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POLYCYSTIC OVARIES AND GLUCOSE TOLERANCE IN HEPATIC GLYCOGEN STORAGE DISEASE. PJ Lee[1], A Patel[2], PC Hindmarsh[2], CGD Brook[2] and JV Leonard[1], International Growth Research Centre, Institute of Child Health[1] and The Middlesex Hospital[2], London, United Kingdom.

Polycystic ovaries (PCO) are found in both lean and obese women in association with hyperinsulinism and insulin resistance. The hepatic glycogen storage diseases (GSDs) are an heterogeneous group of inherited disorders of carbohydrate metabolism characterised by hypoglycaemia, lactic acidosis, hyperlipidaemia and hyperuricaemia in which abnormalities of insulin secretion may be present. We therefore studied 16 female patients with GSDs by performing pelvic ultrasonography and oral glucose tolerance tests (1.75g/kg to maximum 75g glucose) during which samples were drawn every 20 minutes for 2 hours. Fasting could not be standardised and varied between 2 and 3 hours. 8 patients had glucose-6-phosphatase deficiency (GSD Ia); 6 had amylo-1,6-glucosidase deficiency (GSD III); 1 had phosphorylase deficiency (GSD VI); and 1 had phosphorylase b kinase deficiency (GSD IXa). 8 patients were pre-pubertal; mean age was 15.9 years (range 4.5-31.3). The group overall was not obese with age corrected mean body mass index being 111% (range 100-129). All patients except the two youngest (4.5 and 4.6 years) had ultrasonographic evidence of PCO (87.5%). 2 out of 10 adults were symptomatic with oligo- or secondary amenorrhoea. Basal plasma glucose levels varied from 0.4 to 7.9 mmol/l (mean 4.4) with peak values varying between 5.8 and 17.4 mmol/l (mean 9.6). Mean basal insulin was 39.9 mU/l (range 3.4 to 106.2), with mean incremental insulin being 1454.7 mU/L (range 26.9 to 4249.5). The prevalence of PCO in this group is much greater than the 22% seen in the general adult female population. In particular, the early age of onset is quite striking. They display glucose intolerance with abnormal insulin secretion. This supports the hypothesis that insulin may be causative of PCO as well as having important implications for women with GSDs.

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LATE ADRENARCHE IN A PATIENT WITH PSEUDOHYPOPARATHYROIDISM (PHP) : APPLICATION OF OF A NEWLY DEVELOPED ELISA FOR DHEAS. N. Katsumata, Y. Asakura*, H. Maeseka*, K. Tachibana*, K. Nakamura** and S. Suwa*. Department of pediatrics, Hitachi, Ltd. & Hitachi Totsuka General Hospital, Yokohama 244, *Division of Endocrinology and Metabolism, Department of Pediatrics, Kanagawa Children's Medical Center, Yokohama 232, and **Sapporo Immunodiagnostic Laboratory, Sapporo 060, Japan.

The increased production of adrenal androgens or adrenarche, characterized by a disproportional rise in DHEAS, is known to occur prior to pubertal maturation of the hypothalamo-pituitary-gonadal axis or gonarche in normal children. However, the dissociation between adrenarche and gonarche is observed in some pathological conditions such as true precocious puberty. In the present study we report a newly developed ELISA for DHEAS and the late adrenarche in a female patient with PHP. An ELISA for DHEAS was developed by means of DHEA-3-hemisuccinate-HRP conjugate and antiserum against DHEA-3-hemisuccinate-BSA. The sensitivity of the assay was 12.5 pg/well and IC_{50} was about 400 pg/well. The intra- and inter-assay coefficients of variation were <8% and <10%, respectively. There was close correlation between the DHEAS measurements by this ELISA method and the conventional RIA method over a wide range of serum concentrations (r=0.98, n=52). Serum DHEAS levels were determined in 923 normal children aged 5 days through 18 years and the normal range for each age was established. A 13-year-old female patient presented with short stature. She was obese and had round face, short neck, and brachydactyly with with short metacarpal bones. She had low serum calcium and high serum phosphorus concentrations with normal renal function. She did not respond to exogenous PTH in terms of urinary cAMP and phosphorus secretion and was diagnosed as PHP. Although she had had menarche at the age of 12 years and regular menstrual cycles and her breast development was at Tanner's stage IV, she had no axillary and pubic hair. Urinary 17-KS secretion and serum DHEAS level were low, and serum DHEAS levels remained to be lower than the age-matched normal range up to the age of 15 years even after normalization of serum calcium and phosphorus levels. In conclusion, (1) this new ELISA should provide a useful tool to investigate physiological and pathological roles of adrenarche; (2) late adrenarche might be a newly recognized complication of PHP, suggesting resistence of adrenal cortex to the factor(s) which is responsible for adrenarche in PHP.

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HYPERANDROGENISM AS A CAUSE OF EARLY POLYCYSTIC OVARY SYNDROME (PCOS) IN GIRLS WITH CENTRAL PRECOCIOUS PUBERTY (CPP)

Increased adrenal androgens are often observed in girls with CPP. The hyperandrogenism is not affected by the therapy with GnRh analog (GnRHA). We performed an i.v. ACTH stimulation test (0.25mg) in 42 girls with CPP (Tanner 2-3), before (29/42) and during (13/42) therapy with GnRHA. The stimulated levels of 17OH pregnenolone (17OHPreg), 17OH progesterone (17OHP) and the ratio 17OHPreg/17OHP were analyzed and compared to normal values for age and pubertal stage (Lashansky et al JCEM 73: 674,1991). The results revealed 3 patterns of response: a) Enzymatic deficiency (D)-6/42 girls: non-classical (NC)21OHD - 6, NC3βHSDD - 2; b) Exaggerated "adrenarche" i.e. hyper-response to ACTH: 17OHPreg >24nmol/l and 17OHPreg/17OHP <7 - 19/42 girls; c) Normal response - 17/42 girls. Also an ACTH was performed in 12 CPP girls post GnRHA therapy in full puberty. On long term follow up 8/12 had developed early PCOS (ages: 14-16) and revealed abnormal responses: NC3βHSDD was diagnosed in 1 girl and exaggerated "adrenarche" response was found in 7. The remaining 4/12 without PCOS revealed a normal response. According to our data, associated hyperandrogenism is found in a significant number of girls with CPP (59.5% in this report) and remains sustained throughout puberty and thereafter. Hyperandrogenism can be the trigger for the onset of CPP in these patients and can explain the relatively high incidence of early PCOS in girls with CPP.

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HETEROGENEITY OF URINARY STEROID PROFILES IN CHILDREN WITH ADRENAL TUMORS. E.Malunowicz, M.Ginalska-Malinowska, T.E.Romer, A.Wolska, B.Rymkiewicz-Kluczyńska. Child Health Center, Warsaw, Poland.

Adrenocortical tumors in children are rare but important causes of virilization and/or Cushing's syndrome. Other symptoms, including feminization and hyperaldosteronism, are less frequent. We present steroid urine profiles in 8 girls with adrenocortical tumors.

Case	Age (yrs)	Symptoms	17-KS (mg/day)	Urine steroids excreted in high pathological amounts as determined in steroid profile	Histo-pathol.	Tumor size
1.	0.8	Cushing+viriliz.	30.0	Androsterone(AN), DHA, 16-OHDHA, 5-Androstene-3β,16α,17β-triol (An-3-ol)	Ca	9 cm
2.	1.3	Cushing+viriliz.	23.0	AN, DHA, 16-OHDHA, An-3-ol	Ca	4 cm
3.	5.0	virilization	100.0	AN, DHA, 16-OHDHA, An-3-ol	Adenoma	7 cm
4.	3.0	Cushing+viriliz.	12.8	11β-OHAN, THE, THF, aTHF, 5F, ratio THF/THE ↑	Ca	8 cm
5.	13.5	Cushing+viriliz.	26.9	11β-OHAN, THS, THE, THF, aTHF, 6β-OHF Normal ratio THF/THE & ET/AN	Adenoma	5 cm
6.	6.0	virilization	5.0	11β-OHAN	Adenoma	5 cm
7.	3.3	virilization	5.0	11β-OHAN	Adenoma	3 cm
8.	2.6	virilization	5.9	AN, Pregnanediol, Pregnenediol, Pregnene-3β-16α-20triol	Adenoma	4 cm

Assessment of the urinary steroid pattern reveals its heterogeneity and makes it possible to: 1)follow the patients after surgery in terms of possible recurrence of the tumor, 2)exclude CAH as a cause of virilization (cases 6,7,8), 3)confirm the diagnosis of adrenal tumor in patients with only marginally elevated 17-KS but elevated 11β-OHAN (cases 6 & 7), 4)detect patients with adrenal tumor with unusual steroid patterns (case 8). Steroid profiling in urine by capillary gas chromatography has an advantage over the traditional assays of 17-KS, 17-OHCS or serum levels of DHA, DHAS, testosterone, cortisol, 17OH-progesterone for the diagnosis and management of adrenal tumors.

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A POINT MUTATION OF THE ACTH RECEPTOR IN FAMILIAL GLUCOCORTICOID DEFICIENCY.

Familial glucocorticoid deficiency is an uncommon disorder that mimics childhood Addison's disease but with preserved mineralocorticoid function. We have postulated that it might result from defective ACTH receptor function. We studied a patient with this condition using the technique of polymerase chain reaction (PCR) to amplify the ACTH receptor from his genomic DNA using primers based on the sequence of the recently reported ACTH receptor DNA sequence. PCR products were subcloned in plasmids and sequenced using the dideoxy chain termination technique. We consistently found a single base change (G > T) in codon 74 resulting in the substitution of Isoleucine for Serine. This mutation destroys a *Fnu4HI* restriction site which facilitates the study of first degree relatives. Using this restriction site polymorphism we identified the proband and his similarly affected sister as being homozygous mutants, an unaffected brother as being a normal homozygote, and both parents as being heterozygotes for the same mutation. Serine 74 lies in the second transmembrane domain of this receptor and is conserved amongst all members of the ACTH/MSH/cannabinoid receptor family, and thus it appears to play an important part in the recognition of MSH peptides. The study of other families with this syndrome and expression and mutation studies with this receptor should allow us to define more clearly the nature of this interaction.

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QUANTIFICATION OF 17-HYDROXYPROGESTERONE (17OH-P) BY GAS CHROMATOGRAPHY/ISOTOPE DILUTION MASS SPECTROMETRY (GC/IDMS): A REFERENCE METHOD SUITABLE FOR ROUTINE USE

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17-OHP is an important parameter for diagnosis and monitoring of 21-hydroxylase deficiency (21-OHD). Immunoassays bear the risk of falsly elevated 17-OHP values due to cross reactivity or matrix effects. We have developed a rapid, specific **17-OHP assay** using GC/IDMS with a deuterated analog as internal standard (IS): equilibration of plasma with IS is followed by extraction, purification and derivatization (heptafluorobutyrates). **Results:** sensitivity (lower limit of detection 5 pg), accuracy (rel. error < 7.5%), precision (intra- and interassay coeff. of variation < 3.8%). Normal values (mean ± SD, ng/ml): amniotic fluid (15-17th week, n = 5, 1.29 ± 0.53), cord plasma(n = 16, 5.17 ± 3.38), plasma (1-7 days, n = 10, 0.42 ± 0.29; 8-28 days, n = 8, 1.33 ± 0.54; < 7 yrs, n = 12, 0.23 ± 0.20; 8-16 yrs, n = 27, 0.35 ± 0.19; adults, n = 24, 0.79 ± 0.35). Values (ng/ml) at diagnosis of 21-OHD in 3 patients: 24,9 (16th wk of gest.), 22.08 (2 days), 286.6 (41 days). **Conclusions:** 1) We have developed a highly reliable GC/IDMS assay for routine analysis of 17-OHP in amniotic fluid or plasma at all ages. 2) Compared to the literature, our normal values, the first produced by GC/IDMS, are much lower, especially in the neonatal period. Supported by DFG (Wu148/3).