyryl-CoA dehydrogenase activity (bcd-1 locus), a genetic marker on mouse chromosome 5 (8). In this report, we describe the enzymatic defect and absence of SCAD antigen and biosynthesis in multiple tissues from these BALB/cByJ (J) mutant mice. Although Turnbull *et al.* (9) and Farnsworth *et al.* (10) reported a human patient with apparent muscular SCAD deficiency, our observations argue against the presence of tissue-specific SCAD isoforms in mammals.

MATERIALS AND METHODS

Materials. [9,10(n)-^{3}H]- and [15,16(n)-^{3}H]palmitate (280 mCi/mmol) were obtained from New England Nuclear (Boston, MA) or from Research Products International (Arlington Heights, IL). N-ethylmaleimide and substrates used in immunoblotting experiments were purchased from Sigma Chemical Co. (St. Louis, MO) or BioRad Laboratories (Richmond, CA). Pure pig kidney MCAD and pig liver SCAD for antisera preparation and electron transfer flavoprotein were gifts from Drs. Colin Thorpe (11) (University of Delaware, Newark, DE) and Carole Hall (12) (Georgia Institute of Technology, Atlanta, GA). Polyclonal monospecific antisera against purified SCAD and MCAD were prepared as previously described (1).

Mice. BALB/cBy and BALB/cByJ mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and propagated at the Baylor College of Medicine (Houston, TX). All mice were maintained on Wayne rodent food number 8640 (Wayne Pet Food Division, Continental Grain Co., Chicago, IL) and water *ad libitum*.

Preparation of fibroblasts. Ear skin was obtained aseptically and rinsed in α-minimal essential medium (α-MEM; Sigma Chemical Co.). The skin was washed with 1 mL of α-MEM containing 1 mg/mL collagenase without FCS (Hazleton, Lenexa, KS) supplemented with 140 μM penicillin and 86 μM streptomycin. The ear skin was minced, placed in 1.5 mL of collagenase solution, and incubated at 37°C for 3 h, then centri-

Received July 2, 1991; accepted January 24, 1992.
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Supported by USPHS Grant DK33289 and grants from the Muscular Dystrophy Association and the Brent W. Brees/Dows Metabolic Research Fund, all to W.J.R., and USPHS Grant RR02599 to P.A.W. E.F. was supported by funds from the Counseil Regional de Champagne-Ardenne, Association Francaise Contre les Myopathies, and the Philippe Foundation.
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fuged at 800 × *g* for 5 min. The supernatant was discarded and the pelleted cells resuspended in 5 mL of α-MEM, supplemented with 10% FCS and penicillin/streptomycin, and then seeded into culture flasks. Fibroblasts were subcultured 1:4 every 2 wk, with the media changed weekly. Fibroblasts of passage 6 to 20 were used in all experiments.

^{3}H-palmitate oxidations and enzymatic analyses. [9,10(n)-^{3}H]- and [15,16(n)-^{3}H]palmitate oxidations to ^3H_2O by fibroblast monolayers were performed as described previously (13). Mouse liver, muscle and fibroblast mitochondria were isolated as previously described (1, 14, 15). Measurements of mitochondrial acyl-CoA dehydrogenase activities were performed using electron transfer flavoprotein-mediated reduction of dichlorophenolindophenol as described elsewhere (1, 16). In the immunoinactivation studies, 10 μL of rabbit antisera to pig kidney MCAD was incubated with 200–400 μg mitochondrial protein for 5 min at 30°C before adding substrate.

Immunoblotting methods. Mitochondria for immunoblotting experiments were sonicated at 0° in 0.2 mL of 20 mM sodium phosphate buffer, pH 7.4, as previously described (6, 7). The sonicates were centrifuged at 200 000 × *g* for 30 min at 0°. An aliquot of the mixture was assayed for protein by a fluorometric method described earlier (1). The mitochondrial supernatants were dissolved in 0.2 mL of sample buffer containing 8 M urea, 10% glycerol, 5% 2-mercaptoethanol, 62.5 mM Tris HCl, pH 6.8, 2% SDS, and 0.01% bromophenol blue and heated in a boiling water bath for 10 min. An aliquot was placed on a vertical SDS polyacrylamide gel for electrophoresis. Equal amounts of mitochondrial matrix proteins from both Y and J mice were loaded to each lane; SDS-PAGE was performed essentially according to Laemmli (17). Electrophoretic transfer was identical to the Towbin method, except that 0.05% SDS was added to the transfer buffer (18). The proteins were transferred to either nitrocellulose (BioRad Laboratories) or Immobilon membranes (Millipore Corp., Bedford, MA). Antigen was detected on the membrane by the alkaline-phosphatase (Sigma Chemical Co., St. Louis, MO) or horseradish peroxidase (BioRad Laboratories) method.

Immunoprecipitation studies. These studies were performed as described by Ikeda *et al.* (5). Fibroblast monolayers in 25 cm^2 flasks were labeled with [^{35}S]methionine (60 μCi/mL) in methionine-free RPMI 1640 for 12 h and then solubilized in 10 mM EDTA, 0.5% Triton X-100, 0.25% SDS, 2% methionine and 150 mM NaCl, pH 7.2. The extracts were immunoprecipitated by 10 μL of rabbit anti-pig SCAD antiserum or by 10 μL of preimmune antiserum using heat-killed formalin-fixed *Staphylococcus aureus* cells (Boehringer-Mannheim, Indianapolis, IN). The immunoprecipitates were analyzed by 10% SDS-PAGE and revealed by fluorography. In competition experiments, 10 μg of unlabeled pig liver SCAD was added before antiserum addition. MCAD immunoprecipitation studies were performed identically to the SCAD studies, except that the extracts were immunoprecipitated with 10 μL rabbit anti-pig MCAD antiserum, and 5 μg unlabeled pig liver MCAD was added in the competition experiments.

RESULTS

Fibroblast catabolism of tritiated palmitates. Fibroblasts from J mutant mice oxidized [9,10(n)-^{3}H]palmitate to ^3H_2O normally at 96% of control, whereas [15,16(n)-^{3}H]palmitate oxidation was significantly decreased at only 35% of control (Table 1). The residual oxidation noted with the former substrate is comparable to that found in human SCAD-deficient fibroblasts (13).

Mouse tissue mitochondrial acyl-CoA dehydrogenase activities. Our earlier studies in control mouse liver showed that about half of butyryl-CoA dehydrogenation was due to MCAD and half to SCAD under our assay conditions. In the present study, we evaluated the relative contribution of SCAD and MCAD to butyryl-CoA dehydrogenation in two additional tissues: skeletal muscle and fibroblasts (Table 2). In muscle mitochondria from J mice, butyryl-CoA dehydrogenation was 82% of that in Y mice, suggesting that MCAD contributes a greater proportion of activity toward butyryl-CoA in muscle than in liver. However, in the presence of monospecific MCAD antiserum, the J mice had absent SCAD activity with butyryl-CoA, with a similar change in MCAD-catalyzed butyryl-CoA dehydrogenation in both Y and J mice. In fibroblasts from J mice, butyryl-CoA dehydrogenation was 69% of that in Y cells, suggesting that the contribution of MCAD to butyryl-CoA dehydrogenation differs in fibroblasts. Upon addition of MCAD antiserum, butyryl-CoA dehydrogenation was absent in J mice cells, and both Y and J mice had comparable changes in MCAD activity toward butyryl-CoA. There was no contribution of SCAD to apparent octanoyl-CoA dehydrogenation in either tissue, inasmuch as MCAD activity with octanoyl-CoA was identical in both J and Y mice and completely inhibited by MCAD antiserum.

Table 1. *^{3}H-palmitate oxidation by mouse fibroblast monolayers**

Cells	[9,10(n)-^{3}H]palmitate (pmol/mg protein/h)	[15,16(n)-^{3}H]palmitate (pmol/mg protein/h)
Normal controls (Y)	6040 ± 723	4140 ± 492
Mutants (J)	5770 ± 897	1432 ± 98†

* Oxidation of ^{3}H-palmitates was performed as described earlier (13). Fibroblast cultures from two individual mice of each genotype were assayed in duplicate two to three times. Values are mean ± SEM.

† Difference from control significant at $p < 0.01$.

Table 2. *Mitochondrial acyl-CoA dehydrogenase activities in mouse tissue**

	Substrate and additions			
	Butyryl-CoA		Octanoyl-CoA	
Genotype	None	MCAD antiserum	None	MCAD antiserum
Muscle				
Y controls ($n = 3$)	650 ± 191	93 ± 47	4120 ± 965	0 ± 0
J mutants ($n = 6$)	531 ± 167	0 ± 0	4540 ± 615	0 ± 0
% control activity	82%	0%	110%	
Fibroblasts				
Y controls ($n = 4$)	185 ± 31	50 ± 17	816 ± 216	0 ± 0
J mutants ($n = 4$)	127 ± 43	0 ± 0	744 ± 168	0 ± 0
% activity	69%	0%	91%	

* The Y control mice are BALB/cBy and the mutant J mice are BALB/cByJ. The mice were 1 mo old and mitochondria were isolated from muscle as described in Materials and Methods (15) and from fibroblasts as described earlier (1). Antiserum to rabbit anti-pig kidney MCAD (10 μL) was used as previously described (1). Values are pmol/min/mg protein ± S.D.

Kinetic analysis of mouse liver SCAD and MCAD. Apparent Km and V_{max} values of mouse liver SCAD and MCAD for butyryl- and octanoyl-CoA are presented in Table 3. Experiments with J mice estimate V_{max} and Km of mouse MCAD for butyryl-CoA, with a Km of 37 μM. The V_{max} value for butyryl-CoA dehydrogenation in J mice was predictably lower than in Y mice. Experiments with Y mice and MCAD antiserum estimate V_{max} and Km of mouse SCAD for butyryl-CoA; this Km value was 2.7 μM in Y controls. This low Km for butyryl-CoA in Y mice reflects only the affinity of SCAD toward its optimal substrate; as in other studies, SCAD has a higher affinity for butyryl-CoA than does MCAD (3, 4). With butyryl-CoA as substrate, the Km

Table 3. *Apparent Km and V_{max} values for butyryl-CoA and octanoyl-CoA in mouse liver mitochondrial supernatants**

	Substrate and MCAD antiserum					
	Butyryl-CoA (none)		Butyryl-CoA (10 μL)		Octanoyl-CoA (none)	
	Km (μM)	V_{max}	Km (μM)	V_{max}	Km (μM)	V_{max}
Y controls	12†	2090†	2.7‡	600‡	1.2§	6380§
J mutants	37‖	1610‖	0‡	0‡	1.1§	6500§

* Acyl-CoA dehydrogenase activities were measured by the dye-reduction assay in two separate experiments, the results of both were combined, and the apparent Km and V_{max} values were calculated using Eadie-Scatchard plots. Apparent V_{max} values are expressed as pmol/min/mg protein.

† Sum of SCAD and MCAD activities toward toward butyryl-CoA.

‡ Represents only SCAD activity toward butyryl-CoA.

§ Represents only MCAD activity toward octanoyl-CoA.

‖ Represents only MCAD activity toward butyryl-CoA.

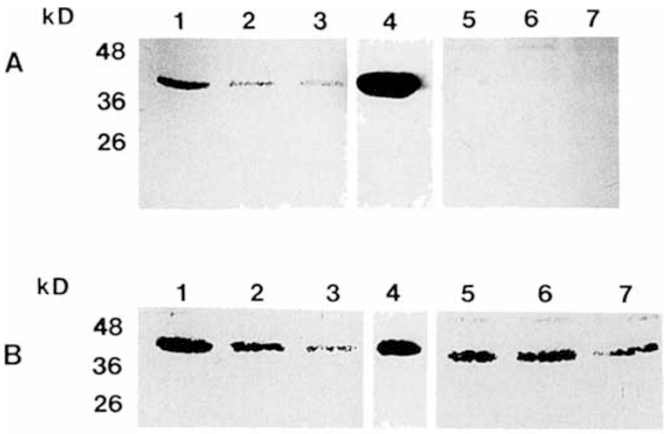

Fig. 1. Immunoblots of SCAD and MCAD antigens in mouse liver mitochondria. *A*, Mouse liver mitochondrial supernatants were electrophoresed in SDS-PAGE (15%) and transferred to a nitrocellulose membrane. Antigen was detected with polyclonal SCAD antiserum and horseradish peroxidase-conjugated goat anti-rabbit IgG. *Lanes 1–3*, three individual Y controls, 200 μg protein each; *lane 4*, pig liver SCAD, 7.5 μg; *lanes 5–7*, three individual J mutants, 200 μg protein each. *B*, As in *A*, except that MCAD antiserum was used. *Lanes 1–3*, Y controls, 200 μg protein; *lane 4*, pig liver MCAD, 7.5 μg; *lanes 5–7*, J mutants, 200 μg protein.

in Y controls was 12 μM, representing the average apparent affinities of both SCAD and MCAD for this substrate.

The V_{max} of J mutants toward butyryl-CoA reflects only MCAD activity toward butyryl-CoA, whereas this value in the Y controls equals the sum of the V_{max} of SCAD and MCAD toward butyryl-CoA, as predicted. The apparent Km of MCAD toward octanoyl-CoA, its optimal substrate, is 1.2 and 1.1 μM in Y and J mice, respectively, similar to those of MCAD from other species (3, 4, 11, 12). In all mice, these similar V_{max} values reflect only MCAD activity toward octanoyl-CoA, because SCAD has no activity toward this substrate in mouse, rat, or human liver (3, 4).

Immunochemical studies of SCAD and MCAD. Immunoblot analysis of mouse liver revealed no SCAD antigen in three J mice (Fig. 1*A*, *lanes 5, 6,* and *7*), whereas three Y mice had easily detectable levels (Fig. 1*A*, *lanes 1, 2,* and *3*). MCAD antigen was present in comparable amounts in both Y and J mice (Fig. 1*B*). Both SCAD and MCAD from mouse liver mitochondria migrated identically to their purified porcine counterparts, suggesting that their mature molecular weights are similar to those reported in other mammals (3, 4, 11). SCAD antigen was also undetectable in J mouse muscle mitochondria, but Y mice had easily detectable levels. As in liver, mouse muscle SCAD had a mobility identical to that of pig liver SCAD (data available on request). Fibroblast mitochondria from J mice also had no SCAD antigen (Fig. 2*A*, *lanes 3* and *4*), whereas SCAD antigen was easily detectable in Y mice (Fig. 2, *lanes 1* and *2*), even after applying 2- to 3-fold more mitochondrial protein from J than from Y mice. Again, MCAD antigen was present in both Y and J mouse fibroblast mitochondria (Fig. 2*B*).

Incubation of fibroblast monolayers with [^{35}S]methionine for 12 h followed by immunoprecipitation with antisera against either SCAD or MCAD revealed no newly synthesized SCAD polypeptide in J fibroblasts (Fig. 3*A*, *lanes 1* and *4*), whereas MCAD was synthesized comparably in both Y and J cells (Fig. 3*B*, *lanes 1* and *4*). Competition experiments with unlabeled antigens confirmed the identities of the newly synthesized poly-

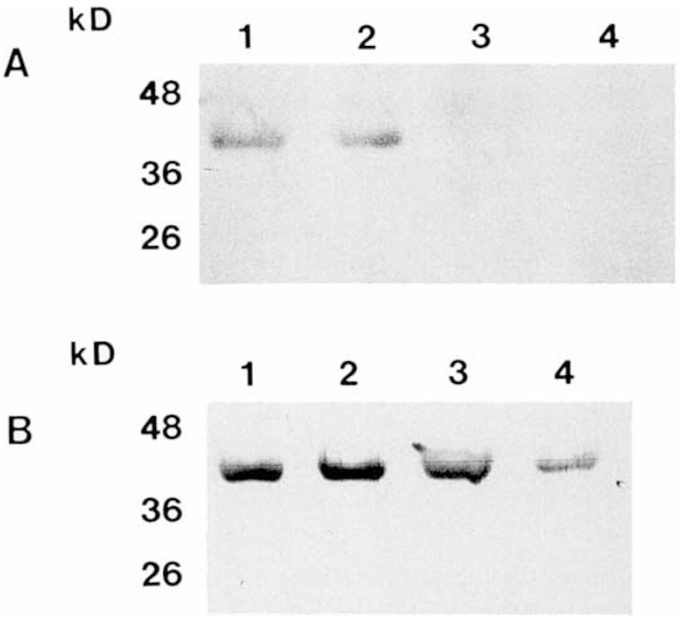

Fig. 2. Immunoblots of SCAD and MCAD antigens in mouse fibroblast mitochondria. *A*, Mouse fibroblast mitochondrial supernatants were electrophoresed in SDS-PAGE (12%) and transferred to a Millipore Immobilon membrane. Antigen was detected with polyclonal SCAD antiserum and alkaline phosphatase-conjugated goat anti-rabbit IgG. *Lanes 1* and *2*, Y controls, 240 and 96 μg protein, respectively; *lanes 3* and *4*, J mutants, 620 and 310 μg protein, respectively. *B*, As in *A*, except that MCAD antiserum was used. *Lanes 1* and *2*, Y controls, 240 and 96 μg protein, respectively; *lanes 3* and *4*, J mutants, 620 and 310 μg protein, respectively.

Fig. 3. SCAD and MCAD biosynthesis in mouse fibroblasts. *A*, Y control (*lanes 1–3*) and J mutant (*lanes 4–6*) fibroblast monolayers in 25 cm^2 flasks were labeled with [^{35}S]methionine (60 μCi/mL) for 12 h and then solubilized. The extracts were immunoprecipitated by 10 μL of rabbit anti-pig SCAD antiserum (*lanes 1, 2, 4,* and *5*) or by 10 μL of preimmune antiserum (*lanes 3* and *6*) using *S. aureus* cells. The immunoprecipitates were analyzed by SDS-PAGE and revealed by fluorography. *Lanes 2* and *5* are identical to *lanes 1* and *4*, respectively, except that 10 μg of unlabeled pig liver SCAD were added before antiserum addition (competition experiment). *B*, As in *A*, except that the extracts were immunoprecipitated with rabbit anti-pig MCAD antiserum and 5 μg of unlabeled pig liver MCAD were added in *lanes 2* and *5*.

peptides as SCAD and MCAD (Fig. 3*A* and *B, lanes 2* and *5*), whereas preimmune serum did not coprecipitate any radiolabeled species (Fig. 3*A* and *B, lanes 3* and *6*).

DISCUSSION

Both SCAD-deficient mice and humans share similiar clinical characteristics, including a tendency toward hypoglycemia, hepatic lipid deposition, and similar organic acidurias (1, 2, 6). In man, SCAD deficiency causes the accumulation and excretion of short-chain acyl-CoA metabolites, such as ethylmalonate, adipate, and methylsuccinate (1, 2). The BALB/cByJ mice also excrete ethylmalonic and methylsuccinic acids, as well as large amounts of *n*-butyrylglycine and *n*-butyrylcarnitine, which are not prominent in human patients (1, 2). This probably results from differences between rodents and humans in glycine conjugation of various acyl-CoA derivatives, particularly butyryl-CoA (19). Butyrylglycine excretion in the mouse is analogous to the isovalerylglycine excretion found in the human disease, isovaleric acidemia, due to isovaleryl-CoA dehydrogenase deficiency (20). In the mouse, the increased flux of butyryl-CoA through this detoxification pathway may explain their relative resistance to acute clinical disease. This increased capacity for glycine conjugation of butyryl-CoA may also reduce intramitochondrial acyl-CoA concentrations, preserving the intracellular carnitine pool and maintaining normal mitochondrial acyl-CoA/reduced CoA ratios.

Fibroblasts from both SCAD-deficient humans (1) and mice oxidize short-chain fatty acids poorly. J mouse fibroblasts oxidized [9,10(n)-^{3}H]palmitate normally, but [15,16(n)-^{3}H]palmitate poorly, as did SCAD-deficient human fibroblasts (13, 21). Because [15,16(n)-^{3}H]palmitate is converted to [3,4(n)-^{3}H]butyrate after seven successive rounds of β-oxidation, oxidation of and tritiated water formation from this substrate require SCAD activity. Because the C_4-C_6 acyl-CoA derived from [9,10(n)-^{3}H]-palmitate are unlabeled, its oxidation should be normal in SCAD-deficient cells. The [15,16(n)-^{3}H]palmitate oxidation in J mice is somewhat lower (35% of control) than might be predicted from the observed butyryl-CoA dehydrogenation activities in the mutant mice tissues (44–82% of control). However, the observed oxidation rates of the ^{3}H-palmitates in intact fibroblasts may not yield valid estimates of tissue or total body β-oxidation flux. These intact fibroblast studies permit rapid identification of SCAD deficiency in both man and mouse, simplifying detection of this disorder and its differentiation from those β-oxidation disorders impairing medium- and long-chain fatty acid oxidation.

In SCAD-deficient mice, liver, muscle, and fibroblast SCAD and MCAD activities were qualitatively similar to those reported in human SCAD deficiency (1, 2). Human liver SCAD and MCAD are identical in electrophoretic mobility and molecular weight to those in human fibroblasts and closely resemble their rat liver counterparts (3, 4, 22, 23). However, Turnbull *et al.* (9) and Farnsworth *et al.* (10) reported an adult patient with an isolated muscular deficiency of SCAD activity and antigen. A potential explanation for the biochemical findings in this patient would involve a mutation of a muscle-specific SCAD isozyme, although there is no direct evidence to support this possibility (9, 10). We found that fibroblasts from this patient had normal SCAD and MCAD activities (1) and oxidized both [9,10(n)-^{3}H]- and [15,16(n)-^{3}H]palmitates normally, arguing against a primary β-oxidation defect in this patient (data available on request). The apparent muscle SCAD deficiency in this patient may be secondary to another as yet unidentified metabolic defect, such as the riboflavin-responsive SCAD deficiency reported in a multiple acyl-CoA dehydrogenation disorder patient by DiDonato *et al.* (24). Furthermore, the SCAD-deficient mice lack SCAD activity in three tissues: liver, muscle, and fibroblasts, suggesting that there is no muscle-specific SCAD isozyme either in this species or, by extension, in man.

Tissue-specific differences in the ratio of SCAD to MCAD activities and the kinetic properties of mouse SCAD may help explain the mild disease noted in the mutant mice. Under our assay conditions, MCAD activity in mouse muscle toward butyryl-CoA appears higher than that in either mouse liver, mouse fibroblasts, or human fibroblasts, representing 82% of total butyryl-CoA dehydrogenation activity in this tissue. The increased MCAD activity toward butyryl-CoA in muscle may explain the lack of clinical symptoms in the J mouse, inasmuch as total body short-chain acyl-CoA dehydrogenation could be largely catalyzed by muscle MCAD. Unfortunately, there are no objective measures of total body short- or medium-chain fatty acid oxidation flux in control or mutant mice to test this hypothesis. Comparing the kinetic parameters of mouse liver SCAD and MCAD to the published values for the corresponding human liver enzymes reveals that the Km of purified human liver MCAD toward butyryl-CoA is 71.4 μM (4), 2-fold higher than the apparent Km of mouse liver mitochondrial MCAD toward butyryl-CoA. This lowered Km (or increased affinity) of mouse liver MCAD for butyryl-CoA might also help explain the mild overall impairment of butyryl-CoA dehydrogenation flux noted in the SCAD-deficient mice (7).

The absence of SCAD antigen in liver, muscle, and fibroblasts from the mutant mouse also argues against the existence of tissue-specific SCAD isozymes in mammals. However, in the human SCAD-deficient patients, SCAD antigen levels varied. Only one SCAD-deficient patient had no detectable SCAD antigen on direct immunoblotting of fibroblasts, whereas the other two had normal amounts of normal molecular weight antigen (25). In biosynthesis and immunoprecipitation experiments, all three patients had normal amounts of newly synthesized enzyme polypeptide; thus, SCAD in the first patient may be extremely labile and rapidly degraded (25, 26). The synthesis and size of SCAD mRNA in three SCAD-deficient patients were also normal (23). These results suggested that the SCAD defects in man are caused by at least two different point mutations, a suspicion that has been recently confirmed (27). In contrast, our data suggest that SCAD gene structure, transcription, or translation is profoundly altered in the mutant J mice. The SCAD-deficient mouse probably has a mutation at or near the Bcd-1 locus on chromosome 5, such that either the mRNA or polypeptide is not synthesized or is extremely unstable. Preliminary molecular studies suggest that there is a deletion in the 5′ region of the SCAD gene in the mutant mice (28). Further experiments are underway to elucidate more precisely this molecular defect.

These results reflect both the advantages and the limitations of studying animal models of human metabolic diseases. Species differences in alternative metabolic or detoxification pathways, enzyme activities, and substrate affinities may yield significant variations in clinical, metabolic and biochemical phenotypes. However, animal models permit a breadth and depth of experimentation clearly impossible with the vast majority of human patients and disorders.

Acknowledgments. The authors thank Drs. Carole Hall and Colin Thorpe for provision of the pure enzymes and Dr. Kay Tanaka for his advice and support.

REFERENCES

1. Amendt BA, Greene C, Sweetman L, Cloherty J, Shih V, Moon A, Teel L, Rhead WJ 1987 Short-chain acyl-CoA dehydrogenase deficiency: clinical and biochemical studies in two patients. J Clin Invest 79:1303–1309
2. Coates PM, Hale D, Finocchiaro G, Tanaka K, Winter S 1988 Genetic deficiency of short-chain acyl-coenzyme A dehydrogenase in cultured fibroblasts from a patient with muscle carnitine deficiency and severe skeletal muscle weakness. J Clin Invest 81:171–175
3. Ikeda Y, Ikeda KO, Tanaka K 1985 Purification and characterization of short-chain, medium-chain and long-chain acyl-CoA dehydrogenases from rat liver mitochondria. J Biol Chem 260: 1311–1325
4. Finocchiaro G, Ito M, Tanaka K 1987 Purification and properties of short-chain acyl-CoA, medium-chain acyl-CoA, and isovaleryl-CoA dehydrogenases from human liver. J Biol Chem 262:7982–7989

5. Ikeda Y, Keese SM, Fenton UA, Tanaka K 1987 Biosynthesis of four rat liver mitochondrial acyl-CoA dehydrogenases: *in vitro* synthesis, import into mitochondria, and processing of their precursors in a cell-free system and in cultured cells. Arch Biochem Biophys 252:662–674
6. Wood PA, Armstrong D, Sauls D, Davisson MT 1988 Screening mutant mice for inborn errors of metabolism. Lab Anim Sci 38:15–19
7. Wood PA, Amendt BA, Rhead WJ, Millington DS, Inoue E, Armstrong D 1989 Short-chain acyl-coenzyme A dehydrogenase deficiency in mice. Pediatr Res 25:38–43
8. Prochazka M, Leiter EH 1986 A null activity variant found at the butyryl-CoA dehydrogenase (Bcd-1) locus in BALB/cByJ subline. Mouse Newslett 75:31
9. Turnbull DM, Bartlett K, Stevens DL, Alberti G, Gibson G, Johnson MA, McCulloch AJ, Sherratt HS 1984 Short-chain acyl-CoA dehydrogenase deficiency associated with a lipid storage myopathy and secondary carnitine deficiency. N Engl J Med 311:1232–1236
10. Farnsworth L, Shepherd IM, Johnson MA, Bindoff LA, Turnbull DM 1990 Absence of immunoreactive enzyme protein in short-chain acylcoenzyme A dehydrogenase deficiency. Ann Neurol 28:717–720
11. Thorpe C, Matthews R, Williams C 1979 Acyl-coenzyme A dehydrogenase from pig liver mitochondria. Biochemistry 18:331–337
12. Hall CL, Kamin H 1975 The purification and some properties of electron transfer flavoprotein and general acyl-coenzyme A dehydrogenase from pig liver mitochondria. J Biol Chem 250:3476–3486
13. Moon A, Rhead WJ 1987 Complementation analysis of fatty oxidation disorders. J Clin Invest 79:59–64
14. Johnson D, Lardy H 1967 Isolation of liver or kidney mitochondria. Methods Enzymol 10:94–96
15. Makinen MW, Lee CP 1968 Biochemical studies of skeletal muscle mitochondria. Arch Biochem Biophys 126:75–82
16. Rhead WJ, Amendt BA, Fritchman KS, Felts SJ 1983 Dicarboxylic aciduria: deficient [1-^{14}C]octanoate oxidation and medium chain acyl-CoA dehydrogenase activity in fibroblasts. Science 221:73–75
17. Laemmli UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 277:680–685
18. Towbin H, Staehelin T, Gordon J 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354
19. Kolvraa S, Gregersen N 1986 Acyl-coA:glycine N-acyltransferase: organelle localization and affinity toward straight- and branched-chain acyl-CoA esters in rat liver. Biochem Med Metab Biol 36:98–105
20. Krieger I, Tanaka K 1976 Therapeutic effects of glycine in isovaleric acidemia. Pediatr Res 10:25–29
21. Rhead WJ 1989 Biochemical screening for inborn errors of fatty acid oxidation in cultured fibroblasts: an overview. Prog Clin Biol Res 321:365–382
22. Ikeda Y, Hale DE, Keese SM, Coates PM Tanaka K 1986 Biosynthesis of variant medium-chain acyl-CoA dehydrogenase in cultured fibroblasts from patients with medium-chain acyl-CoA dehydrogenase deficiency. Pediatr Res 20:843–847
23. Ikeda Y, Tanaka K 1987 Immunoprecipitation and electrophoretic analysis of four human acyl-CoA dehydrogenases and electron transfer flavoprotein using antibodies raised against the corresponding rat enzyme. Biochem Med Metab Biol 37:329–334
24. DiDonato S, Gellera C, Peluchetti D, Uziel G, Antonelli A, Lus G, Rimoldi M 1989 Normalization of short-chain acylcoenzyme A dehydrogenase after riboflavin treatment in a girl with multiple acyl-coenzyme A dehydrogenase-deficient myopathy. Ann Neurol 24:479–484
25. Naito E, Indo Y, Tanaka K 1989 Short-chain acyl-coenzyme A dehydrogenase (SCAD) deficiency: immunochemical demonstration of molecular heterogeneity due to variant SCAD with differing stability. J Clin Invest 84:1671–1674
26. Naito E, Ozasa H, Ikeda Y, Tanaka K 1989 Molecular cloning and nucleotide sequence of complementary DNAs encoding human short-chain acyl-coenzyme A dehydrogenase and the study of the molecular basis of human short-chain acyl-coenzyme A dehydrogenase deficiency. J Clin Invest 83:1605–1613
27. Naito E, Indo Y, Tanaka K 1990 Identification of two variant short-chain acyl-coenzyme A dehydrogenase alleles, each containing a different point mutation in a patient with short-chain acyl-coenzyme A dehydrogenase deficiency. J Clin Invest 85:1575–1582
28. Kelly CL, Hinsdale ME, Wood PA 1990 Cloning and molecular studies of short-chain acyl-CoA dehydrogenase in mice using polymerase chain reaction. Am J Hum Genet 47:A161(abstr)