87 EFFECT OF CRF 1-41 AS COMPARED TO INSULIN HYPOGLYCEMIA (ITT) ON PLASMA CORTISOL AND ACTH IN SUBJECTS WITH INTACT OR DEFECTIVE HYPOTHALAMIC-PITUITARY FUNCTIONS. B. Bauman, A. Pertzelan, Z. Josefsberg, H. Kaufman, Z. Ben Zeev, A.V. Schally & Z. Laron. Beilinson Med. Ctr., Tel Aviv Univ. Sackler Fac. of Med., Israel & Tulane Univ. Med. Sch., New Orleans, USA.

Synthetic CRF 1-41 in a dose of 1 mcg/kg was administered i.v. Plasma cortisol (CO) and ACTH were determined before and at 30, 60, 90 & 120 min. Fourteen children (12M, 2F 12±4.8 yrs) were investigated including 2 normal controls, 6 with IGHD and 6 with MPHD. According to the peak plasma CO during ITT the subjects were classified into 2 groups: Gr. A - intact hypothalamic-pituitary-adrenal (HPA) axis (peak CO > 16 ug/dl; Gr. B - subnormal responders.

Gr.	n	Diagnosis	Peak CO (ug/dl) ITT	Peak CO (ug/dl) CRF	Peak ACTH (pmol/ml) CRF
A	8	Intact HPA	28.4±15.8	26.3±11.1	51.9±46.47
B	4	ACTH Def.	6.9± 5.3	7.3± 6.4	32.7±13.6
	2	CRF Def.	4.6; 11.0	8.9; 25.0	63; 159

The CRF test made it possible to distinguish between a pituitary lesion (ACTH def.) and a hypothalamic defect (CRF def.). There was also a good correlation between the CRF, GH-RH and TRH tests in the same patients. It is concluded that the CRF test is a useful addition to the diagnostic endocrine armamentarium.

88 PREDNISONE/AZATHIOPRINE IMMUNOSUPPRESSIVE THERAPY FOR INSULIN DEPENDENT DIABETES (IDD). Janet H. Silverstein, William J. Riley, Rebecca P. Spillar, Carol Knuth, Douglas Barrett, Kenneth Rand, Diann D. Fisk and Noel K. Maclaren. University of Florida College of Medicine, Departments of Pathology and Pediatrics, Gainesville, Florida, USA.

Six children aged 8-16 years who had clinical IDD for less than 3 weeks, islet cell autoantibodies (ICA) and DR3 and/or DR4 alleles in their HLA phenotype were given immunotherapy, consisting of 4 alternate day I-V boluses of methylpredisolone (30 mg/kg), predisone (2 mg/kg) for 2 months and azathioprine or Imuran (2-3 mg/kg/day) for at least 1 year. Five children were matched to be non treatment controls. Diabetic control was monitored by optimal insulin requirement in U/Kg/day, plasma $HbA1_c$ values, and C-peptide responses to oral Sustacal. Immunotherapy significantly improved the parameters over that of controls. One 9 year old boy was able to discontinue insulin therapy by 4 months of immunotherapy, and has had normal $HbA1_c$ and C-peptide levels 11 months later. One 17 year old girl could be maintained in excellent control 15 months after diagnosis with only 0.2 u/kg of insulin per day. One 14 year old girl was maintained on 0.1 u/kg insulin/day at 6 months of therapy but had increasing insulin requirements beyond 9 months despite continued administration of Imuran. One 8 year old girl who showed a good initial response to therapy by 4 months, had to have Imuran discontinued because of toxicity. Two children who initially presented in diabetic ketoacidosis showed no response to immunosuppression. These results suggest that conventional steroid/azothioprine immunosuppression may provide a less toxic alternative to Cyclosporin in the specific therapy of IDD.

89 GLUCOSE AND INSULIN MODULATE BRAIN OPIATE RECEPTORS. George A.Werther and Annette Hogg. Royal Children's Hospital Dept.of Endocrinology, Melbourne, Australia.

Feeding inhibition by the opiate antagonist naloxone is enhanced in diabetes and blunted by hypoglycaemia. The relative roles of glucose and insulin in these phenomena remain unclear. We investigated possible regulation of the opiate receptor by glucose and/or insulin by examining in vitro effects of glucose(5mM or 10mM) or insulin(1uM), on tritiated naloxone binding to rat brain membranes. In whole brain membranes, glucose 5mM and 10mM increased 3-H-naloxone binding by 10+/-4%(n=4) and 38+/-9%(n=3). Insulin increased 3-H-naloxone binding by 27+/-3%(n=5). In hypothalamus, rich in opiate receptors, glucose(10mM) and insulin(1uM) increased opiate binding by 17+/-3%(n=4) and 29+/-11%(n=3) respectively. Insulin was maximally effective at 0.2nM, enhancing binding by 57%. Glucose and insulin both enhanced the average affinity (Kd) for naloxone binding: control 0.76nM; glucose(10mM) 0.38nM; insulin(1uM)0.32nM. Anti-insulin receptor antibody and insulin analogue D-ala,D-asp-insulin both mimicked the insulin effect, while proinsulin was weakly effective; hGH and IGF did not effect opiate binding. Modest in vivo modulation was demonstrated following intra-ventricular infusion of 10mM glucose, 1uM insulin, or artificial CSF for 6 days: Binding of 3-H-naloxone to hypothalamus was enhanced by 11+/-6% following glucose infusion (n=3) and 14+/-13% following insulin infusion (n=3). Conclusion: Glucose and insulin enhance naloxone binding to brain receptors. The mechanism involves an affinity increase of opiate receptors; insulin acts via its own receptor. These findings suggest that insulin and glucose in the brain may play a role in modulating opiate receptors and their actions.

90 MATURITY OF KETONE BODY PRODUCTION AND UTILIZATION IN THE HUMAN NEONATE. P. Bougnères, U.188 INSERM.

In order to quantify the energetic role of ketone bodies (KB):β-hydroxybutyrate (BOB) and acetoacetate (AcAc), in the neonatal period, 6 infants born at terme were studied within their first 2 days of life, 4-5 hours after being fed. Plasma glucose was 58 ± 5 mg/dl, FFA 810 ± 206 µM, blood AcAc 296 ± 112 µM and BOB 486 ± 67 µM. The kinetics of KB metabolism were measured using the in vivo dilution of the nonradioactive tracer [4,4,4-$^2H_3$] BOB and a gas chromatography-mass spectrometry micromethod validated in fasting rats. The rate of KB production was 17.3 ± 1.4 µmol kg/min., with an almost equal value for KB utilization (values reached in adults only after 7-21 days fasting). KB metabolic clearance rate was 2.8 ± 6.6 ml kg/min. (≤12 ml kg/min in fasted adults with similar utilization rates). From FFA values, we estimated FFA turnover (J.Cl.Inv., 1982 70): 14 ± 2 µmol kg/min., which was correlated with KB production ($p < 0.01$) so that ~ 31 ± 3% of FFA were utilized for ketogenesis (vs 25-35 % in fasted adults). We conclude that (1) the rates of neonatal KB production are similar or superior to those in older children and adults (2) the proportion of FFA converted to KB by the neonatal liver is within the same range as in older subjects (3) the uptake of KB relative to circulating KB concentration is accelerated, indicating an increased capacity of the ketone-consuming neonatal tissues to extract blood KB, mostly at low concentrations.

91 A HIGHLY SENSITIVE RIA SYSTEM FOR HUMAN EPIDERMAL GROWTH FACTOR (EGF). Carlos Callegari, Nilsa P. Laborde, J. Lakshmanan, Jan Alm, C. George-Nascimento, Rosemary D. Leake, Delbert A. Fisher, Harbor-UCLA Med. Ctr., Dept. of Pediatrics, Torrance, CA and Chiron Corp., Emeryville, CA.

Although considerable evidence supports the role of EGF as a modulator of developmental processes, data from adult humans are limited due to the lack of reliable measurement methods. There is no information from infants or children. We developed and characterized a homologous RIA system to implement clinical hEGF studies. Antibodies were raised in NZW rabbits against highly purified Chiron h-EGF. The same preparation was used as standard and radioiodinated with chloramine T. The antibody ($1.5x10^5$ final dilution) was incubated for 48 h at 7°C with standard and samples. Radiolabeled hEGF was added at 24 h and bound-free separation was performed by combined 2nd antibody and 5% polyethylene glycol. Sensitivity of the method is 30 pg; there is no crossreaction with insulin, placental NGF extract, IGF-I, IGF-II or renin up to levels of 100 ng. Within and across assay variations were 7.9 and 12.3%, respectively. Human EGF measured in urine of term newborns expressed in ng/mg of creatinine were 9.9±3.6 ($\bar{x}$±SD) at 0 h and 3.9±1.8 at 24 h after birth ($p<0.05$). These values were lower than levels in normal boys and girls 6-8 yr of age (68±16, $p<0.01$) or normal men (48±13, $p<0.01$). In cord serum hEGF was 0.62±0.36 ng/ml. Conclusion: The present hEGF RIA system provides a reliable and sensitive measurement method which offers an unique possibility for the study of pathophysiological implications of EGF in human development.

92 LOW SERUM SOMATOMEDIN-C (SM-C) IN INSULIN-DEPENDENT DIABETES : EVIDENCE FOR A POST-RECEPTOR DEFECT. Marc Maes, Louis E. Underwood and Jean-Marie Ketelslegers. University of Louvain, Unité de Diabète et Nutrition, Belgium and University of North Carolina, Dept. of Pediatrics, Chapel Hill, N.C., USA.

In poorly controlled insulin-dependent diabetes, serum Sm-C concentrations are low despite high levels of growth hormone (GH), suggesting GH resistance. Whether this resistance might be due to receptor or post-receptor defects remains however unclear. Therefore, the number and affinity constant of liver bovine GH binding sites and the serum Sm-C concentrations, 24 h after injection of graded doses of GH, were determined in hypophysectomized rats injected with saline (controls) or with 40 mg of streptozotocin/kg BW (diabetics). After one week of diabetes there were no significant changes in the affinity constants (diabetics : 0.68 ± 0.04 x $10^9 M^{-1}$ vs controls : 0.92 ± 0.07 x $10^9 M^{-1}$; mean ± SE; n=8; $P < 0.1$) or the numbers of GH binding sites (diabetics : 1.10 ± 0.12 pmol/mg DNA vs controls : 1.26 ± 0.15 pmol/mg DNA; $P < 0.5$). Despite the absence of alterations in GH binding, the maximal serum Sm-C response was severely blunted in the diabetics (2.02 ± 0.32 U/ml) when compared to controls (17.41 ± 2.69 U/ml; $P < 0.01$). The sensitivity (ie ED50) however was the same for control and diabetics rats.

These results strongly suggest that the GH resistant state in poorly controlled insulin-dependent diabetes is due to a post-receptor defect.