Fasting Medium Chain Acyl-Coenzyme A Dehydrogenase–Deficient Children Can Make Ketones

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Medium chain acyl-coenzyme A dehydrogenase (MCAD) deficiency classically presents as hypoketotic hypoglycemia. Underproduction of ketones has been presumed to be the cause of hypoketosis, but this has never been proven. Stable isotope dilution studies of ketone kinetics were performed on three well children with homozygous 985G MCAD deficiency using 1,3-$^{13}C_2$ sodium acetoacetate and 1,2,3,4-$^{13}C_4$ sodium 3-hydroxybutyrate to ascertain the rates of ketone production, interconversion, and use. All children were fasted for 9 to 11.5 hours before the beginning of the study period. Euglycemia was maintained in all cases. Ketone kinetics were calculated using a two–accessible pool model and showed normal ketone production in all three children compared with published control data from children fasted for a similar length of time. There is no evidence for underproduction or overuse of ketones in these MCAD-deficient children, at least when they are well. We propose that another factor, such as fever, may be required to reduce ketone production and result in the biochemical phenotype recognized in unwell children.

MEDIUM-CHAIN acyl-coenzyme A dehydrogenase (MCAD) deficiency presents clinically as hypoketotic hypoglycemia with encephalopathy under conditions of metabolic stress, particularly fasting and viral infections. Between episodes, the child is asymptomatic. Many children who received diagnoses of Reye syndrome in the past may have had MCAD deficiency.[1] Long-term outcome studies show significant morbidity and mortality in the original cohorts with this diagnosis,[2] disappointing for such an easily treated condition. The characteristic plasma profile at presentation demonstrates hypoglycemia, elevated free fatty acids (FFA), and levels of 3-hydroxybutyrate and acetoacetate that are inappropriately low for the concentration of FFA.[3] Low plasma ketone body levels have been assumed because of reduced ketone production, but this has never been proven.

Approximately 1 in 70[4,5] Australians carry the common 985G MCAD mutation, which appears to have a strong founder effect from Northern Europe, similar to that of cystic fibrosis.[6] It was our hypothesis that near-normal ketone production in children with MCAD deficiency might explain the failure to diagnose between 25% and 75% of the predicted cases of MCAD deficiency based on the carrier frequency of this mutation (responsible for more than 90% of MCAD-deficient alleles). The predicted homozygous state for the 985G allele is approximately 1 in 24,500.

In South Australia, we have detected MCAD deficiency in 2 of 20,000 infants in our first year of newborn screening (unpublished data). However, the diagnosis rate in those Australian states that do not screen newborns for MCAD deficiency and other fatty acid oxidation defects is presently significantly less than this. We are concerned that this may be attributable to misconceptions about the ability of children with MCAD deficiency to produce ketones, as well as different exposure to viral pathogens.[7] Although the currently quoted carrier incidence may be incorrect, it is more likely that the diagnosis is being missed. This is supported by newborn screening programs using tandem mass spectrometry, such as ours, which have diagnosed more than the number of cases of MCAD deficiency predicted from the 985G carrier studies.[8]

The present study was designed to evaluate in vivo the relative contributions made by production and use to plasma ketone concentrations, using stable isotope methods, in MCAD-deficient children when well.

A stable isotope/mass spectrometry approach using the "two accessible pools" model[9] of Bougnères and Ferré (Fig 1) was used to investigate ketone turnover in three fasting MCAD-deficient children. Because experiments on normal individuals were not acceptable to the ethics committees, experimental data are compared with data on normal fasting children published by Bougnères and Ferré.[9]

Fasting is essential to evaluate the ability of children to produce and use ketone bodies in response to the biological stimulus to lipolysis and ketogenesis. When well, children older than 1 year with MCAD deficiency appear able to fast for at least 10 hours without any ill effects. An 8- to 10-hour fast is commonly encountered overnight and represents the initiation of fatty acid catabolism.

The mainstay of treatment for MCAD deficiency is avoidance of prolonged fasting and glucose supplementation at times of metabolic stress to reduce or abolish fatty acid catabolism. Dietary restriction of foods containing fat, particularly those high in medium-chain triglycerides, may be appropriate but is not universal. A better understanding of ketone kinetics in fasting MCAD-deficient patients should provide the basis for rational therapy.

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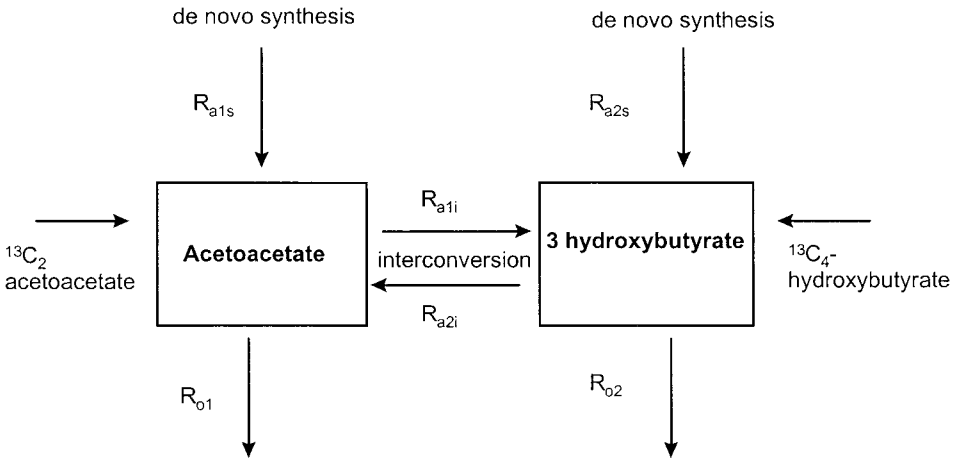

Fig 1. Two–accessible pool model of ketone turnover, adapted from Bougnères and Ferré.[9]

PATIENTS AND METHODS

Patients

Stable isotope–dilution studies of ketone kinetics were performed on three children with MCAD deficiency; all were homozygous for the 985G mutation.

Because of the inherent dangers in fasting children with this condition for prolonged periods,[10] the children were awakened at a previously specified time for a carbohydrate-containing drink or snack on the night before the study. They presented to the hospital the next morning, and a sampling line was inserted one hour after application of EMLA cream (Astra Pharmaceuticals, North Ryde, Australia). Fasting time to study commencement varied from 9 to 11.5 hours. Blood samples were collected at baseline and every 10 minutes throughout the 2-hour infusion period. Children were observed closely from admission to discharge, which took place after they had eaten and euglycemia was confirmed. The total period of fasting ranged from 11.5 to 13.5 hours.

Physical parameters, including age at presentation and fasting periods of the children in this study, as well as isotope infusion rates, are summarized in Table 1. Blood was collected from an indwelling venous catheter for estimation of plasma glucose, (FFA, 3-hydroxybutyrate, and acetoacetate estimation as well as mass spectroscopic studies of stable isotope enrichment.

The ethics committees of the Royal Children's Hospital and the Royal Alexandra Hospital approved these studies for MCAD-deficient children but withheld consent for control children. The results obtained in MCAD-deficient patients were compared with the two normal children whose ketone body kinetics were studied using the two–accessible pool approach, reported by Bougnères and Ferré, who had fasted for a similar length of time (Table 2). The clinical characteristics of these children are summarized in Table 3.[9]

Table 1. Age, Sex, Weight, Age at Diagnosis, Fasting Period, Biochemical Parameters, and Infusion Rates of the Patients Studied

Characteristic	MCAD 1	MCAD 2	MCAD 3
Age	6 yr	13 mo	11 mo
Sex	Male	Female	Female
Weight	22.45 kg	11.7 kg	9.2 kg
Age at diagnosis	6 mo	9 mo	5 mo
Fasting period (this study)	11½-13½ h	9½-11½ h	10-12 h
Mean plasma glucose (mmol/L)	3.2	3.7	5.0
Mean plasma FFA (mmol/L)	1.15	1.02	3.62
Mean plasma 3OHB (mmol/L)	0.3	0.2	0.24
$^{13}C_2$ acetoacetate infusion rate (μmol/kg/min)	0.34	0.35	0.42
$^{13}C_4$ 3-hydroxybutyrate infusion rate (μmol/kg/min)	0.67	0.62	0.60

Materials

The sodium salt of 1,2,3,4-$^{13}C_4$ 3-hydroxybutyrate and 1,3-$^{13}C_2$ ethyl acetoacetate were purchased from Tracer Technology (Somerville, Australia). 1,2,3,4-$^{13}C_4$ ethyl acetoacetate was purchased from Merck Sharp and Dohme Isotopes (division of Merck Frosst, Canada, Montreal, Canada).

The sodium salt of 1,3-$^{13}C_2$ acetoacetate was prepared by hydrolysis of an aqueous solution of labeled ethyl acetoacetate with a 10% molar excess of 1 mol/L sodium hydroxide, as previously described.[11] 1,3-$^{13}C_2$ sodium acetoacetate prepared in this way was also used to synthesize 1,3-$^{13}C_2$ 3-hydroxybutyrate for use as a standard using a modification of the method of Passingham and Barton.[12] Isotope infusion rates are summarized in Table 1.

Mass Spectrometric Studies: Atom Percent Enrichment

One hundred microliters of the supernatant from isovolumetric perchloric acid–collected blood was added to 100 μL of 0.7 mol/L tripotassium phosphate, mixed, and centrifuged. One hundred microliters of this supernatant was added to 10 μL each of two internal standards, 6.15 mmol/L dimethylmalonic acid and 5.34 mmol/L 3,3-dimethylglutaric acid, and 20 μL of 6 mol/L hydrochloric acid. Sodium chloride crystals were added. The organic acids were extracted twice

Table 2. Ketone Body Kinetics at Steady State, Calculated According to Two–Accessible Pools Approach for Control Children Fasted for 13 Hours

Rate (μmol/kg/min)	Subject 1	Subject 2
R_{a1s} (ACAC synthesis)	9.9	8.6
R_{a2s} (3OHB synthesis)	1.1	0.3
Total ketone body synthesis ($R_{a1s} + R_{a2s}$)	11.0	8.9
R_{a2i} (interconversion ACAC→3OHB)	2.5	2.6
R_{a1i} (interconversion 3OHB→ACAC)	1.0	0.8
R_{o1} (use of ACAC)	8.5	6.8
R_{o2} (use of 3OHB)	2.5	2.0
Total ketone body use ($R_{o1} + R_{o2}$)	11.0	8.8

Data from Bougnères and Ferré.[9]

Table 3. Age, Weight, and Blood Glucose and Ketone Body Concentrations of Six Published Control Patients

Parameter	1	2	3	4	5	6
Age (mo)	48	42	47	52	40	37
Weight (kg)	16	15.5	16	16	15	13.5
Duration of fasting (h)	13	13	16	20	22	22
Mean blood glucose (mmol/L)	4.2	4.1	4.8	4.9	2.6	2.3
Mean blood ACAC (mmol/L)	0.03	0.03	0.288	0.744	0.820	0.850
Mean blood 3OHB (mmol/L)	0.052	0.074	0.295	1.516	2.557	2.670

Data from Bougnères and Ferré.[9]

into ethyl acetate. The solvent extracts were pooled and evaporated to dryness under nitrogen. The residue was derivatized using 27 μL of bis-trimethylsilyltrifluoroacetamide containing 1% trimethylsilyl and 3 μL pyridine that had been dried over potassium hydroxide, incubated at 60°C for 30 minutes. One microliter of this derivatized sample was injected onto the gas chromatograph/mass spectrometer for analysis, performed on a Hewlett Packard MSD-5890 gas chromatograph/HP MSD-5971 mass spectrometer.

Calculations

A modification of the two–accessible pool model of Bougnères and Ferré[9] was used for calculations of ketone kinetic parameters. This is a steady-state model and is represented in diagrammatic form in Fig 1. A single-pool model cannot be used here because there are two interconverting pools. 3-Hydroxybutyrate and acetoacetate interact together too slowly to be considered together. The published model was modified to allow matrix equations to be used for calculation of rates of synthesis, interconversion, and use of acetoacetate and 3-hydroxybutyrate from the enrichments of the various isotopes.[11]

Statistical analyses of the results obtained in control and MCAD-deficient children were performed using a two-sample *t* test, MINITAB statistical software (Minitab Inc, State College, PA).

RESULTS

All children remained euglycaemic during the study. Plasma glucose, FFA, and ketone levels for each child are shown in Table 1. Mean values for all patients of glucose, FFA, and 3-hydroxybutyrate throughout the study were 4.24, 1.93, and 0.26 mmol/L, respectively.

Steady state was reached in all cases under the conditions of the study, according to the atom percent enrichment (APE) curves (Fig 2). APE data were then used for calculation of ketone body kinetics for the three MCAD-deficient children, according to our modification of the two–accessible pool model (Table 4).

There is no difference between the rates of total ketone body production in the control and MCAD-deficient groups (T = 0.5, P = .67, 95% confidence interval [CI] −5.03 to 6.4) or in the rates of total ketone body use (T = 1.28, P = .33, 95% CI −4.15 to 7.6). However, there are differences in the ketone body kinetics of the two groups. In the control patients, acetoacetate is the major ketone body synthesized, representing between 90% and 97% of total ketone body synthesis. In MCAD-deficient patients, 3-hydroxybutyrate is the major ketone body synthesized, with de novo synthesis of acetoacetate representing between 35% and 49% of total ketone body production. This difference in percentage of acetoacetate synthesized is significant (T = 9.63, P = 0.011, 95% CI 28.5 to 74.5), suggesting altered intracellular redox potential.

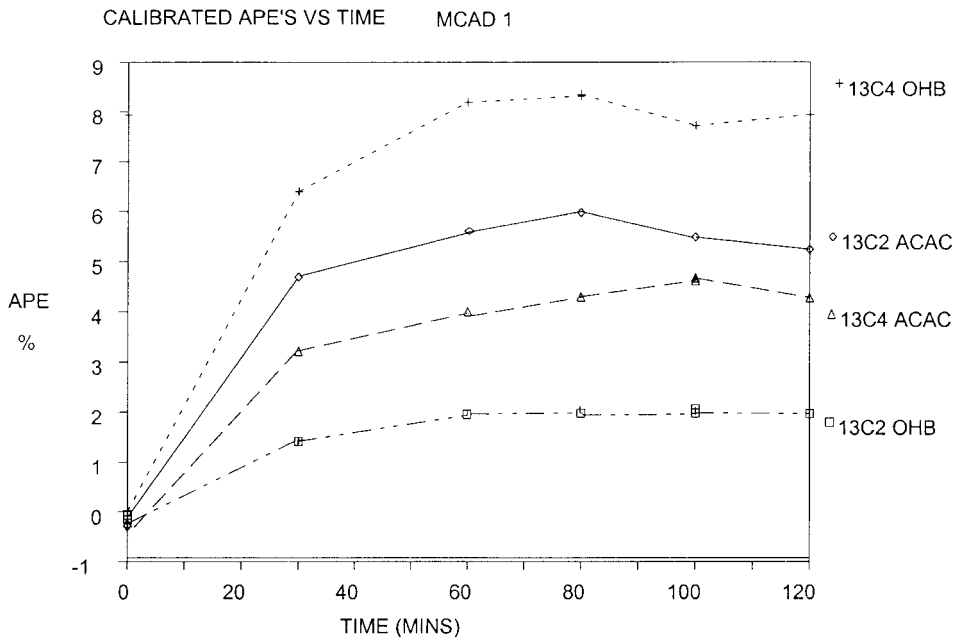

Fig 2. Enrichments of acetoacetate (ACAC) and 3-hydroxybutyrate(OHB) during the study of MCAD-deficient patient 1. Time 0 is the beginning of the tracer infusion.

Table 4. Rates of Acetoacetate and 3-Hydroxybutyrate Production, Interconversion, and Use in Children With MCAD Deficiency, Calculated Using the Two–Accessible Pool Approach

Rate (μmol/kg/min)	MCAD 1	MCAD 2	MCAD 3
R_{a1s} (ACAC synthesis)	3.21	4.51	5.86
R_{a2s} (3OHB synthesis)	5.94	6.24	6.07
Total ketone body synthesis ($R_{a1s} + R_{a2s}$)	9.15	10.75	11.93
R_{a2i} (interconversion ACAC→3OHB)	3.55	4.38	7.35
R_{a1i} (interconversion 3OHB→ACAC)	3.88	5.05	9.47
R_{o1} (use of ACAC)	3.89	5.54	8.41
R_{o2} (use of 3OHB)	6.28	6.19	4.55
Total ketone body use ($R_{o1} + R_{o2}$)	10.17	11.73	12.96

Abbreviations: ACAC, acetoacetate; 3OHB, 3-hydroxybutyrate.

In control children, the rates of ketone body production, as published, are equal to the rates of ketone body use and are assumed to include a correction factor for infusion. We have not corrected our results for infusion rates; therefore, in the MCAD-deficient children, ketone use exceeds ketone production by an average of 10%.

DISCUSSION

The only published data for pediatric ketone kinetics using a two-tracer technique come from studies performed in six children by Bougnères and Ferré.[9] These "control" children had suspected but undocumented fasting hypoglycemia and were later proved to be normal on an endocrine and metabolic basis. This study involved the use of two–accessible pool model kinetics to calculate the rates of ketone production, interconversion, and use after dual-isotope infusion, similar to that used in our study. The only difference between their study and ours was a different isotope of 3-hydroxybutyrate—[4,4,4-$^{2}H_3$]3-hydroxybutyrate compared with [1,2,3,4-$^{13}C_4$]3-hydroxybutyrate.

We have shown that the ketone production rates in well, mildly fasted MCAD-deficient children, while euglycemia is maintained, are at least equal to those of published control children. This suggests that MCAD-deficient children have enough residual ketone-synthesizing capability to maintain ketone production under normal circumstances and supports our hypothesis that near-normal ketone production may mask the presence of MCAD deficiency. Failure to diagnose MCAD deficiency is therefore most likely because of failure to present with a characteristic biochemical profile.

The postulated incidence of MCAD deficiency and the significant morbidity and mortality associated with diagnosis after catastrophic presentation[1,2] make it worthy of inclusion in newborn screening programs.

Given the adequate ketone production under normal fasting conditions, something must change with added metabolic stress. There is no evidence, from these studies, of increased ketone use. We have failed to determine a single causative virus at presentation of MCAD deficiency (unpublished observation).

Work from other inborn errors of metabolism caused by missense mutant proteins suggests that one of the most likely explanations is an external factor, such as fever. Recent work by Gregerson et al[13] has shown that the common 985G mutation of the MCAD enzyme produces a structural mutant that is thermolabile. In vitro, it is associated with a dramatic reduction in activity at increasing temperatures.[14] Attempts to study the activity of the 985G mutant MCAD enzyme in cultured cells at elevated temperatures have been unsuccessful (personal communication, N. Gregersen). However, accelerated degradation, correlated with relative loss of protein and activity, has been demonstrated in other disease-causing missense mutants, most notably phenylalanine hydroxylase.[15]

If fever, rather than the presence of the mutant enzyme, is the most important factor in reducing ketone production, significant benefit may be gained by the use of antipyretics in febrile MCAD-deficient children, or indeed children with any fatty acid oxidation defect. This is currently standard therapy in our clinic because the number of affected children is presently too small to study this issue in a controlled study.

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