

▲1009 INTRINSIC REGULATION OF GLYCOGEN PARTICLES: LIVER v. SKELETAL MUSCLE. Philip A. Gruppuso, David L. Brautigam (Spons. by Robert Schwartz). Dept. of Pediatrics and Biochemistry, Brown Univ., Providence, RI

Regulation of glycemia requires the precise control of glycogen metabolism. Regulation intrinsic to the protein-glycogen complex can be studied by "flash activation" of glycogenolysis. In this procedure, the addition of Ca^{++} and MgATP to purified rabbit skeletal muscle glycogen particles resulted in conversion of 80% of phosphorylase (P) to the active form in 1 min, followed by return to baseline (5%) by 2 min. Using ^{32}P -ATP, phosphorylation of P, β -subunit of phosphorylase kinase (PK), glycogen synthase (GS) and an unidentified 32kDa protein was detected by PAGE-SDS. Activation of P and ^{32}P labeling were Ca^{++} -dependent and temporally parallel, as were inactivation and dephosphorylation. Unidentified proteins at 69kDa and 19kDa were labeled independently of Ca^{++} and were not dephosphorylated. In contrast, hepatic glycogen particles could not be "flash activated", even with attempts to further optimize conditions. Labeling showed no Ca^{++} -dependent phosphorylation, but addition of cAMP-dependent protein kinase phosphorylated α - and β -subunits of PK (as well as GS and the 32kDa protein) in liver, as it had in skeletal muscle preparations. Nonetheless, "flash activation" of hepatic glycogen particles did not occur even upon addition of cAMP or the protein kinase catalytic subunit. We conclude: 1) Only Ca^{++} and MgATP are needed to trigger glycogenolysis in muscle glycogen particles; 2) Additional unknown factors are required in liver glycogen particles, even following cAMP-dependent phosphorylation; 3) There are fundamental differences in the regulation of liver and muscle glycogen metabolism.

●1010 METABOLISM OF C-PEPTIDE IN THE PREGNANT RHESUS MONKEY. Philip A. Gruppuso, John B. Susa, Prabhat Sehgal, Bruce Frank, Robert Schwartz, Dept. Pediatrics, Brown University, Providence, RI, New England Primate Center, Eli Lilly Co.

The assessment of fetal and maternal B-cell function by C-peptide measurement requires an understanding of its metabolism in pregnancy. We gave ^{125}I -tyrosylated-C-peptide (I-CP) to 6 pregnant Rhesus monkeys by bolus followed by constant infusion. This resulted in a steady state in the maternal circulation of immunoprecipitable cpm, as measured using excess C-peptide antiserum. At the end of the 2 or 4 hr infusion fetuses were exsanguinated via the umbilical vein. In the umbilical venous plasma (UVP) 3-13% of total cpm were immunoprecipitable. UVP immunoprecipitable cpm represented 1.4-5.8% of maternal immunoprecipitable cpm at delivery. Medium pressure gel filtration chromatography of UVP showed that the immunoprecipitable cpm were due to a fragment of I-CP approximately 25 amino acids long. The predominant immunoprecipitable peak in maternal plasma was intact I-CP; a peak corresponding to the fetal immunoprecipitable peak was also present. Simultaneous maternal arterial and uterine vein samples showed that the degradation of I-CP occurred across the uterus. Comparison to studies in 3 post-partum animals indicated that pregnancy increased the rate of appearance of I-CP metabolites. Further, when I-CP was incubated with trophoblastic cells in culture, degradation to the fetal immunoreactive species was observed. We conclude that pregnancy alters maternal C-peptide metabolism, owing largely to placental metabolism. Though placental passage was insufficient to alter interpretation of fetal measurements, the placenta may degrade fetal C-peptide.

1011 HYPERINSULINISM: A REVERSIBLE CAUSE OF HYPERTROPHIC CARDIOMYOPATHY. James P. Gutai, J. Peter Harris, David J. Dabbs. East Carolina University School of Medicine, Depts. Ped. and Path., Greenville, N.C.

The role of insulin in cardiac muscle growth has been controversial. *In vitro* studies have had equivocal results but insulin has been implicated as an etiologic factor in the hypertrophic cardiomyopathy seen in the infant of a diabetic mother. We have recently cared for an infant with neonatal hypoglycemia secondary to endogenous organic hyperinsulinism (blood glucose 22mg/dl; insulin 92uU/ml) that was refractory to diazoxide, chlorothiazide and glucocorticoids. Despite a 95% pancreatectomy on day 30 which showed islet hypertrophy/hyperplasia with overall features of endocrine cell dysplasia, the hypoglycemia persisted and was marginally controlled with the same drugs. At 4 months of age, the patient presented with a severe biventricular hypertrophic cardiomyopathy and marked congestive failure (RV end diastolic pressure = 18; LV end diastolic = 23 mmHg; Septal wall thickness = 8 mm, LV free wall = 12mm) Based upon the hypothesis that hyperinsulinism might be an etiology and the refractoriness to standard anticongestive therapy, the remaining pancreas was removed. One year later there has been nearly complete resolution of the cardiomyopathy (RV end diastolic pressure = 5, LV end diastolic = 12mmHg; Septal thickness = 5mm, LV free wall = 7mm). The child is normoglycemic without medication. This patient's clinical course suggests a role for insulin in cardiac growth/hypertrophy. We recommend that infants with hyperinsulinism undergo a cardiac evaluation.

1012 LACK OF RELATIONSHIP BETWEEN HYPOGLYCEMIC AWARENESS AND CATECHOLAMINE (CAT) RESPONSE IN INSULIN DEPENDENT DIABETES (IDD) Robert P Hoffman, Carol Singer-Granick and Dorothy J Becker, Dept. Pediatrics, Children's Hospital and University of Pittsburgh, Pittsburgh, PA

We assessed hypoglycemic symptoms in 29 children aged 15.4±2.4 yrs (x±SD) with IDD duration 7.9±3.7 yrs. without clinical evidence of autonomic neuropathy. An intravenous insulin bolus of 0.15-0.75 u/kg according to basal plasma glucose (PG) was given before and again after 3 days of intensive insulin therapy (IIT). Oral questionnaires regarding hypoglycemic symptoms were completed at each time blood samples were taken for measurements of PG and CATS. A hypoglycemic awareness score (HAS) was assigned to each patient. All but one patient reported symptoms during each test. These occurred prior to hypoglycemia (PG>65 mg%) in 73% before and 52% after IIT. The initial symptoms (usually hunger) appeared at 38±26 min. before and 35±18 min. after IIT after PG decrements of 121±81 (47±28%) and 88±64 mg% (46±26%) resp. (NS). The CAT increments at this time were very small (174±55, range 0-802 and 70±89, 0-262 pg/ml resp.). The maximum HAS correlated negatively with PG nadir, before (r=-.69, p<.001) but not after IIT. The max HAS correlated with peak CAT response before (r=.42, p<.05) and after (r=0.42, p<.05) IIT. In patients with PG nadir <65 mg%, there were no differences in HAS or glucose recovery before and after IIT despite the fact that CAT response was significantly lower after IIT with some patients never achieving an increment >250 pg/ml. Thus hypoglycemic unawareness is not common in children with IDD and symptoms occur prior to the onset of hypoglycemia with minimal if any CAT increments.

●1013 A SINGLE DOSE OF PANTOTHENATE, CYSTEINE, AND CARNITINE INCREASES LIVER COENZYME A AND ACETYL CoA LEVELS IN NORMAL INFANT MICE AND SUBSTANTIALLY REVERSES VALPROATE-INDUCED HEPATIC DYSFUNCTION: POSSIBLE CLINICAL SIGNIFICANCE. Jean Holowach Thurston, Richard E. Hauhart, Washington U. Medical Center, Children's Hospital, Dept. of Pediatr., St. Louis, MO.

We have previously reported that valproate (VAL) reduced coenzyme A (CoA) and acetyl CoA (AcCoA) levels in the livers (LV) of 4-8-d-old mice. In this study, we attempted to increase LV CoA and AcCoA synthesis in normal mice by s.c. injection of pantothenate (B5, 2 mmol/kg), L-cysteine (CYST, 1 mmol/kg) and L-carnitine (CARN, 2.5 mmol/kg). Effects in VAL-treated mice were also examined. B5 + CYST + CARN had a significant effect

Measurement	Control (N=4)	B5+CYST+CARN (N=7)	Change	P
CoA, $\mu\text{mol/kg}$	114 ± 4	135 ± 5	+18%	0.027
AcCoA, $\mu\text{mol/kg}$	22.5 ± 1.4	33.9 ± 1.2	+51%	<0.001
Acid-Sol CoA Esters	25.7 ± 4.0	34.7 ± 3.1	+35%	NS
Total, $\mu\text{mol/kg}$	162 ± 5	203 ± 7	+25%	0.005

on LV CoA metabolism in normal mice (Table). B5 + CYST without CARN or CARN alone had no effect. In VAL-treated mice (15 mg/kg) (N=6) injection of B5 + CYST + CARN increased depressed LV CoA and AcCoA levels 58% (P<0.001), and 70% (P=0.006), respectively; plasma [β -OHB] increased 128%, P<0.001.

CoA and AcCoA are essential cofactors in over 100 synthetic and degradative reactions. Increasing levels of CoA and AcCoA in LV may have clinical relevance not only to VAL-associated hepatotoxicity, but also to other conditions where there is sequestration of CoA (as acyl-CoA) and inhibition of LV metabolism because of CoA and AcCoA depletion.

●1014 SODIUM BENZOATE (SB) INCREASES FREE TRYPTOPHAN (TRP) IN BLOOD AND SEROTONIN (5-HT) FLUX IN CORTEX OF OTC DEFICIENT *Spf* MICE. Susan L. Hyman, Joseph T. Coyne, Ijaz Qureshi, Mark L. Batshaw. Depts. Peds., Psych. and Kennedy Inst. Johns Hopkins Meds Inst., Balto. and Hopital Ste. Justine, Univ. Montreal.

We have previously shown that both hyperammonemia and SB used to treat hyperammonemia increase central 5-HT flux in rats and mice (Pediat Res 19:309A, 1985; 20:325A, 1986). In this study we injected ip. 1 or 5 mmol/kg SB or sodium acetate (Ac) in hyperammonemic *Spf/y* and normoammonemic *CD-1/y* mice (n=5/condition) with sacrifice after 1 h. Compared to *CD-1* mice, *Spf* (all conditions) had significantly higher cortical levels of the 5-HT precursor Trp (p<.001), of 5-HT (p<.01), and of its metabolite 5-hydroxyindoleacetic acid (HIAA), (p<.001). SB did not change plasma ammonium levels but further increased cortical Trp (p<.005) and HIAA (p<.01) in *CD-1* mice. The effect of increasing SB from 1 to 5 mmol/kg was to increase cortical Trp (p<.001) and HIAA (p<.005) in *Spf* and Trp (p<.05) in *CD-1* mice. Plasma benzoate levels ranged from 1.8 to 4.1 mM 1 h post 5 mmol/kg SB. Serum free Trp levels doubled in both *Spf* (25±4.2 vs. 13.7±3.9 uM, p<.001) and *CD-1* mice (23.9±4.2 vs. 11.0±1.8uM, p<.001) following 5 mmol SB. No change occurred following injection of 1 and 5 mmol/kg Ac or sodium phenylacetate, which is also used to treat hyperammonemia. We suggest that SB competes for albumin binding sites with Trp, resulting in increased free Trp in blood, Trp transport across the blood-brain barrier, and increased serotonin flux. It is possible that the clinical symptoms of SB intoxication are related to this alteration in serotonin metabolism.