Lactic Acidosis and Mitochondrial Dysfunction in Two Children with Peroxisomal Disorders

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Summary: Mitochondrial myopathies and defects in oxidative phosphory-
lation have been described in some patients with peroxisomal disorders. Although
peroxisomes and mitochondria play a role in the β-oxidation of fatty acids, the
metabolic interactions between the two are not well defined. Defects in
peroxisomal β-oxidation are associated with extracellular accumulation of very
long-chain fatty acids and may be accompanied by alterations in the intracellular
pool of fatty acyl-CoAs, which are known to alter mitochondrial function. This
study was initiated to examine alterations in the intracellular pool of acyl-CoAs
and mitochondrial function in two children with generalized disorders of
peroxisomal function and clinical lactic/pyruvic acidaemia. Fibroblasts were
cultured from skin biopsies obtained from one child with neonatal adrenoleuko-
dystrophy (NALD) and another with rhizomelic chondrodysplasia punctata
(RCDP). Fibroblast lactate oxidation was significantly inhibited in NALD by
76% and RCDP by 92% compared to control values of 1.9 ± 0.1 nmol/min
per mg protein. Pyruvate dehydrogenase (PDH) (mean $\pm$ SEM; activity nmol/
min per mg protein) was: NALD 0.55 ± 0.02 ($p < 0.01$), RCDP 0.44 ± 0.02
($p < 0.01$), and controls 0.83 ± 0.02. The acid-insoluble (long-chain and very
long-chain) acyl-CoA levels (mean $\pm$ SEM; pmol/mg protein) were: NALD
129 ± 69 ($p < 0.01$), RCDP 65 ± 15 ($p < 0.05$), and control 45 ± 7. These two
patients with generalized peroxisomal disorders exhibited an increase in
intracellular acyl-CoA species accompanied by decreased PDH activity and
clinical lactic/pyruvic acidaemia.

Isolation and biochemical characterization of peroxisomes began less than thirty
years ago. Peroxisomes have multiple biosynthetic functions and, like mitochondria,
play a role in the β-oxidation of fatty acids (Wanders et al 1990). The main function

of the peroxisomal oxidation system is the β-oxidation of very long-chain fatty acids (VLCFA), prostaglandins, dicarboxylic acids, xenobiotic fatty acids and hydroxylated 5-β-cholestanoic acids (Schulz 1991). The products of peroxisomal β-oxidation, chain-shortened acyl-CoAs, enter the mitochondrial β-oxidation pathway, promoting oxidative phosphorylation and supplying acetyl-CoA for ketogenesis.

The metabolic interaction between peroxisomes and mitochondria is not well defined. Goldfischer (1973) observed hyperlactataemia in two patients with peroxisomal disorders. In addition, abnormalities in the mitochondrial electron-transport chain in Zellweger syndrome have been described (Kelley and Corkey 1983; Trijbels et al 1983; Roels et al 1988), and patients with peroxisomal disorders have been reported to develop mitochondrial myopathies (Sarnat et al 1983; Wolff et al 1986). Abnormal oxidative phosphorylation has also been described in an infant with Zellweger syndrome (Muller-Hocker et al 1984).

Defects in peroxisomal β-oxidation are accompanied by an increase in the extracellular pool of fatty acids as indicated by increased plasma concentrations of VLCFA. It is not known whether there is a concurrent increase in the intracellular pool of acyl-CoAs that could affect mitochondrial function. Acyl-CoA molecules appear to have regulatory effects on a variety of reactions and have been shown to inhibit ureagenesis, gluconeogenesis and mitochondrial energy metabolism (Corkey and Deeney 1990). Long-chain acyl-CoAs have also been shown to inhibit the activity of pyruvate dehydrogenase complex, an enzyme composed of pyruvate dehydrogenase (E_1; EC 1.2.4.1), dihydrolipoyl transacetylase (E_2; EC 2.3.1.12) and dihydrolipoyl dehydrogenase (E_3; EC 1.6.4.3) (Kiechle et al 1990b; Moore et al 1992). This study examines alterations in the intracellular pool of acyl-CoAs in cultured skin fibroblasts from two patients with peroxisomal disorders and their relation to mitochondrial metabolism.

METHODS

Patient 1: N.R. was born on 26 May 1983 following a full-term gestation. Birth weight was 3.13 kg (50th percentile), length 54 cm (95th percentile) and head circumference 34.5 cm (50th percentile). He was evaluated at 7 weeks of age because of failure to thrive and was noted to have hypotonia and enlarged anterior and posterior fontanelles with diastasis of the sagittal suture. Serum electrolytes were: sodium = 145 mmol/L (139–146), potassium = 6.5 mmol/L (4.0–6.2), chloride = 111 mmol/L (96–106) and bicarbonate = 19 mmol/L (24–30). Serum lactic acid and pyruvic acid were elevated (Table 1). Hepatomegaly was present at age 4 months and serum aminotransferase levels were elevated (Table 1). Percutaneous liver biopsy showed abnormal lobular architecture with fibrous septa extending from portal tracts into the surrounding parenchyma. By 8 months of age he could not visually fix on objects and had wandering nystagmus. Ophthalmological evaluation revealed bilateral punctate cortical and plaque-like nuclear changes, optic atrophy and retinal degeneration.

A skin biopsy was performed at 1 year of age and fibroblasts were cultured, harvested and assayed for the activity of the peroxisomal membrane enzyme, dihydroxyacetone phosphate acyltransferase (DHAP-AT) (Datta et al 1984). The

Table 1 Results of clinical laboratory tests

Test	Reference range	NALD	RCDP
Serum AST (U/L)	20–45	97	44
Serum ALT (U/L)	3–20	39	14
Plasma very long-chain fatty acids			
C_{26} (μg/ml)	0.33 ± 0.18	1.49	0.36
C_{26}/C_{22}	0.01 ± 0.01	0.22	0.02
Plasma phytanate (μmol/L)	<9.6	9.8	12.2
Plasma carnitines			
Free (μmol/L)	35–55	9	42
Total (μmol/L)	45–65	17	50
Urine acylcarnitine profile	N[a]	N	N
Urine organic acids	ND[b]	Adipic, suberic	ND
Serum lactate (mmol/L)	0.6–1.8	2.8	2.1
Serum pyruvate (μmol/L)	30–70	200	130

NALD = neonatal adenoleukodystrophy; RCDP = rhizomelic chondrodysplasia punctata
[a]N = within the reference range determined by mass spectrometry at Duke University Medical Center, Pediatric Metabolism Laboratory
[b]ND = none detectable

activity was 30% of control (Wilson et al 1986). Levels of C_{26} VLCFA and the ratio of C_{26}/C_{22} assayed at 20 months of age were elevated (Table 1). The phospholipid composition of erythrocytes was measured when the child was 2.5 years old. The erythrocyte phosphoethanolamine plasmalogen levels were 4% as compared to reference values of 12% (Wilson et al 1986) (Table 1). These results confirm the diagnosis of neonatal adrenoleukodystrophy (NALD; McKusick 20237).

The child is currently enrolled in special education classes with a level of development equivalent to a young toddler. He has not yet developed clinical or laboratory evidence of adrenal insufficiency (normal Cortrosyn stimulation test at age 8.5 years).

Patient 2: G.B. was born on 29 November 1989 by Caesarean section because of failure to progress and fetal distress. Gestational age was determined by physical examination at 33 weeks, birth weight 2.13 kg (< 5th percentile) and length 43 cm (< 5th percentile). She had limb disproportion and radiographs showed rhizomelic shortening of the extremities, metaphyseal splaying, and punctate epiphyseal stippling diagnostic of rhizomelic chondrodysplasia punctata (RCDP; McKusick 21510). Bilateral nuclear cataracts were present.

At 1 month of age a sample of blood was sent to the Kennedy Institute for Handicapped Children (Baltimore, Maryland, USA) for analysis of erythrocyte plasmalogens and plasma VLCFA. Red blood cell plasmalogen levels were measured as dimethylacetal (DMA) and the ratio of DMA to the respective chain-length fatty acid was calculated. The mean value of C_{16}-DMA/C_{16} fatty acid was 0.002 (reference range 0.065 ± 0.010) and C_{18}-DMCA/C_{18} fatty acid was 0.003 (reference range 0.195 ± 0.035). The ratio C_{26}/C_{22} VLCFA was only slightly higher than normal (Table 1). A second sample was obtained at age 20 months and assayed for phytanic

acid (Table 1). The depressed red blood cell plasmalogens, near normal levels of VLCFA and mildly elevated phytanic acid are consistent with the diagnosis of RCDP.

The infant underwent cataract surgery at 2 months of age and has been wearing corrective lenses. Serum electrolytes have been normal; however, serum lactic acid and pyruvic acid were found to be elevated at age 31 months (Table 1). Growth and development are delayed and she currently attends an early intervention school where she receives occupational and physical therapy.

Fibroblasts: Skin biopsies were performed on these two patients after obtaining informed consent from the parents and following guidelines established by the William Beaumont Hospital Human Investigation Committee. Biopsy specimens obtained from remnants of human foreskin removed from healthy infants undergoing routine circumcision and from adult volunteers were used as controls. Skin biopsy specimens were subcultured in Eagle's Minimum Essential Medium (MEM) (Mediatech, Washington, DC, USA) supplemented with 15% fetal bovine serum (Biocell, Carson, CA, USA), 100 U/ml penicillin G potassium, 100 µg/ml streptomycin sulphate (Gibco, Grand Island, NY, USA) and 100 µU/ml insulin (Sigma, St Louis, MO, USA). Fibroblasts were grown as monolayer cultures and maintained in a 37°C humidified incubator containing a mixture of 95% air and 5% CO_2. Medium was replaced twice a week and cells passed during their log growth phase. Cultures were routinely screened for mycoplasma contamination by Mycotrim TC (Hanna Biologicals Inc., Alameda, CA, USA).

Lactate oxidation: An assay was developed to screen for mitochondrial metabolic dysfunction by measuring oxidation of lactate by fibroblasts. Metabolism of D, L-[1-^{14}C]lactate was measured in fibroblast monolayers grown to confluence in 25-cm^2 tissue flasks containing MEM. Fibroblasts were washed twice with phosphate-buffered saline then treated with phosphate-buffered saline containing 25 µmol/L L-lactate (Sigma) and 0.1 µCi/ml D,L-[1-^{14}C]lactate (American Radiochemical Co., St. Louis, MO, USA) and slowly shaken at 37°C for 2 h 40 min. Filter paper moistened with 0.2 ml of 100 g/L sodium hydroxide, used to collect atmospheric $^{14}CO_2$, was suspended in the flask with an airtight stopper directly above the fibroblast monolayer. Each filter was transferred into scintillation vials and the amount of $^{14}CO_2$ liberated was determined. The cells were then harvested for protein determination. Activity was expressed as nmol $^{14}CO_2$ released per min per mg cellular protein. Background activity determined using flasks with no cells and containing only 0.1 µCi D,L-[1-^{14}C]lactate in 1 ml phosphate-buffered saline was subtracted from both the control and patient results.

Pyruvate dehydrogenase (PDH): Fibroblasts were harvested 3–4 days after reaching confluence. Two 150-cm^2 tissue flasks, containing approximately 3.0×10^7 cells, were washed three times with calcium-free and magnesium-free phosphate-buffered saline (pH 7.4). After the final wash, 2 ml homogenization buffer containing 25 mmol/L sodium fluoride, 4 mmol/L dichloracetic acid, 1 mmol/L dithiothreitol and 0.25 mmol/L sucrose in 40 mmol/L phosphate-buffered saline (pH 7.4) was added to

the monolayers. The cells were transferred into 5-ml cryogenic vials. The sample was homogenized using a glass Potter–Elvehjem homogenizer at setting 6 for 6 seconds. The cell homogenate was further frozen and thawed three times in a dry-ice–acetone bath and centrifuged for 15 min at 35 000g in a refrigerated Beckmann J2-21 centrifuge. The supernatants were maintained on ice and assayed immediately for PDH activity and total protein determination (Smith et al 1985). The phosphorylation state of the PDH complex was stabilized by treatment of the fibroblasts with dichloracetic acid to inhibit PDH kinase (EC 2.7.1.99) (Whitehouse et al 1974) and sodium fluoride to inhibit the PDH phosphatase (EC 3.1.3.43) (Hucho et al 1972).

A radiochemical method based on the specific decarboxylation of $[1\text{-}^{14}C]$pyruvate to $^{14}CO_2$ has been employed in the measurement of PDH activity. However, $[1\text{-}^{14}C]$pyruvate is unstable and undergoes spontaneous non-enzymatic decarboxylation that may reduce the sensitivity of the assay (Williams et al 1978; Constantopoulos and Barranger 1984; Kiechle et al 1990a). An assay was developed by Schofield et al (1980) to overcome these problems using *in situ* generation of $[1\text{-}^{14}C]$pyruvate from $[1\text{-}^{14}C]$lactate and is modified as described by Buffington et al (1984), who added two phosphorylation/dephosphorylation inhibitors of PDH complex, NaF and DCA, to measure PDH activity at the time of homogenization.

The assay mixture containing 50 mmol/L potassium phosphate buffer (pH 7.4), 2.5 mmol/L MgCl$_2$, 1.5 mmol/L β-nicotinamide-adenine dinucleotide, 500 μmol/L CaCl$_2$, 80 μmol/L thiamine pyrophosphate, 100 mU/ml phosphotransacetylase, 0.2 mmol/L coenzyme A (CoASH), 1 mmol/L dithiothreitol and the fibroblast suspension (20–100 μl) containing 0.05–0.25 mg protein was preincubated for 10 min at 37°C. The reaction was started by adding 10 IU L-lactate dehydrogenase, 1.1 mmol/L L-lactate and 0.1 μCi/ml D,L-$[1\text{-}^{14}C]$lactate to the assay mixture for a total volume of 0.5 ml. Filter paper moistened with 0.2 ml of 100 g/L sodium hydroxide, used for trapping $^{14}CO_2$, was then suspended in a vial with an airtight stopper directly over the assay mixture. This linear reaction (Hansen 1982) was stopped after 60 min at 37°C by addition of 3 mol/L H$_2$SO$_4$. Incubation continued for 30 min at 37°C and the filter paper was transferred to a vial containing scintillation fluid for the determination of $^{14}CO_2$ liberated.

Experiments were performed in triplicate. 'Blank' values were obtained from identical assay tubes without CoASH and β-nicotinamide-adenine dinucleotide. PDH activity is expressed as nmol $^{14}CO_2$ released per min per mg of homogenate protein. Values are expressed as the mean $\pm$ SEM.

Citrate synthase and catalase: For the determination of citrate synthase (EC 4.1.3.7), catalase (EC 1.11.1.6), acyl-CoA synthetase (C$_4$ to C$_{11}$, EC 6.2.1.2; C$_6$ to C$_{20}$, EC 6.2.1.3), acyl-CoA hydrolase, and acyl-CoA levels, the fibroblasts were prepared in the same manner. Frozen packed cells were dispersed in 3 volumes of ice-cold 0.25 mol/L sucrose–0.01 mmol/L potassium phosphate buffer (pH 7.4) and homogenized for 40 s with a Tissue-Tearer at the lowest setting. Aliquots of homogenate were used for protein assay, enzyme assay, and CoA determinations. Standard spectrophotometric assays were employed for assay of citrate synthase (EC 4.1.3.7) (Shepherd et al 1969) and catalase (EC 1.11.1.6) (Johansson and Hakan Borg 1988).

Acyl-CoA synthetase: Incubation mixture (0.25 ml) containing 100 mmol/L Tris-HCl buffer (pH 8.0), 150 mmol/L KCl, 15 mmol/L $MgCl_2$, 1 mmol/L CoASH, 10 mmol/L adenosine triphosphate and 1 mmol/L [1-^{14}C]palmitate–0.2 mmol/L albumin was added to tubes containing 0.12 mg of fibroblast homogenate. The reaction proceeded in a linear manner for 20 min at 37°C and was terminated by addition of 0.5 ml Dole reagent (Hosaka et al 1981). Unreacted substrate was removed by repeated heptane extractions. Aliquots of the remaining aqueous layer were dissolved in scintillation fluid and counted.

Acyl-CoA hydrolase: The assay was performed by incubating 20 μg tissue in 0.7 ml of reaction mixture which contained 100 mmol/L Tris-HCl buffer (pH 7.4), 1.25 mmol/L 5,5'-dithiobis-(2-nitrobenzoic acid), and 40 μmol/L acyl-CoA. The reaction of released CoASH with 5,5'-dithiobis-(2-nitrobenzoic acid) was monitored at 412 nm by the rate analysis program of the Milton-Roy Diode Array Spectrophotometer (Berge and Farstad 1979). The acyl-CoA substrates were prepared as 0.5 mmol/L stock solutions in deionized water. Concentrations were confirmed by absorption at 260 nm.

CoA levels: Immediately after preparation, aliquots of fibroblast homogenate were extracted with ice-cold perchloric acid (6% v/v, final concentration) to separate soluble CoA moieties (CoASH and acetyl-CoA) from acid-insoluble long-chain and very long-chain acyl-CoA moieties. The acid-insoluble acyl-CoA pellets were washed, resuspended, hydrolysed, and neutralized as previously described (Moore et al 1982). The acid-soluble supernatant was neutralized and assayed directly for CoASH plus acetyl-CoA and, after treatment with *N*-ethylmaleimide, for direct analysis of acetyl-CoA (Allred and Guy 1969). The sensitive fluorimetric enzymatic cycling method of Veloso and Veech (1974) was used to assay neutralized fractions. Free CoASH content was estimated by subtracting acetyl-CoA content from total acid-soluble content.

Total protein: Protein content was determined by the bicinchoninic acid assay (Smith et al 1985).

Statistical significance: Statistical significance was assigned to data with $p < 0.05$ as determined by the unpaired Student's *t*-test.

RESULTS

Fibroblasts from the patients and two infant controls were screened for their ability to oxidize lactate (Table 2). The infant control values represent the average of two controls each assayed in triplicate. Lactate oxidation was significantly decreased in NALD (76% inhibition) and RCDP (92% inhibition) compared to the infant control value of 1.9 ± 0.1 nmol/min per mg protein. These results suggested that the mitochondrial oxidation of pyruvate to CO_2 by PDH was decreased in both of the patients' fibroblasts. Therefore, PDH activity was determined. PDH activity was significantly decreased in the NALD fibroblasts (34% inhibition) and the RCDP

Table 2 **Lactate oxidation and pyruvate dehydrogenase activity in fibroblasts**

Fibroblasts	Lactate oxidation	Pyruvate dehydrogenase
	(nmol/min per mg protein)	
Control	1.9 ± 0.1	0.83 ± 0.02
NALD	$0.45 \pm 0.015^{a,b}$	0.55 ± 0.02^{c}
RCDP	0.14 ± 0.003^{a}	0.44 ± 0.02^{c}

Results from a representative experiment were performed in triplicate. The results represent the mean $\pm$ SEM. Similar results were obtained in at least 3 additional experiments
[a] $p < 0.0005$, compared with infant controls
[b] $p < 0.05$, compared with RCDP
[c] $p < 0.01$, compared with infant controls

fibroblasts (47% inhibition) as compared with the infant control value of 0.83 ± 0.02 nmol/min per mg protein (Table 2). The location of PDH on the inner mitochondrial membrane suggests that the decrease in activity observed in NALD and RCDP is attributable to mitochondrial dysfunction. Since PDH activity can be inhibited *in vitro* by a variety of acyl-CoA species (Kiechle et al 1990b; Moore et al 1992), the acyl-CoA concentration in the fibroblasts was determined.

The distributions of CoA species for infant and adult controls and patient fibroblasts are shown in Table 3. The infant controls and patients were more closely age-matched and their results were compared. Fibroblasts derived from NALD had acid-insoluble acyl-CoA levels, including acyl-CoA with chain length $> C_8$, almost 3-fold higher than those found in infant control fibroblasts. NALD fibroblasts also demonstrated a 2.2-fold increase in acetyl-CoA content; in contrast, the free CoASH in these fibroblasts was only 6% of infant control fibroblasts. Total CoA (acid-insoluble acyl-CoA + acetyl CoA + CoASH) was elevated almost 2-fold in NALD. The increased esterified CoA and decreased free CoASH result in dramatic increases in both acetyl-CoA/CoASH and acid-insoluble acyl-CoA/CoASH ratios in NALD fibroblasts.

Table 3 **Fibroblast coenzyme A levels**

CoA species	Infant controls	Adult controls	NALD	RCDP
		(pmol/mg protein)		
Acid-insoluble acyl-CoA	45 ± 7	45 ± 11	129 ± 69^{a}	65 ± 15^{b}
CoASH + acetyl-CoA	163 ± 37	154 ± 40	261 ± 64	194 ± 72
Acetyl-CoA	115 ± 40	108 ± 24	258 ± 77^{a}	137 ± 45
CoASH	48	46	3	57
Total CoA	208	199	390	259
Ratios				
Acetyl-CoA/CoASH	2.40	2.35	86.0	2.40
Acyl-CoA/CoASH	0.94	0.98	43.0	1.14

Acid-insoluble acyl-CoA species represent those with chain length $> C_{8:0}$
Metabolite levels are expressed as pmol/mg protein. The data are means of 3 or more determinations $\pm$ SD
[a] $p < 0.01$, [b] $p < 0.05$, when compared with infant controls

RCDP fibroblasts had a total CoA content 25% greater than that observed for infant controls. The most significant change was a 44% increase in the long-chain acyl-CoA level. Despite the modest enhancement in the CoA content, the acetyl CoA/CoASH ratio was identical for both infant control and RCDP fibroblasts, but the long-chain acyl-CoA/CoASH was slightly elevated in RCDP fibroblasts. It should be noted that this study did not include quantitation of acid-soluble short- and medium-chain acyl-CoA species. Under most conditions these acyl-CoA species represent a very minor component of the total CoA pool (Moore et al 1988).

Fibroblasts from both patients contained normal or enhanced levels of both mitochondrial (citrate synthase) and peroxisomal (catalase) marker enzymes (Table 4). In addition, fibroblast homogenates were assayed for levels of enzymes involved in formation (acyl-CoA synthetase) and degradation (acyl-CoA hydrolase) of intracellular acyl-CoA species (Table 4). Acyl-CoA synthetase was determined with a single substrate, palmitic acid (16:0), while three substrates, octanoyl-CoA (8:0), palmitoyl-CoA (16:0), and erucoyl-CoA (22:0), were employed in the acyl-CoA hydrolase assays. As seen in Table 4, the NALD fibroblasts had infant control levels of acyl-CoA synthetase as well as medium- and long-chain acyl-CoA hydrolase; however, the very long-chain acyl-CoA hydrolase activity was elevated 2.8-fold compared with the infant control. The RCDP fibroblasts exhibited a 36% decrease in palmitoyl-CoA synthetase (EC 6.2.1.3) activity and a 2.7-fold increase in the very long-chain acyl-CoA hydrolase activity.

DISCUSSION

Two children with peroxisomal disorders were found to have moderate lactic and pyruvic acidaemia, prompting this investigation of the interrelationship of mitochondrial and peroxisomal function. Lactic and pyruvic acidaemia are not described in patients with peroxisomal disorders, but previous findings suggest that these patients have altered mitochondrial function (Sarnat et al 1983; Wolff et al 1986; Muller-Hocker et al 1984). Goldfischer et al (1973) first reported that patients with Zellweger syndrome lacked peroxisomes. Mitochondrial morphology was not

Table 4 Fibroblast enzyme levels

Activity	Infant controls	Adult controls	NALD	RCDP
		(nmol/min per mg protein)		
Catalase	6.85 ± 1.40	7.63 ± 1.80	11.98 ± 2.39	7.60 ± 3.77
Citrate synthase	29.80 ± 2.65	36.52 ± 9.17	27.10 ± 13.16	34.90 ± 2.40
Acyl-CoA synthetase	2.48 ± 0.56	1.98 ± 0.68	2.64 ± 0.71	1.58 ± 0.50[b]
Acyl-CoA hydrolase				
$C_{8:0}$	18.93 ± 6.50	16.21 ± 7.22	14.02 ± 7.55	13.36 ± 6.77
$C_{16:0}$	22.59 ± 11.53	16.84 ± 4.61	17.23 ± 9.74	16.25 ± 7.21
$C_{22:0}$	1.88 ± 1.41		5.32 ± 2.71[a]	5.02 ± 1.89[a]

Enzyme activities are expressed as nmol/min per mg protein. The data are means of 3 or more determinations $\pm$ SD
[a] $p < 0.01$, [b] $p < 0.05$, when compared with infant controls

described but cytochemical studies of intact brain mitochondria demonstrated reduced capability to oxidize succinate.

Clinical and biochemical results suggest that our patients with peroxisomal disorders and acidosis have mitochondrial dysfunction. The conversion of radiolabelled lactate to radiolabelled CO_2 appears to represent an excellent screening assay for potential metabolic errors that may lead to clinical lactic acidosis (Kerr 1991). Reduced PDH activity is the most common cause of congenital lactic acidosis and affected children present with a variable clinical picture (Robinson et al 1987). The NALD and RCDP patients both demonstrated decreased lactate oxidation and decreased PDH activity.

Biochemical studies utilizing mitochondria in liver and muscle cells from patients with peroxisomal disorders have confirmed a defect in the electron-transport chain before the oxidative step involving cytochromes (Versmold et al 1977; Trijbels et al 1983). Alterations in the biosynthesis of cell membranes (Poulos 1989; Naider and Moser 1990; Wanders et al 1990) and the accumulation of amphiphilic very long-chain acyl-CoA in peroxisomal disorders may lead to a disturbance in the structure of the inner mitochondrial membrane causing defects in the electron-transport chain (Trijbels 1983). The results of our studies utilizing fibroblasts show that the reactions localized to the inner mitochondrial membrane, i.e. the conversion of lactate to CO_2 and PDH activity, are suppressed in NALD and RCDP. These results imply that the heterogeneous clinical presentation of patients with peroxisomopathies may be related to the degree of associated secondary mitochondrial dysfunction.

Mitochondrial dysfunction may occur secondary to the intracellular accumulation of acyl-CoA species (Corkey et al 1988; Corkey and Deeney 1990). In the NALD and RCDP patients the acid-insoluble acyl-CoA concentration (long-chain and very long-chain species) is significantly increased compared to the infant and adult controls. Palmitoyl-CoA in the micromolar concentration range alters a variety of mitochondrial functions, like the activity of the pH-dependent anion-conducting channel (Halle-Smith et al 1988), steady-state transmembrane potentials (diLisa et al 1989), PDH activity (Smith and Saggerson 1979; Lai et al 1989; Kiechle et al 1990a,b; Moore et al 1992), and other mitochondrial dehydrogenases (Lai and Cooper 1991). These experiments measuring PDH activity were performed in the absence of adenosine triphosphate, the substrate for the PDH kinase, excluding this enzyme as the mediator of the inhibition of mitochondrial PDH activity (Smith and Saggerson 1979; Lai et al 1989; Lai and Cooper 1991). Other acyl-CoA species have been reported to inhibit (butyryl-CoA) or stimulate (malonyl-CoA, acetoacetyl-CoA, propionyl-CoA) PDH kinase activity by covalent acylation (Rahmatullah and Roche 1985). The acetyl-CoA/CoASH is markedly increased in NALD compared to infant control (Table 3). This increased ratio is sufficient to activate PDH kinase *in vitro*, phosphorylate the enzyme complex and reduce activity (Denton et al 1978). This mechanism alone or in combination with mitochondrial damage induced by amphiphilic acyl-CoA may contribute to the decreased PDH activity observed in NALD. However, in RCDP this ratio is equivalent to the control, suggesting that the elevation in long-chain and very long-chain acyl-CoA species alone may be responsible for the inhibition of PDH activity.

To complement the above findings, we investigated the activity of a group of enzymes with the potential of altering mitochondrial and peroxisomal acyl-CoA pools. Homogenate acyl-CoA synthetase activity was assessed using palmitic acid as substrate (Table 4). Palmitoyl-CoA synthetase has a mitochondrial (21%), peroxisomal (16%), and microsomal (60%) distribution in liver (Bronfman et al 1984). There is evidence that separate long-chain and very long-chain acyl-CoA synthetases are found in peroxisomes (Lageweg et al 1991). Lazo et al (1988) propose that deficiency of the peroxisomal very long-chain acyl-CoA synthetase is responsible for the impaired oxidation of very long-chain fatty acids by fibroblasts from patients with X-linked adrenoleukodystrophy. Our NALD patient had normal levels of the palmitoyl-CoA synthetase in spite of dramatically elevated intracellular acyl-CoA levels. It is possible that the elevated acyl-CoA pool is due primarily to very long-chain acyl-CoAs that may alter the very long-chain acyl-CoA synthetase activity by feedback inhibition. The RCDP patient had significantly reduced levels of long-chain acyl-CoA synthetase, which may have prevented the dramatic acyl-CoA accumulation seen with the NALD patient.

The activity of acyl-CoA hydrolase was elevated in both NALD and RCDP in the presence of very long-chain erucoyl-CoA substrate (Table 4). It is likely that this specific elevation was induced by accumulated very long-chain acyl-CoAs. Moore et al (1988) observed an increase in medium-chain acyl-CoA hydrolase activity subsequent to medium-chain acyl-CoA accumulation due to valproic acid (2-propylpentanoic acid) feeding, and Kurooka et al (1971) correlated increased long-chain acyl-CoA hydrolase activity with the increased long-chain acyl-CoA content due to alloxan diabetes. As the long-chain acyl-CoA hydrolase activity (determined with palmitoyl-CoA) was not elevated in patients, it appears that there is a specific very long-chain acyl-CoA hydrolase enzyme. In light of the proposed role of acyl-CoA hydrolases as regulators of intracellular CoA pools (Berge and Aarsland 1985), it appears that both patients are hydrolysing very long-chain acyl-CoAs in order to maintain a normal CoASH concentration; the RCDP patient has achieved this metabolic goal, while the NALD patient, with a severely restricted CoASH pool, has been unsuccessful. Another indication of the metabolic response to the stress of accumulating acyl-CoAs is the expanded total CoA content found in the NALD fibroblasts. Increases in total CoA have also been correlated with enhanced acyl-CoA hydrolase activities (Moore et al 1988; Kurooka et al 1971).

These two patients with peroxisomal disorders, NALD and RCDP, and moderate hyperlactataemia have been shown to exhibit decreased PDH activity which may be related to alterations in the pool of acyl-CoA species within the cell. These patients also exhibit alterations in the activity of enzymes involved in regulating the size of the acyl-CoA pool. These results suggest that the heterogeneity observed in the clinical presentation of the various peroxisomal disorders or within a specific peroxisomal disorder, as has been confirmed by complementation studies (Roscher et al 1989), may be related to the degree of mitochondrial dysfunction.

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ANNOUNCEMENT

The **First European Paediatric Congress**, organized under the auspices of 17 European National Paediatric Societies, will be held in Paris, France on March 9–12, 1994, at the Palais de Congres, Port Maillot. Symposia will be organized around six themes:

Endocrinology/Diabetes, Gastroenterology/Nutrition, Genetics/ Metabolism, Immunology, Neurology and Perinatology.

Plenary sessions, satellites symposia and posters will complement the six theme-related symposia. For further information, contact the Organizing Secretariat:

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