1033 URINARY ALBUMIN EXCRETION RATE (AER) IN TYPE I DIABETES MELLITUS. Thomas Roe, Gertrude Costin and Francine Kaufman. Univ. So. Calif., Childrens Hosp. of Los Angeles, Dept. of Ped., Los Angeles, CA.

Persistent albuminuria (AU) is a sign of incipient diabetic nephropathy. The AER is usually measured in timed urine collections; 24 hr or overnight. When obtained from outpatients these collections are cumbersome. We have evaluated the use of single voided urine (SVU) samples from diabetic outpatients as a screen for AU. Albumin was measured by RIA and expressed as mg/g creatinine (cr). To set normal limits for SVU AER, urine from 52 non-diabetic outpatients, age 3-20 yr was tested. The upper limit of normal (mean+2SD) was 40 mg/g cr. One or more SVU samples from 147 diabetics, age 2-26 yr were tested. Persistently elevated levels were found in 23/147 (15.7%). Ten of the 23 with high SVU AER had 24 hr AER measured; 9/10 were abnormal. A significant correlation (corr. coeff.=0.85, $p<0.01$) was found between the AER measured in 24 hr and SVU samples. There was a progressive increase, with duration of diabetes, in the per cent of patients with elevated SVU AER: 0-4 yr=6.4%, 4-8 yr=13.5%, 8-12 yr=22.6%, 12-16 yr=41.7%. Mean glycohemoglobin (HgA1) levels were calculated from values obtained over the last 4 yr. In patients with mean HgA1 levels under 10%, only 9.2% had high SVU AER while 36.4% of those with mean HgA1 over 10% had high SVU AER.

CONCLUSIONS: SVU AER provides a convenient economical screening procedure for AU. The frequency of elevated values increases with duration of disease as expected. High mean HgA1 values appear to be correlated with elevated AER in type I diabetics.

1034 LEAD (Pb)-CALCIUM (Ca) INTERACTIONS IN CULTURED OSTEOCLASTIC BONE CELLS (OC). John F. Rosen, Joel G. Pounds. Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY; Brookhaven National Laboratory, Upton, NY.

Perturbations in intracellular Ca homeostasis, produced by Pb at low concentrations, may be an unifying hypothesis to explain Pb toxicity at the cellular level. Such perturbations may place the regulation of cellular processes out of the physiological range of normal control through changes in cytosolic free Ca concentration. The present study was undertaken to characterize more fully Ca:Pb ratios in different structural compartments of OC. Bone cells, enriched for OC, were prepared by a collagenase digestion of murine calvaria. After OC grew to confluence in 7 days, intracellular Ca:Pb ratios were calculated using kinetic analyses of dual label ^{45}Ca and ^{210}Pb washout curves by desaturation techniques that employed a validated 3 compartment model. The ratio of Ca:Pb half-times and rate constants were <4 thereby indicating similar intracellular pathways for these two metals. However, the kinetic distribution of Ca and Pb in OC was not symmetrical; the Ca:Pb ratio for two rapidly exchanging compartments was 40:1; for the most slowly exchanging compartment, which includes mitochondrial Ca and Pb, the Ca:Pb ratio was 7:1. The Ca:Pb ratios for the steady state flux across the plasma membrane was 35:1; and the ratio was 30:1 across the mitochondrial membrane. These observations reveal both similarities and differences in the intracellular homeostasis of Ca and Pb in OC. These quantitative relationships should be considered for the evaluation of Pb effects on intracellular Ca homeostasis and Ca functions in OC, other cell types and in cell-free systems.

●1035 GROWTH HORMONE (GH) THERAPY: EFFECTS ON IN VITRO ADIPOCYTE INSULIN (I) SENSITIVITY CORRELATE WITH ANTHROPOMETRIC RESPONSE. M. Rosenbaum, J.M. Gertner, J.W. Temeck, R.L.Leibel. Dept Pediatrics, Cornell Med Coll; Rockefelier U. New York, NY 10021

GH therapy is associated with adipose tissue redistribution from an android (abdominal; abd) to a gynecoid (gluteal; glt) pattern. In 6 GH-deficient children we studied the anatomic site-specific effects of GH Rx on adipocyte in vitro response to I and adrenergic stimulation, and their correlation with anthropometric changes. Adipose tissue samples obtained from abdominal and gluteal sites in fasting subjects by subcutaneous needle aspiration before (T1) and after 3 months (T2) of met-hGH (0.1 mg/kg TIW) were incubated for 2 h with 14C-glucose and 3H-palmitate plus I (10 or 100 uU/ml), isoproterenol (α-1 adrenergic agonist, 10^{-7}M), norepinephrine (β-2/α-1 agonist, 10^{-7}M), met-hGH (10 or 100 ng/ml), or dibutyryl cAMP (10^{-3}M). Rates of lipogenesis and lipolysis were calculated from absolute and relative rates of isotope accumulation after a 2 h incubation. Cell size was determined by osmium fixation. Anthropometry was performed at T1 and T2. There were no consistent differences in response to adrenergic stimulation or to the lipogenic effects of I between T1 and T2. However, there was a decrease in the antilipolytic effect of I in the abd fat depot following GH Rx which correlated with changes in height, weight, abd cell size, and the abd/glt cell size ratio as summarized:

Incubation (abdomen)	Anti-lipolysis* Mean(SEM)	Correlation Coefficient (Probability) Δ Height	Δ Weight	Δ Abd Cell Size	Abd:Glt Cell Size
I 10 uU/ml	-58.9(65.0)	.79(.06)	.74(.09)	-.71(.11)	-.91(.01)
I 100 uU/ml	-46.3(39.8)	.83(.04)	.79(.06)	-.77(.07)	-.94(.006)

* - % delta basal lipolysis.

Conclusion: GH Rx desensitizes abd subcutaneous adipocytes to the antilipolytic effects of I. This effect is strongly correlated with gynecoid redistribution of adipose tissue and the growth response. These data increase our understanding of the role of growth hormone in fat accretion. They may also be predictive of growth response to GH Rx.

1036 HIGH FETAL PLASMA OSTEOCALCIN (OC) AND POSITIVE RELATIONSHIP OF OC TO PLASMA PHOSPHATE (P) IN FETAL (F) AND MATERNAL (M) SHEEP USING A HOMOLOGOUS RIA. Richardus Ross, Mei Chen (Spon. by R.C.Tsang) U. of Cincinnati Med. Ctr., Dept. Ped., Cincinnati, OH.

Plasma concentrations of the bone-specific protein OC correlate with bone formation rate. OC may regulate the mineralization process. We isolated OC from ovine bone and used an homologous RIA to investigate plasma OC in chronically catheterized M and F sheep during the last month of gestation. OC was purified from adult cortical bone by sequential gel filtration, anion exchange chromatography and SDS PAGE. Characterization was on the basis of amino acid analysis, including presence of γ-carboxyglutamic acid and its conversion to glutamic acid upon decarboxylation. N-terminal aminoacid sequencing (20 residues) revealed hydroxyproline at amino acid position 9. The protein demonstrated a typical stoichiometry of binding to hydroxylapatite (HTP) of 64mg protein/mg HTP and retained this property when radioiodinated. The RIA has a sensitivity of 1 ng/tube and intra and inter assay CV of 7% and 12.4%. Mean (+SEM) plasma OC in 51 samples from 10 pregnant sheep of 6.3±0.8ng/ml was not different from 23 non-pregnant sheep (5.8±1.1ng/ml) but was markedly lower than the corresponding F value of 141±8 ng/ml ($p<0.0001$). There was no correlation between F and M values and neither F nor M values correlated with gestation, total plasma Ca or ionized Ca. However, both F and M plasma OC was directly correlated with plasma P (F:r= 0.45, $p<0.01$; M r=0.54, $p<0.001$). We suggest there is 1) lack of placental transfer of OC, 2) positive regulation of bone turnover by P during pregnancy, 3) plasma OC is useful in the study of fetal mineral metabolism.

●1037 HEPATIC DRUG METABOLISM IS INCREASED IN POORLY CONTROLLED INSULIN DEPENDENT DIABETES MELLITUS (IDDM). Paul Saenger, Albert Einstein Coll. Med., Dept. Peds. Bronx, New York.

In addition to increased glycosylation of hemoglobin, abnormalities of other heme proteins such as cytochrome P-450 might also occur in IDDM. We reported previously that cytochrome P-450 dependent drug metabolism is altered in IDDM using antipyrine (AP) as marker drug. AP is a reliable measure of hepatic drug metabolism. Its elimination from plasma is largely determined by hepatic drug oxidizing enzyme capacity. AP kinetics and urinary excretion of AP metabolites were measured in 14 patients (8-21 years) with IDDM in poor control (nl renal function) and compared to age matched controls. Improvement in diabetic control achieved by increases in insulin dose (mean 1.1 U/kg/day), more frequent insulin doses combined with better dietary control lead to normalization of AP halflife and metabolism. ($t_{\frac{1}{2}}$ halflife):

	AP $t_{\frac{1}{2}}$ (hr)	AP Clearance (ml/min/kg)	Hgb A_1 (%)
Diab. High Hgb A_1	4.6±.18	1.3±.16	13.2±2.1
near nl Hgb A_1	7.8±.6	0.82±.1	8.2±1
Controls	8.1±1.0	0.80±.19	< 7.8

All three urinary metabolites of AP were increased to a similar extent in poorly controlled IDDM: N-dimethyl AP ↑60.5±5%, 4-hydroxy AP ↑54±4%, 3-hydroxymethyl AP ↑68±6%. Separate cytochrome P-450 isoenzymes regulating AP metabolism appear to be stimulated to a similar extent in poorly controlled IDDM.

Conclusions: Poorly controlled IDDM leads to increased hepatic drug metabolism affecting all three isoenzymes involved in AP metabolism. These data suggest that improved metabolic control normalizes hepatic drug metabolism in IDDM.

1038 IN VIVO PHENYLALANINE HYDROXYLASE ACTIVITY. Claude Sansaricq, Selma E. Snyderman and Ann Arcay. NYU Med. Ctr., Dept. of Pediatrics, NYC, NY.

The enzyme phenylalanine hydroxylase is almost exclusively limited to the liver although some activity may be found in the kidney and the intestinal mucosa. Determination of this enzyme activity necesitates, therefore, the use of an invasive procedure (liver biopsy). Milstien and Kaufman have measured tritiated water produced from a pentadeuterated phenylalanine in rats and penta tritiated phenylalanine in a single patient to obtain in vivo activity of this enzyme. We have studied phenylalanine hydroxylase activity in rabbits using a carbon 4 tritium tagged phenylalanine which yields one mole each of tyrosine and tritiated water. A load of 150 mg/kg of phenylalanine and 10 mcc/kg of tritiated phenylalanine was given. The tritiated water formed was determined for 3 hours. The reaction is linear up to 4 hours, $Y=885.2618X + 17463.15$; $r=0.980963$ p .001. After informed consent from the involved parties, and approval by the institutional Research Review Committee were obtained, the test was carried out on one adult control, one obligate heterozygote mother and her adult PKU daughter. The production of tritiated water was .439 umole/ml of plasma/hour for the control, 0.125 for the obligate heterozygote and less than 1% of the control value for the affected patient. The radiation exposure is minimal and was calculated to be less than a chest X-Ray. We conclude that phenylalanine hydroxylase activity can be safely determined in vivo with a load of tagged ring 4 phenylalanine.