Insulin-resistant hyperglycaemia complicating neonatal onset of methylmalonic and propionic acidaemias.

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ONLINE REPORT

Insulin-resistant hyperglycaemia complicating neonatal onset of methylmalonic and propionic acidaemias

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Summary *Background:* Insulin-resistant hyperglycaemia may occasionally complicate the clinical course of organic acidaemias. *Study Design:* Clinical observation. *Results:* Two term infants, one suffering from acute early-onset methylmalonic acidaemia, the other suffering from acute early-onset propionic acidaemia, presented acutely with dehydration, ketoacidosis, and hyperammonaemia. Urinary organic acid, plasma amino acids, and blood and plasma acylcarnitine analysis allowed the diagnosis of methylmalonic and propionic acidaemias. The detection of the novel c.481G>A (p.Gly161Arg) and the known c.655A>T (p.Asn219Tyr) *MUT* gene mutations identified the first patient as affected by methylmalonic acidaemia *mut* type. The high increase of propionylcarnitine after carnitine administration in both patients suggested a greatly elevated metabolic intoxication. Both newborns showed insulin-resistant hyperglycaemia. Patient 1 died, but patient 2, after a strong reduction of glucose administration, survived. To our knowledge, this is the only patient with this complication who survived. *Conclusion:* Insulin-resistant hyperglycaemia complicating neonatal onset of methylmalonic and propionic acidaemias is probably a marker of a serious disease. One patient with this complication survived after a strong reduction of glucose administration. Even if this is probably only a partial intervention, we hypothesize that in this situation a reduction of glucose administration can reduce almost the risk of persistent hyperglycaemia. Further studies are required to confirm our hypothesis.

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References to electronic databases: *Propionic acidaemia: OMIM #606054. Methylmalonic aciduria due to methylmalonyl-CoA mutase deficiency: OMIM #251000.*

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Abbreviations

C_3	propionylcarnitine
C_4DC	methylmalonylcarnitine
EEG	electroencephalogram
MCM	methylmalonyl-CoA mutase
MMA	methylmalonic acidaemia
MMCoA	methylmalonyl-CoA
MRI	magnetic resonance imaging
NICU	neonatal intensive care unit
PA	propionic acidaemia
PC	pyruvate carboxylase
PCC	propionyl-CoA carboxylase
PDH	pyruvate dehydrogenase

Introduction

Propionic and methylmalonic acidaemias (PA and MMA) in their severe neonatal-onset forms are disorders with a high mortality and poor outcomes (Deodato et al 2006; Hori et al 2005). PA (OMIM 606054) is caused by a deficiency of propionyl-CoA carboxylase (PCC), a mitochondrial biotin-dependent enzyme that catalyzes the first step in the catabolism of propionyl-CoA. Both *PCCA* and *PCCB* genes, encoding the alpha and beta subunits of PCC, have been correlated with PA. Isolated MMA is mostly caused by a deficiency of methylmalonyl-CoA mutase (MCM) (OMIM 251000; MMA *mut* type), a vitamin B_{12}-dependent enzyme that converts 1-methylmalonyl-CoA to succinyl-CoA. Mutations in the *MUT* gene, encoding MCM, may be responsible for partial or complete enzyme deficiency (mut^- and mut^0 biochemical phenotype, respectively). Both PA and mut MMA are characterized by a greatly increased concentration, respectively, of propionyl-CoA and methylmalonyl-CoA, resulting, respectively, in an increased amount of propionic and methylmalonic acids in blood and urine (Fenton et al 2001).

Usually, the first signs of these diseases are feeding refusal, drowsiness, dehydration, hypotonia of the trunk and hypertonia of upper and lower limbs. Later, seizures, lethargy, cerebral oedema and finally coma may appear. Laboratory investigations show usually ketosis or ketoacidosis, hyperammonaemia, moderate hypocalcaemia, neutropenia, thrombocytopenia, hyperlactacidaemia and hypoglycaemia (Fenton et al 2001). The diagnosis is usually confirmed by blood or plasma acylcarnitine profile, urinary organic acid analysis, enzyme assay, or molecular analysis.

Emergency treatment includes rehydration, stimulation of diuresis, correction of acidosis, a hypercaloric nutritional intake of glucose to prevent further protein and fat catabolism, specific vitamins, and oral or intravenous carnitine to assist the excretion of organic metabolites. Treatment with sodium benzoate and arginine hydrochloride is suggested if ammonium levels are high.

An unusual complication of MMA and PA is a severe insulin-resistant hyperglycaemia (Boeckx and Hicks 1982; Ciani et al 2000; Lehnert et al 1980). Few cases are described at neonatal and paediatric ages, associated with high mortality (Boeckx and Hicks 1982; Ciani et al 2000; Lehnert et al 1980). We describe two patients affected by MMA and PA respectively with severe neonatal onset and insulin-resistant hyperglycaemia. To our knowledge, the patient with PA described here is the only patient with this complication who survived.

Material and methods

GC-MS and MS/MS analysis

Urinary organic acids were measured with an Agilent 6890N gas chromatograph coupled to a 5973 mass spectrometry system (Agilent Technologies, Palo Alto, CA, USA). Acylcarnitine analysis was carried out on an API 4000 spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA, USA) according to the conventional method as previously reported (Chace et al 1993; Millington et al 1990) with some modifications (la Marca et al 2003). Data were acquired with Analyst software and analysed automatically using ChemoView (Applied Biosystems).

Molecular analysis

Molecular analysis was performed after the parents' informed consent had been obtained. Unfortunately, parents of patient 2 affected by PA denied informed consent for mutation detection in *PCCA* and *PCCB* genes. Genomic DNA of patient 1 and his parents was isolated from whole blood using QIAamp DNA kit (Quiagen, Hilden, Germany). Specific primer sets were designed to amplify all MUT exons and intron-exon junctions. PCR products were checked on a 1.5% agarose gel and purified using the QIAquick gel extraction kit (Quiagen, Hilden, Germany). Mutation detection was performed by nucleotide sequencing on an ABI PRISM 310 genetic analyser using BigDye terminator chemicals (Applied Biosystems).

Consent to publication was obtained from the parents of both patients reported and from the Institutional Review Board.

Clinical reports

Patient 1

Patient 1, male, was born to non-consanguineous parents after a normal pregnancy and delivery. Birth weight was 3240 g; gestational age was 41^{+2} weeks; Apgar index was 9–9 at 1 and 5 min, respectively. He had a physiological course for the first 24 h of life. Weight loss was 420 g in 48 h and lazy feeding was noted. At the third day of live he showed tachypnoea and general conditions were worsening, and the newborn was transferred to our neonatal intensive care unit (NICU).

Intravenous glucose (7 mg/kg per min) and bicarbonate infusion were started to correct the metabolic acidosis (pH 7.102; base excess -24.3 mmol/L; HCO_3^-

7.9 mmol/L). Blood spot acylcarnitine profile revealed increased propionylcarnitine (C_3) concentrations of 12.2 μmol/L (normal value <3.3 μmol/L) and methylmalonylcarnitine (C_4DC) concentrations of 0.54 μmol/L, at the upper limit of the normal range (<0.54 μmol/L), plasma amino acids showed glycine concentration of 751 μmol/L (normal 158–337 μmol/L). Coagulation profile was normal. Urine organic acid analysis showed an increased excretion of lactic acid (306 μmol/mmol creatinine, normal <25), methylmalonic acid (2919 μmol/mmol creatinine, normal <2) and methylcitric acid (30 μmol/mmol creatinine, normal <2). Plasma homocysteine was normal. The clinical and laboratory investigations suggested the diagnosis of methylmalonic acidaemia type mut. The diagnosis was then confirmed by molecular analysis of the *MUT* gene. The proband resulted a compound heterozygote for the known c.655A>T (p.Asn219Tyr) and the novel c.481G>A (p.Gly161Arg) genetic lesions, both localized in exon 3. The two mutations were inherited from the patient's father and mother, respectively. The novel c.481G >A, leading to the p.Gly161Arg amino acid substitution, was not found in 120 sequenced alleles of unrelated control subjects.

Treatment with *N*-carbamylglutamate (150 mg/kg per day) and arginine hydrochloride (360 mg/kg in 2 h) was started to correct hyperammonaemia (559 μg/dl) and L-carnitine (200 mg three times daily) was added. After carnitine administration, C_3 increased to 78.6 and C_4DC to 1.19 μmol/L. Repeated blood gas analysis showed a persistent severe metabolic acidosis despite the treatment with bicarbonate. After 12 hours of treatment, ammonium decreased to 188 μg/dl. At day four, clinical conditions worsened, the infant was intubated and mechanically ventilated, and sodium benzoate (250 mg/kg per day) was added because the ammonium level increased further (214 μg/dl). Intravenous glucose infusion was increased until 12 mg/kg per min was reached and 2 g/kg of intravenous lipids were added, to increase the caloric intake to more than 356 kJ/kg (85 kcal/kg) and to promote anabolism. However, early severe hyperglycaemia was observed; insulin therapy was unsuccessfully started and progressively increased to a dose of 1 U/kg per h. Intravenous glucose infusion was therefore reduced quickly to 7 mg/kg per min. Insulin therapy was further increased until 4.8 U/kg per h, but glucose plasma concentrations increased until 48.3 mmol/L even if glucose infusion was further reduced to 5 mg/kg per min and an oral feeding giving a glucose contribution of 4.5 mg/kg per min was added (Fig. 1). At the same time general conditions worsened, a dramatic increase of lactate concentration was noted, blood acylcarnitines (C_3 295 μmol/L, C_4DC 1.26 μmol/L) and urine organic acids profiles (lactic 4398 μmol/mmol creatinine, methylmalonic 5296 μmol/mmol creatinine) demonstrated the catastrophic metabolic decompensation, and the following day the infant died after a pulmonary haemorrhage.

Patient 2

Patient 2, female, was born to non-consanguineous parents after a normal pregnancy and delivery. Birth weight was 3080 g; gestational age was 38^{+4} weeks; Apgar index was 9–10. From the second day of life, lazy feeding and poor reactivity were noted. Laboratory

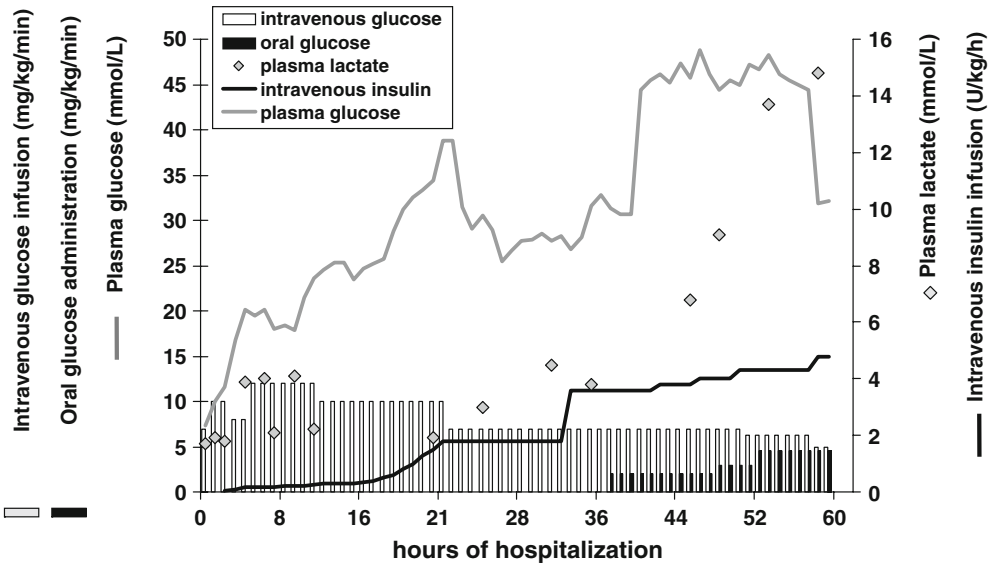

Fig. 1 Metabolic profile (glucose and lactate plasma concentrations) during glucose and insulin administration in patient 1

investigations revealed metabolic acidosis (pH 7.08, HCO_3^- 5.3 mmol/L, BE -21.3 mmol/L) and hyperammonaemia (770 μg/dl). Oral feeding was stopped and intravenous glucose infusion (8 mg/kg per min), treatment with sodium benzoate (250 mg/kg) and arginine hydrochloride (360 mg/kg) in 2 h were started, and the newborn was transferred to our NICU at third day of life.

On admission the infant was in fairly good general condition, spontaneously breathing, but generalized seizures with hypertonic upper and lower limbs required treatment with phenobarbital. Cerebral ultrasound showed signs of cerebral oedema. Intravenous lipids (2 g/kg) were added to the glucose infusion in order to increase the caloric intake to around 293 kJ/kg (70 kcal/kg), and to promote a faster metabolic detoxification oral *N*-carbamylglutamate (150 mg/kg per day) was added. Blood acylcarnitines (C_3 6.18 μmol/L), and urine organic acids profiles (lactic 285 μmol/mmol creatinine, 3-hydroxy-propionic 553 μmol/mmol creatinine, methylcitrate 36 μmol/mmol creatinine, tiglylglycine 25 μmol/mmol creatinine) suggested the diagnosis of propionic acidaemia. L-Carnitine (100 mg/kg per day), sodium bicarbonate and sodium phenylbutyrate (250 mg/kg per day) were added. Warming was stopped and the neonate went spontaneously into hypothermia reaching 34.5°C. Plasma ammonium concentrations decreased to 254 μg/dl within 6 h and normalized after 12 h. At this point the infant was re-warmed to 37°C. Twenty-four hours after carnitine administration, C_3 increased to 80.1 μmol/L. Because of the severe increase of glucose plasma concentrations, insulin therapy was unsuccessfully started and rapidly increased to a dose of 1.2 U/kg/h (Fig. 2). Simultaneously, serious hyponatraemia appeared. Liver function (coagulation profile, total protein concentration) was normal. After 24 h of hospitalization, clinical conditions worsened, and the infant was intubated for recurrent apnoea and pulmonary haemorrhage. Plasma concentrations of 3-hydroxybutyrate and acetoacetate at 36 h of hospitalization were 839 and 844 μmol/L, respectively.

On the basis of the earlier experience at hour 40 of hospitalization we significantly reduced and then stopped the intravenous glucose infusion, increasing the oral glucose administration. Lactate concentrations were only mildly increased. Glucose concentrations decreased and normalized in 6 hours; insulin was rapidly stopped and an improvement of clinical conditions was observed. In the following days, thrombocytopenia and leukopenia were noted. *N*-Carbamylglutamate, sodium benzoate and arginine hydrochloride were progressively reduced and suspended on the 10th day of life.

Cerebral magnetic resonance imaging (MRI) at 19 days of life showed wide alteration of signal in the white substance with low differentiation between it and the cerebral cortex reduced in thickness. A video-electroencephalogram (EEG) at 12 days of life showed the presence of multifocal paroxystic anomalies. The girl showed a slowly but continuously improving neurological case history. She began to be bottle-fed and she was discharged at 29 days of life. At 2 months of life a cerebral MRI showed a signal reduction in the white matter and a diffuse cerebral hypotrophy on T2-weighed images. The child was in good general

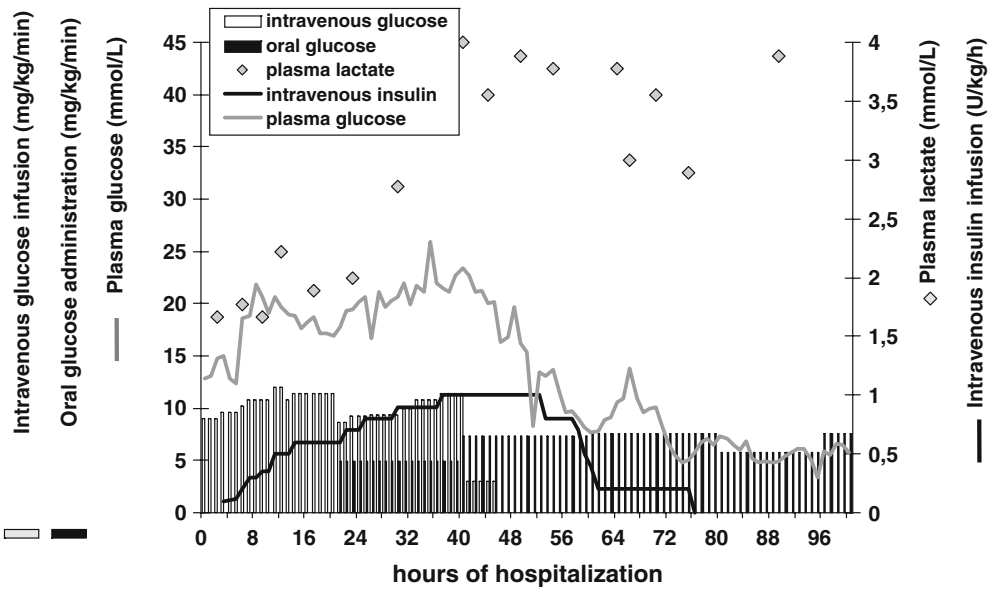

Fig. 2 Metabolic profile (glucose and lactate plasma concentrations) during glucose and insulin administration in patient 2

condition and responsive, but she presented axial hypotonia with head control only for short time.

Discussion

Patients with early-onset MMA and PA often demonstrate abnormal carbohydrate homeostasis. In MMA, hypoglycaemia occurs in about 40% of affected patients (Worthen et al 1994). Many mechanisms were proposed to explain this phenomenon. The intracellular accumulation of CoA as methylmalonyl-CoA (MMCoA) could produce insufficiency of this coenzyme and impair carbohydrate metabolism (Oberholzer et al 1967). Other studies suggested that the excess of MMCoA inhibits the pyruvate carboxylase (PC) activity, which prevents gluconeogenesis and provokes hypoglycaemia (Scrutton et al 1965). Moreover MMCoA was observed to inhibit the synthesis of malate, a key step in gluconeogenesis and the main transmitochondrial shuttle of reducing equivalents (Fig. 3) (Halperin et al 1971). In patients with PA the accumulation of propionyl-CoA (PCoA) inhibits mainly pyruvate dehydrogenase (PDH), resulting in a fall of acetyl-CoA levels. This reduces PC activity due to a lowered allosteric activation by acetyl-CoA and accounts for the observed inhibition of gluconeogenesis and consequent hypoglycaemia (Martin-Requero et al 1983).

Treatment of acute neonatal onset includes a high intake of glucose to prevent protein and fat catabolism. If hyperglycaemia appears, insulin infusion can theoretically be very useful because, as a potent anabolic hormone, it may suppress catabolism and promote protein and lipid synthesis, facilitating the uptake of harmful amino acid precursors (Wendel et al 2006). However, insulin-resistant hyperglycaemia in such patients has been occasionally reported (Boeckx and Hicks 1982; Ciani et al 2000; Lehnert et al 1980). Boeckx and colleagues (Boeckx and Hicks 1982) described a newborn affected by MMA complicated by a severe insulin-resistant hyperglycaemia, hypocalcaemia, metabolic acidosis. The patient died on the 8th day of life, and the authors hypothesized that another biochemical defect was responsible for the hyperglycaemia, such as an insulin-receptor defect. Another case of lethal PA associated with severe hyperglycaemia was described by Lehnert and colleagues (1980). Ciani and colleagues (2000) described an apparently previously healthy 12-year-old girl admitted to the intensive care unit with progressive loss of consciousness associated with ketoacidosis, hyperammonaemia, hyperglycaemia and lactic acidosis, and a consequent diagnosis of insulin-dependent diabetes mellitus. This patient died three days after the hospitalization, but the post-mortem examination brought a diagnosis of MMA. Her older brother died at the age of 15 years

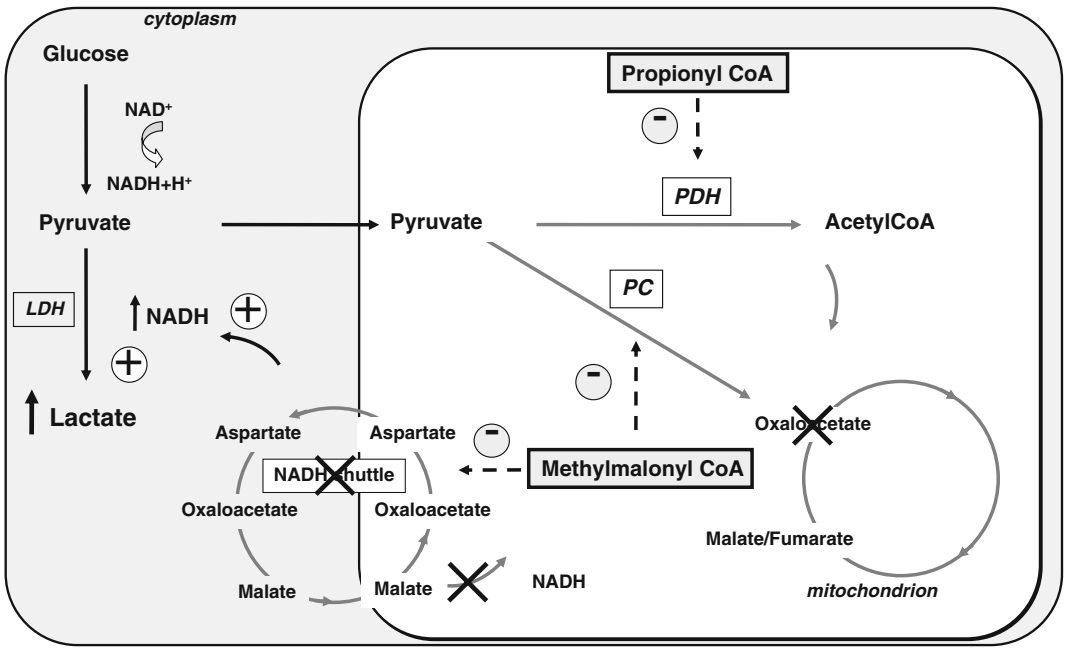

Fig. 3 Biochemical consequences of intramitochondrial pools of acyl-CoA esters, such as propionyl and methylmalonyl-CoA. The dashed arrow and the symbol (α) show the inhibitory effects of propionyl-CoA on pyruvate dehydrogenase (PDH) activity and of methylmalonyl-CoA on pyruvate carboxylase (PC) activity and, through the reduced synthesis of oxaloacetate, on the malate—aspartate shuttle. The symbol (+) indicates that the shuttle inhibition produces an elevation in the NADH/NAD$^+$ ratio and consequently an elevation of the lactate/pyruvate ratio

with the diagnosis of Reye syndrome complicated by hyperglycaemia.

Until now, all reported patients with organic acidaemias associated with severe insulin-resistant hyperglycaemia have died within a few days, suggesting that hyperglycaemia could be a marker of more serious metabolic decompensation.

Here, we report two clinical cases, one of MMA and the other of PA, both with acute neonatal onset associated with insulin-resistant hyperglycaemia. Both the newborns showed a severe form of these diseases, as confirmed by the clinical course, by elevated values of plasma acylcarnitines after carnitine administration, and by the urinary organic acids analysis and from *MUT* gene mutations identified in the newborn with MMA, described as patient 1. This patient was compound heterozygous for the c.655A>T (p.Asn219Tyr) and c.481G>A (p.Gly161Arg) mutations, both localized in the MCM N-terminal barrel domain containing the active site. It is known that more than 75% of mut^0 mutations affect the barrel domain, while the mut^- mutations affect residues located in the C-terminal cofactor-binding domain (Acquaviva et al 2005). The p.Asn219Tyr is reported as a frequent mutation among caucasian mut^0 MMA patients (Acquaviva et al 2001). The Gly161Arg affects a highly conserved residue of the MCM barrel domain and the substitution of a glycine with an arginine is predicted to cause a hydrophobicity change at buried site, resulting in a destabilization of the barrel domain (PolyPhen server: http://www.bork.embl-heidelberg.de/PolyPhen; accessed January 2009). The absence of the c.481G>A (p.Gly161Arg) substitution in 120 control alleles, the high phylogenetic conservation of the Gly161 amino acid and the bioinformatic data strongly suggest that the c.481G>A (p.Gly161Arg) is a disease-causing mutation. However, the formal proof of pathogenetic mechanism for this mutation will require further biochemical investigations, such as overexpression and kinetic analysis of the mutant enzyme. Although biochemical studies on fibroblast from patient 1 were not carried out, the severe clinical picture and the detected *MUT* gene mutations involving highly conserved residues of the barrel domain lead us to hypothesize that this patient is affected by the mut^0 phenotype.

PA and MMA represent different diseases that affect different metabolic pathways. Moreover, these metabolic defects affect enzymes that play a key role in different organs (PC in liver and PDH in muscle), with different effects on glucose metabolism. This probably explains the different outcomes of the two infants.

Patient 1 developed a persistent lactic acidosis concomitant with progressive hyperglycaemia. These complications can be explained by the intramitochondrial storage of MMCoA, which inhibits PC, and the synthesis of malate. This produces an inhibition of the malate—aspartate shuttle, the main system for transfer of reducing equivalents into mitochondria (Bremer and Davis 1975). The shuttle inhibition produces an elevation in the cytoplasmic redox potential and $NADH/NAD^+$ ratio and consequently an elevation of the concentration ratios of lactate/pyruvate. This means that intramitochondrial pyruvate utilization is reduced and that cytoplasmic pyruvate is converted to lactate (Fig. 3) (Barron et al 1998). The glucose concentrations in patient 1 did not diminish despite an attempt (probably belated) to reduce the glucose administration.

Also the newborn with PA described as patient 2 showed insulin-resistant hyperglycaemia. Glucose concentrations were less high but her clinical history was less dramatic than patient 1 and she survived. To our knowledge, this is the only newborn with this complication to survive. Indeed, lactic acidosis did not occur, probably because PCoA does not inhibit malate synthesis. This means that the cytoplasmic redox potential and NADH/NAD ratio were normal and therefore pyruvate was not converted to lactate. The hyperglycaemia of this newborn resolved and clinical conditions improved rapidly only with a strong reduction of intravenous glucose administration, replaced with a progressive increase of oral glucose. Oral glucose was better tolerated than intravenous glucose, probably because oral glucose enters the liver via an insulin-independent mechanism. Besides, the liver of patients with PA is able to oxidize glucose, shifting glucose via PC to oxaloacetate.

We cannot claim that a strong reduction of glucose infusion in patient 1 would have avoided his death, as happened to the patient 2. In fact the death of patient 1 was due predominantly to lactic acidosis secondary to the failure of PC activity. However, it can be assumed that also in patient 1, a drastic reduction of glucose administration would probably have allowed to control hyperglycaemia and to reduce lactate concentrations.

This clinical evolution enables us to comprehend that hyperglycaemia in this case was not due to an underlying disease associated with PA (as supposed in other patients) (Boeckx and Hicks 1982), but rather to a transitory and reversible intolerance to high glucose concentrations. For this reason, we suggest that hyperglycaemia could be a seriousness marker.

The clinical course of patient 2 was characterized by serious hyponatraemia. Hypertonicity secondary to hyperglycaemia was probably the major cause of

hyponatraemia. In fact hyponatraemia is relatively common in diabetic children (Walters and Hughes 1984), because hyperglycaemia promotes both an osmotic diuresis and an osmotic shift of water from within the cells to the extracellular fluid. This in turn decreases the plasma sodium concentration by dilution. Hyponatraemia can promote the development of cerebral oedema (Hale et al 1997) and it therefore may have played a role in the clinical neurological outcome. We are not able to explain the development of pulmonary haemorrhage in reported patients. Coagulation profile and liver function allowed us to exclude a relationship with liver failure or disseminated intravascular coagulation. However, in infants suffering from congenital organic acidaemias, pulmonary congestion has sometimes been observed (Ozand et al 1994).

Regarding the mechanism of this hyperglycaemia, in our patients we were not able to measure the glucose production and therefore we are not able to specify whether hyperglycaemia was due to an inappropriate hepatic endogenous glucose output or to a reduced muscular or adipose glucose uptake, even if this option appears more likely. Moreover, we must not overlook that both newborns received intravenous lipid infusion. It is well known that fatty acids induce insulin resistance in muscle (Kruszynska et al 2002). It is therefore possible that lipid infusion may have accentuated the hyperglycaemia. The high levels of ketone bodies were probably due more to the insulin resistance than to the enzymatic defect.

In conclusion, this clinical observation, together with literature data, prompts us to suspect that the occurrence of insulin-resistant hyperglycaemia can identify newborns at higher risk of death. The outcome of both reported infants allows us to suggest, in newborns with this complication, reduction of the glucose infusion, which in patients with MMA could lead to lethal hyperlactacidaemia. It is likely that even if we had drastically reduced the intake of glucose in newborn patient 1, this would not have prevented the infant's death. However, we hope to undertake further research to assess whether a drastic reduction of glucose intake in these patients might allow at least avoidance of complications secondary to a persistent hyperglycaemia.

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