901 SERUM FREE AND TOTAL INSULIN IN JUVENILE DIABETES MELLITUS.

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Simple rapid polyethylene glycol precipitation of serum makes it possible to measure free (FI) and total insulin (TI) in insulin-treated patients by standard radioimmunoassay. Six diabetics, ages 8-19 years, were studied because of suspected overinsulinization with rebound hyperglycemia. Patients were hospitalized and received their usual insulin and dietary regimen. Blood was sampled hourly, for 24 hours, for glucose, free and total insulin.

Pt.	Insulin Dose U/kg/day	Mean BS mg/dl	Mean FI μU/ml	Mean TI μU/ml
A	.72	115 ± 46	18 ± 5	>500
B	.77	139 ± 60	34 ± 12	306 ± 50
C	.80	191 ± 23	29 ± 7	--
D	1.16	275 ± 89	24 ± 11	233 ± 46
E	1.20	227 ± 36	30 ± 9	--
F	1.38	168 ± 68	56 ± 16	>500

FI varied between patients from 3-12% of TI. Neither TI nor FI correlated positively with insulin dose. FI did not correlate with TI in individual patients, but data suggests that binding of administered insulin is instantaneous; equilibrium between bound and free insulin pools is variable and slow.

A better understanding of those variables is required for rational adjustment of insulin therapy.

902 DEFICIENT KETOGENESIS IN NEONATAL HYPOGLYCEMIA

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To investigate the reasons for the high incidence of neonatal hypoglycemia, hormone and metabolic fuel responses to postnatal fasting were examined in 24 term-AGA, 9 preterm-AGA, 6 term-SGA, and 4 preterm-SGA infants. At 8 hours of age or when plasma glucose (GLU) was <40mg%, blood was drawn for determinations of GLU, gluconeogenic substrates, fat-derived fuels, and hormones. Fuel and hormone levels in the preterm-AGA and 2 SGA groups were similar to term-AGA infants: lactate 2.9±.22mM (m±SEM), pyruvate .16±.01mM; alanine .48±.02mM; free fatty acids 1.4±.07mM; b-OH-butyrate .32±.04mM; acetoacetate .05±.01mM; insulin 10±9uU/ml; growth hormone 16±3ng/ml; cortisol 8±1.3ug/dl. Asymptomatic hypoglycemia (GLU ≤40mg%) occurred in 18% of AGA and 80% of SGA infants. In the 14 hypoglycemic infants, ketone levels were low (b-OH-butyrate .21±.04mM) despite low insulin (8±1.3uU/ml) and high free fatty acid levels (1.2±.14mM).

These results suggest that capacity for hepatic ketogenesis is low during postnatal fasting. This may be a primary factor limiting the ability of neonates to tolerate fasting. In addition, at times of hypoglycemia, ketones are not available as fuel for CNS metabolism. This raises concerns about whether the currently accepted definition of hypoglycemia in the neonatal period provides sufficient margin for safety.

903 REGULATION OF NEONATAL GLUCONEOGENESIS: PHOSPHOENOLPYRUVATE CARBOXYKINASE (GTP)-FERROACTIVATOR DEVELOPMENT AND DISTRIBUTION IN THE GUINEA PIG.

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The key gluconeogenic enzyme, phosphoenolpyruvate carboxykinase (GTP) [PEPCK], is subject to hormonal regulation by a mechanism involving enzyme synthetic and/or degradative rates. PEPCK activity can also be affected by ions. Fe^{2+} has been shown to be involved in the activation of the enzyme in vivo. A protein necessary for the activation of purified PEPCK by Fe^{2+} has been identified in guinea pig tissue. Distribution of this PEPCK-Ferroactivator parallels that of the enzyme, being highest in liver [8550±1640 units/gm (M±SD), n = 6] to no activity in adipose tissue. 96% of PEPCK-Ferroactivator is found in cytosol and 4% in the mitochondria although cytosolic PEPCK activity is 43% and mitochondrial PEPCK is 57% of the total liver cell activity. Study of the development of PEPCK-Ferroactivator in fetal guinea pig liver reveals low levels (15% of adult) by the 40th day of gestation with an increase in activity to term when neonatal and maternal levels are equal. The maternal to fetal ratio of liver ferroactivator (units/gm) correlates with gestational age (r = 0.84, n = 15) in a linear regression y = 13.43 - 0.18x. Increase in cytosolic PEPCK activity and hence gluconeogenesis occurs rapidly postpartum by a mechanism not yet completely elucidated. The existance of PEPCK-Ferroactivator activity which is involved in the rapid activation of PEPCK by Fe^{2+} in neonatal liver suggests that Fe^{2+} activation may be important in regulating neonatal gluconeogenesis.

904 HEPATIC INSENSITIVITY TO INSULIN IN THE NEONATAL LAMB

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We have shown persistant endogenous hepatic glucose production during exogenous glucose infusion is characteristic of the newborn lamb. Glucose turnover at steady state glucose conc. falls to 0.56±1.08mg/kg/min(M±SEM,n=4) when glucose infusion reaches 22 mg/kg/min with a pl. insulin response of 270±108μU/ml. In order to separate pancreatic from hepatic responses to test the hypothesis that there is intrinsic hepatic insensitivity to insulin, 8 newborn sheep (age 4.4±1 d.) were infused with both glucose and insulin. Two hours after the initiation of a glucose infusion (6 mg/kg/min) a simultaneous infusion of sodium pork insulin was begun. After four hours of both, glucose turnover at steady state glucose conc. was determined by prime constant infusion with $^{3}H_{6}$-glucose. $^{14}C_{U}$-lactate was given to assess gluconeogenesis from labelled glucose. Control animals received 0.45% NaCl.

Infusion Gluc. mg/kg/min	Ins. mU/kg/min	During Steady State Pl. Gluc. mg/dl	Pl. Ins. μU/ml	Turnover mg/kg/min	$\frac{^{14}C dpm}{^{3}H dpm}$
0(12)	0	115±17	23±6	7.45±.74	0.42±.02
5.67±.09(7)	0	182±19	32±6	4.76±.70	[14]
6.09±.11(8)	1.25/6.25	56±13	137±31	2.48±.51	0.21±.02

Insulin infusion diminished glucose production and gluconeogenesis in the newborn lamb at plasma insulin levels significantly higher than those required to produce glucose suppression in our older animals. The neonatal lamb liver is relatively insensitive to the glucoregulatory activity of insulin.

905 EFFECTS OF HYPOXIA ON METABOLIC RESPONSE TO GLUCOSE (Glc) LOAD IN LOW BIRTHWEIGHT INFANTS (LBWI).

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Hypoxia is known to alter intestinal function in the newborn. To determine the protracted effects of hypoxia on tolerance to a Glc load, 9 infants under 2500 g who had developed respiratory distress with cyanosis at delivery or within 8 hr after birth were studied. Tests were carried out at a mean age of 19 days,at least 24 hr after clinical improvement and cessation of oxygen therapy. All infants had tolerated formula feedings providing between 90 to 110 kcal/kg/day for at least 4 days. Following a 4-hr fast, 1.75 g/kg Glc as a 10% solution was administered by n.g. tube. Capillary blood for Glc, bicarbonate (Bic) and lactic acid (LA) determinations were drawn prior to, and at 30, 60 and 120 min after Glc administration. Blood Glc increased from a baseline of 71±4 (mg/dl,means±SEM) to 139±14 at 30 min and 173±24 at 60 min and fell to 108±14 at 120 min. Simultaneously, Bic fell from 24±1 at 0 time to 17±2 mEq/l at 30 min ($p<0.01$) and returned to baseline at 60 min: 20±2 (NS). LA did not change during the 2-hr period. There was an inverse correlation between Glc and Bic ($r=-.365$, 31 df, $p<0.05$). These findings suggest that LBWI recovering from hypoxia may develop transient metabolic acidosis after Glc intake. This alteration could provide an explanation to the poor growth observed in LBWI recovering from hypoxia.

906 UREA CYCLE ENZYMES AND OROTIC ACID (OA) IN REYE'S SYNDROME (RS).

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Assays of liver from patients with RS reveal ornithine transcarbamylase (OTC) deficiency which may be isolated, or may occur in combination with carbamyl phosphate synthetase (CPS) deficiency. The correlation between these activities and clinical features of RS is unclear. Blockage of the urea cycle predominating at the OTC step would result in excessive formation of OA, whereas a co-existing block at the CPS step would tend to limit formation of OA from carbamyl phosphate. OA was determined directly in urine using high pressure liquid chromatography. Urine from consecutive 12-hr periods was assayed during acute and recovery phases of RS in a patient with isolated OTC deficiency (A), a patient with combined OTC and CPS deficiency (B), and in normal controls (C). A and B had normal renal functions. OA concentrations in C were 5 to 10 μM. In A, OA was in excess of 2000 μM (312 mg/L) during the acute stage of illness. OA crystals formed in these specimens. OA in A decreased to normal levels during the 4-day recovery period. In B, OA concentrations during the acute phase were 2 to 3 times normal (23-30 μM), returning to normal within 5 days. These observations indicate that extreme elevations of OA may occur at the onset of RS with isolated OTC deficiency, whereas in RS with combined OTC and CPS deficiency OA elevation may be modest. These data suggest that OTC and CPS measured in vitro may correlate with OA formation during the active stage of RS.