

Retrospective biochemical screening of fatty acid oxidation disorders in postmortem livers of 418 cases of sudden death in the first year of life

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Objective: Fatty acid oxidation (FAO) disorders are frequently reported as the cause of sudden and unexpected death, but their postmortem recognition remains difficult. We have devised a biochemical protocol in which informative findings in liver tissue are microvesicular steatosis, elevated concentrations of C₈-C₁₆ fatty acids, glucose depletion, and low carnitine concentration.

Study design: We analyzed 27 cases representing five FAO disorders and compared the results with those obtained in a retrospective blinded analysis of 418 cases of sudden infant death (313 SIDS, 45 infections, and 34 accidents and abuse).

Results: All cases of accidents and abuse correctly tested negative. Among the others, 25 (6%) showed at least two abnormal findings. Of these, 14 closely matched the biochemical profiles seen in specific FAO disorders. These included 2 cases with medium-chain acyl-CoA dehydrogenase deficiency, 4 cases consistent with glutaric acidemia type 2, 4 cases with either very long-chain acyl-coenzyme A dehydrogenase deficiency or long-chain 3-hydroxy-acyl-coenzyme A dehydrogenase deficiency, and 4 cases predicted to be affected with carnitine uptake defect.

Conclusion: The results of this study support the view that approximately 5% of all cases of sudden infant death are likely caused by an FAO disorder. (*J Pediatr* 1998;132:924-33.)

Inherited defects of fatty acid oxidation represent a rapidly expanding class of metabolic diseases.¹⁻⁵ More than 25 enzymes and transporters are involved in

See editorial, p. 913.

this pathway, and up to 18 distinct inherited disorders have been reported in human beings. The mitochondrial oxidation of fatty acids is critical in supplying energy during periods of fasting once glycogen stores have been depleted. The heart, skeletal muscle, and liver are particularly dependent on this pathway,⁴ and characteristic manifestations of FAO disorders are metabolic decompensation during fasting, cardiac and skeletal myopathy, and hepatic failure.¹⁻³

CUD	Carnitine uptake defect
FAO	Fatty acid oxidation
GA2	Glutaric acidemia type 2
LCHAD	Long-chain 3-hydroxy acyl-coenzyme A dehydrogenase
MCAD	Medium-chain acyl-coenzyme A dehydrogenase
SIDS	Sudden infant death syndrome
VLCAD	Very long-chain acyl-coenzyme A dehydrogenase

A sudden and unexpected death triggered by fasting intolerance and fulminant metabolic decompensation is a frequently reported outcome of this group of disorders.⁵⁻⁸ When the diagnosis of an FAO disorder is made, it is not uncom-

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mon to find a family history of sudden death in one or more siblings.⁹ Because many sudden and unexpected deaths occur before the child is 1 year old, they may fulfill the diagnostic criteria for sudden infant death syndrome.¹⁰

The involvement of FAO disorders as causes of SIDS remains controversial. The first observation was made in 1976 on the basis of the finding of hepatic fatty infiltration in 5% of 200 SIDS cases.¹¹ In 1984, the diagnosis of medium-chain acyl-coenzyme A dehydrogenase deficiency in a SIDS victim was reported.¹² Several studies in the following years indicated that FAO disorders might account for up to 5% of SIDS cases.⁵⁻⁸ Since the recognition of the A985G common mutation in the MCAD gene,¹³ several studies investigating the expression of this allele in SIDS cases have reported its incidence in the homozygous state as less than 1%.¹⁴⁻¹⁸ Unfortunately, a serious repercussion of the publication of these studies was the tendency to ignore all other FAO disorders as possible causes of sudden death when the common MCAD mutation was not detected.

To date, the effort to investigate the collective contribution of FAO disorders to sudden and unexpected death in early life, with or without a diagnosis of SIDS, has involved the analysis of body fluids, including dried spots of urine¹⁹ and blood²⁰ collected during newborn screenings, or postmortem urine and vitreous humor.²¹ These approaches are not always possible to implement because of the frequent unavailability of postmortem samples. To overcome this problem, we have developed a diagnostic protocol for the biochemical screening of FAO disorders in frozen postmortem liver specimens.²² We report here the retrospective and blinded application of this protocol to the analysis of 418 cases of sudden and unexpected death that occurred in the first year of life, 313 of which were diagnosed as SIDS.

METHODS

Confirmed Cases with FAO Disorders

We examined frozen liver specimens from 27 cases with proven FAO disorder

Table I. Cause-of-death groups and distribution of abnormal findings

Group	No. of cases	Not informative	Results of initial screening*	
			Single	Multiple
SIDS	313	259	40	14
Infectious diseases				
Pneumonia	29	18	8	3
Sepsis	9	0	5	4
Meningitis	1	0	0	1
Myocarditis	6	4	1	1
Accidents	26	22	4	0
Abuse	8	6	2	0
Congenital anomalies				
Brain	5	4	1	0
Heart	7	3	3	1
Lungs	5	2	2	1
Cardiomyopathy	3	1	2	0
Other causes	6	4	2	0
Subtotal (non-SIDS)	105	64	30	11
Total	418	323	70	25
(%)		(77)	(17)	(6)

*Not including total carnitine determination in liver homogenate.

Table II. Scoring of liver fatty infiltration in positive controls

FAO disorder	No. of cases	Fatty infiltration of the liver*			
		Negative (-)	Mild (+)	Moderate (++)	Diffuse (+++)
MCAD deficiency	10	0	0	2	8
GA2	4	0	0	0	4
VLCAD deficiency	5	0	2	1	2
LCHAD deficiency	5	1 [†]	0	2	2
Carnitine uptake defect	3	0	1	0	2
Total	27	1	3	5	18
(%)		(4)	(11)	(18)	(67)

*For scoring criteria, see Methods section.

[†]For details, see reference 33.

Table III. Summary of abnormal findings in positive controls

FAO disorder	No. of cases	No. of abnormal findings				
		0/4	1/4	2/4	3/4	4/4
MCAD deficiency	10	0	0	3	5	2
GA2	4	0	0	0	4	0
VLCAD deficiency	5	0	0	1	3	1
LCHAD deficiency	5	1	0	2	2	0
Carnitine uptake defect	3	0	0	1	2	0
Total	27	1	0	7	16	3
(%)		(4)		(26)	(59)	(11)

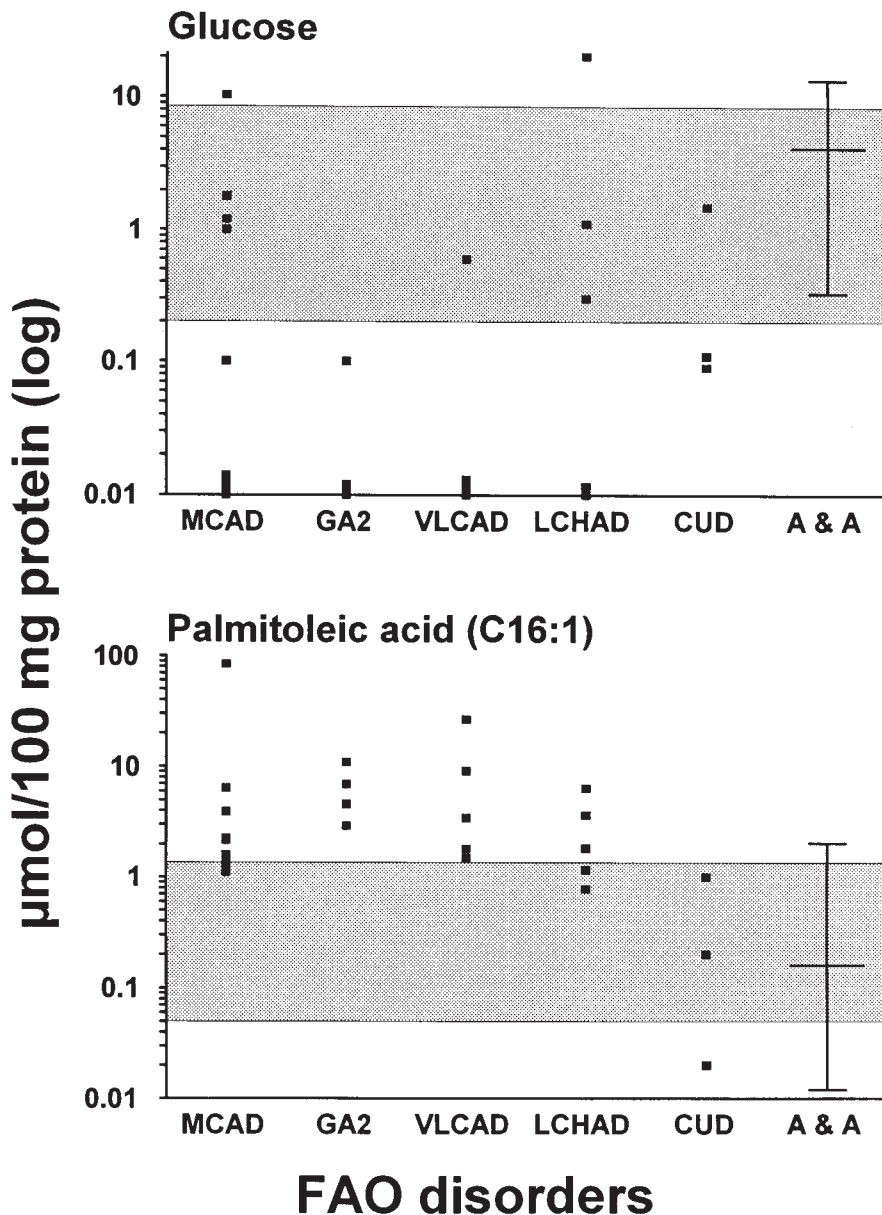


Fig. 1. Glucose (top) and palmitoleic acid (bottom) concentrations in postmortem liver specimens from 27 confirmed cases affected with an FAO disorder and 34 cases of death in the first year of life caused by accidents or abuse (A & A). Results are presented on a log scale. Gray areas correspond to the 5th to 95th percentile range of control values.²² In the A & A group, vertical bar corresponds to full range of values, and horizontal bar, to median value. MCAD, Medium chain acyl-CoA dehydrogenase deficiency; LCHAD, long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency; VLCAD, very long-chain acyl-CoA dehydrogenase deficiency.

ders, either prospectively diagnosed in our laboratory or provided by other investigators for the purpose of this study. The 27 cases included 10 cases with MCAD deficiency, 4 cases with either α - or β -electron transfer flavoprotein deficiency or electron transfer flavoprotein-ubiquinone oxidoreductase deficiency (collectively referred to as GA2), 5 cases

with very long-chain acyl coenzyme A dehydrogenase deficiency, 5 cases with long-chain 3-hydroxy acyl-coenzyme A dehydrogenase deficiency, and 3 cases with carnitine uptake defect. Nineteen of these cases were initially diagnosed as cases of sudden and unexpected death (15 SIDS). Biochemical results of 12 of these cases have been partially reported

in previous studies,^{22,23} or as case reports.²⁴⁻²⁷

Population Study

This study was approved by the Yale Human Investigation Committee (HIC no. 5125). Among the cases of sudden death throughout the state of Maryland from 1985 to 1993, 442 frozen liver samples were obtained from the Brain and Tissue Bank for Developmental Disorders at the University of Maryland. The release of these samples was contingent upon an anonymity agreement that no information except sex, race, age at the time of death, liver histologic findings, and cause of death would be disclosed and that no effort would be made by the investigators to contact the families of retrospectively diagnosed cases. All autopsies were performed by pathologists of the Office of the Chief Medical Examiner of the State of Maryland. These samples were randomly coded by personnel not directly involved in this study and identified only by a number until all analyses were completed.

Twenty-five samples were later excluded because of insufficient tissue available, age greater than 1 year, or unavailability of the autopsy report. Bile samples were also obtained from 32 of the cases enrolled toward the end of the study.²⁵

Four hundred eighteen liver samples were included in this study (Table I). After a complete postmortem examination, 313 of these cases (75%) were given a diagnosis of SIDS by the case pathologist. The remaining 105 cases were categorized as summarized in Table I. The 34 cases of accidents and abuse were designated as the negative control group, because it was assumed that none of these infants had an underlying FAO disorder.

Methods of Analysis

Liver tissue slides were scored as to the presence or absence of microvesicular fatty infiltration on histologic examination with hematoxylin-eosin and oil red O stains. Fatty changes were scored as diffuse (>2/3 of hepatocytes affected, shown in Tables II, IV, and V as +++), moderate (1/3 to 2/3, ++), mild (<1/3, +), and negative (-). Quantitative analyses of glucose and C₈-C₁₈ free fatty acid levels were per-

formed by gas chromatography and gas chromatography/mass spectrometry.²² Total carnitine concentrations^{28,29} were determined in a sample if at least one abnormal result was found in the initial screening, in all accident and abuse cases, and in 37 randomly chosen SIDS cases without any abnormal results. Bile acylcarnitine profiles were performed by using electrospray tandem mass spectrometry.²⁵ Finally, 309 of the 418 cases were assayed by polymerase chain reaction for the MCAD deficiency common mutation A985G, including 24 of the 25 cases with multiple abnormal results.¹⁵

RESULTS

Positive Control Group

The positive control group consisted of 27 infants and children with known FAO disorders. With one exception,²⁶ the age at the time of death ranged from 2 days to 5 years. Diffuse microvesicular fatty infiltration of the liver was seen in 67% of the cases (Table II). Although all cases with MCAD deficiency and GA2 had moderate to diffuse liver steatosis, one third of the remaining cases showed only mild or negative fatty changes. In partial disagreement with a recent report of the postmortem findings in 15 cases with LCHAD deficiency,³⁰ our findings tend to underscore the unreliability of steatosis as the sole criterion for suspecting a possible underlying FAO disorder during the postmortem evaluation of a case of sudden death.

Biochemical findings consistent with an FAO disorder include elevated concentration of palmitoleic acid ($C_{16:1}$ fatty acid) and glucose depletion (Fig. 1). The metabolic profiles obtained from frozen liver specimens in the known cases with FAO disorders were readily distinguishable from our previously established reference values (shown as gray areas in Figs. 1 to 4). These profiles corresponded with the 5th to 95th percentile range in a group of 100 specimens randomly selected from the population study before the completion of any of the analyses²² and from the accidents and abuse control group (Fig. 1).

The total liver carnitine concentration was reduced in 9 of 27 cases (Fig. 2). The

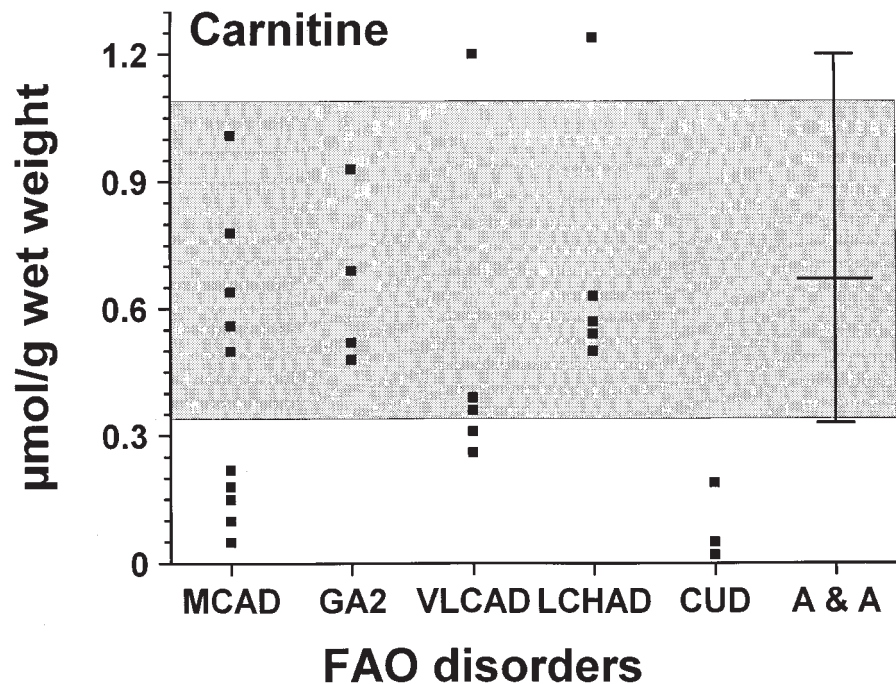


Fig. 2. Total carnitine concentration in postmortem liver specimens from 27 confirmed cases affected with an FAO disorder and 34 cases of death in the first year of life caused by accidents or abuse. Gray area corresponds to range of control values.²⁹

esterified fraction in the tissue homogenate was negligible (data not shown). Although the overall range was lower in the FAO disorder group, a substantial overlap with the control groups existed. However, this parameter was instrumental in the correct identification of cases affected with CUD and therefore represents a critical component of the diagnostic protocol used in this study.

The level of *cis*-4-decenoic acid ($C_{10:1}$), a pathognomonic marker in plasma of individuals with MCAD deficiency and GA2,³¹ was elevated in 8 of 10 and 3 of 4 cases with these respective disorders (Fig. 3). Although 3 cases tested negative for this parameter, they were readily identifiable as possible FAO disorders by the finding of microvesicular fatty infiltration, elevated concentration of palmitoleic acid, undetectable glucose, and, in one, low carnitine concentration. The concentration of octanoic acid was also elevated in a similar proportion of cases (Fig. 3).

The levels of tetradecadienoic acid ($C_{14:2}$) and tetradecenoic acid ($C_{14:1}$) were elevated in the liver homogenate of infants with VLCAD deficiency (Fig. 4).

The level of the $C_{14:1}$ species, which often is considered to be a specific biochemical marker of VLCAD deficiency in plasma,³² was actually elevated in 13 of 24 disease control samples, not including the CUD cases.

In one case of LCHAD deficiency,³³ the findings for all diagnostic criteria were negative. On the other hand, the acylcarnitine profile obtained from postmortem bile was typical of the disorder.²⁵ The results of the four other cases were clearly abnormal in multiple parameters, including moderate to diffuse fatty infiltration (4 of 5 cases), elevated $C_{16:1}$ concentration (3 of 5 cases), and glucose depletion (3 of 5 cases). Overall, it was very difficult to differentiate these cases from those with VLCAD deficiency.

The metabolite profiles in three CUD cases showed glucose depletion (2 of 3 cases), low-normal concentrations of C_8 - C_{18} fatty acids, and low carnitine concentration. The one case of CUD with a normal glucose level was believed to involve an FAO disorder because of an extremely low carnitine concentration (0.05 µmol/gm wet

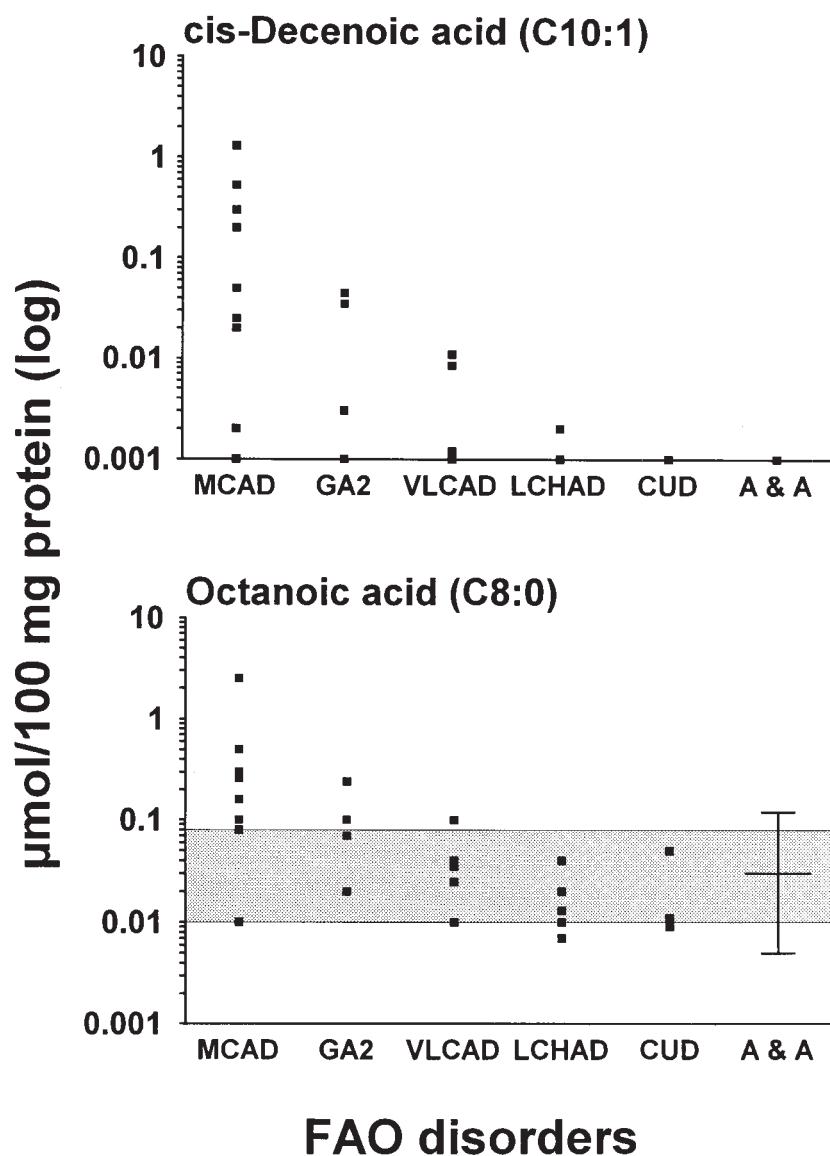


Fig. 5. Cis-4-decenoic acid (top) and octanoic acid (bottom) concentrations in postmortem liver specimens from 27 confirmed cases affected with an FAO disorder and 34 cases of death in the first year of life caused by accidents or abuse. Results are presented on a log scale. Gray area corresponds to range of control values.²²

weight) in association with marked fatty infiltration of the liver and cardiac muscle.²⁷

In summary, 3 of the 27 cases with known FAO disorders tested positive for all four parameters, whereas 7 cases (26%) showed only two abnormal results (Table III). On the basis of these results, the cutoff for the biochemical screening of the population study was set as any combination of at least two abnormal findings among the four diagnostic criteria.

Sudden Death Cases

Ninety-five livers (23%, Table I) showed at least one abnormal result in the initial screening on the basis of three criteria: elevated levels of C₁₀-C₁₆ unsaturated fatty acids (33 cases, 8%), glucose depletion (60, 14%), and steatosis of the liver (37, 9%). These samples were then analyzed for carnitine content, and 16 cases (17% of those previously found to have at least 1 abnormal finding) showed a concentration below 0.34 mmol/gm wet weight.

After a low carnitine concentration was included as a fourth criterion for determining an abnormal pattern, individual cases were suspected of having a possible underlying FAO disorder when the findings for at least two of the four diagnostic criteria were abnormal. None of the cases with autopsy-listed cause of death as accidents and abuse showed abnormal test results for more than one parameter and accordingly were all classified as negative before their causes of death were disclosed (Table I).

Sudden Death Cases Diagnosed as SIDS

The cause of death in 313 of the 418 autopsy reports was listed as SIDS (Table I). In this group the male to female ratio was 1.3:1 and the mean age at time of death was 3 months. The racial distribution was 50% black, 49% white, and 1% other. In the remaining 104 cases (non-SIDS group), the male to female ratio was 1.5:1, which was increased relative to the SIDS group as a result of male predominance in the accidents and abuse subgroup (1.8:1). The mean age at time of death was 5 months. The racial distribution was 54% black, 44% white, and 2% other.

Fourteen cases (4%) showed two or more abnormal criteria suggestive of an FAO disorder (Table IV). Among these 14 cases, the mean age at time of death was 3 months and the racial distribution was equal to that of the entire study group. In one white boy, who died at age 5 months (no. 367, Table IV), the biochemical findings strongly suggested either MCAD deficiency or GA2. A diagnosis of MCAD deficiency was confirmed by evidence that the patient was homozygous for the A985G mutation. Another case (#202) showed less pronounced abnormalities, but a possible diagnosis of MCAD deficiency also was considered because he was found to be heterozygous for the A985G mutation of the MCAD gene. Although no additional genotyping was performed to characterize the presence of a second mutant allele, the association of this genotype with the abnormal biochemical findings is unlikely to represent a coincidence because the only other heterozygote found among

309 of the 418 cases included in this study was a SIDS case with a completely uninformative profile (0/4 abnormal findings). Two additional cases (#s 74 and 338) had profiles showing elevated $C_{10:1}$ concentration (0.009 and 0.02 $\mu\text{mol}/100$ mg protein, respectively; range in 14 patients with MCAD and GA2, <0.001 to 1.3; median, 0.03), glucose depletion, and fatty changes also suggestive of possible MCAD deficiency or GA2. In addition, one of these cases had highly elevated $C_{14:1}$ and $C_{16:1}$ concentrations. Because both cases were homozygous normal for the MCAD A985G mutation, these cases were considered more likely to have had GA2, although an MCAD genotype non-G/non-G cannot be excluded a priori.

Case no. 233 tested abnormal in all categories but had only mild fatty changes of the liver. The concentration of octanoic acid was not elevated. On the basis of experience gathered from the analysis of the positive controls, we suspected a diagnosis of either VLCAD deficiency (considering the low carnitine concentration) or LCHAD deficiency, but not GA2. Finally, three of the abnormal SIDS cases (nos. 47, 57, and 401, Table IV) revealed fatty changes, glucose depletion, and low carnitine concentrations, a profile consistent with that observed in three confirmed cases with CUD. In one of these families, an older sibling had also died of SIDS in the first year of life. The results in the remaining six cases were not sufficiently specific to allow a presumptive diagnosis. These cases may belong to a category of other known FAO disorders that were not included in the disease controls of this study, particularly disorders of the carnitine cycle. In the future, it may be possible to extend our method of comparison to confirmed cases to include such disorders.

Non-SIDS Sudden Death Cases

In the non-SIDS group, 11 cases showed abnormal results for two or more criteria, suggestive of a possible FAO disorder (Table V). Of particular interest was the high proportion of cases (9 of 45 [20%]) that were classified as infections and showed multiple abnormal results.

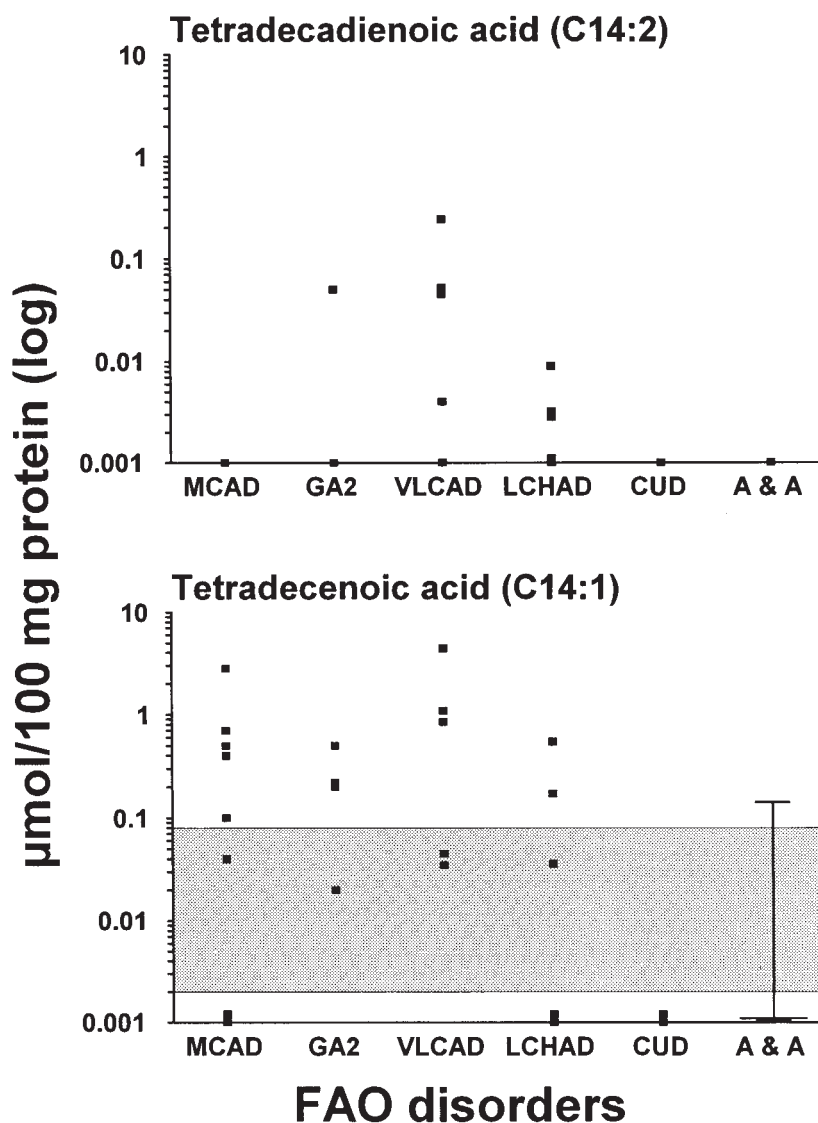


Fig. 4. Tetradecadienoic acid (top) and tetradecenoic acid (bottom) concentrations in postmortem liver specimens from 27 confirmed cases affected with an FAO disorder and 34 cases of death in the first year of life caused by accidents or abuse. Results are presented on a log scale. Gray area corresponds to range of control values.²²

The male to female ratio was 1.2:1, the mean age at time of death was 4 months, and there was a predominance of black infants (7 cases).

Three cases (nos. 69, 193, and 238, Table V) each had three abnormal findings with profiles suggestive of possible MCAD deficiency or GA2. Because they did not carry the MCAD A985G mutation, these cases were tentatively considered more likely to have had GA2. In one case (no. 238), the reported cause of death was bronchopulmonary dysplasia as a result of prematurity and congenital brain

abnormalities. These findings were consistent with a possible diagnosis of GA2.³⁴ Three cases (nos. 216, 350, and 440) were given a possible diagnosis of either VLCAD deficiency or LCHAD deficiency. In one case (no. 216), the concentration of tetradecadienoic acid ($C_{14:2}$) was 0.52 $\mu\text{mol}/100$ mg protein, more than double the highest value detected in all disease controls (range in 10 VLCAD and LCHAD cases, <0.001 to 0.24; median, 0.004). The concentration of tetradecenoic acid ($C_{14:1}$, 0.27 $\mu\text{mol}/100$ mg protein) was also clearly elevated. In an-

Table IV. SIDS cases with multiple abnormal results

Case No.	Cause of death	Age (mo)	Gender/race	Unsaturated fatty acids	Glucose	Total carnitine	Steatosis	MCAD alleles	Abnormal findings	Possible diagnosis
47	SIDS	2	F/AA	/	0.17	0.15	+	A/A	3/4	CUD
57	SIDS	2	M/AA	/	<0.01	0.23	+++	A/A	3/4	CUD*
61	SIDS	2	M/C	C _{10:1}	/	/	++	A/A	2/4	?
74	SIDS	5	F/C	C _{10:1}	< 0.01	/	++	A/A	3/4	GA2
158	SIDS	4	M/C	/	0.06	/	+++	A/A	2/4	?
167	SIDS	2	F/AA	/	<0.01	0.19	-	A/A	2/4	?
176	SIDS	2	F/C	C _{16:1}	/	/	++	A/A	2/4	?
181	SIDS	3	M/AA	/	<0.01	0.18	-	A/A	2/4	?
196	SIDS	1	M/AA	C _{10:1}	/	0.15	+	A/A	3/4	?
202	SIDS	1	F/C	/	0.10	0.26	-	A/G	2/4	MCAD
233	SIDS	4	F/C	C _{10:1} C _{14:1} C _{16:1}	0.15	0.21	+	A/A	4/4	VLCAD/LCHAD
338	SIDS	6	F/AA	C _{10:1} C _{14:1} C _{16:1}	<0.01	/	++	A/A	3/4	GA2
367	SIDS	5	M/C	C _{10:1} C _{14:1} C _{16:1}	<0.01	0.22	+++	G/G	4/4	MCAD
401	SIDS	4	M/C	/	<0.01	0.05	+	A/A	3/4	CUD

*A sibling died suddenly in the first year of life, and the diagnosis was SIDS.

AA, African-American; C, Caucasian (non Hispanic); F, female; LCHAD, long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency; M, male; MCAD, medium-chain acyl-CoA dehydrogenase deficiency; VLCAD, very long-chain acyl-CoA dehydrogenase deficiency.

Reference values: C_{10:1}, none detected (detection limit 0.001 μmol/100 mg protein); C_{14:1}, none detected (detection limit: 0.001 μmol/100 mg protein); C_{16:1}, ND-1.36 (median 0.21) μmol/100 mg protein; glucose, 0.2-8.5 (median 2.27) μmol/100 mg protein (detection limit: ≈0.01); carnitine, 0.69 ± 0.26 μmol/gm wet weight (abnormal: <0.34). Normal findings shown as "/".

Fatty liver: -, negative; +, mild microvesicular changes; ++, moderate microvesicular changes; +++, diffuse microvesicular changes.

Table V. Non-SIDS cases with multiple abnormal results*

Case No.	Cause of death	Age (mo)	Gender/race	Unsaturated fatty acids	Glucose	Total carnitine	Steatosis	MCAD alleles	Abnormal findings	Possible diagnosis
69	Sepsis	11	M/C	C _{10:1}	<0.01	/	+	A/A	3/4	GA2
163	Sepsis	3	M/AA	C _{10:1} C _{16:1}	/	/	++	ND [†]	2/4	?
193	Pneumonia	4	F/C	C _{10:1}	/	/	+++	A/A	3/4	?
216	Sepsis	9	F/AA	C _{14:2} C _{14:1}	<0.01	/	+++	A/A	3/4	VLCAD/LCHAD
220	Congenital anomalies	1	M/AA	C _{16:1}	/	/	++	A/A	2/4	?
238	Congenital anomalies	4	M/C	C _{10:1} C _{16:1}	/	0.29	++	A/A	3/4	GA2
239	Sepsis	<1	F/C	/	0.03	0.01	+++	A/A	3/4	CUD
310	Myocarditis	10	F/AA	/	<0.01	0.26	+	A/A	2/4	?
350	Pneumonia	2	M/AA	C _{14:1} C _{16:1}	<0.01	/	+++	A/A	3/4	VLCAD/LCHAD
440	Pneumonia	2	M/AA	/	<0.01	0.23	+++	A/A	3/4	LCHAD [‡]
480	Meningitis	2	F/AA	/	<0.01	/	++	A/A	2/4	?

*See legend to Table IV for details.

[†]Not determined (tissue not available);

[‡]Based on acylcarnitine analysis of postmortem bile.²⁵

other of these three cases (no. 440), a bile specimen was available and revealed an acylcarnitine profile essentially identical to those detected in three confirmed cases with LCHAD deficiency²⁵ (and unpublished results). Although a method for the measurement of LCHAD activity in post-

mortem liver has been proposed recently,³⁵ the lack of knowledge of the exact interval between the time of death and the collection of the tissue precluded us from pursuing enzymatic studies to confirm the diagnosis. In several other cases, no liver tissue was available for enzymatic or mol-

ecular studies after completion of the biochemical testing.

Finally, the sample from one patient who died of sepsis, as determined by post-mortem examination, showed the combination of marked fatty changes, normal C₈-C₁₈ free fatty acids, glucose depletion,

and low carnitine concentration (0.01 $\mu\text{mol/gm}$ wet weight, the lowest value observed among all positive controls, the population study, and all CUD cases to our knowledge reported in the literature). This is strongly suggestive of a diagnosis of CUD. The remaining five cases had insufficiently informative profiles, and no tentative diagnosis was made.

DISCUSSION

Analysis of fatty acids, glucose level, carnitine concentration, and histologic findings for steatosis can be performed on liver samples obtained at autopsy for the biochemical diagnosis of at least five FAO disorders. In view of the occasional occurrence of uninformative results for individual tests, our findings underscore the necessity of relying on multiple independent diagnostic criteria that are evaluated collectively to provide an effective biochemical screening of FAO disorders. In particular, our results suggest that no single test is likely to be sufficiently specific to be used alone. On the other hand, 26 of 27 confirmed cases with MCAD deficiency, VLCAD deficiency, LCHAD deficiency, GA2, and CUD could be effectively identified as abnormal. The specificity of this approach is supported by the fact that none of the 34 cases in the accidents and abuse group were positive for more than one criterion.

Our finding of two probable (1 confirmed) cases with MCAD deficiency among 313 infants with SIDS (0.6% of all SIDS cases) indicates that MCAD deficiency is a relatively rare single cause of death among cases diagnosed as SIDS in an ethnically diverse and predominantly urban American population. These data are not inconsistent with the absence of homozygosity for the A985G mutation in 1236 SIDS cases reported from another ethnically diverse urban population,¹⁴ or with the absence of MCAD deficient cases reported in several smaller studies.¹⁵⁻¹⁸ To conclude from these studies that MCAD deficiency is not an appreciable cause of SIDS requires caution in two regards. First, because the A985G mutation is more common among populations of northern

European origin,⁵⁶ the contribution of MCAD deficiency to SIDS cases is likely much greater in regions such as the American Midwest.⁸ Among white SIDS cases, the incidence of MCAD deficiency in our study was 1.3%, which corresponds to approximately 50 cases per year in the United States. Morbidity and death in this group are preventable with treatment. Second, mutation-based studies used to screen for the A985G mutation fail to differentiate between affected compound heterozygotes and unaffected carriers. Five of nine MCAD deficiency cases detected by tandem mass spectrometry on newborn screening were found to be compound heterozygotes for A985G and a second mutation,³⁷ indicating that screening for the A985G mutation alone may significantly underestimate the number of affected cases.

The anonymous nature of the study protocol barred us from pursuing further information and testing family members to confirm the diagnosis of the cases with possible CUD.³⁸ CUD is an important diagnosis to make because the often fatal complications of fasting intolerance and cardiomyopathy can be prevented with oral carnitine supplementation and avoidance of fasting. To our knowledge, the possibility of a contribution of CUD to cases of sudden death in early life in general or SIDS in particular has not been seriously investigated. Four cases demonstrated a metabolic profile consistent with that seen in CUD, thus further investigations are needed to evaluate this hypothesis. Prospective studies with the option to interact with the family of an index case will allow confirmatory testing by assay of carnitine uptake in parental skin fibroblasts.^{27,38} An independent confirmation of the diagnosis would also allow the exclusion of other metabolic disorders that may lead to secondary carnitine deficiency as a result of renal loss.³⁹

The finding of a possible FAO disorder in 9 of 45 cases with infections is potentially relevant to future investigations. Each of these cases presented with sudden and unexpected death, a diagnosis of possible SIDS was considered, and the alternative diagnosis (infection) was given after autopsy and review. It is well established that death of patients with

FAO disorders predominately occurs during fasting, which usually is associated with an infectious, often viral, illness.¹⁻³ Of the nine cases suspected of having an FAO disorder among the infection group, three were given a diagnosis of pneumonia, four of sepsis, and one each of myocarditis and meningitis, possibly reflecting pathologic findings of the acute infection that served as the trigger to a fatal episode of metabolic decompensation. Consistent with this observation, 7 of 15 cases with LCHAD deficiency described by Tyni et al.⁵⁰ showed postmortem evidence of pneumonia.

In most cases of FAO disorders, the prognosis is favorable once the diagnosis has been made and treatment established.^{1-3,9} Because all known FAO disorders are inherited in an autosomal recessive manner with a 25% recurrence risk, it is common to discover siblings who are affected but clinically free of symptoms, once a diagnosis is made in an index case. The authors are aware of families in which the diagnosis of an FAO disorder was not recognized in the case of the sudden death of a child (or the diagnosis was not properly communicated to the family), and a second sibling died of the same disorder. In fact, one of the SIDS cases with a CUD profile in our study had had an older sibling whose cause of death was diagnosed as SIDS despite diffuse fatty infiltration of the liver. Cases such as these underscore the need for more effective screening to identify patients with FAO disorders among sudden death cases.

The biochemical protocol described here can be readily used as a screening test to identify cases of sudden and unexpected death suspected to have a possible underlying FAO disorder. Once recognized, these cases can be further investigated by several methods including molecular analysis, enzyme activity assays, immunoblotting, and parental carnitine transport studies. This protocol can be expanded to include additional diagnostic parameters, specifically carnitine and acylcarnitine analyses of postmortem bile.²⁵ Bile specimens were available for only 32 of the 418 cases, yet one of them was instrumental in establishing a diagnosis of LCHAD deficiency.

Financial and practical concerns make routine screening in all cases of pediatric sudden death unrealistic. We therefore propose that pathologists, pediatricians, and geneticists regularly inquire about risk factors for the presence of a possible FAO disorder and submit for screening those cases thus identified. These risk factors include the finding of fatty infiltration of the liver and other organs, family history of sudden death, Reye's syndrome, or myopathy, and especially a history of lethargy, vomiting, or fasting (decreased caloric intake) before death.¹⁻³ Ideally, a frozen specimen should be preserved in all cases, to be discarded at a later time if no risk factors are identified and, of course, when a precise cause of death has been established. Considering the high incidence of profiles suggesting an FAO disorder in the infections group (20%), we particularly recommend screening for FAO disorders in all infants with sudden and unexpected death in whom an infection has been identified, especially when associated with any degree of fatty infiltration of the liver.

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