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IN VIVO TIME COURSE OF MUSCLE PHOSPHOCREATINE, PHOSPHORUS, AND ADENOSINE TRIPHOSPHATE DURING TREATMENT OF RICKETS. Charles E. Mize, Ronald Corbett, Ricardo Uauy, Ray Nunnally, Susanna Williamson. University of Texas Health Science Center at Dallas, Dept. Pediatrics & Radiology, Dallas, Texas

To assess potential skeletal muscle changes of high energy phosphate compounds and inorganic phosphate (P) in early childhood rickets, a non-invasive but direct estimate of the relative concentrations in gastrocnemius muscle of P, phosphocreatine (PCr), & adenosine triphosphate (ATP) was measured by magnetic resonance spectroscopy (MRS) in an infant, age 10 months. He had generalized weakness and low serum P (3.5mg/dl) and Ca (6.7mg/dl). Therapy was begun with vitamin D & dietary P & Ca, and weekly 31-P MRS spectra were obtained in an Oxford 30 cm, 1.9T magnet. Initially, PCr was approximately 50% reduced compared to that in gastrocnemius of a normal 6-month infant. Tissue P, PCr & ATP gradually normalized, preceding return of serum P & Ca to normal. Data below are serum (Ser) values, & tissue peak heights (mm) and peak height ratio:

Week	P	PCr	Σ ATP	PCr/ATP-β	Ser Ca	Ser P
1	2	48	51	3.15	5.3 mg/dl	3.7 mg/dl
2	8	95	72	3.91	5.0	4.2
3	25	104	85	5.79	6.9	3.4
Control	31	83	69	8.38	(10.5)	(5.6)

Functional muscle tone & strength gradually improved in concordance with MRS spectral return to a normal pattern. High energy phosphate depletion may explain the hypotonia of rickets. MRS may uniquely allow definition of individual muscle phosphorus components over time.

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USE OF AN INTRACRANIAL PRESSURE MONITOR IN CEREBRAL EDEMA COMPLICATING DIABETIC KETOACIDOSIS. Ian Ocran, Michael A. Bressack, Ron G. Rosenfeld, Raymond L. Hintz, Darrell M. Wilson. Stanford University School of Medicine, Stanford University Hospital, Department of Pediatrics, Stanford, CA.

Cerebral edema(CE) is a frequently fatal complication of diabetic ketoacidosis(DKA) in children. We describe the use of an intracranial pressure(ICP) monitor in the management of CE in a 3 year old girl with DKA who was successfully treated for glucose and electrolyte derangements by conventional fluid therapy and IV low-dose insulin, but deteriorated neurologically. She became comatose, bradycardic, and hypertensive. Head CT disclosed CE and an ICP monitor was placed. She was treated by fluid restriction and received 5 infusions of mannitol(300mg/kg/dose) for ICP>20 torr not relieved by sedation, which were followed by rapid improvement in clinical status and ICP. The mean change in ICP in the hour after mannitol was -10.7 torr(range -4 to -21). The patient recovered without neurological sequelae. Interestingly, clinical signs did not adequately predict when ICP was dangerously high. Although the correlation between Glasgow coma score (3-deepest coma, 15-normal) and ICP was statistically significant (r=-.52,p<.01), a score of 7, for example, was associated with ICPs ranging from 10 to 30 torr. Neither pulse nor BP was a clinically or statistically significant predictor of ICP(r=.29, p>0.1;r=.12,p>0.1, respectively). Since the pathogenesis and therapy of this lethal condition are controversial, and our data indicate that clinical signs are inadequate predictors of elevated ICP, the use of an ICP monitor can reduce uncertainty when managing this complication of DKA.

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A NOVEL METABOLIC PATHWAY FOR 25-HYDROXYVITAMIN D<sub>2</sub> IN A MAMMALIAN KIDNEY. Satyanarayana G. Reddy

Kou-Yi Tserng, Rahul Ray, Brian DeCosta, Michael F. Holick. CVRU at Cleve. Metro Gen. Hosp. and VA Med. Ctr., Depts. of Peds and Pharmacology, Cleveland, OH and Human Nutrition Research Center at Tufts Univ., Boston, MA, (Spon. by R. Marshall). Two new metabolites of vitamin D<sub>2</sub> were produced by perfusing kidneys, isolated from D-sufficient normocalcemic rats with pharmacological concentration (2x10<sup>-6</sup>M) of 25-hydroxyvitamin D<sub>2</sub> [25(OH)D<sub>2</sub>]. They were isolated and purified from the lipid extract of the perfusate using high performance liquid chromatography. By means of ultraviolet absorption spectrophotometry, mass spectrometry and specific chemical reactions, the new metabolites were identified as 24,25,28-trihydroxyvitamin D<sub>2</sub> [24,25,28(OH)<sub>3</sub>D<sub>2</sub>] and 24,25,26-trihydroxyvitamin D<sub>2</sub> [24,25,26(OH)<sub>3</sub>D<sub>2</sub>]. Along with the two new metabolites 24,25-dihydroxyvitamin D<sub>2</sub> [24,25(OH)<sub>2</sub>D<sub>2</sub>] was isolated and it was also demonstrated that the two new metabolites were formed from 24,25(OH)<sub>2</sub>D<sub>2</sub>. Thus, our results indicate that 25(OH)D<sub>2</sub> is first hydroxylated at C-24 to form 24,25(OH)<sub>2</sub>D<sub>2</sub> which is then further hydroxylated at C-28 and C-26 to form 24,25,28(OH)<sub>3</sub>D<sub>2</sub> and 24,25,26(OH)<sub>3</sub>D<sub>2</sub> respectively. In order to demonstrate the formation of the new metabolites under physiological conditions, <sup>3</sup>H-25(OH)D<sub>2</sub> was biologically produced by perfusing livers isolated from D-deficient rats with <sup>3</sup>H-vitamin D<sub>2</sub> (15Ci/mmol). Using <sup>3</sup>H-25(OH)D<sub>2</sub>, we demonstrated the formation of the new metabolites when a concentration of 25(OH)D<sub>2</sub> as low as (8x10<sup>-10</sup>M) was used as the substrate. Thus our study describes for the first time, a novel metabolic pathway for 25(OH)D<sub>2</sub> in a mammalian kidney under physiological conditions.

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AMMONIUM SULFATE DECREASES THE INHIBITORY EFFECT OF ATP ON LUNG PHOSPHOFRUCTOKINASE (PFK) ACTIVITY. Gerald T. Reinersman and Robert E. Kimura (Spons by G. Chan ).U of Utah, Salt Lake City, UT. Significant aerobic lactate production and glutamine oxidation occur in developing rat lung and intestine. In intestine, the inhibitory effect of ATP on PFK has been reported to be eliminated by the presence of

NH<sub>4</sub><sup>+</sup>, an end product of glutamine oxidation. The association between aerobic lactate production and glutamine oxidation may be mediated by activation of PFK by NH<sub>4</sub><sup>+</sup> despite the presence of high ATP associated with aerobic conditions. In order to determine if lung PFK is controlled in a similar manner we compared the effect of NH<sub>4</sub><sup>+</sup> on developing rat lung and intestine PFK activity in the presence of 2 mM ATP. PFK activity was determined on particle free tissue homogenates by measuring the disappearance of NADH in the presence of 2 mM fructose-6-phosphate, 2 mM ATP, and excess aldolase, alpha-glycerol phosphate dehydrogenase, and triose phosphate isomerase at 27° and pH8.0. PFK activity is expressed as μmoles fructose-6-phosphate utilized/min/gram wet tissue. Data are mean±sd, n≥4.

LUNG	AGE	ADULT	SUCKLING PUP	
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	PFK	% of maximal act	PFK	% of maximal act
0 mM	1.31±0.40	27±6	1.58±0.48	28±6
10 mM	4.96±1.03	100	4.64±1.22	100
JEJUNAL MUCOSA				
0 mM	0.7±0.2	8±2	0.6±0.2	6±2
10 mM	12.5±4.4	100	11.4±1.0	100

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> stimulated lung PFK activity in the presence of 2 mM ATP by 3.7 fold compared to 18 fold for intestinal PFK activity. There was no difference between suckling and adult rats for either lung or intestinal PFK. This suggests that there may be an association between aerobic lactate production and glutamine oxidation in developing rat lung.

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MITOCHONDRIAL NADH-CoQ OXIDOREDUCTASE (COMPLEX I) DEFICIENCY IN MAN. William J. Rhead, Brad A. Amendt, Cheryl Greenberg, and Frances Booth. Dept. of Pediatrics, Univ. of Iowa City, IA, and Depts. of Pediatrics and Child Health, Univ. of Manitoba, Winnipeg, Man.

Two brothers, aged 11 and 13 years, have seizures, growth and developmental delay and intellectual regression. Physical findings include mild facial coarseness, bilateral ptosis, sensorineural hearing loss, hypotonia, weakness, incoordination, and hyporeflexia. Their mother has late onset sensorineural hearing loss but is otherwise normal. Abnormal laboratory results include elevated plasma and CSF alanine and lactate (4-6mM), low dibasic and neutral amino acids and mild cerebral atrophy; muscle from the younger boy contains ragged red fibers. Lactate to pyruvate ratios in fibroblasts incubated with glucose were normal (Dr. Brian Robinson). Dr. Thomas Perry of Vancouver has ruled out lysinuric protein intolerance or renal tubular dysfunction. In the younger boy's fibroblast mitochondria, ATP synthesis with pyruvate and malate was undetectable (<1% of control) and with succinate, 70% of control, suggesting deficient activity of Complex I of the electron transport chain. In another child with hypotonia, necrotic lesions of the cerebral cortex, hepatomegaly with severe fatty change, lactic acidosis and early death, we found Complex I activity to be 20% of control, as did Dr. Robinson. These patients demonstrate the clinical and biochemical heterogeneity of disorders involving the electron transport chain in man and the utility of mitochondrial studies in fibroblasts.

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SJÖGREN-LARSSON SYNDROME (SLS): DEFICIENT FATTY ALCOHOL: NAD<sup>+</sup> OXIDOREDUCTASE (FAO) ACTIVITY IN CULTURED FIBROBLASTS.

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We investigated lipid metabolism in cultured skin fibroblasts from SLS patients and normal controls. Intact SLS fibroblasts incubated in the presence of 14C-palmitate accumulated more radioactive hexadecanol (HD) than normal, whereas incorporation of radioactivity into other neutral lipids and phospholipids was unaltered. The rate of HD synthesis and its utilization for glycerol ether synthesis were normal in SLS cells. The intracellular half-life of radioactive HD loaded into SLS fibroblasts was 70 minutes compared to 15 minutes in normal cells. Oxidation of HD to fatty acid was decreased in intact SLS fibroblasts to 12-32% of normal. Total FAO activity, the enzyme catalyzing this reaction, in normal cells was 59.8 ± 14.8 pmol/min/mg protein (range 36.6-79.6, n=9) and 7.8 ± 3.8 pmol/min/mg (range 4.3-13.1, n=4) in SLS cells. FAO is partially inhibited by palmitoyl CoA. Palmitoyl CoA-inhibitable FAO activity was decreased in SLS fibroblasts to 1% of normal (normal: 32.4 ± 8.3 pmol/min/mg protein; SLS: 0.4 ± 0.7 pmol/min/mg). Fibroblasts from two SLS heterozygotes had intermediate levels (46%;48%) of FAO activity. FAO was normal in cells from patients with X-linked ichthyosis, multiple sulfatase deficiency and Refsum disease. These studies suggest that SLS is due to FAO deficiency.