Production and Disposal of Medium-chain Fatty Acids in Children with Medium-chain Acyl-CoA Dehydrogenase Deficiency

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Summary: The effect of fasting on plasma concentrations of fatty acids has been determined in four children with medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. In addition, the in vivo rate of octanoate oxidation was measured, using [1-13C]octanoate. In the three older children (1.5-11.2 years), fasting for up to 18 h stimulated lipolysis, as reflected by the increasing concentration of free fatty acids, but with little rise in concentrations of medium-chain fatty acids, octanoate, decanoate and cis-4-decenoate. In an infant (0.5 year), lipolysis was greater and was accompanied by rising concentrations of medium-chain fatty acids. After 13.5 h there was a rapid increase in the concentration of decanoate and cis-4-decenoate. The calculated in vivo rate of octanoate oxidation was substantial in all patients studied (6.4-13.1 μmol/kg per h) despite very low MCAD activity in vitro. It is concluded that under basal conditions the in vivo oxidation rate of medium-chain fatty acids is near normal in the four children studied with MCAD deficiency.

Medium-chain acyl-CoA dehydrogenase (MCAD; EC 1.3.99.3) deficiency (McKusick 201450) is a potentially lethal inborn error of fatty acid oxidation characterized by episodes of lethargy, hypoketotic hypoglycaemia and coma. Between episodes patients are generally asymptomatic (Roe and Coates 1989). Although about 85% of patients have the same mutation, G985, there appears to be significant phenotypic heterogeneity (Editorial 1991). The mean age at which patients present is 15 months, with wide a range; affected individuals can become ill in the neonatal period but may not present until 4 years (Roe and Coates 1989; Wilcken et al 1992). In the second decade, recurrent illness is less frequent and the need for hospitalization appears to be rare (Roe and Coates 1989). The reason for this is not clear but may be related

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to the relative rates of lipolysis and β-oxidation at different ages. In this study we have examined the effects of fasting on plasma medium-chain fatty acid concentrations and on the in vivo rate of oxidation of octanoate in children with MCAD deficiency.

METHODS

[1-13C]octanoate (sodium salt) was obtained from Aldrich, UK, and sodium bicarbonate (NaH13CO3) from Cambridge Isotopes, USA. Plasma medium-chain fatty acids were extracted, derivatized and analysed using gas chromatography–mass spectrometry (GC-MS), as described previously (Heales et al 1991). The plasma enrichment (IEpl) of [1-13C]octanoate was calculated using GC-MS configured for selective-ion monitoring (m/z and (m + 1)/z) (Wolfe 1992). The system was calibrated using [1-12C]octanoate and [1-13C]octanoate standards. The enrichment of CO2 in expired air (IEa) was determined by isotope ratio mass spectrometry (IRMS) (Wolfe 1992). Isotopes for intravenous use were dissolved in sterile saline (0.9% w/v), prepared and sterilized by the Pharmacy Department, Northwick Park Hospital, Middlesex. These studies were approved by the joint ethical committee of the Hospitals for Sick Children, London. Informed consent was obtained from parents and also patients where appropriate.

Plasma concentrations of total free fatty acids (FFAs) were determined by an enzyme-linked kit (Wako, Germany). FFAs, in the presence of acyl-CoA synthetase and free CoA, are converted to acyl-CoA molecules. The latter are oxidized, by acyl-CoA oxidase, to 2,3-trans-enoyl-CoA and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide brings about the oxidative condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)aniline with 4-aminoantipyrine to form a purple coloured adduct. The absorbance, at 550 nm, of this adduct is directly proportional to the FFA concentration. This assay has broad chain length specificity: 6–20 carbon atoms for both saturated and unsaturated fatty acids.

Patients with MCAD deficiency: (A) Female, 10.9 years, homozygote for G985 mutation. (B) Male, 11.2 years, homozygote for G985 mutation. (C) Male, 0.5 years, homozygote for G985 mutation. (D) Male, 1.5 years, homozygote for G985 mutation. (E) Male, 9.1 years, homozygote for G985 mutation. (F) Female, 10.1 years, compound heterozygote. The defect was confirmed by the electron transport flavoprotein-linked enzyme assay (Professor D. Turnbull, Newcastle, UK).

Fasting test: Blood was taken at regular intervals to determine the effect of fasting on plasma concentrations of octanoate (C8:0), decanoate (C10:0) and cis-4-decenooate (C10:1) and total FFAs in patients A to D. Each child was carefully monitored and remained well throughout. The maximum duration of fast was 18 h. The effect of fasting on plasma medium-chain fatty acids was also determined in a control group of 5 children (0.6–7.1 years, 3 male and 2 female) who were being fasted (13–22 h) because a metabolic defect was suspected clinically but in whom none was identified.

Stable isotope studies: The in vivo rate of octanoate oxidation was determined in subjects A, B, E and F. The children were fasted overnight (approximately 12 h) and

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