capacity, and had an additional somatic inactivating mutation on the same allele. Somatic inactivation of the deleterious *SAMD9* allele likely caused expansion of the clone with two mutations ("adaptation by inactivation"), and was possibly associated with the absence of hematologic abnormalities.

FC92

**HIGH DHEAS AT AGE 7 ARE ASSOCIATED TO HIGHER GLYCEMIA DURING PUBERTY BUT NOT METABOLIC SYNDROME**

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**Objectives:** Premature adrenarche (PA) has been recently identified as a risk factor for metabolic diseases. This risk may depend on ethnic background,birthweight and infancy weight gain. In a longitudinal cohort (Growth and Obesity Cohort,GOCS n=969 both sexes) children with high DHEAS (HD=biochemical adrenarche) at 7 y,were fatter and more centrally obese than their counterparts (ND) (AJCN,2013).Aim:To determine whether HD at age ~7yr in girls determines a higher prevalence of metabolic Syndrome or its components during puberty.
**Methods:** Girls from the GOCS cohort with anthropometry since birth (2003).From 2006, a clinical evaluation and a complete metabolic profile at Tanner B2/B4.HD defined by DHEAS (RIA, µg/dl) >75$^{th}$ percentile (girls>42.0 µgr/dl).Metabolic syndrome accordingly to IDF 2007. Statistics: Generalized linear models and survival analysis were used to assess the relation between HD and anthropometric and metabolic profile at B2(n=401) and B4(n=357).Adjusting by infancy weight change(>0.67),mother´s age at menarche,maternal height and education.
**Results:** Girls who displayed HD,at tanner B2 were taller,had higher BMI and were younger (8.8 (CI 95%CI;7.9-9.3)vs.9.3(95%CI;9.1-9.6)than girls with normal DHEAS (ND).None of the girls met the blood pressure criteria. No differences were observed in the risk of metabolic syndrome or its components (Summarized in Table).However, glycemia > 100 mg/dl was present in higher percent of HD girls at Tanner II and Tanner IV. *p<0.05**p>0.005

| | Tanner II | | Tanner IV | |
|---|---|---|---|---|
| | ND | HD | ND | HD |
| Metab.Synd. n(%) | 2 (0.7) | 3(3.8) | 2(0.8) | 2(2.6) |
| WC > 90thperc. | 27(8.6) | 11(12.9) | 34(11) | 11(13.2) |
| TG> 150 mg/dl | 34(12) | 12(15) | 40(16.6) | 16(20.7) |
| HDL<40 mg/dl | 57(20.0) | 19(23.8) | 36(14.4) | 10(12.5) |
| Glycemia>100mg/dl | 4(1.4) | 5(6.3)* | 4(1.7) | 7(9.1)** |
| BMI>2SDS | 37(12.2) | 20(20.4)* | 32(12.5) | 18(18) |
| mean glycemia mg/dl | 88.3 ±7.1 | 89.9±7.8* | 86 ±6.6 | 87 ±8.7* |

**Conclusions:** HD conferred a risk of higher glycemia and the rest of the components of metabolic syndrome appeared to ameliorate with advanced pubertal stage. Follow-up of this cohort is necessary to address prospectively the interrelationships of HD, early growth ,adiposity, sex steroids and markers of metabolic risk (Fondecyt 1140447 & 1120326, WCRF:2010/245).

FC93

**ALTERED STEROID AND DRUG METABOLISM BY A P450 OXIDOREDUCTASE VARIANT FOUND IN APPARENTLY NORMAL POPULATION**

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**Objectives:** A broad spectrum of human diseases, including abnormalities in steroidogenesis, is caused by mutations in the NADPH P450 oxidoreductase (POR). POR transfers electrons from NADPH to several small molecules, non-P450 redox partners and microsomal cytochrome P450 proteins (CYPs). Our aim was to check if POR variations from non-clinical samples, by seeking 1000 genomes database, can be disruptive. Y607C variant (rs72557954, NM_000941.2:c. 1820A>G) has been reported in population studies and prevalent in south Asians, but was predicted to be likely pathogenic. We performed detailed enzymatic and biochemical characterizations of Y607C variant to study its effect on different substrate and redox partners.
**Methods:** We analysed the ability of POR wild type (WT) and Y607C variant to reduce ferricyanide, MTT, cytochrome c and drug, and steroid metabolizing CYP450. POR WT and Y607C were expressed and produced as recombinant proteins while CYP19A1 and CYP3A4 were produced as His-tagged recombinant proteins and purified by affinity chromatography. The effect of mutation on cofactor (FAD/FMN) binding and activity under varying substrate and cofactor conditions was performed.
**Results:** We found varied effects of Y607C mutation on reduction activity of different substrates. As compared to WT, Y607C variant showed 66% cytochrome c and 91 % ferricyanide reduction activity but had only 13 % MTT reduction activity. Y607C did not affect POR flavin content but NADPH binding was severely affected. With varying NADPH concentration, Y607C showed ~95% decrease in supporting CYP19A1 and CYP3A4 activity. This mutation was later identified in patients with POR deficiency.
**Conclusions:** Identification of severe effects of this mutation on both drug and steroid metabolizing CYP450s indicates that likely pathogenic mutations may be found in apparently normal (non-clinical) population. Their combination as compound heterozygotes or homozygous may lead to severe impact on both steroid and drug metabolism by modification of its redox partners activities. Variations in POR need to be evaluated individually. Most importantly, advanced identification of disease causing variants in POR will help in

understanding the POR deficiency in patients if the same mutations are later identified.

FC94

**BIOAVAILABILITY OF ORAL HYDROCORTISONE CORRECTED FOR BINDING PROTEINS AND MEASURED BY LC-MS/MS USING SERUM CORTISOL AND SALIVARY CORTISONE**

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**Objectives:** The assessment of absolute bioavailability of oral hydrocortisone is complicated by its saturable binding in the therapeutic range to cortisol binding globulin (CBG). Ninety percent of serum cortisol circulates bound to CBG, 5% to generic binding proteins, such as albumin and α-1 glycoprotein, and only 5% is unbound or 'free'(1). CBG has high affinity for cortisol but lower capacity whereas albumin has a lower affinity and higher capacity(2). An increase in the free fraction results in an increase in clearance with dose and therefore less than dose proportional increases in Cmax and AUC(3). Derendorf reported absolute bioavailability to be 0.96 (CI 0.82 to 1.09) using a radioimmunoassay to measure cortisol in serum (3); however immunoassays give variable results and LC-MS/MS is now becoming the gold standard method for measuring steroids. We have measured absolute bioavailability of hydrocortisone using serum cortisol and salivary cortisone measured by LC-MS/MS.
**Methods:** 14 healthy male dexamethasone suppressed volunteers were administered 20mg hydrocortisone either intravenously or orally by tablet. Samples of serum and saliva were taken and measured for cortisol and cortisone by LC-MS/MS. Serum cortisol was corrected for saturable binding using published data (2) and PK parameters derived using the program WinNonlin.
**Results:** The mean (95% CI) bioavailabilities of oral hydrocortisone calculated from serum cortisol, corrected serum cortisol and salivary cortisone were 1.00 (0.89-1.14); 0.89 (0.75-1.05); and 0.93 (0.83-1.05), respectively.
**Conclusions:** The data confirm that after oral administration hydrocortisone is completely absorbed(3). The data derived from serum cortisol corrected for protein binding and that from salivary cortisone are similar supporting the concept that salivary cortisone reflects serum free cortisol levels and that salivary cortisone can be used as a non-invasive method for measuring the pharmacokinetics of hydrocortisone.
**Refs:** 1. Lewis JG, et al. Clin Chim Acta 2005; 359:189-194. 2. Lentjes & Romijn. J Clin Endocrinol Metab 1999; 84:682-687. 3. Derendorf et al. Journal of Clinical Pharmacology 1991; 31:473-476
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FC95

**SIMULTANEOUS PROFILING OF 17 STEROID HORMONES USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY IN NEWBORN AND EARLY INFANCY**

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**Objectives:** Many of the steroidogenic disorders present in newborn or early infancy with ambiguous genitalia and/or salt-wasting.Quantification of endogenous hormonal steroids and their precursors is essential for the diagnosis. Rapid changes in steroid hormone concentrations due to fetal adrenal zone involution, low-concentration analytes and assay interference by endogenous or placental steroids are main diagnostic challenges of this critical life period. Analysis of serum steroids by liquid chromatography tandem mass spectrometry (LC-MS/MS) can provide rapid, highly specific, and sensitive simultaneous analysis of multiple steroid analytes.
**Methods:** We employed LC-MS/MS to measure a panel of 17 steroids of 300 healthy full-term newborns and babies (150 males, 150 females) grouped in five age groups (3-7th, 8-14th, 15-28th, 29-90th, 91-180th days). Nonparametric statistical approaches were used to generate the mean, 2.5th-97.5th percentile distributions for age groups. 17OH-Progesterone+21-deoxycortisol)/cortisol, 11-deoxycortisol/cortisol, 17OH-pregnenolone/cortisol, cortisol/cortisone and testosterone/dihydrotestosterone (only in boys) ratios were calculated as biomarkers of specific enzyme deficiencies.
**Results:** Reference intervals of 17 steroids measured simultaneously by LC-MS/MS were established.17-hydroxylated C21-glucocorticoids , non-17-hydroxylated C21-mineralocorticoid precursors (pregnenolone and progesterone) and C19-androgens (dehydroepiandrosterone, dehydroepiandrosterone-sulphate, Δ4-androstenedione) showed most significant age-related changes from 3 to 180 days of life.However C21-mineralocorticoids (aldosterone, corticosterone, 11-deoxycorticosterone) remained constant during the first 6 months of life.Biomarkers of 21-α-hydroxylase, 11-β-hydroxylase, 3-β-hydroxylase activities were in favor of cortisol increase.
**Conclusions:** Implementation of LC-MS/MS based steroid panel will provide shortly correct diagnosis.Therefore, the establishment of age-related reference intervals for newborn babies and early infancy in this study will allow rapid, noninvasive, accurate diagnosis and differential diagnosis, guide sex assignment, genetic counseling, predicting prognoses and prevent the potential life-threatening outcomes of impaired steroidogenesis.