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Accumulation of Odd-Numbered Long-Chain Fatty Acids in Fetuses and Neonates with Inherited Disorders of Propionate Metabolism

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ABSTRACT. Fetuses affected with propionic acidemia incorporate great amounts of odd-numbered long-chain fatty acids (OLCFA) into their body lipids. This is due to abundant supply with precursor amino acids of propionyl-CoA throughout pregnancy. After birth, the lower provision of precursor amino acids during dietary treatment compared with fetal life results in a decline of propionyl-CoA production and therefore OLCFA synthesis. However, the observed decrease of OLCFA may also partly reflect the recovery from acute ketoacidotic episodes that the patients experienced soon after birth as long as they were undiagnosed. In a patient with vitamin B_{12}-responsive methylmalonic aciduria treated prenatally with large doses of vitamin B_{12} given to the mother, the cord plasma lipids contained normal amounts of OLCFA. This indicates that prenatal therapy led to an increased flux of propionyl-CoA through the defective methylmalonyl-CoA mutase step. Thus, in addition to the quantification of a decline in methylmalonic acid in maternal urine, OLCFA in cord blood lipids might be a further parameter for evaluating prenatal treatment in patients with vitamin B_{12}-responsive methylmalonic aciduria. (*Pediatr Res* 29: 403–405, 1991)

Abbreviations

PA, propionic acidemia
MMA, methylmalonic aciduria
OLCFA, odd-numbered long-chain fatty acid

The relative abundance of OLCFA in body lipids of patients with defective propionate metabolism (PA, MMA) can be used for monitoring these patients, inasmuch as it is influenced by the clinical management (1). Increased accumulation of OLCFA has already been found in newborns who had died with one of these disorders shortly after birth (2–5). Here we show that in affected fetuses increased OLCFA production from the precursor amino acids of propionyl-CoA is a process that starts early in fetal life. It appears that excess accumulation of OLCFA in a fetus with vitamin B_{12}-responsive MMA could be reduced by prenatal therapy.

PATIENTS AND METHODS

Patients. Fetuses Ho 1 and Ho 2 were at risk for PA and amniocenteses were performed in the 16th gestational wk. In both fetuses, PA was diagnosed prenatally by means of the determination of methylcitrate in amniotic fluids and by enzymatic studies in cultured amniocytes. Activity of propionyl-CoA carboxylase measured as enzyme-dependent incorporation of ^{14}C-bicarbonate into acid-nonvolatile products (6) in cultured amniocytes was 14 and 5.3 pmol/mg protein × min, respectively (controls: mean ± SD, 1297 ± 668; range, 472–2629; n = 14). In each fetus, abortion was induced at wk 21 of gestation. After abortion, erythrocytes, plasma, and whole blood, as well as liver, brain, and s.c. tissue, were obtained and immediately frozen at −20°C.

M.B., the second child of a healthy and unrelated couple, was born at term. The first child had suffered from ventricular septal defect and had died at 3 wk of age of unknown cause. M.B. presented with vomiting, hypotonia, respiratory insufficiency, and coma at 5 d of age. He was severely hyperammonemic (1.8 mmol/L). The gas chromatography-mass spectrometry of urine suggested a diagnosis of PA, which was confirmed later by enzymatic analysis in cultured fibroblasts. Activity of propionyl-CoA carboxylase in fibroblasts was 7.0 pmol/mL protein × min (controls: mean ± SD, 1087 ± 356; range, 564–2005; n = 21). At d 9, continous arteriovenous hemofiltration and dietary management was begun in the University Children's Hospital, Innsbruck. At d 11, 13, 23, and 44, the fatty acid composition in lipids of red cell membranes and plasma was studied. Despite a successful treatment of initial coma, the patient died at the age of 3 mo during a metabolic crisis.

M.Y., a boy, is the second child of healthy consanguineous Turkish parents. Pregnancy and delivery were normal. A 3-y-old daughter is healthy; two children had died of unknown causes in Turkey at 2 wk of age. The patient presented at 9 d of age with hyperammonemia (650 μmol/L) and a slight metabolic acidosis and was treated with peritoneal dialysis. Gas chromatography of the urine showed a high excretion of methylmalonic acid (7.2 mol/mol creatinine). After peritoneal dialysis lasting for 3 d, he was started on a low-protein regimen supplemented with nontoxic amino acids, L-carnitine, and vitamin B_{12}. It appeared that he suffered from vitamin B_{12}-responsive MMA. Later, the diagnosis was confirmed in the patient's fibroblasts. Incorporation of ^{14}C-propionate without hydroxycobalamin added was about 10% that of controls and showed a 3.5-fold increase with supplemented hydroxycobalamin (100 μg/L of culture medium). At the age of 20 mo, the boy was doing well. At d 9 and 24, the fatty acid composition in red cell and plasma lipids was studied.

In a pregnancy at risk for vitamin B_{12}-responsive MMA, prenatal diagnosis performed gas chromatography-mass spectrometry analysis of amniotic fluid obtained at 16 gestational wk and by measuring [^{14}C]propionate incorporation into cultured amniocytes (15% of control values; Dr. W. J. Kleijer, Rotterdam) demonstrated that the fetus was affected. For prenatal therapy,

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the mother was given high oral doses of vitamin B_{12} (22.5 mg/d in three doses) starting in the 27th wk of gestation. On this treatment, the maternal urinary methylmalonate excretion was around 10.3 mmol/mol creatinine, which leveled off during the last 5 wk of gestation (7). A clinically healthy female infant was born by spontaneous delivery at 41 wk of gestation. After birth, an increase in methylmalonate excretion was observed, reaching 2.1 mol/mol creatinine on the 3rd d of life. Administration of hydroxycobalamin by intramuscular injection (1 mg/2 d) was started on d 4. By this measure, methylmalonate excretion was successfully reduced. The OLCFA content was measured in the plasma lipids of cord blood.

The diagnosis of vitamin B_{12}-responsive MMA was confirmed in the patient's fibroblasts by Dr. B. Fowler, Manchester, UK. Incorporation of ^{14}C-propionate without hydroxycobalamin added was about 15% that of controls and reached the control range with supplemented hydroxycobalamin (1000 μg/L of culture medium).

Methods. Red blood cells were isolated from EDTA-blood and washed as described (8). Plasma and erythrocytes were stored at −20°C until analysis. The cord blood of controls was collected into EDTA-tubes, and erythrocytes and plasma were separated within 24 h. Fetal tissues and tissues from deceased prematures were stored at −20°C until analysis.

The work-up of the red cell lipids was exactly as described previously (8).

Lipids were extracted from 1.0 mL plasma with chloroform/methanol (9). The different lipid classes were separated on silica gel plates (Merck, Darmstadt, Germany) with a run of petroleum/ether/acetic acid (75/25/1, vol/vol/vol) and a run of chloroform/methanol/water (65/25/4, vol/vol/vol). The bands containing sterol esters, triglycerides, and phospholipids were identified by comparison with appropriate standards and removed by scraping. The fatty acids were transesterified with methanol and hydrochloric acid (10).

The tissues (1–20 mg wet wt) were homogenized by use of a microdismembrator (Braun Melsungen, Germany) under liquid nitrogen (maximal power, 0.5 min). The tissue homogenates were extracted with 7.5 mL methanol/chloroform (2/1, vol/vol). After standing overnight in the dark at room temperature, 2.5 mL chloroform and 2.5 mL water were added. After shaking, the mixture was allowed to stand for 15 min on ice. Then it was centrifuged at 1500 × *g* (20 min, room temperature) and the supernatant was discarded. The lipid extracts (lower layers) were evaporated to dryness at 35°C. The residue was transesterified by base catalysis with sodium methoxide as described previously (8).

Analysis of the fatty acid methyl esters by capillary column gas liquid chromatography was exactly as we have described (8). Fatty acids of 14–22 carbon length were identified by comparison with authentic standards.

In each sample, the proportions of the individual OLCFA—15- and 17-carbon saturated and 17-carbon monounsaturated fatty acids (C 15:0, C 17:0, C 17:1)—as well as their sums were calculated and expressed as a percentage of the total C14–C22 fatty acids in the sample.

RESULTS

Different tissues of two fetuses of 21 wk gestation affected with PA contained much more OLCFA than tissues from premature or full-term controls (Table 1). Among the specimens, the OLCFA content was highest in plasma sterol esters, liver, and s.c. tissue and lowest in brain.

The OLCFA levels in red cell and plasma lipids of two newborns with PA and MMA are shown in Table 2. Plasma lipids in the PA patient contained higher amounts of OLCFA than those in the MMA patient. Both patients experienced a severe metabolic episode before they were diagnosed. In each lipid fraction studied, the high OLCFA level present shortly after birth declined substantially during the first 4–6 wk of life while patients were on treatment.

We found normal OLCFA contents in cord blood lipids of a child with vitamin B_{12}-responsive MMA who was treated prenatally with large doses of vitamin B_{12} given to the mother (Table 3). By extrapolation from our data in fetuses with PA and newborns with PA and MMA, as well as from the increased urinary methylmalonate excretion of the mother before institution of B_{12}-therapy, this might indicate that at least during the last weeks of fetal life the production of excess OLCFA was reduced.

Table 1. *OLCFA content in tissues from two fetuses (21 wk of gestation) affected with PA and from premature and term controls (% total C14–C22 fatty acids)*

Tissue	Fetus HO 1	Fetus HO 2	Controls (mean ± SD; range)
Red cell lipids	3.5		1.0 ± 0.2 (0.66–1.20)*
Whole venous blood		6.6	0.9; 1.2†
Plasma lipids			
Phospholipids	4.8		1.0 ± 0.1 (0.73–1.14)*
Triglycerides	5.6		1.9 ± 0.6 (0.90–2.94)*
Sterol esters	8.3		2.1 ± 0.8 (1.23–3.32)*
Liver	5.8	6.2	1.0; 1.1‡
Brain	2.9	3.5	
Subcutaneous tissue	5.2	5.8	1.6; 1.8‡

* Cord blood at term, $n = 7$.
† Cord blood at term, $n = 2$.
‡ Deceased prematures, 26 gestational wk, $n = 2$.

Table 2. *Alterations with age of % of OLCFA in red cell and plasma lipids in two newborns with PA and MMA*

		OLCFA (% of total fatty acids)			
			Plasma		
Disorder	Age (d)	Red cell lipids	Phospholipids	Triglycerides	Sterol esters
PA	11	2.7	8.2	11.8	8.0
	13	2.8	5.3	8.8	8.5
	23	2.2	3.8	4.6	3.7
	44	1.7	2.5	2.5	1.5
MMA	9	3.1	3.5	4.8	5.6
	24	1.8	2.4	2.8	1.7

Table 3. *OLCFA content in cord plasma lipids of prenatally treated newborn with vitamin B_{12}-responsive MMA and normal controls (% total fatty acids)*

Lipid fraction	MMA patient	Controls* (range)
Phospholipids	1.1	0.73–1.14
Triglycerides	2.2	0.90–2.94
Sterol esters	1.5	1.23–3.32

* Controls: cord blood at term, $n = 7$.

DISCUSSION

We showed that fetuses affected with a disorder of propionate degradation incorporate great amounts of OLCFA into body lipids. At approximately 20 wk of gestation, the OLCFA content in various lipids of fetuses with PA was very high and was similar to that found shortly after birth. Due to a transplacental gradient of free amino acids in favor of the fetus providing the fetus with optimal amino acid nutrition (for ref. see 11), relatively high concentrations of the free precursor amino acids—valine, isoleucine, methionine, and threonine—exist in fetal blood. This results in the formation of large amounts of propionyl-CoA in the

tissues, which under the highly anabolic conditions of fetal life augments OLCFA production and incorporation into newly synthesized lipids. The fact that part of propionyl-CoA is eliminated from the fetus as propionate derivatives offers the possibility of prenatal diagnosis of PA and MMA by means of the determination of methylcitric acid in amniotic fluid (12). Another class of propionyl-CoA-derived compounds found in amniotic fluids of fetuses with PA and MMA are methyl-branched dicarboxylic acids (13).

Elevated OLCFA were also found in erythrocyte and plasma lipids of newborns with PA and MMA. By extrapolation from our data, a higher accumulation of OLCFA could be expected for fetuses with PA than MMA. This is in line with the finding that the concentration of methylcitric acid in amniotic fluids of fetuses with PA is somewhat higher than in those with MMA (14) suggesting that the former have higher elevations of propionyl-CoA.

After birth, two facts may account for the observed decline of OLCFA in plasma and red cell lipids: *1*) the physiologic shift in the fatty acid pattern of plasma lipids during the first 4 wk of age (15), which results in a reduction of the percentage of monounsaturated fatty acids—here heptadecenoic (C 17:1) acid and *2*) the lower provision of precursor amino acids during dietary treatment compared with fetal life leading to a decline in propionyl-CoA production. However, in the present cases the decrease could at least partly reflect recovery from the acute ketoacidotic episodes (see Fig. 2 of ref. 1) that both neonates experienced before first blood samples were taken.

It is likely that substantial amounts of propionyl-CoA and methylmalonyl-CoA during prenatal life cause injury to the fetus. In that respect, the highly active flux of propionyl-CoA into the OLCFA synthesis might have a beneficial and detoxicating effect by reducing the fetal propionyl-CoA pool. However, it cannot be answered whether great amounts of OLCFA in brain lipids, per se, are responsible for impaired neurologic development in those patients, as has been proposed (5).

The OLCFA might be regarded as a storage form of propionyl-CoA accumulating already in fetal life. During periods of strong tissue catabolism, such as in early days of life, the stimulated breakdown of adipose tissue might importantly contribute to the accumulation of extensive amounts of toxic propionyl-CoA in the mitochondria, and thus contribute to the severe illness of these patients.

Prenatal treatment of a fetus affected with vitamin B_{12}-responsive MMA during the last 13 wk of gestation with large doses of vitamin B_{12} given to the mother reduced the increased maternal excretion of methylmalonic acid and led to a normal OLCFA content in plasma lipids at birth. The pharmacologic vitamin dose was given to diminish the accumulation of various metabolites including propionyl-CoA by an increased flux through the defective methylmalonyl-CoA mutase step. Until now, the success of prenatal treatment could be followed only indirectly by monitoring methylmalonic acid in maternal urine (16). Although it was suggested that the more important effect of vitamin B_{12}-treatment was on fetal metabolism, it could not satisfactorily be clarified whether the decrease in maternal methylmalonic acid excretion could have resulted from an effect on fetal metabolism alone or on fetal and maternal metabolism combined (16). The normal OLCFA content of cord plasma lipids in our patient appears to demonstrate more directly that in fact the metabolic block in the fetus was improved by vitamin B_{12}-treatment. Thus, OLCFA in cord blood lipids after birth might be an additional parameter for the evaluation of effectiveness of prenatal treatment of fetuses with vitamin B_{12}-responsive MMA and perhaps also with holocarboxylase synthetase deficiency (17).

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