P1-d1-155 Adrenal 1

Genotype-dependent differences in renin-aldosterone response, urinary Na/K ratio and growth in pseudohypoaldosteronism patients with mutations in epithelial sodium channel (ENaC) subunit genes

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Multi-system pseudohypoaldosteronism (PHA) is a rare syndrome of aldosterone unresponsiveness characterized by symptoms of severe salt losing caused by mutations in one of the genes that encode α, β or γ subunit of epithelial sodium channels (ENaC). We examined long-term changes in the renin-aldosterone response in patients with different mutations. Four PHA patients were followed-up for 7-22 years. Patient A with a heterozygous Gly327Cys missense mutation in dENaC is a mild case and patients B, C and D are severe cases. Two additional patients with renal PHA served as controls. In patient A, serum aldosterone and plasma renin activity (PRA) decreased with age, PRA reaching near normal values at age 7. In contrast, patients B-D showed a positive correlation between age and aldosterone (r>0.86 for all). In patient B with Arg508stop mutation, aldosterone reached 166 nmol/L. 17-hydroxyprogesterone (17-OHP) screening for classical congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is part of many newborn screening programs worldwide. However, sensitivity does not reach 100 %, mainly due to a high variability of 17-OHP levels in healthy newborns and thus high cut-off values. Recently, the glucocorticoid receptor (GR) N363S variant has been linked to relatively low degree of virilization and serum 17-OHP. We also did not detect differences regarding GR genotypes and steroid profile in LC-MS/MS. Lack of significant genotype effects remained stable even after subgrouping samples for gestational and postnatal age. However, retrospective genotyping of five CAH children reported previously with screening failure revealed N363S heterozygosity. In patient B with Arg508stop mutation, aldosterone reached 166 nmol/L.
at age 19 (>300 fold higher than normal). Urinary Na/K ratios normalized gradually with age in all patients. Growth curves of the patients were reflective of the severity of PHA and compliance with salt therapy. Functional expression studies in oocytes showed that ENAc with gGly327Cys mutation, as observed in patient A, showed nearly 40% activity of the wild type ENAc. In contrast, stop mutation as in patient B reduces ENAc activity to less than 5% of the normal. Our results demonstrate distinct genotype-phenotype relationships in PHA patients. The degree of ENAc function impairment affects differently the renin-aldosterone system and urinary Na/K ratios. The differences observed are age dependent and PHA form specific.

Compound heterozygosity (Phe79Ile and Arg448His) in the CYP1B1 gene resulting in late-onset Congenital Adrenal Hyperplasia in Danish trzygotic triplets

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Congenital Adrenal Hyperplasia (CAH) is in 90% of cases caused by mutations in the 21-hydroxylase encoding gene (CYP21). In 5% CAH is caused by mutations in the 11beta-hydroxylase encoding gene (CYP11B1). 11beta-hydroxylase deficiency is associated with cortisol insufficiency, symptoms of androgen excess and, in two thirds of the patients, hypertension. We hereby present compound heterozygosity in the CYP11B1 gene in three 8.3 years old Danish children who were trzygotic triplets (two boys and one girl) who presented with pubic hair (stage PH2-3) and penile growth (stage G2) in the boys, and with breast development (stage B3) in the girl. They all showed accelerated growth, markedly advanced bone age, and were normotensive. The 24-hour urinary steroid profile revealed elevated tetrahydro-11-deoxycortisol (THS) excretion, highly indicative of 11beta-hydroxylase deficiency. Blood sampled showed slightly elevated basal ACTH, highly elevated deoxycortisol (THS) excretion, highly indicative of 11beta-hydroxylase deficiency. 24-hour urinary steroid profile revealed elevated tetrahydro-11-deoxycortisol (THS) excretion, highly indicative of 11beta-hydroxylase deficiency. Blood sampled showed slightly elevated basal ACTH, highly elevated deoxycortisol excretion, highly indicative of 11beta-hydroxylase deficiency.

Atypical clinical presentation in a girl with type 1 autoimmune polyglandular syndrome

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Autoimmune Polyglandular Syndrome type 1 (APS1) is a monogenic autosomal recessive disorder associated with organ and non-organ-specific autoimmune manifestations. Diagnosis (at least two of the following disorders): chronic mucocutaneous candidiasis, hypoparathyroidism and/or primary adrenal insufficiency. The AIRE gene has been mapped on chromosome 21q22.3. We describe a 14.1yrs old girl from Northern Italy who developed subsequent y,mucocutaneous candidiasis (1yr), IDDM(3.6yr), intestinal dysfuction (3.9 yrs), epilepsy (4.7yrs), hypoparathyroidism (5.3yrs), ocular myasthenia (5.6yrs) and precocious puberty (6.6yrs). Autoimmune pattern showed the presence of ICA, GADA, IAA, thyroperoxidase (TPO), L-aminooacid decarboxylase (AA DC), thyrotophan hydroxylase (TPHA), adren al cortex (ACA), 21-hydroxylase (21OHA), and steroid-producing cells (STCA) autoantibodies. Cranial MRI evidenced cortical displasia of right insula and atrophy of hippocampus. The immunohistochemical examination of duodenal mucosa showed a marked reduction in the number of enterochromaffin mucosal cells containing serotonin and TPH. At 7.6yr a transient elevated ACTH and plasmatic renin was found (clinical Addison disease). At last examination adrenal function was normal but ACA and 21OHA persisted high. She had a partial LH and FSH response to LHRH test (6.6yrs). HLA typing was: DR2/DR4-DQB1*0502/DQB1*0201. DNA sequencing revealed the homozgyous R139X mutation on exon 3 of AIRE gene. This patient presents several unusual features: a) temporal-lobe epilepsy, rarely associated with AS1, could be an autoimmune manifestation related to GADA, b) intestinal dysfuction with impaired bowel motility may be due to an autoimmune process hampering serotonin production; c) STCA in APS1 are usually responsible for premature ovarian failure; our patient, atypically, presented precocious puberty, that may be due to hypothalamic activating autoantibodies; d) our patient presented the R139X mutation of AIRE, originally described in Sardinians; being the family from Northern Italy we suggest that the same mutation may be present in different geo-ethnic groups.

Is fasting early morning salivary cortisol a reliable method with which to investigate children treated with inhaled steroids for potential adrenal suppression?

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Introduction: Recently there has been concern about adrenal suppression in children treated with inhaled steroids for asthma. However it remains unclear at what dose of inhaled steroids this may occur and how best to screen or investigate when there is clinical concern. Measuring salivary cortisol is an attractive potential method as it is cheap & non-invasive.

Aims: To establish whether measurement of fasting early-morning salivary cortisol is useful clinical tool for assessing possible adrenal suppression in asthmatic children treated with inhaled steroids.

Methods: Basic laboratory work to validate a salivary cortisol assay, establish its relationship to plasma cortisol, establish the stability of samples in the post and to investigate potential interference with the assay by inhaled steroids was undertaken. Age-specific reference ranges for fasting early morning cortisol were then established in 155 healthy children. These were compared with samples obtained from 55 children treated with varying doses of inhaled steroids.

Results: Fasting early morning cortisol levels were inversely related to the dose of inhaled steroid ( r = 0.36, p = 0.007). The higher the dose of inhaled steroid, the lower the cortisol. This was apparent at total daily doses of 800mcg budesonide/beclometasone & 400mg fluticasone. In children on inhaled steroids 18.5% had salivary cortisol levels < 2.0 nmol/l (approximates to plasma cortisol <150 nmol/l) compared to 7.8% of controls (p<0.05). And 7.8% of asthmatics had a cortisol <1.5nmol/l (approximates to plasma cortisol <100 nmol/l) compared to 3.2% of controls.

Conclusions: A dose related reduction in early morning salivary cortisol was found in children on inhaled steroids (at relatively modest doses). Significantly higher proportions of children on inhaled steroids had cortisol levels below that which may trigger clinical concern but there was considerable overlap with controls. Whilst potentially useful in identifying individuals who may need further investigation salivary cortisol alone may not reliably predict adrenal suppression.
Enhanced cortisol catabolism due to induction of cytochrome P450 3A4 in epileptic children taking oxcarbazepine (OXC)

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In contrast to other antiepileptic drugs, OXC so far has not been known to induce hepatic cytochrome P450 enzymes, which would enhance steroid catabolism. Our index-patient was a 6y-old boy with Addison’s disease, who was also treated with OXC for temporal lobe epilepsy. He required high supplementation doses of hydrocortisone (>20mg/m²/day). Nevertheless, he had grossly elevated ACTH levels (>300pg/mL) and hyperpigmentation. An i.v. hydrocortisone-test confirmed enhanced cortisol (F) elimination (90min post-injection concentration of 40% on OXC vs. 59% off OXC) and excessive amounts of urinary 24hr 6β-OH-cortisol/F. After switching from OXC to levetiracetam, all symptoms, weight, biochemical features and hydrocortisone doses (10mg/m²) normalised. This observation prompted the comparative study of 24hr urinary steroid profiles using gas-chromatography-mass-spectrometry in 6 males with epilepsy on OXC and 6 healthy male controls. Their 24hr urinary 6β-OH-cortisol/F amounts were not significantly reduced in patients on OXC, the cytochrome P450 3A4 activity was induced and the formation of 45mg hydrocortisone [22mg/m²/day] was also treated with OXC for temporal lobe epilepsy. He required high supplementation doses of hydrocortisone (>20mg/m²/day). Nevertheless, he had grossly elevated ACTH levels (>300pg/mL) and hyperpigmentation. An i.v. hydrocortisone-test confirmed enhanced cortisol (F) elimination (90min post-injection concentration of 40% on OXC vs. 59% off OXC) and excessive amounts of urinary 24hr 6β-OH-cortisol/F. After switching from OXC to levetiracetam, all symptoms, weight, biochemical features and hydrocortisone doses (10mg/m²) normalised. This observation prompted the comparative study of 24hr urinary steroid profiles using gas-chromatography-mass-spectrometry in 6 males with epilepsy on OXC and 6 healthy male controls. Their mean (SD) urinary excretion rates and those of the index-patient, who was on 45mg hydrocortisone [22mg/m²], 100ug fludrocortisone and 25mg DHEA on both occasions, are presented in the table.

All units in ug/day

<table>
<thead>
<tr>
<th>Controls</th>
<th>OXC</th>
<th>% Diff. in OXC</th>
<th>Index-Patient on OXC (16.4y)</th>
<th>Index-Patient off OXC (16.9y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum C21 (log)</td>
<td>10177.0 (3349.9)</td>
<td>7878.6 (4402.0)</td>
<td>-22.5</td>
<td>17276.5 (15068.5)</td>
</tr>
<tr>
<td>Fcomb/BSA</td>
<td>61.27 (52.17)</td>
<td>41.21 (15.97)</td>
<td>-32.8</td>
<td>77.14 (83.77)</td>
</tr>
<tr>
<td>Sum C19 (log)</td>
<td>3.74 (0.36)</td>
<td>3.47 (0.17)</td>
<td>-7.2</td>
<td>4.06 (3.85)</td>
</tr>
<tr>
<td>6-OH-F</td>
<td>249.35 (130.21)</td>
<td>320.38 (142.05)</td>
<td>+28.5</td>
<td>2192.31 (294.77)</td>
</tr>
<tr>
<td>Ratio 6β-OH-F/F</td>
<td>2.32 (0.50)</td>
<td>4.67 (1.25)**</td>
<td>+101.3</td>
<td>13.37 (1.80)</td>
</tr>
<tr>
<td>Ratio 6β-OH-F/(THF+aTHF)</td>
<td>0.07 (0.02)</td>
<td>0.15 (0.05)**</td>
<td>+114.2</td>
<td>0.36 (0.04)</td>
</tr>
</tbody>
</table>
| Ratio 6β-OH-F/(THF+aTHF+THE) | 0.03 (0.01) | 0.07 (0.03)** | +133.3 | 0.18 (0.02) | **p=0.002, *p=0.015, *p=0.02

Although the sum of urinary C21 steroids, Fcomb/BSA and the sum of C19 steroids were not significantly reduced in patients on OXC, the cytochrome P450 3A4 elimination pathway towards 6β-OH-cortisol was significantly increased. These results confirm that OXC increases elimination of glucocorticoids via induction of cytochrome P450 3A4. Neurologists and endocrinologists should be aware that epileptic patients on OXC who also take glucocorticoids may require greater glucocorticoid doses to reach the desired treatment effect.

Illness and Mortality

Five patients, 4 are alive and well, but one (1.1) has cerebral damage related to severe hypoglycaemia and prolonged seizures during childhood. Hyponatraemia without associated hyperkalaemia was noted at presentation in 7 of 8 patients (renin normal in 3, elevated in 2, not measured in 3). All surviving patients required at least one hospital admission with adrenal decompensation, with hyponatraemia. Additionally, disorders included severe eczema (2), asthma (3), congenital heart lesions (3), and renal anomalies (2). FGD is a potentially lethal disease which can present at post mortem (when the diagnosis may be missed) and is associated with morbidity and death despite treatment. FGD should be considered in children presenting with unexplained hypoglycaemia, low sodium levels but normal potassium. We stress the importance of teaching families “sick day” rules for increasing hydrocortisone therapy during illness and providing them with an Adrenal Insufficiency card detailing the emergency treatment required.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at Presentation</th>
<th>Gene/ Mutation</th>
<th>Initial Plasma Nat</th>
<th>Follow up Plasma Nat</th>
<th>Initial presenting features</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 M 5.6 y</td>
<td>MC2R/S741</td>
<td>125</td>
<td>119</td>
<td>Hypoglycaemia, convulsions, hypopigmentation</td>
<td>Epilepsy; cognitive impairment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2 F 3.5 y</td>
<td>NK</td>
<td>128</td>
<td>NA</td>
<td>Hypoglycaemia, fever, acidosis, vomiting, diarrhoea, convulsions, coma</td>
<td>Died 3.5 y; adrenal cortex hypoplastic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.3 M 7 m</td>
<td>MC2R/S741</td>
<td>134</td>
<td>133</td>
<td>Hypoglycaemia, vomiting, diarrhoea, convulsions, coma</td>
<td>Well</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1 M 5w3d</td>
<td>NK</td>
<td>133</td>
<td>122</td>
<td>Jaundice, diaphoresis, dehydration, hyperpigmentation</td>
<td>Died 10 w; adrenals considered normal size at post mortem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.2 M 10 d</td>
<td>NK</td>
<td>129</td>
<td>136</td>
<td>Jaundice, sudden collapse, hyperpigmentation</td>
<td>Died 3.2 y; very small adrenal glands at post mortem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.3 F 2 w</td>
<td>MC2R/S741</td>
<td>138</td>
<td>133</td>
<td>Hypoglycaemia, prolonged jaundice, poor feeding, heart murmur</td>
<td>Well</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 M 3 m</td>
<td>MC2R/S741</td>
<td>129</td>
<td>133</td>
<td>Hypoglycaemia, convulsions, hypopigmented nevi, collapse</td>
<td>Well</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 F 3 w</td>
<td>MRAP/M11</td>
<td>134</td>
<td>132</td>
<td>Hypoglycaemia, jaundice, lethargy, shock, neonatal hepatitis, hyponatraemia</td>
<td>Well</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† NR 135-145 mmol/L.

Age at diagnosis during life ranged from 11 days to 6.6 years (median 80 days). Three patients have died; two were diagnosed posthuminously (1.2, 2.1) and one (2.2) died despite being on glucocorticoid therapy. Of the remaining five patients, 4 are alive and well, but one (1.1) has cerebral damage related to severe hypoglycaemia and prolonged seizures during childhood. Hyponatraemia without associated hyperkalaemia was noted at presentation in 7 of 8 patients (renin normal in 3, elevated in 2, not measured in 3). All surviving patients required at least one hospital admission with adrenal decompensation, with hyponatraemia. Additionally, disorders included severe eczema (2), asthma (3), congenital heart lesions (3), and renal anomalies (2). FGD is a potentially lethal disease which can present at post mortem (when the diagnosis may be missed) and is associated with morbidity and death despite treatment. FGD should be considered in children presenting with unexplained hypoglycaemia, low sodium levels but normal potassium. We stress the importance of teaching families “sick day” rules for increasing hydrocortisone therapy during illness and providing them with an Adrenal Insufficiency card detailing the emergency treatment required.
Intravenous pamidronate was administered to 64 patients [42F:8M] were recruited. A 10 ml blood sample (fasted) was taken for biochemical analysis. Height, weight, bone age, clinical diagnosis of EA. Fifty patients [42F:8M] were followed up for up to 23 years were studied. Twelve patients from 5 families who were followed up for up to 23 years were studied. The diagnosis of EA was confirmed by hormonal and molecular studies in all patients. Most of the boys presented with signs of adrenal insufficiency such as salt wasting and failure to thrive during the neonatal period. Aldosterone deficiency usually preceded cortisol deficiency requiring early mineralocorticoid therapy. Serum cortisol levels performed in the first weeks of life ranged from very low to high levels (< 0.1 to > 64.4 μg/dl). Five boys showed signs of precocious sexual development during infancy and childhood (e.g. enlargement of penis and testes). In 4 patients the initial diagnoses were erroneous. Molecular analysis of the NROB1 gene identified point mutations in 6 patients from 2 families who manifested impaired mental development, contiguous gene deletion was found. This study highlights the protean manifestations of adrenal hypoplasia congenita (AHC) due to different molecular defects and emphasizes the value of genetic testing in boys presenting with salt-wasting or with houston cortisol deficiency. A high index of suspicion is required in order to avoid misdiagnosis and to facilitate appropriate management.

The main objective of this study was to determine the efficacy and safety of pamidronate in improving bone mineralization and reducing fracture incidence in osteogenesis imperfecta (OI).

**Material and Methods:** Intravenous pamidronate was administered to 64 children (from 21 months to 10 years old) with severe OI, in a 1 mg/kg single daily dose for 3 sequential days in 4 months' intervals, over a 24-48 months duration. Clinical status, biochemical characteristics including bone turnover markers, the bone mineral density of the lumbar spine and femoral neck, and radiologic changes were assessed regularly during treatment.

**Results:** The number of fractures decreased from median of 8 (range 4-11) to 0 fractures/year (range 0-4) (P<0.05). After 16 months of treatment, there was significant improvement in bone mineral density (BMD-DXA) z-scores of the lumbar spine from median of -5.90 (range -7.01 to -4.76) to -2.70 (range -4.46 to -1.98) (P<0.001). Serum alkaline phosphatase (ALP) (bone formation marker) decreased from a median of 731.0 U/l (range 438-998 U/l) to 183U/l (range 95-256 U/l) (P<0.001), implying a significant reduction in bone turnover and its resorption and increase in bone mineralization. There was no improvement either in their height growth velocities or in their standard deviation scores. Mobility and ambulation improved in all but 5 children.
A novel loss-of-function mutation, Gln459Arg, of the calcium-sensing receptor gene: Unusual heterogeneity of phenotypes

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Loss-of-function mutation in the calcium-sensing receptor gene (CASR) decreases the sensitivity of the G protein-coupled parathyroid CaSR and leads to PTH-induced hypercalcemia and hypocalcuria. Heterozygous mutations cause familial benign hypocalciuric hypercalcemia (FHH), which in most patients is characterized by moderate, asymptomatic hypercalcemia. In contrast, homozygous mutations lead to severe primary hyperparathyroidism in neonates with severe hypercalcemia and failure to thrive. We report on two related consanguineous families in which a novel CaSR mutation led to both autosomal recessive and dominant FHH. Fourteen subjects of two related families of Arab-Bedouin descent were analyzed. The proband (case 1) was a 5-year-old male with a history of recurrent hospital admissions due to abdominal pain and hypercalcemia (13 mg/dL), which was first discovered at the age of 2 years. Due to increased levels of intact PTH (100 pg/mL, <65 pg/mL), parathyroidectomy was recommended. Further analysis revealed low urinary excretion of calcium (FeCa = 0.001), which suggested a diagnosis of FHH. Sequencing of CaSR revealed a novel missense mutation in exon 4 of both alleles that predicts a Gln459Arg replacement in the fifth extracellular loop of the protein. The mutant protein was normally expressed in HEK293 cells but retained only 30 to 50% of the calcium-dependent activity of wild-type CaSR. Both parents and two sibs were heterozygous for this mutation and were normocalcemic. In family 2, the proband (case 2, first cousin of case 1), as well as the father and two sibs, were heterozygous for the Gln459Arg mutation. Serum 25(OH)D was normal in tested subjects. We hypothesize that Gln459Arg causes only a very modest reduction in CaSR activity, which may explain the mild biochemical findings in the heterozygous family members and the moderate phenotype of the homozygous proband. Our study demonstrates the heterogeneity of the biochemical phenotypes of CaSR mutations, even within a family, and provides evidence for FHH inheritance as an autosomal recessive disorder.
Vitamin D (Vit D) is one of the important elements for bone metabolism. The need for Vit D is increased during periods of rapid growth. In order to determine the frequency of Vit D deficiency and its correlation with different factors, 313 healthy children and adolescents (192 females and 121 males aged 8 — 18 years) with no Vit D supplement after infancy, were enrolled into the study. The serum levels of 25-hydroxyvitamin D3 [25(OH)D3] (RIA), iPTH (IRMA), calcium, phosphorus, phosphatase alkaline, creatinine, sodium, potassium, fasting blood sugar, T4, T3 uptake and TSH were measured. Vit D status was designated according to the serum level of 25(OH)D as: severe deficiency < 12.5 nmol/L; deficiency, ≥12.5 and <25; insufficiency, ≥25 and <50; normal ≥50 and <250. Frequency of different states of Vit D levels is illustrated in the table.

<table>
<thead>
<tr>
<th>Vit D state</th>
<th>Female %</th>
<th>Male %</th>
<th>total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe deficient</td>
<td>37.5</td>
<td>5.0</td>
<td>24.9</td>
</tr>
<tr>
<td>Deficient</td>
<td>29.2</td>
<td>24.0</td>
<td>27.2</td>
</tr>
<tr>
<td>Insufficient</td>
<td>17.7</td>
<td>38.8</td>
<td>25.9</td>
</tr>
<tr>
<td>Normal</td>
<td>15.6</td>
<td>32.2</td>
<td>22.0</td>
</tr>
</tbody>
</table>

The concentration of 25(OH)D in males (mean±SD, mmol/L) (65.8±68.1) was significantly more than females (31.1±44.3) (P<0.0001), and it significantly higher in pubertal than subpubertal subjects 77.2±77.9 and 37.6±49.4 respectively (P<0.0001). Vit D had significant negative correlation with BMI and height SDS (P=0.002 and P=0.006 respectively), but did not have significant seasonal variation. The subjects with low 25(OH)D did not have any sign of rickets in wrist and hand radiograph. Conclusion: Subclinical Vit D deficiency was more prevalent in females and during puberty. More obese and taller children need more Vit D. Normal values of Vit D should be reevaluated according to the level that causing PTH rising.

Albight twins with parental PSEUDOHYPOPARATHYROIDEA" was the first reported example of hormonal resistance in humans with epigenetic imprinting process of GNAS1 gene. Monozygotic twins with mutation of GNAS1 have not been reported. Twin boys were delivered full term uneventfully. Neonatal screening reported slightly elevated TSH level in twin K (2 muI/l) leading to the diagnosis of congenital hypothyroidism in both twins with normally localized thyroid. Resistance to TSH was demonstrated. At the age of 6 months both exhibited differential methylation status at each of the four GNAS DMRs through nucleotide sequence analysis of bisulfite-modified genomic DNA revealed that two of the patients had broad methylation defects, which is consistent with observations in sporadic PHP-Ib. The two patients from the consanguineous family showed slighty elevated TSH level in twin K (2 muI/l) leading to the diagnosis of congenital hypothyroidism in both twins with normally localized thyroid. Resistance to TSH was demonstrated. At the age of 6 months both exhibited cutaneous lesions, a large plaque in the popliteal area for K, multiple papules on the trunk and on the scalp for F. Microscopic examination demonstrated subcutaneous calcinosis with an intradermal oesoma. Biological examination demonstrated, hypercalcaemia (K: 2.73 mmol/l; F: 2.64 ; N: 2.2 -2.6), hyperphosphorinaemia (K: 1.9 mmol/l ; F: 2.1 ; N: 1.1-1.6), and normal level of PTH (K : 35 pg/ml; F : 59 ; N: 7-53) in both twins. The association was suggestive of pseudo-hypoparathyroidism. Both children were slightly overweight and have lunar facies, and relative 4th and 5th brachydactylas. The PTH increased progressively with age (26 m, K : 197.7 pg/ml ; F : 402.9 ; N: 9-78). The Gs activity was decreased on erythrocytes (58%, N: 85-115%; Dr Basayau, Rouen, France). Height and weight progression of the twins are identical. The dermatological expression is still more severe in twin K. GNAS1 gene was sequenced and a c.ins 345 T mutation of the 5th exon was found. This is a “de novo” mutation located on the maternal allele. It is important to report the first clinical variation of expression in monozygotic twins. The clinical expression may be regarded as quite similar for thyroid, growth, weight and short bone growth. Dermatological expression is similar for age at onset of expression. The variation of cutaneous expression in term of localization and severity is poorly understood and is likely the reflect of heterogeneous imprinting of GNAS1 in skin. The epigenetic factors acting on skin imprinting are not known.

Pseudohypoparathyroidism (PHP) is characterized by hypocalcemia, hyperphosphatemia, and elevated PTH levels due to end-organ resistance to this hormone. Patients with PHP type Ib (PHP-Ib) typically exhibit isolated renal resistance to PTH and lack features of Albright’s ostesodystrophy (AHO) and show epigenetic abnormalities of GNAS. Microdeletions at the NESP55 differentially methylated region (DMR) of this gene and the closely linked STX16 (encoding syntaxin-16) are detected in patients with autosomal dominant PHP-Ib (AD-PHP-Ib). We now investigated Turkish PHP-Ib patients with respect to GNAS imprinting and searched for the previously described microdeletions. Seven patients (4, F, 3 M) with PHP-Ib from six families were included in the study (Table). Clinical findings at presentation included symptoms related to hypocalcemia, i.e. seizures, tetany or spasms in five patients and routine laboratory examination without any hypocalcemic symptoms was observed in two patients. With the exception of two affected siblings from a consanguineous kindred, all patients were apparently sporadic cases. Differential methylation status at each of the four GNAS DMRs through nucleotide sequence analysis of bisulfite-modified genomic DNA revealed that two of the patients had broad methylation defects, which is consistent with observations in sporadic PHP-Ib. The two patients from the consanguineous family showed an isolated A/B methylation defect and both turned out to be carriers of the 3 kb STX16 microdeletion, which they had inherited from their healthy mother, who showed a normal GNAS methylation pattern. We did not detect any methylation defect at this locus in the remaining three patients. About half of the investigated patients thus showed no epigenetic GNAS abnormalities indicating other genetic or epigenetic defects are the cause of PTH-resistance in these patients.

**P1-d1-167 Bone 1**

**Subclinical vitamin D deficiency in healthy children and adolescents**

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**P1-d1-169 Bone 1**

**Genetic testing of Turkish patients with pseudohypoparathyroidism type Ib**

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**Poster Presentations**
Incidence of vitamin D deficiency rickets in eastern part of Turkey

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Turkey, especially its eastern part, has been accepted as endemic for vitamin D deficiency rickets (VDR). In a study performed by our team in the region at the beginning 2000’s, the incidence of VDR was 6.09% in children aged between 0-3 years. In 2005, Ministry of Health initiated free vitamin D supplementation campaign in nationwide for every infant to eradicate the VDR. In this study, we aimed to investigate the incidence of VDR in children aged between 0-3 years. Between March 2007 and February 2008, 49133 children aged 0-3 years who were brought to different pediatric outpatient clinics in Erzurum were evaluated for VDR. The diagnosis of VDR was made by radiological and biochemical findings in the cases who were initially suspected of having clinically VDR. During one year period, 39(0.079%) of the 49133 were diagnosed as having VDR. Of these, 29 (74.4 %) were boys and 10 (25.6 %) were girls. The majority (69%) of the cases with rickets was diagnosed in spring, 25% of cases (10/39) was under 6 months of age. While 23 (59%) of the cases were breastfed, the others were fed with cow’s milk and/or additional food. None of the cases with rickets was taking vitamin D supplementation. 53% of the mothers were illiterate. 89% of families of the cases had lower income than minimum rate. 89% of cases had veiled mothers. The most frequent physical findings were rachitic rosary, enlargement of the wrists and ankles. Laboratory findings of the cases were compatible with VDR; serum Ca 7.5±1.9 mg/dL, PO4 4.4±1.3 mg/dL, ALP 134±823, 25OH D3 5.8±2.9 ng/mL, iPTH 240±106 pg/mL. We concluded that although VDR has been a continuing childhood health problem, nationwide free vitamin D supplementation campaign initiated by the Government appear to be effective on the way of completely eliminating of VDR.

Study of osteogenesis in achondroplasia: From laboratory to clinical evidence

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Achondroplasia is the most frequent osteodysplasia, characterized by a disorder of chondroblastic proliferation and maturation in cartilaginous growth plate of long bones. The achondroplastic osteoblasts also presented an impaired osteogenesis. They incorporate a large amounts of calcium already in the early stages of the process and they express a low level of alkaline phosphatase with a consequent inadequate mineralization. We have clinically verified if achondroplastic patients submitted to lengthening of the lower limbs (ACH) present some ossification difference in respect of a control group of patients submitted to lengthening for congenital limb-length discrepancy (CG). We analyzed an index of bone consolidation, internationally used, expressed by the ratio between the number of days between the application and removal of fixators and centimeters obtained (healing index, HI). We tested and compared the ossification times in achondroplastic patients (ACH) and in (CG). We considered 26 ACH (11 males and 15 females) and 25 CG (12 males and 13 females) underwent at surgery after 1994 and 2007. The lengthening skeletal segments were respectively 26 femurs and 10 tibiae in ACH and 18 femurs and 16 tibiae in CG. To assess the increase in length, measurements were performed on X-rays of the lower limbs of individual patients. The mean HI, estimated for the 26 femurs of ACH, is 37.3 ± 7.9 days for cm (range 27.1-65.5), while for 14 femurs of CG is 56.9 ± 17.7 days for cm (range 36.5-91.6). The mean HI, estimated for the 10 tibiae of ACH is 40.4 ± 7.8 days for cm (range 30.1-52.3), while in 13 tibiae of CG patients is 69.7 ± 14.6 days for cm (range 46.4-95.7). The lowest HI found in ACH compared to CG is essentially a result of altered osteogenesis as demonstrated in cell cultures.

Contribution of vitamin D supplementation to the vitamin D status of infants in the age of fortified milk

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In line with European guidelines, infant formulas in France have been vitamin D fortified with a vitamin D3 content of approximately 400 IU/L. New guidelines for the prevention of vitamin D deficiency and rickets have subsequently been published and the vitamin D supplement for formula-fed infants was reduced to 800 to 400 IU per day. To contribute to the evaluation of the need for vitamin D supplementation, a study was conducted to assess vitamin D3 intake (cholecalciferol) provided by sunlight exposure and fortified milk, and intake provided by supplementation prescribed in form D2 ergocalciferol. This study was conducted in a population of Caucasian infants. The vitamin D2 intake, the type and quantity of milk received, time spent outdoors and clothing modalities were recorded. Vitamin D status was assessed on serum (25-OH)D levels. The 25(OH)D3 level was low (< 10 ng/ml) for 5/36 infants, all in summer for 26 cases. The contribution of 25(OH)D2 vitamin D supplementation (26 boys and 19 girls) were included, during winter for 19 cases and during summer for 26 cases. The contribution of 25(OH)D2 vitamin D supplementation (mean: 18 ng/ml; range: 2-47) was approximately equal to vitamin D intake from sun exposure and 25(OH)D3 fortified milk (mean: 21 ng/ml; range: 5.5-32). Three infants had a low 25(OH)D2 level and one had a level of 2 ng/ml. The 25(OH)D3 level was low (< 10 ng/ml) for 5/36 infants, all included during summer. The most original result of this study is the equivalent contribution to the infant’s vitamin D status of D3-intake by fortified milk and sun exposure and D2-intake by the prescribed vitamin D supplement. The vitamin D status appeared to be satisfactory under these conditions, with extreme values within the locally defined normal range.

The muscle-bone unit in obese children

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Background and Aims: A close relationship exists between muscle and bone strength, termed the ‘muscle-bone unit’. It has been demonstrated in tennis players, healthy children, SGA and GHD children. Published data on the muscle-bone unit in obese children are yet unavailable. An increase in fat mass causes an increase in muscle mass in children. On the other hand sedentary behaviour and physical inactivity lead to an increase in fat mass and decrease in muscle mass. Thus we hypothesized that a loss of bone strength could result. We aimed at investigating the relationship between muscle and bone strength in children with obesity.

Methods: We investigated 47 (19 female) obese children (mean values: age [yr]=11.7; height [SDS]=0.91; BMI [SDS]=4.6) in a structured follow-up scheme which included pQCT (XCT 2000) measurements of the forearm and DEXA ( Lunar DXP Pro) measurements of total body composition.

Results: We found significant associations of muscle mass (MM) (DEXA) [SDS]=0.26; muscle area (MA) (pQCT) [SDS]=0.3; bone mineral content (BMC) (DEXA) [SDS]=1.6; bone area (BA) (pQCT) [SDS]=2.67; cortical density (CD) (pQCT) [SDS]=0.35; cortical area (CA) (pQCT) [SDS]=0.33; and strength strain index (SSI) (pQCT) [SDS]=0.8. We found a close correlation between (a) MM and BMC, R²=0.8329; (b) MA (pQCT) and MM (DEXA), R²=0.9457; (c) MA and CA, R²=0.6431; and (d) MA and SSI, R²=0.6678 (all p<0.001).

Conclusions: Muscle mass and BMC are high in obese children. The close relationship known between muscle and bone strength is also observable in
obese children. The lack of physical activity, which plays an important role in the pathogenesis of obesity, has, however, no influence on bone strength in this group.

P1-d1-174 Bone 1
Spontaneous corticosteroid-induced osteoclastogenesis in children with 21-hydroxilase deficiency
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21-OHD children need chronic glucocorticoid (cGC) therapy since diagnosis (at birth or later) to replace congenital deficit of cortisol synthesis. cGC therapy is the most frequent and severe form of drug-induced osteoporosis, but its pathogenesis is not completely understood. GCs cause a rapid decline in bone mass due to a striking remodelling imbalance of osteoclasts (OCs) and osteoblasts (OBs) number, by exerting an anti-inflammatory effect on OCs in vivo. Furthermore, GCs enhance expression of RANKL, a cytokine involved in OCs formation and activation, and inhibit in vitro the production of osteoprotegerin (OPG), the decoy receptor for RANKL, by stromal cells and OBs. We investigated the osteoclastogenetic potential of peripheral blood mononuclear cells (PBMCs) obtained from 18 children (9 F), aged 3-16 years, affected by 21-OHD (molecular diagnosis) on long-term GC therapy and from 25 children (C) who never received GCs and without risk factors for osteoporosis. PBMCs from pts (9 with classical forms) and C were cultured for 15-17 days in presence/absence of recombinant human M-CSF (25 ng/ml) and RANKL (30 ng/ml). At the end of the culture period, mature OCs were identified as tartrate-resistant acid phosphatases positive (TRAP+) multinucleated cells, containing three or more nuclei. Freshly isolated PBMCs from pts and C were stained for CD11b, CD14, and CD51/CD61 and analyzed by flow cytometry. Spontaneous osteoclastogenesis, without adding M-CSF and RANKL, and significantly higher OCs resorbing activity occurred in 21-OHD pts. Conversely, MCSF and RANKL were essential to trigger and sustain osteoclastogenesis of PBMCs from C. Furthermore, only in 21-OHD pts we identified a percentage of CD51/CD61 positive cells, which are OC or OB precursors (OCPs) strongly committed. In conclusion, our data showed that cGC therapy increases the number of circulating OCPs, enhancing spontaneous OCs formation in vitro and therefore suggests a new mechanism of cGC-induced osteoporosis.

P1-d1-175 Pancreas 1
MODY type 2 in Greig Cephalopolysyndactyly syndrome (GCPS) as part of a contiguous gene deletion syndrome
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GCPS (OMIM 175000) is a rare syndrome affecting limb formation and craniofacial development, with an autosomal mode of inheritance. The syndrome originates from a deletion in the 7p13 region, including the GLI3 gene. Atypical cases with additional symptoms including mental retardation (MR) are related to the loss of genes closely linked to the GLI3 locus. We describe a 7-year old girl with GCPS based on typical signs of macrocephaly, a broad flat face, contractures of the feet, with MR. Following febrile convulsion-associated hyperglycemia, a 7-year old girl with GCPS was identified. Genetic analysis for the presentation of MODY type 2. Although GCK gene was found to be deleted in five patients with atypical GCPS in a previous study, only one of them presented with borderline high blood glucose levels. We describe the first case of MODY type 2 in a patient with GCPS due to contiguous gene deletion syndrome.

P1-d1-176 Pancreas 1
Using an insulin secretagogue to target the insulin secretory defect in Turner's syndrome
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Women with Turner syndrome (TS) have been shown to have an 11.5% relative risk of type 1 diabetes mellitus (DM) and 4.3% relative risk of type 2 DM. However it has been recently highlighted that the defect of glucose homeostasis has been observed even in younger, non-obese women with TS and that the pattern of insulin secretion seems more likely to be due to beta-cell dysfunction or insufficiency, reminiscent of maturity onset diabetes of the young. Consequently traditional categorisation of DM may not be completely appropriate in TS. We hypothesised that Repaglinide, an insulin secretagogue targeting the first phase of insulin response (FPIR), should be a treatment suitable for diabetic TS patients and therefore tested its effect. We measured FPIR to intravenous glucose overload (IVGTT) in eight diabetic TS patients. All subjects exhibited reduced FPIR compared to non-diabetic Turners women in a reference population. Repaglinide was administered for 11-14 weeks in association with other anti-diabetic drugs and an IVGTT was repeated after optimising drug dosage. As showed in Table 1, a significant improvement in glucose homeostasis was observed with a rise in FPIR by over 50% after treatment (P<0.028) and significant reduction in Glucose AUC (AUCC P< 0.046).

<table>
<thead>
<tr>
<th>Date shown as Mean (SD)</th>
<th>Pre-Treatment</th>
<th>Post-Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>33.1 (8.4)</td>
<td>29.5 (8.1)</td>
</tr>
<tr>
<td>Fasting BM (mmol/L)</td>
<td>10.6 (5.2)</td>
<td>7.3 (2.5)</td>
</tr>
<tr>
<td>Fasting Insulin (mUI/L)</td>
<td>11 (6.3)</td>
<td>14.6 (7.9)</td>
</tr>
<tr>
<td>AUC glucose</td>
<td>156.8 (42)*</td>
<td>119.8 (35)*</td>
</tr>
<tr>
<td>FPIR</td>
<td>22.3 (12.3)*</td>
<td>57.4 (31.8)*</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>8.3 (1.9)</td>
<td>7.5 (1.3)</td>
</tr>
</tbody>
</table>

* P < 0.05

The drug was generally well tolerated, diarrhoea has been observed as side effect, only in two cases. We have shown that the early beta cell insufficiency in TS is responsive to medical therapy. Our experience has emphasized the theory that TS patients exhibit a particular form of diabetes which requires specific treatments of which Repaglinide has been shown to be effective.

P1-d1-177 Pancreas 1
Resistin, adiponectin, leptin levels in adolescents with cystic fibrosis
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Patients with Cystic Fibrosis (CF) have significant improved survival and quality of life, due to progress in diagnosis and follow-up. However, especially in adolescents, several complications related to endocrine and nutritional assess are described, since CF is characterized by weight loss and chronic low-grade inflammation.

Leptin plays an important role in energy balance and may be affected by hormonal and metabolic derangement associated with chronic disease. Serum concentrations of leptin are decreased in children with CF and associated with...
clinical conditions and body composition. Adiponectin is higher in adult CF patients but no data are reported in CF children. Furthermore no data are available in literature about Resistin levels in CF. 24 patients (14 M; 10 F, age 13.3±2.6 years) affected by mild CF disease were selected between the patients in peripubertal age following these criteria: no CFDR, no pathological OGGT; no acute disease; no lung and/or bowel transplantation; good nutritional status. These patients were compared with health controls matched for sex and age. BMI: CF 17.92±3.29 (M/F: 17.26±2.97; 18.84±3.65), Controls 22.42±1.15 HOMA IR: CF 1.2±0.76, Controls 1.84±0.78 (HOMA B%:CF 1.49±1.14, Controls 2.3±1.25 (Leptin (ng/ml): CF 8.71 ± 9.77 (M/F:3.67±5.49; 10.44±11.88), Controls: 3.97±4.52 (M/F: 3.97±4.52; 6.16±6.73) Adiponectin (µg/ml): CF 11 ± 3.84 (M/F:11.81±3.99; 8.56±4.17), Controls 8.63±1.67 (M/F:7.8±1.52; 9.88±1.04) Resistin (ng/ml): CF 2.05±0.87 (M/F:2.07±0.89; 2.11±1), Controls 0.93±0.42 (M/F:0.93±0.29; 0.92±0.62) HOMA IR and HOMA B% are in the normal range but lower than in controls, expression of a lower insulin-resistance with a lower insulin secretion. Leptin levels and BMI had a statistically significant correlation (r 0.72, p 0.000069). Adiponectin levels were mildly lower in CF than in controls, possible expression of low malnutrition typical of CF. Furthermore these are the first data in pediatric CF. Resistin levels more elevated in CF than in controls may be explained by chronic inflammatory pattern typical of these patients.

**P1-d1-179** Pancreas 1

**Protein sensitive hyperinsulinaemic hypoglycaemia due to a novel mutation in the short-chain L-3-hydroxyacyl-CoA dehydrogenase (HADH) gene with normal acylcarnitines and urine organic acids**

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Short-chain L-3-Hydroxyacyl-CoA dehydrogenase (SCHAD), encoded by HADH (formally known as HADHSC) catalyses the penultimate reaction in the β-oxidation of fatty acids. So far, three patients with mutations in *HADH* and hyperinsulinaemic hypoglycaemia (HH) have been reported. The acylcarnitine profile in these reported patients showed increased hydroxybutyrylcarnitine and raised urinary 3-hydroxyglutarate. We report a novel mutation in *HADH* associated with protein sensitive HH. The index case was born at term with a birth weight of 3500 grams to consanguineous parents. There was no neonatal hypoglycaemia. At 4 months of age she had a tonic-clonic convolution due to hypoglycaemia. Her hypoglycaemia responded to diazoxide but episodes of hypoglycaemia recur even on diazoxide but consuming high protein fed. Investigations confirmed HH (blood glucose level of 1.8mmol/l with simultaneous serum insulin level of 58mU/l) associated with undetectable serum ketone bodies. The acylcarnitines and urine organic acids were normal on each occasion tested. This patient was severely protein sensitive (accounting for the unexplained episodes of hypoglycaemia) but not leucine sensitive with a normal serum ammonia level. As the parents were consanguineous and the proband was negative for mutations in the *ABCC8*, *KCNJ11* and GLUT 1 genes the *HADH* gene was sequenced. A novel variant M188V (562A>G) in exon 5 of the gene was identified. The parents and two unaffected siblings were all heterozygous for the variant. The variant is novel and the residue is conserved across species. We conclude that patients with HH due to mutations in the *HADH* gene may have normal acylcarnitines and urine organic acids. Mutations in the *HADH* gene are associated with increased protein sensitivity. Understanding the molecular mechanisms of how mutations in the *HADH* gene cause HH and protein sensitivity will provide new insights into pancreatic beta-cell physiology.
Heterozygous mutations of the TCF1 gene, encoding the Hepatocyte Nuclear Factor 1a (HNF1a), have been found in patients with Maturity Onset Diabetes of the Young 3 (MODY3), hepatocellular adenomas (HCA)/liver adenomatosis (LA) and gynecological tumors. We report a young woman with HNF1a mutations who presented with co-existing MODY3, LA and uterine leiomyomas. This was a 26-year-old woman admitted to the hospital after a road accident. Computer tomography revealed more than 10 hepatic lesions, as well as a uterine tumour. She was on metformin for non-autoimmune diabetes mellitus since she was 14 years old. Her 49-year-old mother also had DM from the age of 14 years, and a history of removal of uterine leiomyomata. Biopsy of a liver lesion of the patient revealed HCA and confirmed the diagnosis of LA. The uterine tumour was also removed and histology showed leiomyoma. Genomic DNA was isolated from peripheral blood lymphocytes of the patient and her mother, from a liver tumor from the patient and from the uterine tumors of both the patient and her mother. These DNA samples were screened for mutations in the 10 exons of TCF1 gene and the exon-intron boundaries, by direct sequencing of PCR products. Sequencing of the blood and tissue DNA samples revealed a deletion of three nucleotides in exon 3 (c.682_684delGAG) resulting in an in-frame deletion of amino acid 228 of the TCF1 gene (p.E228del) in the region of the DNA-binding domain of the HNF1a protein, which is highly conserved among various species. No other mutation was detected in any of the samples examined. Our findings indicate that TCF1 germ line mutations can lead to MODY3, HCA and uterine tumors in the same patient, suggesting a common role of this transcription factor in all these disorders. They also indicate that systematic screening for liver adenomatosis and uterine tumors in MODY3 patients and their family members is suggested.
Type 1 diabetes (T1D) is a multifactorial autoimmune disease with both environmental and genetic component contributing to the disease outcome. Several studies have suggested that reactive oxygen species (ROS) are involved not only in diabetes development but also in development of severe microangiopathic complications like diabetic retinopathy (DR) and diabetic nephropathy (DN). The aim of our study was to investigate well described polymorphic markers in antioxidant enzymes superoxide dismutase (MnSOD) and glutathione-S-transferases M1 and T1 (GSTT1 and GSTM1) in association with microangiopathic complications. One hundred and twenty five patients with T1D were included in the study. They were divided in two groups: patients with chronic complications of diabetes (case subjects) and without complications (control subjects), matched by sex, age and duration of diabetes. Genotyping was conducted using Real-time PCR for Ala(-9)Val polymorphism in MnSOD and multiplex PCR for determination of GSTM1 and GSTT1 null genotypes. Pearson Chi-Square test revealed significant differences in allele and genotype frequencies for the two groups. We found a positive association of MnSOD genotype Val/Val (OR = 2.52, 95% CI = 1.02-6.24, p = 0.042) and GSTM1 null genotype (OR = 2.5, 95% CI = 1.05-6.33, p = 0.035) with diabetic retinopathy but not with diabetic nephropathy. Polymorphisms in antioxidant enzymes could be useful genetic markers for identification of patients with T1D at an increased risk for developing diabetic retinopathy.

Conclusions: Regardless of sex, diabetes duration, metabolic control and insulin dosage, DM1 results in elongation of the corrected QT value in diabetic for adolescents, what is a well-known risk factor associated with sudden death. The elongation of the isovolumetric relaxation time observed in DM1 adolescents may reflect early stage of left ventricular diastolic dysfunction.

Cardiovascular disease and the development of coronary artery disease play a pivotal role in increasing mortality in patients with diabetes. The aim of the present study was to determine the presence of subclinical atherosclerosis (measured as carotid intima-media thickness [cIMT]) and to study possible associated risk factors in children and adolescents with type 1 diabetes. For this purpose, 114 diabetic subjects, 20 males and 20 females, were recruited. Their age ranged from 11-30 yr, while duration of diabetes ranged from 3-25 yr. Twenty normal healthy subjects, matched for age, sex and body mass index (BMI) with patients were included as controls. Carotid intima media thickness (cIMT) was measured using ultrasound in patients and controls at 3 main segments on each side: the distal 1.0 cm of the common carotid proximal to the bifurcation, the bifurcation itself and the proximal 1.0 cm of the internal carotid artery. The wall maximum thickness was calculated at each site. The mean aggregate cIMT (mean of the 12 sites) was higher in diabetics than controls (0.60 ± 8.59 mm vs 0.41 ± 2.91 mm, p<0.001). Moreover, it was higher in patients with positive family history of type 2 diabetes than in those with negative family history (mean 0.75 ± 2.3 mm vs 0.59 ± 3.5 mm, p=0.01). cIMT was found to positively correlate with: age in both diabetics and controls (r=0.53; p=0.003, r=0.46, p=0.007 respectively), body mass index (BMI) in both diabetics and controls (r=0.33, p<0.04, r=0.36, p=0.04 respectively. As cardiovascular morbidity is high in diabetes, non-invasive methods for monitoring vascular changes as cIMT might be useful in clinical practice for early diagnosis of subclinical atherosclerosis which can allow for strategies designed to reduce the cardiovascular event rate in those patients.

chronic inflammation, as in CF, may be associated with insulin-resistance. We evaluated insulin-resistance and a possible role of adiponectin, and resistin in the regulation of glucose metabolism in CF. Fifty-seven patients, subdivided in 3 groups: prepubertal (aged 6-11.7yr, n: 6), pubertal (aged 12-17.4yr, n: 19) and adult (aged 19-39.3yr n:32), and healthy subjects as adult controls (aged: 21-31,1 yr, N: 28) were enrolled. C-peptide, glycated Hb, C reactive protein (CRP), resistin, adiponectin were assayed in serum. OGTT was done in prepubertal patients and both OGTT and IVGTT in the elder patients. OGTT was classified according to the CF International criteria, the First Phase Insulin Response (FPIR) and HOMA index according to ISPED criteria. Acute insulin response (AIR) was assessed also as well as FEVI, Schwan- man score and genotype. Data are mean±SEM. HOMA was altered in one patient, glucose/insulin ratio in 13 patients. Based on OGTT, 26 patients were normal, 21 had impaired glucose tolerance (IGT), 8 had CFRD without fasting hyperglycaemia and one had CFRD with fasting hyperglycaemia. Based on FPIR, 15 patients were normal, and 42 had insulin deficiency. Crosstabulation analysis according to age showed the highest prevalence of normal OGTTs in puberty and IGT increased with age. FPIR decreased from puberty to adulthood. Considering genotype, heterozygosis for the ∆F 508 mutation was protective towards developing insulin deficiency with respect to homozygosis, confirmed by a significant difference in FPIR and AIR. Adiponectin was lower in the homozygotes. In adult patients adiponecin was lower compared with controls (10757,8±807 vs 15445,7±1691 mg/ml, p<0.05), whereas resistin was increased (4,39±0,4 vs 3,04±0,3 ng/ml). In CF, resistin correlated with CRP (R=−0,5; p: 0.06) and FEVI (R =−0,47; p: 0.00). In conclusion, CF patients develop insulin deficiency, can present mild insulin...
Increased weight gain has been reported in type 1 diabetes prior to disease onset (accelerator hypothesis) and as a side-effect of intensified insulin therapy. Pediatric studies are complicated by the age- and gender-dependence of the BMI and by the time-trend of increasing obesity prevalence in recent years in many countries. By March 2008, the DPV database includes 116 046 pediatric patients with type 1 diabetes, age <20 years, on stable insulin regimen, accumulated between 1995 and 2007 in 201 diabetes centers in Germany and Austria. Standardized patient records are documented locally at each participating center. Data are pseudonymized and transferred for central analysis to Austria. Standardized patient records are documented locally at each participating center. Data are pseudonymized and transferred for central analysis to Austria.

From the age of 6 month on no hypoglycemic crises occurred under formula supplemented with simple and complex carbohydrates. This supplementation was necessary. Apart from the known symptoms of neonatal lupus our child underwent a 3-hour hyperinsulinemic-euglycemic (40 mU/m2/min) clamp, a minuminsulin modified minimal model FSIVGTT, and a 3-hour OGTT. Correlations were established using Spearman’s rank correlations. Two clamp derived formulas were considered, that were highly correlated (r=0.85). The index derived from the FSIVGTT was highly correlated with both clamp measures (r=0.69, 0.74). We considered 9 different indices derived from the OGTT. Of these, 4 indices showed high correlation with the clamp results: ISI Matsuda (r=0.63, 0.69), ISI Belfiore (r=0.62, 0.64), SI OGTT (r=0.62, 0.62) and Log sum insulin, the most closely correlated index (r=−0.67−0.80). Fasting indices of IS had slightly lower correlations with clamp results: HOMA-IR (r=−0.55, -0.56), QUICKI (r=0.55, 0.57), and INSO (r=−0.59, -0.63). Measurement of IS using the clamp, the FSIVGTT, and OGTT derived indices were highly correlated in this group of children. In particular, the Log sum insulin index was the most strongly correlated with clamp results, and appears to provide more valid information than either HOMA-IR or QUICKI. This suggests that OGTT derived indices provide valid, clinically feasible methods of estimating IS in youth.

Given the rise in childhood obesity and its association with insulin resistance, valid methods to measure insulin sensitivity (IS) in youth are needed. Surrogate estimates from the oral glucose tolerance test (OGTT) have not been well studied in children and adolescents. The objective of this study was to examine the correlation between four different methods of measuring IS in a group of children: the hyperinsulinemic-euglycemic clamp, the frequently sampled intravenous glucose tolerance test (FSIVGTT), various indices derived from the OGTT, as well as fasting indices (HOMA-IR, QUICKI, and fasting insulin [INSO]). Twenty healthy children (mean (SD) age: 9(2) years) were studied: 9 boys and 11 girls. Their mean (SD) BMI z-score was 1.5 (0.8). No partici- pant had impaired fasting glucose/glucose tolerance or diabetes. Each child underwent a 3-hour hyperinsulinemic-euglycemic (40 mU/m2/min) clamp, an insulin modified minimal model FSIVGTT, and a 3-hour OGTT. Correlations were established using Spearman’s rank correlations. Two clamp derived formulas were considered, that were highly correlated (r=0.85). The index derived from the FSIVGTT was highly correlated with both clamp measures (r=0.69, 0.74). We considered 9 different indices derived from the OGTT. Of these, 4 indices showed high correlation with the clamp results: ISI Matsuda (r=0.63, 0.69), ISI Belfiore (r=0.62, 0.64), SI OGTT (r=0.62, 0.62) and Log sum insulin, the most closely correlated index (r=−0.67−0.80). Fasting indices of IS had slightly lower correlations with clamp results: HOMA-IR (r=−0.55, -0.56), QUICKI (r=0.55, 0.57), and INSO (r=−0.59, -0.63). Measurement of IS using the clamp, the FSIVGTT, and OGTT derived indices were highly correlated in this group of children. In particular, the Log sum insulin index was the most strongly correlated with clamp results, and appears to provide more valid information than either HOMA-IR or QUICKI. This suggests that OGTT derived indices provide valid, clinically feasible methods of estimating IS in youth.
A novel mutation of the thyroid transcription factor-1 gene causing congenital hypothyroidism with resistance to TSH
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Resistance to TSH (RTSH [MIM 275200]) is a heterogeneous condition defined by variable degree of insensitivity to biologically active TSH molecule. The etiology of RTSH has been poorly clarified, although some cases were reported to be associated with gene mutations in TSHR, PAX8, or GNAS1. In this report, we describe a patient with RTSH who had a novel mutation of TITF1. The patient was Japanese boy with congenital hypothyroidism detected by newborn screening. He suffered from recurrent lower respiratory infection in his infancy and subsequently developed choreoathetosis. To our knowledge, this is the first documentation of RTSH in a patient having TITF1 mutation. We conclude that TITF1 mutation can cause RTSH.
Levothyroxine administration through nasogastric feeding tubes

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In neonates, levothyroxine may be administered through a nasogastric feeding tube. Although in several EU countries registered L-thyroxine solutions are commercially available, this is not the case in the Netherlands. Current practice in the Netherlands is therefore to make a suspension of ground tablets in water and to administer it on a metal spoon, because there are anecdotal reports that levothyroxine may adsorb to plastics. This study evaluates the adsorption of levothyroxine to nasogastric feeding tubes. Two 15 mcg/mL suspensions were prepared in the pharmacy laboratory (levothyroxine tablets suspended in water and in 4.2.% bicarbonate, respectively). These were compared to a 15 mcg/mL reference solution of pharmaceutical grade levothyroxine dissolved in water pH=10. Levothyroxine concentrations of the samples were quantified using HPLC both directly after preparation, and after one hour instillation in a gastric feeding tube (VYGON Corp. Nutrisafe 2, PUR, 8 Ch.). All samples were homogenized and diluted with methanol (1:1) before HPLC analysis. The experiment was repeated with a levothyroxine solution commercially available in Germany (L-thyroxin Henning 100 mcg/mL, Sa-nofi-Aventis). The solubility of levothyroxine tablets suspended in water was very poor, with a levothyroxine recovery of 1,8 mcg/mL (12%) compared to the reference solution. In alkal (pH=8) solubility was increased. Instillation of the two tablet suspensions in nasogastric feeding tubes led to a further loss of the drug to 1,4 mcg/mL (9%). This phenomenon was not observed in the commercial solution, with 98% drug recovery after instillation in a gastric feeding tube. The current practice of levothyroxine administration to young children in the Netherlands leads to a large variability in the amount of drug actually delivered to the patient. This is most likely caused by poor solubility of the drug in water or by negative influences of the tablet matrix, and not by adsorption to plastics. Access to the commercial preparation is desirable.

Polymorphism A/G at position 49 of exon 1 of the CTLA-4 gene and antithyroid antibodies in children with Hashimoto's thyroiditis

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The genetic predisposition to antithyroid antibodies production is well known. The CTLA-4 gene is considered as one of the strongest genetic factors determining this predisposition. The aim of the study was to evaluate the association between the polymorphism A/G at position 49 of exon 1 of the CTLA-4 gene and antithyroid antibodies level in young patients with Hashimoto’s thyroiditis.

Material and methods: One hundred caucasian children were examined: 45 with Hashimoto’s thyroiditis (average age 14,9 years, range: 8,1-17,9), and 55 healthy controls matched for age, sex and ethnic origine. In children in the control group the thyroid dysfunction and antithyroid antibodies presence were excluded, as well as other autoimmune diseases. TSH value, and antibo

P1-d1-199 Thyroid 1

Multiple endocrine neoplasia 2A: Prophylactic intervention

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A good genotypic-phenotypic correlation is observed in type 2 multiple endocrine neoplasm (MEN). Specific mutations of the RET proto-oncogene give rise to syndromic variants of MEN [MEN 2A, MEN 2B and familial medullary thyroid carcinoma (FMTC)], and are in turn correlated to the prognosis, patient age at onset of the tumor, and aggressivity of the lesion. The current recommendations comprise prophylactic thyroidectomy in carriers, at an early age: grade II risk (mutations in codons 618, 620, 630, 634, 891) between 2-5 years of age; and grade III risk (mutation in codon 918) between 1-6 months of age. We present two families with MEN 2A (the index case in both instances being the mother with medullary thyroid carcinoma and mutation in C634R in exon 11 of the RET proto-oncogene), in which carrier studies were made among the relatives (father and offspring). In the first family prophylactic radical thyroidectomy was performed in the first daughter at 28 months of age, revealing a 3-mm microcarcinoma and C cell hyperplasia in part of the thyroid parenchyma. In a second daughter thyroidectomy was performed at 23 months of age, with the identification of hyperplasia. In the second family prophylactic radical thyroidectomy at 18 months of age revealed a 2-mm medullary microcarcinoma in the right thyroid lobe, and C cell hyperplasia in the rest of the thyroid parenchyma. Despite these very young ages, malignancy was already seen to be present The discovery of new mutations and the study of new affected families will improve genotypic-phenotypic correlation, and will allow us to establish more precise interventional protocols Cooperation among different specialists is needed (pediatric endocrinologists, endocrinologists, pediatric surgeons and geneticists) to ensure very close follow-up of the MEN 2A index cases, and early intervention.

P1-d1-198 Thyroid 1

Relationship between sodium iodide symporter and thyroid peroxidase and proapoptotic (TIA1 and TIA1-1) markers detection in thyrocytes from adolescents with thyroid diseases

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The course of Graves‘ disease (GD) is associated with the inflow of lymphocytes to the thyroid gland and dysregulation of the immune system characterized by reaction to thyroid antigens (peroxidase, thyroglobulin, TSH receptors and Na⁺/I⁻ symporter). After activation they shift to the infiltrated thyroid gland, thus leading to the production of cytokines which can stimulate activity of thyrocytes and increase expression on intracellular proapoptotic markers such as TIA1 and TIA-1. The aim of this study was to estimate sodium iodide symporter (NIS) and thyroid peroxidase (TPO) expression in thyrocytes from patients with GD and no-toxic multinodular goitre (NTMG). The investigation was performed on thyroid cells isolated from postoperated thyroid tissues from 12 patients aged 12-18 years old with GD and 12 cases aged 13-18 years old with NTMG. Detection of NIS and TPO was performed by immunohistochemistry using mAb-47 and anti-NIS antibodies in DAB chromogene and marked by Mayer’s hematoxylin. Additionally, TPO identified by Western Blot method with mAb-47. Analysis of apoptotic markers in thyrocytes was performed using antibodies to TIA1 and TIA-1 by immunohistochemistry. The analysis of expression of NIS and TPO in thyroid follicular cells was higher in patients with Graves‘ disease in compared to their detection in patients with NTMG. In addition, degree of thyroid antigen
expression positive correlated with amount of proapoptotic markers (TIAR, $p<0.001$; TIA-1, $p<0.025$). We conclude that elevated expression of NIS and TPO in Graves’ disease is associated with higher stimulation and activation of apoptosis in thyroid follicular cells during autoimmune process.

**P1-d1-199 Thyroid 1**

**Congenital central hypothyroidism due to a TSHβ gene mutation**

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2University Hospital Mainz, Department of Pediatrics, Mainz

Congenital central hypothyroidism (CCH) is a rare disease occurring in 1:2000 to 1:50 000 newborns. Congenital central hypothyroidism is usually missed by newborn screening because of low TSH levels. However, delayed substitution of L-thyroxine may lead to psychomotor delay of the affected newborns. We report on the first child of nonconsanguineous German parents, who developed a newborn infection within the first week of life and was treated with intravenous antibiotics for 5 days. After initial improvement, feeding problems occurred and the child became progressively sleepy. This was first thought to be due to the infection. On day 19 thyroid function tests revealed isolated central hypothyroidism with elevated basal and TRH stimulated proactin levels excluding an inactivating TRH receptor gene mutation. All other hormone axes were normally functioning. Further mutational analysis revealed a frame-shifting 1bp deletion (C105V) in exon 3 of the TSHβ subunit gene. The child was immediately started on a replacement therapy and improved significantly after a few days. Psychomotor development at age 7 months appeared to be normal. We conclude that TSHβ subunit gene mutation is a rare cause of CCH which usually remains undetected by the newborn screening. Therefore, thyroid function tests should be performed early if clinical signs of hypothyroidism are present.

**P1-d1-200 Thyroid 1**

**Long-term levothyroxine therapy in young adults with congenital hypothyroidism: Effects on cardiovascular system**

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Background: Congenital hypothyroidism is a common thyroid disorder in children, and is routinely treated with life-long levothyroxine (LT4) replacement therapy. Several studies concerning overt as well as subclinical hypothyroidism and hyperthyroidism have demonstrated that thyroid hormones may impact on the cardiovascular system, however, there are no data concerning the effects of long-term LT4 administration in patients with congenital hypothyroidism. Objective: The aim of the study has been to evaluate whether long-term LT4 replacement therapy in young adults with CH is associated with cardiovascular abnormalities. Patients and Methods: Thirty young adults with congenital hypothyroidism aged 18.1±0.2 years and 30 age and sex-matched controls underwent cardiac and carotid Doppler ultrasound and symptom-limited cardiopulmonary exercise testing. Hypothyroidism was diagnosed by neonatal screening and LT4 treatment was initiated within the first month of life and carefully adjusted to maintain TSH levels in the normal range and free thyroid hormone levels in the high-normal range. Results: Compared with controls, hypothyroid patients exhibited left ventricular (LV) diastolic dysfunction, impaired exercise capacity, and increased intima-media thickness. At multiple regression analysis, the number of episodes of LT4 treatment was initiated within the first month of life and carefully adjusted to maintain TSH levels in the normal range. Comparing with controls, hypothyroid patients exhibited lower exercise capacity, increased intima-media thickness. Such abnormalities occur despite careful replacement therapy and appear related to unphysiological fluctuations of TSH levels, with attendant episodes of subclinical hyperthyroidism and, more frequently, subclinical hypothyroidism.

**P1-d1-201 Thyroid 1**

**Longitudinal evaluation of patients with a R450H mutation of the TSH receptor gene**

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3Gunma University Graduate School of Medicine, Pediatrics and Developmental Medicine, Gunma

The purpose of this study was to clarify the phenotype of patients with a R450H mutation of the TSH receptor (TSHR) gene; the mutant receptor has previously demonstrated moderately impaired function in vitro and the mutation has been frequently identified in Japanese patients with resistance to TSH. We performed a clinical investigation of five homozygous and three heterozygous Japanese patients. Six of eight patients were referred because of hyperthyrotropinemia on neonatal screening. In the two remaining patients with heterozygous mutation, hyperthyrotropinemia was diagnosed after screening. At the first examination, serum TSH/LT4 levels of the homozygous and heterozygous patients were 12.6-38.5 µU/ml (elevated)/1.1-1.3 ng/dl (normal) and 4.7-7.4 µU/ml (slightly elevated)/1.1-1.7 ng/dl (normal), respectively. Treatment with L-T4 was started in all homozygous patients, and then an increased dose of L-T4 was necessary to maintain a normal TSH. During adolescence, one homozygous patient had high level of TSH (54.8 µU/ml) with low level of FT4 (0.6 ng/dl; normal range, 0.7-1.5) in the absence of L-T4 for 1 month, suggesting that patients with partially compensated TSH resistance might develop uncontrolled resistance to TSH if untreated. Only one homozygous patient was treated with L-T4 from the age of 25 months because of TSH levels higher than 10 µU/ml (11.9 µU/ml). In the two remaining heterozygous patients, the TSH levels were 3.1-7.5 µU/ml after the age of 6 months. For the eight patients, ultrasonography and 123I scintigraphy showed thyroid glands of normal size and location. Clinical assessments including intelligence tests of all affected patients revealed normal growth and development. Early treatment should be recommended for patients with hyperthyrotropinemia and a homozygous R450H mutation, even when thyroid hormone levels are normal. However, further research is needed to decide whether replacement therapy is necessary for heterozygous patients.

**P1-d1-202 Thyroid 1**

**Long-term follow-up of a patient with pituitary resistance to thyroid hormones: Comparison of D-thyroxine and triiodothyroacetic acid treatments**

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Patients with pituitary resistance to thyroid hormones (PRTTH) exhibit features of hypothyroidism due to normal sensitivity to thyroid hormones in the peripheral tissues. Treatment of PRTTH is controversial and different agents including high dose T3, atomelom, somatostatin, steroids, D-Thyroxine (D-T4) and triiodothyroacetic acid (TRIAC) have been tried with various success. Short-duration of these trials and lack of comparison of different agents are the main reason for uncertainty in the treatment of PRTTH. Here, we present a long-term (9 years) clinical and biochemical follow-up of a patient with PRTTH under the treatment of D-T4 initially (for 1.5 years) followed by TRIAC for 6 years (Table 1). An 11.5 year-old girl was evaluated for goitre, palpitations and nervousness. She was born at 8 months of gestation with a birth weight of 1800 grams and grew poorly until 2 years of age, then she had grown better. She had goiter, palpitations, heat intolerance, sleep disorders, nervousness and frequent stools for 3 years. Thyroid function tests were consistent with PRTTH. The mutational analysis revealed a heterozygous missense mutation of the TSHR gene at codon 245 in exon 7 (R245Q) and a nonsense (silent) mutation at codon 245 in the index and the mother who also diagnosed to have PRTTH. The patient was started on D-T4 treatment since she exhibited clinical

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Ala373Ser). Missense mutations have been found (Novel heterozygous Thr725Pro, and other dyshormonogenesis with negative PDT. Average thyroxine dose before SCH. Among subjects with permanent SCH, 19 had thyroid hypoplasia, 6 these children, 18 (37.5%) had transient SCH, and 30 (62.5%) had permanent SCH. Results: All children to whom performed a PDT not to overlook heterozygous cases. A

<table>
<thead>
<tr>
<th>Thyroid volume (ml)</th>
<th>Before treatment</th>
<th>D-T4 treatment (1.5 years)</th>
<th>Triac treatment (6 years)</th>
<th>Off treatment</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4 (µg/dl)</td>
<td>19.7</td>
<td>22.9±1.4</td>
<td>13.1±2.1</td>
<td>19.8</td>
<td>(5.1-13.5)</td>
</tr>
<tr>
<td>s-T4 (ng/dl)</td>
<td>2.6</td>
<td>3.96±1.5</td>
<td>2.28±0.4</td>
<td>4.2</td>
<td>(0.9-2.0)</td>
</tr>
<tr>
<td>T3 (ng/ml)</td>
<td>3.61</td>
<td>3.11±0.5</td>
<td>2.04±0.5</td>
<td>2.21</td>
<td>(0.8-2.0)</td>
</tr>
<tr>
<td>s-T3 (ng/ml)</td>
<td>12.7</td>
<td>14.7±3.4</td>
<td>5.98±0.8</td>
<td>6.98</td>
<td>(2.6-5.1)</td>
</tr>
<tr>
<td>TSH (U/L)</td>
<td>3.09</td>
<td>2.47±0.8</td>
<td>1.41±0.4</td>
<td>1.42</td>
<td>(0.27-6.3)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>116</td>
<td>111±10</td>
<td>102±5</td>
<td>96</td>
<td>(85-91)</td>
</tr>
</tbody>
</table>

* Mean of multiple measurements during treatment periods has been given for all parameters.

P1-d1-204 Thyroid 1

Raised TSH serum levels in children born prematurely: A follow-up study

Francesca Pellegrini1; Giorgio Radetti1; Davide Calebiro1; Livia Renzuillo1; Petra Wanker1; Luca Persani1

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Non autoimmune hyperthyrotropinemia has been previously reported with a certain prevalence among children born prematurely. The natural history of the raised TSH in these children is however not known. We evaluated therefore 26 children born at a gestational age of 32.1 ± 1.6 weeks, appropriate for weight and length, for the first time at the age of 8.1 ± 2.1 years (phase 1) and subsequently at the age of 10.5 ± 1.9 years (phase 2). At each visit FT3, FT4, TSH, TPO-Ab, TG-Ab were evaluated and a thyroid ultrasound was performed. Iodine deficiency was excluded. Mean TSH serum level was similar in both phases (2.7 ± 1.0 vs 3.0 ± 1.9 mU/l; NS; normal range: 0.4-3.6 mU/l), however it was above the upper normal limit in 4 (15.4%) subjects at phase 1 (range 3.9-5.2 mU/l) and in 6 (23.7%) subjects at phase 2 (range 3.7-5.2 mU/l). There was no statistical difference between the two frequencies. During the follow-up 17 patients remained euthyroid, 2 showed a persistently raised TSH, 3 normalized and in 4 TSH increased above the upper normal limit. Free T4 and FT3 were always in the normal range (phase 1 vs phase 2: FT4 12.6±1.9 vs 12.2±1.6 µg/dl; FT3 4.2±0.4 vs 3.9±0.4 ng/ml) and TPO- and TG-Abs were absent. Ultrasound showed a normal thyroid structure in all children. Mean thyroid volume increased during the follow-up study (p=0.025), but remained significantly lower than that of matched controls (phase 1: -0.67 ± 0.49 SDS; p=0.000; phase 2: -0.25 ± 0.54 SDS; p=0.005). We confirm that children born prematurely frequently show non autoimmun hyperthyrotropinemia. The small thyroid volume together with its normal structure would support the concept of a partial refractoriness of the gland to TSH action or secretion of TSH isoforms with reduced bioactivity.

P1-d1-205 Thyroid 1

Presentation and course of differentiated thyroid carcinoma in pediatric patients: Comparison between pre-pubertal children and adolescents

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Background: Differentiated thyroid carcinoma (DTC) is uncommon in children and adolescents. Since the prevalence is low in prepuberty, clinical studies tend to include prepubertal children within the larger group of adolescents. Objective: To analyze separately the clinical characteristics of prepubertal patients diagnosed with DTC, and to determine whether differences exist in their presentation, course, and outcome. Methods: The records of 27 patients (10 prepubertal;17 pubertal) diagnosed and followed in our tertiary pediatric endocrine clinic from 1986 to 2007 were reviewed. Age at diagnosis, extension of tumor, treatment modality, course, and outcome were analyzed.

Results: At diagnosis the prepubertal group was distinct with regard to the prevalence of positive family history (p=0.037) and degree of tumor invasion: extrathyroid extension (p=0.012), lymph nodes involvement (p=0.09) and lung metastases (p=0.09). The extent of surgery and permanent post-operative complications (vocal cord paralysis, 2 patients; hypoparathyroidism, 2 pati-
Both proapoptotic cytokines, IL-6 and IL-1beta are considered to be involved in the pathogenesis of autoimmune thyroid disease as well as in nonendocrine autoimmune diseases such as juvenile chronic arthritis (JCA). The cytotoxic mechanisms lead to thyroid damage and hypothyroidism (hyperT) in the course of chronic autoimmune thyroiditis (AIT) or thyroid stimulation in Graves disease (GD), leading to hyperthyroidism (hyperT). Serum concentrations of IL-6 and IL-1beta in groups of patients with hypo- and hyperthyroidism (vs control) were evaluated at the onset of disease (before treatment introduction). We studied 26 children: 9 children with hyperT (7 girls and 2 boys), age 11.3±3.2 years; mean hormone values: TSH 44.9 uUI/mL, FT4 0.61 ng/dL, FT3 2.33 pg/mL; ATPO 2691.7 IU/mL, 12 children with hyperT (9 girls and 3 boys, age 12.7±5.1 years; mean hormone values: TSH 0.0 uUI/mL, FT4 4.16 ng/dL, FT3 18.95 pg/mL; TRAb 29.7 U/L). A group of 5 healthy subjects (2 girls and 3 boys, age 13.1±6.4 years) were also studied as a control group. Serum concentrations of IL-6 were low in all groups (below 1.6 pg/mL) compared with high values in sera and synovial fluids of patients with JCA (in many of them more than 100 pg/mL). IL-1beta levels were significantly higher in hyperT (2.23 pg/mL) compared to control (1.43 pg/mL) (p=0.037) and were not significant to hyperT (1.59 pg/mL). There was no difference between hyperT and hyperT. These results support the significant role of IL-1beta in the late hypothyroid phase of AIT rather than in late phase of extremely severe GD thus supporting the involvement of different proapoptotic factors in these autoimmune thyroid disorders of young patients.

Background: High plasma ghrelin levels have been reported in Prader-Willi syndrome (PWS) and could play a role in early-onset obesity. However, little is known about plasma ghrelin in these children during the first years of life characterized by a failure to thrive.

Objective: To investigate total plasma ghrelin levels in children with PWS and in controls from 2 months to 17 years. Subjects and methods: Forty children with PWS (24 boys 16 girls, median age 3.6 years [2.0 - 17.2 years], median BMI 0.3 Z-score [-4.0 -+4.4]) were compared to 84 controls (57 boys 27 girls median age 4.2 years [0.3-17.1]) median BMI 0.1 Z-score [-1.5 -+1.9]). Children with PWS were then divided into 2 groups according to age and GH treatment.

Results: Median plasma ghrelin levels were significantly higher in children with PWS compared to controls at any age (568 vs. 173, p<0.0001) and decreased with age in both groups (p<0.0001). In the whole group of PWS, we found an inverse relationship between ghrelin and BMI Z-score (p=0.0032), insulin (p=0.0001), HOMA-IR (p=0.0002), leptin (p=0.0027) and lean mass (p=0.04). Plasma ghrelin levels were significantly higher in children with PWS than in controls, both in the youngest children below 3 years who were not receiving GH (771 vs. 233 p<0.0001) and in the children older than 3 years all of whom were treated with GH (428 vs. 159 p<0.0001). In young children with PWS, we did not find any relationship between ghrelin and BMI Z-score, insulin, HOMA-IR.

Conclusions: Plasma ghrelin levels in children with PWS are elevated at any age, particularly during the first years of life, thus preceded the development of obesity.
Atherosclerosis represents a progressive and slow process that seems to start in early childhood. Several studies have shown significantly increased carotid intima media thickness (cIMT) in children with type 1 diabetes, hypertension, familiar hypercholesterolemia and obesity, but no data are available in pre-pubertal children with a sole familiar history of cardiovascular events (FHPCe). The aim of this study was to evaluate cIMT in children with a parental history of premature cardiovascular risk and the relationship between cIMT and other known risk factors (insulin resistance (IR), oxidant status and lipid profile) involved in structural vascular changes. In 16 pre-pubertal children with a sole familiar history of premature cardiovascular events as a sole and independent risk factor for cardiovascular risk presented an increased cIMT that is not influenced by alteration in glucose metabolism and/or oxidant-antioxidant status and/or lipid profile; in these children probably hereditary and genetic predisposition play a pivotal role in the pathogenesis of increased cIMT.

Being born large for gestational age (LGA) has an increased risk of developing insulin resistance. Hypoadiponectinemia is associated with insulin resistance. The aim of this study was to evaluate insulin resistance, body composition and adiponectin levels in LGA born non-obese children at prepubertal ages. Twenty-one (8F, 13M) LGA born non-obese children (mean age 5.5±2.3 yrs) were evaluated with respect to insulin, glucose, IGFBP-1, leptin, adiponectin levels and body composition by DEXA. Their data were compared to that of non-obese 58 (26 F, 32 M) appropriate for gestational age (AGA) children (mean age 5.9±0.9 yrs). Insulin resistance was evaluated as HOMA-IR. LGA children were taller than AGA children (p=0.005) but had similar weight SDS (-0.31±0.7 vs -0.25±0.7). There were no significant differences in leptin, IGFBP-1, glucose and insulin levels, HOMA-IR and body composition between LGA and AGA born children. However, adiponectin level was significantly lower in LGA born (8.6±4.3 µg/mL) than AGA born (21.9±3.3 µg/mL) children after controlling for age, sex and BMI (p=0.008). In conclusion, LGA children have lower adiponectin levels than AGA children in spite of similar BMI and insulin levels. Adiponectin is a better indicator of insulin resistance in LGA born children at prepubertal ages. Being born LGA is per se a risk factor for hypoadiponectinemia even in the absence of obesity.

Atherosclerosis represents a progressive and slow process that seems to start in early childhood. Several studies have shown significantly increased carotid intima media thickness (cIMT) in children with type 1 diabetes, hypertension, familiar hypercholesterolemia and obesity, but no data are available in pre-pubertal children with a sole familiar history of premature cardiovascular events (FHPCe). The aim of this study was to evaluate cIMT in children with a parental history of premature cardiovascular risk and the relationship between cIMT and other known risk factors (insulin resistance (IR), oxidant status and lipid profile) involved in structural vascular changes. In 16 pre-pubertal children with a sole familiar history of premature cardiovascular events as a sole and independent risk factor for cIMT (0.93±0.020 vs 0.32±0.06 mm; p=0.001) compared to healthy subjects. hs-CRP was not different between the two groups (0.65±0.62 vs 0.47±0.38 mg/L; p=0.7). Furthermore, no significant differences were found in term of fasting insulin levels (6.96±3.62 vs 8.66±5.22 IU/mL; p=0.5); I/G (16.28±10.63 vs 13.38±9.55; p=0.4) and HOMA-IR (1.29±0.82 vs 0.57±0.38; p=0.055) between children with FHPCe and controls. In addition, in children with FHPCe, cIMT was not correlated to indices of IR, oxidant status and lipid profile. In conclusion, pre-pubertal children with precocious history of cardiovascular risk presented an increased cIMT that is not influenced by alteration in glucose metabolism and/or oxidant-antioxidant status and/or lipid profile; in these children probably hereditary and genetic predisposition play a pivotal role in the pathogenesis of increased cIMT.

Being born large for gestational age (LGA) has an increased risk of developing insulin resistance. Hypoadiponectinemia is associated with insulin resistance. The aim of this study was to evaluate insulin resistance, body composition and adiponectin levels in LGA born non-obese children at prepubertal ages. Twenty-one (8F, 13M) LGA born non-obese children (mean age 5.5±2.3 yrs) were evaluated with respect to insulin, glucose, IGFBP-1, leptin, adiponectin levels and body composition by DEXA. Their data were compared to that of non-obese 58 (26 F, 32 M) appropriate for gestational age (AGA) children (mean age 5.9±0.9 yrs). Insulin resistance was evaluated as HOMA-IR. LGA children were taller than AGA children (p=0.005) but had similar weight SDS (-0.31±0.7 vs -0.25±0.7) and BMI SDS (-0.01±0.8 vs -0.25±0.7). There were no significant differences were found in term of fasting insulin levels (6.96±3.62 vs 8.66±5.22 IU/mL; p=0.5); I/G (16.28±10.63 vs 13.38±9.55; p=0.4) and HOMA-IR (1.29±0.82 vs 0.57±0.38; p=0.055) between children with FHPCe and controls. In addition, in children with FHPCe, cIMT was not correlated to indices of IR, oxidant status and lipid profile. In conclusion, pre-pubertal children with precocious history of cardiovascular risk presented an increased cIMT that is not influenced by alteration in glucose metabolism and/or oxidant-antioxidant status and/or lipid profile; in these children probably hereditary and genetic predisposition play a pivotal role in the pathogenesis of increased cIMT.

P1-d2-210 Obesity and Fat 2
Adiponectin as an early indicator of insulin resistance in non-obese prepubertal children born large for gestational age
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To investigate the relationship between ghrelin and adiponectin levels and growth of infants, total and free ghrelin and adiponectin levels were studied in human milk and fasting serum samples of 25 healthy lactating women aged 20-31 years who had vaginal delivery and their infants at the 1st and 4th months of delivery. All ghrelin and adiponectin levels were analyzed by ELISA kit. Body weight and body mass index (BMI) of infants and their mothers at birth and during study period were also recorded. While there were no significantly changes in 1st and 4th months total ghrelin levels (TGHRL) of breast milk and the serum of infant (p=0.05), maternal TGHRL was found significantly decreased at 4th month (p<0.05). Free ghrelin levels (FGHRL) in breast milk, and both infant and maternal serum FGHRL, were found significantly increased at 4th month (p<0.05). The mean adiponectin level in the serum of infant was decreased at the 4th month (p<0.05). The other adiponectin levels were found unchanged during the study period. While breast milk TGHRL were similar with maternal serum levels at the 1st and 4th months, both breast milk FGHRL were found significantly higher than infant and maternal serum FGHRL (p<0.05). BMI at birth was negatively correlated with breast milk TGHRL of 1st month (r=-0.41; p<0.05). There was a positive correlation between breast milk FGHRL of 1st month and delta body weight, BMI at 4th month and maternal delta FGHRL (r=0.44; p<0.05; 0.47; p<0.05; 0.44; p<0.05, r respectively). Breast milk FGHRL of 4th month was positively correlated with delta body weight of infant (r=0.43; p<0.05). No relationship could be determined between breast milk ghrelin and adiponectin levels and infant or maternal serum levels at 1st and 4th months (p>0.05). These findings suggest that the ghrelin in breast milk might have a significant role in growth of infants without any effect on serum ghrelin levels of infants.
P1-d2-213 Obesity and Fat 2

B-type natriuretic peptide (BNP) in asymptomatic obese children; Is there a relationship with cardiac parameters?

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Objective: Obesity in childhood and, in particular, adolescence is associated with increased risk for cardiovascular disease in adulthood. Here, it was aimed to assess (1) the changes in Nt-pro-BNP, epicardial fat tissue, and inti-ma-media thickness of carotid artery in childhood obesity, all of which can be used for early diagnosis of cardiovascular complications in adults, (2) the correlations of these markers within each other and myocardial performance index and to evaluate the utility of the 3 parameters in the follow-up of childhood obesity.

Patients and Methods: Twenty cases (10 male, mean age: 10.25±0.53) with the diagnosis of obesity (patients group) and 20 controls (10 male, mean age: 10.47±0.78) with innocent murmurs (control group) were recruited for the study. In all of the patients, the serum NT-pro-BNP were measured and, by using transthoracic echocardiography, the thicknesses of epicardial fat tissue and intima-media of carotid artery were evaluated.

Results: The mean left ventricular mass index was detected 39.87±8.5 gm2 in the obesity group and 36.7±6.28 gm2 in the control group (p=0.05). The mean NT-pro-BNP was measured 109.25±48.53 pg/mL in the obesity group and 51.96±22.36 pg/mL in the control group (p=0.001), while the mean thickness of epicardial fat tissue was detected 5.66±0.32 mm in the obesity group and 2.9±0.09 mm in the control group (p=0.001) and the mean carotid artery intima-media thickness was found 0.07±0.01 mm and 0.05±0.01 mm in the control group (p=0.001). No statistically significant correlation was found between NT-pro-BNP levels and left ventricle mass index, left and right ventricle diastolic functions, epicardial fat and intima media thickness in obese patients (p=0.05; r: -0.29; -0.24; -0.18; 0.32, 0.19, respectively).

Conclusion: In childhood and adolescent obese patients, there is not a correlation between NT-pro-BNP and cardiac parameters. Because of this reason, there is no need to routinely monitor NT-pro-BNP levels during clinical follow-up of asymptomatic obese patients.

P1-d2-214 Obesity and Fat 2

Hypothalamus syndrome caused by tryptophane hydroxylation impairment

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The hypothalamus is phylogenetically very ancient and has often been denoted as the head ganglion of the autonomic nervous system because of the profound sympathetic and parasympathetic effects. Disruption of this area causes the so-called hypothalamic syndrome with a variety of endocrine and metabolic disorders. Little is known about the pathophysiology of this syndrome and evidence of a structural hypothalamic lesion has not been found (1).

A three-year-old girl was referred to our hospital because of obesity and behaviour problems, disturbance of sleep rhythm and episodes of hypothermia. Physical examination showed a obese girl with evidently breast development (Tanner 2).

Additional investigation revealed elevated values of LH, FSH and prolactin during a GnRH test. A TRH test also shows an abnormal pattern of TSH. Cortisol was measured in the saliva during 24 hours and revealed an inverted day-night concentration.

Based on the endocrine disorders and the neurological abnormalities a hypothalamic disorder was considered and advanced metabolic research for inborn errors performed. A defect in the biogenic amines metabolism in the CSF with a reduced rate of 5-hydroxyindoleacetic acid (5-HIAA) was found due to a tryptophane hydroxylation impairment.

To the best of our knowledge, a relationship between tryptophane hydroxylation deficiency and hypothalamic disorder has not been reported previously.

P1-d2-215 Obesity and Fat 2

Increased RBP-4 and lipocalin in children born after IVF: An early marker of the insulin resistance phenotype?

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Obesity and Fat 2

Assisted reproduction techniques (IVF) have been widely used during the last few decades. However, few long-term prospective studies of children conceived after IVF have been performed. Our earlier findings demonstrated elevated blood pressure and triglyceride levels in IVF children compared to controls, while no differences in the occurrence of the metabolic syndrome were observed. However, nontraditional metabolic risk factors, such as leptin, adiponectin, high sensitivity CRP (hsCRP), interleukin-6 (IL-6), and retinol binding protein 4 (RBP4) and neoptrofil gelatinase-associated lipocalin (NGAL) have not been studied in IVF children as yet. The aim of the study was to study the non-traditional metabolic risk factors in children born after IVF. Patients and methods: 100 children born after IVF (47 boys) and 59 spontaneously conceived controls (30 boys), aged 4-14 y, were studied prospectively. All children underwent physical examination and had fasting glucose, insulin, lipid profile, IL-6, hsCRP, adiponectin, leptin, NGAL, and RBP4 determined.

Results: As a group, IVF children had significantly higher RBP4 (p=0.009) and NGAL (p=0.028) levels than controls. Studied in subgroups, when SGA children and twins were excluded, IVF girls had higher RBP4 (p=0.019) than controls. Furthermore, IVF singletons at puberal age had significantly higher hsCRP (p=0.048) and IL-6 (p=0.032) levels than controls. There were no statistically significant differences in leptin and adiponectin levels between the two groups. In our study, significantly higher RBP4 and NGAL levels in IVF children than controls were found, suggesting an early insulin resistance phenotype in these children. These results are in accordance with our previous findings of higher triglycerides and blood pressure in IVF children than controls. Further prospective studies should be performed in IVF children to determine the natural course of their metabolic risk factors profile.

P1-d2-216 Obesity and Fat 2

Cannabinoid receptor, CB1, expression in primary adipocyte cultures in lean and obese pre-pubertal children, in relation to High Molecular Weight (HMW) adiponectin, HOMA Insulin Resistance (IR) and waist circumference

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1University of Patras School of Medicine, Division of Pediatric Endocrinology and Diabetes, Patras, Greece; 2University of Patras School of Medicine, Department of Anatomy, Histology and Embryology, Patras, Greece; 3Karamandaneio Childrens Hospital, Department of Pediatric Surgery, Patras, Greece

Cannabinoid receptor, CB1

Childhood obesity is associated with insulin resistance and the metabolic syndrome. CB1 is an endocannabinoid receptor associated with increased food intake and lipogenesis. HMW adiponectin is an adipokine biomarker for insulin sensitivity. We aimed to study the expression levels of CB1 in pre- (p) and mature (m) adipocytes from obese and lean children in association with HMW adiponectin, HOMA IR and waist circumference. Primary cultures of pre- and mature adipocytes were developed from routine surgical biopsies of subcutaneous abdominal adipose tissue from 36 healthy lean (BMI <85%) and 17 healthy obese (BMI ≥95%) pre-pubertal children in 2 groups (A: 2 months-7 yrs and group B: 8-12 yrs). CB1 expression was studied at the mRNA (mR) level with RT-PCR and at the protein level (P) with western immunoblotting. Serum HMW adiponectin and insulin, high sensitivity CRP (hsCRP), interleukin-6 (IL-6), and retinol binding protein 4 (RBP4) and neutrophil gelatinase-associated lipocalin (NGAL) have not been studied in IVF children as yet. The aim of the study was to study the non-traditional metabolic risk factors in children born after IVF. Patients and methods: 100 children born after IVF (47 boys) and 59 spontaneously conceived controls (30 boys), aged 4-14 y, were studied prospectively. All children underwent physical examination and had fasting glucose, insulin, lipid profile, IL-6, hsCRP, adiponectin, leptin, NGAL, and RBP4 determined.

Results: As a group, IVF children had significantly higher RBP4 (p=0.009) and NGAL (p=0.028) levels than controls. Studied in subgroups, when SGA children and twins were excluded, IVF girls had higher RBP4 (p=0.019) than controls. Furthermore, IVF singletons at puberal age had significantly higher hsCRP (p=0.048) and IL-6 (p=0.032) levels than controls. There were no statistically significant differences in leptin and adiponectin levels between the two groups. In our study, significantly higher RBP4 and NGAL levels in IVF children than controls were found, suggesting an early insulin resistance phenotype in these children. These results are in accordance with our previous findings of higher triglycerides and blood pressure in IVF children than controls. Further prospective studies should be performed in IVF children to determine the natural course of their metabolic risk factors profile.
older lean while it was $S$ decreased in the mature adipocytes of the obese in both age groups. Serum HMW adiponectin was $S$ decreased and HOMA IR was increased in the lean and obese of the older age group. WC was at a high risk percentile in the obese boys. In the obese subjects the decreased HMW adiponectin levels in association with the increased HOMA IR and high risk WC might play a role in the development of metabolic syndrome. The increased CBI P in the older lean pre-pubertal children may reflect a physiologic mechanism which enhances fat deposition in preparation for the increased energy expenditure of puberty. The reduced mR and P of CBI in the older obese pre-pubertal children though may be the body’s attempt to reduce lipogenesis in the abdominal region to limit the development of excessive insulin resistance during puberty.

**P1-d2-217 Obesity and Fat 2**

**Echocardiographic epicardial adipose tissue in obese children: A new indicator of insulin resistance**

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**Aim:** Until now, the association between subepicardial fat (SAT), insulin resistance, and intima-media thickness has not been evaluated in obese children. In this study, we evaluated whether echocardiographic SAT is related to insulin resistance, and intima-media thickness in obese children.

**Patients - methods:** A total of 46 obese patients (10.2 ± 2.46 years of age, 25 male patients) and 30 age- and gender-matched control subjects (10.8 ± 3.11 years of age, 13 male patients) were included in this study. The criterion for diagnosing obesity was defined as the body mass index (BMI) being over 97% percentile of the same gender and age. Serum triglyceride, low- and high-density lipoprotein, cholesterol, and glucose and insulin levels were measured in the fasting state. The estimation of insulin resistance was made using a homeostasis model assessment index. Each subject underwent a transthoracic echocardiogram and the SAT thickness was measured during end-diastole from the parasternal long-axis views.

**Results:** The obese patients had significantly higher SAT thickness and intima-media thickness values compared to the subjects in the control group (5.7 ± 1.4 vs. 3.02 ± 0.66 mm, 0.78 ± 0.015 vs. 0.05 ± 0.01, p<0.001, respectively). Simple linear regression analysis showed no significant correlation between epicardial adipose tissue and BMI SDS (r=0.08, p=0.585), insulin resistance (r=0.170, p=0.264) whereas there was significant correlation between epicardial adipose tissue and intima-media thickness (r=0.379, p=0.02) in obese patients group. In addition intima media thickness was significantly correlated with insulin resistance (r=0.379, p=0.01). At an optimal cut off point, 4.1 mm SAT thickness determined insulin resistance with 90% sensitivity and 61% specificity.

**Conclusions:** Our study showed that the epicardial fat was significantly related to intima-media thickness. Assessment of SAT (>4.1 mm) and intima-media thickness during routine echocardiographic examination, a non-invasive method, might be used in predicting insulin resistance in obese children easily and reliably.

**P1-d2-218 Obesity and Fat 2**

**BMI changes during childhood and adolescence as predictors of amount adult subcutaneous and visceral adipose tissue in men - The good study**

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It is unclear how body mass index (BMI) changes during childhood and adolescence predict adult body composition. We have investigated the impact of BMI changes during different developmental periods for adult body composition and fat distribution. Detailed growth charts were retrieved for the men participating in the population-based Gothenburg Osteoporosis and Obesity Determinants (GOOD) study (n=612). Body composition was analysed using Dual X-Ray Absorptiometry and adipose tissue areas using abdominal computer tomography at 18-20 years of age. The main finding in the present study was that the amount of adult subcutaneous adipose tissue of the trunk was predicted both by BMI changes during late childhood (4-10 years of age) and adolescence (10-19 years of age) while the amount of adult visceral adipose tissue was predicted by BMI changes specifically during adolescence. Subjects with increases in BMI Z-score of >1 SD during late childhood had larger amount adult subcutaneous adipose tissue (+83%; p=0.001) than subjects with unchanged BMI Z-score, but unaffected amount of visceral adipose tissue. In contrast, during adolescence, subjects with increases in BMI Z-score of >1 SD had both larger subcutaneous (+138%; p<0.001) and visceral adipose tissue areas (+91%; p<0.001) than subjects with unchanged BMI Z-score. BMI increases during adolescence predicted the amount of adult visceral adipose tissue independent of prepubertal BMI. Early childhood (1-4 years of age) BMI changes were positively associated with adult lean mass but not with adult fat mass. The amount of visceral adipose tissue in men was associated with BMI changes specifically during adolescence, while the amount of subcutaneous adipose tissue was associated with BMI changes during both late childhood and adolescence.

**P1-d2-219 Obesity and Fat 2**

**LDL receptor in a Druze kindred - Clinical, biochemical and genetic characteristics**

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Familial hypercholesterolemia (FH) is an autosomal dominant disease caused by mutation in the LDL receptor gene. The heterozygous frequency is about 1/500 and the homozygous frequency is about 1/1,000,000. The average age of FH patients ranging from 6 to 7 years old and 9 years old from a Druze kindred presented with cutaneous and tuberous xanthomas, and with failure to thrive. LDL-cholesterol levels ranged between 800-900 mg/dl. Analysis of mutation in the LDL receptor gene was done for 48 members of the extended family. Genomic DNA was extracted from the family member’s peripheral blood, and from cord blood of a newborn sibling. LDLR exon 4 was sequenced directly. The Y188X mutation was detected in three index patients. Restriction enzyme analysis confirmed the DNA sequence in these patients. We identified other heterozygous family members as Y188X carriers. Using the cord blood of the new-born sibling we diagnosed him as heterozygous for the LDL receptor mutation. Interestingly 3 infants with normal LDL-cholesterol levels were diagnosed as heterozygous carriers based on DNA analysis. Identifying the mutation in this large Druze family enabled us to diagnose carrier children who would otherwise be...
missed because of phenotype and genotype discrepancy and who are prone to develop symptoms of atherosclerotic cardiovascular disease in the third or fourth decade of their life. To the best of our knowledge this is the first report of using cord blood for DNA identification of FH and it is a possible tool for early diagnosis.

**P1-d2-220 Obesity and Fat 2**

**Isoflavones treatment for familial hypercholesterolemia (FH) in children**

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It has been reported that soy protein maintains a cholesterol-lowering effect, which was attributed to its isoflavone content. We evaluated the effects of soy-derived isoflavones in children with FH. 12 FH children (8 F/4M; aged 5.3-11.2 y) have completed a randomized placebo-control study. Inclusion criteria were cholesterol above 200 mg/dl and LDL above 130 mg/dl on two measurements. Children with endocrine diseases and those with BMI-SDS > 95th percentile were excluded. Isoflavones’ containing candies were used to improve compliance. Following an AHA step-1 diet over 12 weeks, participants with persistent hypercholesterolemia were randomly assigned to three 8-weeks long interventions: placebo, 16mg/d and 48mg/d of isoflavones, with a washout time of two weeks between the intervention periods. A monthly report of intake was obtained throughout the study. By candies counting we ascertained a compliance of at least 80%. Isoflavones had no effect on thyroid hormones, sex-hormones and SHBG. Mean TSH level was significantly decreased during 16mg-isofalavones intervention. Neither low nor high doses of isoflavones had any effect on lipid profile in the children (Table). Isoflavones have no effect on lipid profile in children with FH. Although considered the preferred approach in children, dietary intervention yielded no benefit in our study. Table: The difference (%) in the children (Table). Isoflavones have no effect on lipid profile in children with FH.

<table>
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<td>-9 ± 7</td>
<td>-4 ± 11</td>
<td>15 ± 12</td>
<td>12 ± 16</td>
</tr>
<tr>
<td>APO-B</td>
<td>-3 ± 5</td>
<td>4 ± 4</td>
<td>2 ± 6</td>
<td>1 ± 5</td>
</tr>
<tr>
<td>LP(a)</td>
<td>5 ± 10</td>
<td>13 ± 6</td>
<td>5 ± 9</td>
<td>11 ± 6</td>
</tr>
</tbody>
</table>

O.Si.M.E. study Group: Domenico Viggiano, Antonio Fasolini, Norma D’Alessio, Natalia Avellino, Maria Carmela Vorga, Antonio Giosue Prisco, Felice Sorrentino. All the members are Primary Care Pediatricians of the National Health Service Unit “Salerno 1”, Italy.

**P1-d2-221 Obesity and Fat 2**

**Waist-to-Height ratio is a reliable tool to predict insulin resistance and metabolic syndrome in obese children: A population-based survey in a primary care setting**

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1Gaetano Rummo Hospital, Pediatric Endocrinology Unit, Benevento, Italy; 2on Behalf of the O.Si.M.E. Study Group, National Health Service Unit “Salerno 1”, Nocera Inferiore, Italy; 3Umberto I Hospital, Biochemistry Unit, Nocera Inferiore, Italy; 4University of Chieti, Department of Pediatrics, Chieti, Italy

The presence of metabolic syndrome (MS) in children and its close relationship to obesity are well demonstrated. The majority of studies have been hospital-based or have dealt with racially homogeneous cohorts, and thus are not exempted from selection bias or confounding factors. Furthermore, the prevalence in free-living individuals is not correctly estimated. Other studies aimed to identify one or some clinical or biochemical predictive factors for MS, such as Body Mass Index (BMI), Waist Circumference (WC), Waist-to-Height Ratio (WHR) or Insulin-Resistance (IR), the latter showing probably the best correlation. We conducted a primary care-based study in order to determine the prevalence of MS and to identify its predictive factors a cohort of free-living obese children and adolescents. 415 subjects were enrolled with obesity as unique selection criterion. The entire cohort was screened for MS (at least two findings other than obesity: fasting hyperglycaemia (FH), low levels of HDL cholesterol (LHC), hypertriglyceridaemia (HTg) and hypertension (HT)). The overall prevalence of MS was 30.8%, without a significant difference between prepubertal and pubertal subjects. The major findings (other than obesity) were LHC (46.2%), HT (23.6%), HTg (22.2%) and FH (16.6%). The influence of puberty was evident only concerning the prevalence of LHC (42.4% vs 55.1%; p = < 0.01). The only significant clinical parameter related to MS was WHR, directly related with IR (assessed by HOMA) and with the same predictive power for MS, as indicated by ROC curve (figure). Our data clearly indicate that MS can be present in a significant percentage, even in a cohort of “healthy” obese children and that a simple, no-cost parameter easily assessed at the time of clinical evaluation could identify at risk subjects.

In view of the increasing prevalence, important predictors of overweight in childhood and adolescence need to be determined. Depressive symptoms have been described both as a predictor and as a complication of overweight. Therefore, the aim of this study is to assess the association between depressive symptoms in childhood and overweight in adolescence. The TRacking Adolescents Individual Lives Survey (TRAILS) is a population-based cohort study among 2000 adolescents, presently aged 14-16 years. These adolescents have been assessed biennially from the age of 11 years. At ages 11 and 15, we have assessed weight, height, skinfold thicknesses, and depressive symptoms through the Youth Self Report and Child Behavior Checklist questionnaires. Body fat percentage and waist circumference have been obtained at age 15. Results regarding the first 1037 participants showed that at age 11, clinical depressive symptoms were present in 10.9% of girls and 9.9% of boys. Mean BMI at age 15 was 21.28 kg/m2 in girls and 20.18 kg/m2 in boys. Results from linear regression analyses showed no significant results in boys. The
Results regarding girls are listed in the table:

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Continuous predictor: B depressive symptoms at age 11 (95% CI), P-value*</th>
<th>Dichotomous predictor: Adjusted difference between depressed and non-depressed (at age 11) children (95% CI), P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI at age 15 (kg/m²)</td>
<td>0.18 (-0.06 - 0.41), P = 0.14</td>
<td>0.83 (0.24 - 1.42), P = 0.01</td>
</tr>
<tr>
<td>Sum of four skinfolds at age 15 (mm)</td>
<td>2.01 (-0.21 - 4.23), P = 0.08</td>
<td>4.81 (-0.94 - 10.55), P = 0.10</td>
</tr>
<tr>
<td>Body fat at age 15 (%)</td>
<td>0.44 (0.05 - 0.83), P = 0.03</td>
<td>1.27 (0.28 - 2.25), P = 0.01</td>
</tr>
<tr>
<td>Waist circumference at age 15 (cm)</td>
<td>0.72 (0.01 - 1.42), P = 0.05</td>
<td>2.06 (0.27 - 3.84), P = 0.02</td>
</tr>
</tbody>
</table>

* Adjusted for BMI at age 11.

In conclusion, clinically relevant depressive symptoms at age 11 in girls are associated with an increased BMI, body fat percentage, and waist circumference at age 15.

The prevalence of obesity is increasing in children worldwide. Although environmental factors have had a large impact, there is much evidence to support the fact that 40-70% of body fat is inherited. To identify the genetic basis of severe childhood obesity, we have recruited over 3000 severely obese children (BMI sds>3) to the Genetics of Obesity Study (GOOS). 50% of the GOOS cohort have a positive family history of obesity, consistent with the major genetic component to the obesity in these children. Taking a candidate gene approach, we have identified seven monogenic causes of obesity. Pathogenic mutations in the melanocortin 4 receptor gene (MC4R) represent the commonest monogenic cause of obesity (103/2699 unrelated probands) and are present in 4-5% of the children with severe obesity. Mutations in the leptin and leptin receptor gene are found in up to 3% of children. Most of these mutations are found in homozygous form and are associated with hypogonadotropic hypogonadism and impaired linear growth. Heterozygous mutations in pro-opiomelanocortin (POMC)(Y221C, H143Q, R236C) are found in 1% of patients and result in hyperphagia and an increase in lean mass as well as fat mass. The detailed clinical phenotype of these disorders is reported. Only 16% of patients in the GOOS cohort have any form of developmental delay or syndromic features. Rare mutations in TrkB are associated with development mental delay, hyperactivity and impaired short-term memory. In summary, we report the genetic findings in the GOOS study which account for 7% of the patients recruited. There is considerable heterogeneity in the metabolic and endocrine phenotypes associated with severe childhood obesity, suggesting that other genes and mechanisms remain to be found.

ADMA (asymmetrical dimethylarginine), the metabolite of methylated L-arginine, is a competitive nitric oxide (NO) synthase antagonist. It decreases the production and availability of endothelium-derived NO. Increased ADMA concentrations have been described in adults with obesity, hypertension and arterial occlusive diseases. This study was planned to search whether increased ADMA levels are present in obese children, related with insulin resistance and high atherosclerosis risk. In this prospective study, 65 obese and 45 non-obese children were evaluated. Fasting glucose, insulin, lipid profile, lipoprotein a, apolipoprotein A and B levels were measured. Height and body weight were taken, physical examination and pubertal staging were performed. Mean age of patients was 11.13±2.86 and 10.41±2.66 years in obese and controls respectively (p<0.05). The mean BMI of obese patients was 28.01±4.19, and 18.18±2.99 kg/m² in controls (p<0.05). The mean ADMA level was 0.95±0.40 in obeses, which was significantly higher than 0.68±0.21 micromol/L in controls (p<0.001). The mean HDL was lower, LDL was significantly higher in obeses than controls (HDL: 51.1±12.09 and 59.5±13.62 mg/dL (p<0.001), and LDL: 91.38±26.58 and 80.88±21.17 mg/dL (p=0.025) in obese and control groups respectively). HOMA-IR was also significantly higher in obeses than controls; 3.42±1.80 and 2.61±1.31 respectively (p=0.03). The mean FGIR was significantly lower in obeses than 7.36±5.24 and 9.97±7.67 in controls (p=0.044). ADMA levels were correlated with presence of acantosis nigricans (Pearson correlation=0.207, p=0.032) and lipoprotein a (Pearson correlation=0.207, p=0.032) levels. ADMA levels were not correlated with BMI, FGIR and pubertal stage. There was also no correlation between ADMA and HOMA-IR levels. In conclusion, ADMA levels in obese children are higher than non-obese controls. It is positively correlated with presence of AN, which is an indicator of insulin resistance at tissue level and lipoprotein a, of which high level is a risk factor for cardiovascular diseases.
BIB may be effective to control body weight in PWS patients with morbid obesity. This study shows that, when non-invasive pharmacological therapies fail, the use of a bilio-pancreatic diversion can be effective. In the two oldest patients (20.6 and 30.1 yrs) only a slight BMI reduction was obtained with their first balloon and a BMI stabilization was achieved after treatment interruption. BMI tended to increase in every patient, except for the third one who, after treatment interruption, showed a slight BMI reduction at 14.6 yrs. Later, BMI increased to 23.6 kg/m² at the end of the third treatment BMI was 23.6 kg/m². The third patient (12.4 yrs) inserted BIB three times in 3 years: at the end of his first treatment BMI was 29 kg/m². The patient inserted a new balloon twice, other two times and one four times. We obtained excellent results in the two youngest patients. One was treated for the first time at 8.1 yrs (BMI: 26.66 ± 0.033) and at the end of his third treatment, slightly in excess of the 95 percentile for Body Mass Index (BMI) was diagnosed as being obese. Results: Leptin levels were significantly higher in obese adolescents than control group (p<0.001). Lipid profiles, atherosclerotic markers, and homeostatic levels did not differ significantly between obese and control groups. Arteria carotis intima media thickness in obese children significantly higher than controls (p=0.0006). There were no similar correlation in healthy adolescents. We found positively moderate correlations between leptin leves and carotid artery intima media thickness in obese children (r=0.26, p=0.02). Conclusion: Common carotid artery intima-media thickness (IMT) is considered a factor of cardiovascular risk and an early marker of coronary artery disease. Thicker IMT and positively relationship between leptin leves and carotid artery intima media thickness in obese children suggest that high leptin level is associated with vascular changes related to early atherosclerosis. However, positively relationship between leptin level and Apo A level in obese children suggest high leptin level could be preventive vascular changes related to early atherosclerosis.

Objective: Atherosclerotic Cardiovascular Diseases is the most common cause of death in the world. A high leptin concentration, in particular, is found in obese individuals and is strongly associated with vascular changes related to early atherosclerosis. We investigated relationship between leptin levels and atherosclerotic markers, homeostatic levels and arteria carotis intima media thickness. We aimed to determine whether high leptin levels is associated with vascular changes related to atherosclerosis in obese adolescents children.

Subjects and Methods: Forty-eight obese children were included in this study. Thirty healthy children defined as a control group. Lipid profile, atherosclerotic markers, leptin and homocysteine levels and common carotid artery intima media thickness determined in both groups. Adolescents who were above the 95 percentile for Body Mass Index (BMI) were diagnosed as being obese.

Obsesity in Prader Willi Syndrome (PWS) is progressive and severe. A drastic body weight reduction is mandatory to reduce the risk of cardio-respiratory and metabolic complications. The insertion of a Bioenterics Intragastric Balloon (BIB) in the gastric cavity represents an effective alternative to the more complex and invasive bariatric surgery. Recently, we reported the risks and benefits of BIB for treatment of morbid obesity in 12 PWS patients during 6 months. The aim of this study was to assess long-term effects of BIB treatment. Five patients out of them (3M, 2F), aged 16.1±6.2yrs (8.1±30.1yrs), underwent multiple treatment with BIB. Two patients repeated the treatment twice, other two three times and one four times. We obtained excellent results in the two youngest patients. One was treated for the first time at 8.1yrs (BMI: 44.4 kg/m²); 6 years later he stopped his fourth treatment with a BMI of 33.8 kg/m². The second patient (9.4 yrs) had a BMI of 39.1 kg/m²; at the end of his second treatment BMI was 23.6 kg/m². At 14.6yrs, 2 yrs after last BIB, BMI was 29 kg/m². The third patient (12.4yrs) inserted BIB three times in 3 years: starting BMI was 39.3 kg/m²; and at the end of his third treatment, slightly increased to 40.2 kg/m². At 17.5yrs, his BMI was 49.16 kg/m² and he underwent a bilio-pancreatic diversion. In the two oldest patients (20.6 and 30.1yrs) only a slight BMI reduction was obtained with their first balloon and a BMI stabilization was observed during the subsequent treatments. During the free intervals or after treatment interruption BMI tended to increase in every patient. However, some complications occurred: acute gaseous gastric distension due to ingestion of a fizzy drink; balloon rupture; recurrent diarrhea and aerophagy. This study shows that, when non-invasive pharmacological therapies fail, BIB may be effective to control body weight in PWS patients with morbid obesity, particularly if started in early childhood. Careful clinical follow-up and close collaboration with parents are crucial to avoid severe complications caused by unrestrained food intake despite BIB.

Changes in blood glucose and insulin levels were associated with modifications in serum ghrelin levels with the fractions AG and NAG being oppositely correlated. Partial correlation between glucose and insulin levels with the fractions AG and NAG was 0.39 and r=-0.27 respectively, p<0.05). No correlations between glucose and TG, AG or the AG/TG ratio were seen at any timepoint.

**P1-d2-228 Obesity and Fat 2**

**Is leptin level associated with vascular changes related to early atherosclerosis in obese adolescents?**

**Isil Ozgüven**; Betul Eryas; Ali Aykan Ozguven; Mine Ozkol; Eco Onur

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While the majority of energy homeostasis studies focus on central melanocortin action, peripheral effects of melanocortins and their receptors are not well established. Alpha-melanocyte stimulating hormone (α-MSH) is a posttranslational product of the POMC prohormone and the pituitary pars intermedia lobe melanotrophs are considered to be the major source of circulating α-MSH in most mammals. Recent evidence shows that α-MSH plays a role in thermal regulation by increasing free fatty acid oxidation (FAO) and increase of glucose intake in skeletal muscle through the activation of MC5R by activation of the PKA-AMPK pathway. In this study, we aimed to investigate peripheral α-MSH levels in 1) children with simple obesity 2) lean children 3) children with hypothalamic obesity and 4) patients with craniofacial hypoplasia (CP) to learn more about the role of peripheral, human α-MSH in obesity and CP. Fast serum α-MSH measured by radioimmunoassay with no cross-reactivity to ACTH. Furthermore we measured fasting leptin, insulin and glucose. Interestingly in patients with hypopituitarism or CP very low to zero α-MSH le-
levels were measured (healthy 26.6 fmol/ml vs hypopituitarism 8.4 fmol/ml vs craniohypophysectomy 7.7 fmol/ml). Compared to patients with simple obesity, patients with CP significantly lower (p<0.001) fasting serum α-MSH levels, but there were no significant differences of α-MSH levels in obese children compared to lean children. Low α-MSH levels in CP did not increase one hour after ingestion of a 500 kcal mixed liquid meal. CP patients had higher fasting insulin, insulin resistance index HOMA and leptin levels compared to patients with simple obesity and similar BMI. The low serum α-MSH levels in patient groups, which have low- or non-functioning pituitaries, verify that the pituitary is the critical source for circulating α-MSH. The very low α-MSH levels in CP can be explained by their pituitary or hypothalamic damage and might contribute to severe obesity associated with low thermogenesis.

**P1-d2-230 Obesity and Fat 2**

**Expression profiles of human adipocyte differentiation using the SGBS cell model**

Petra Böttner; Matthias Blüher; Martin Wabitsch; Wieland Kies; Antje Körner

1University of Leipzig, University Hospital for Children & Adolescents, Leipzig, Germany; 2University of Leipzig, Dept. of Internal Medicine III, Leipzig, Germany; 3University of Ulm, Dept. of Pediatrics, Ulm, Germany

The increase in fat mass in obesity results not only from hypertrophy but also from hyperplasia from differentiation of preadipocytes into mature adipocytes. Current information on transcriptional control of adipocyte differentiation is mainly derived from mouse model systems. We differentiated human preadipocytes of the SGBS cell line into mature adipocytes and characterized the alterations in gene expression patterns using whole genome microarrays. 248 genes were significantly (FDR 0.05) regulated more than 5-fold during the differentiation process. Amongst these genes we recovered well known markers of adipogenesis such as adiponectin, C/EBPα or PPARγ. Altogether 31% of the upregulated genes were associated with lipid metabolism. Principal component analysis and cluster analysis correctly grouped early differentiation stages vs. late differentiation stages. SGBS data were then compared to microarray data from differentiating mouse 3T3-L1 cells and human primary adipocytes of different fat depots. SGBS expression patterns showed highest similarity with primary subcutaneous adipocytes, as expected. These data indicate that SGBS cells are a valid model of human adipocyte differentiation and can be applied to identify new regulators of adipogenesis as well as adipocyte biology.

**P1-d2-231 Obesity and Fat 2**

**Estrogens influence endothelial function as measured by Peripheral Arterial Tonometry (PAT) index**

Amit Bhangu; Sunil Sinha; Michael Rosenbaum; Steven Shelov; Svetlana Ten

1Infants’ and Children’s Hospital of Brooklyn, Pediatrics, Brooklyn, United States; 2New York Presbyterian Medical Center at Columbia, Pediatric Endocrinology, New York, United States

**Background:** Factors like Obesity, insulin resistance and estrogens are known to influence arterial endothelial function.

**Objective:** To study the affect of estrogens on the endothelial function in 7th grade school children (12.2, 0.57 yrs).

**Methods:** Endothelial function in 102 healthy subjects was evaluated by using Endo-PAT device; then divided into 4 quartiles (Q) on the basis of their PAT index. Q1 PAT < -1SDS (n=16,boys 10), Q2 PAT Mean = -1SDS (n=39,boys 16), Q3 PAT Mean to +1SDS (n=33,boys 13) and Q4 PAT > +1SDS (n=4,boys 5). Height, weight, waist circumference (WC), body fat, BMI, BP, lipids, glucose, insulin, adiponectin, estrone (E1), estradiol (E2), DHEAS & estrone sulfate (ES) were measured.

**Results:** Fasting glucose, insulin, QUICKI, lipids were normal in all 4 quartiles. BMI, WC, body fat increased with quartiles. E2 levels in Q3 & Q4 were higher & lowest in Q1 (p<0.01 respectively). The E1 levels were lower in Q1 highest in Q3 (p<0.01). ES levels were the highest in the Q3 & were different from Q1 (p<0.001) (Table). DHEAS levels were not statistically different in any of these groups. The adiponectin levels were increased and highest in the Q4 quartile (p <0.001).

**Conclusion:** Better endothelial function was associated with higher BMI, Estrone, Estradiol, Estrone Sulfate. These findings underline the role of estrogens in maturation of endothelial function during childhood.

**P1-d2-232 Obesity and Fat 2**

**Effect of growth and body mass on intestinal absorption vs. endogenous synthesis of cholesterol in children and adolescents**

Uta Ceglarek; Martin Fiedler; Anke Baumann; Joachim Thiery; Wieland Kies; Antje Körner

1University of Leipzig, Institute of Laboratory Medicine, Clinical Chemist, Leipzig, Germany; 2University of Leipzig, University Hospital for Children & Adolescents, Leipzig, Germany

Serum concentrations of plant derived sterols (phytosterols) are closely related to intestinal cholesterol absorption whereas cholesterol precursors such as lanosterol are markers for endogenous cholesterol synthesis. Studies from adults indicate that cholesterol absorption and synthesis are associated with body weight. The aim of our study was to investigate the relation between growth and BMI with sterol metabolism in children. We determined free and esterified phytosterol concentrations of brassicasterol, stigmasterol, campesterol and beta-sitosterol as well as lanosterol (marker of endogenous cholesterol synthesis) and total cholesterol in 521 serum samples (285 girls, 256 boys, aged of 7 to 17 years) with BMI-SDS range from -3.2 to 3.1 applying liquid chromatography-tandem mass spectrometry. Samples were categorized in two age groups (<12 and ≥ 12 years). Ratios of phytosterols and lanosterol to total cholesterol were calculated. Serum concentrations of phytosterols and campesterol did not differ between boys and girls. Phytosterol and total cholesterol levels were significantly lower, whereas the lanosterol concentrations were significantly higher in older boys≥12 years (P<0.001). For girls we did not identify age-dependent changes in phytosterol concentrations. In both sexes, phytosterol levels significantly decreased from pubertal stage 1 to 5 (P<0.001). BMI showed the strongest influence on serum sterol concentrations in girls and boys. Lean children (BMI-SDS < -1) had significantly higher phytosterol concentrations and lower lanosterol concentrations compared to children with BMI-SDS ≥1. In contrast the total cholesterol concentration was not associated by BMI in this normal population childhood cohort. In summary, endogenous cholesterol synthesis appears to increase during puberal development. The BMI showed the strongest modulating effect on sterol homeostasis with higher phytosterol and lower lanosterol concentrations in lean children representing markers of cholesterol absorption and endogenous cholesterol synthesis, respectively.
The nucleotide pyrophosphatase/phosphodiesterase-1 (ENPP-1) gene encodes a membrane-bound glycoprotein that inhibits the insulin-receptor tyrosine kinase activity, resulting in reduced insulin sensitivity. Variants on the ENPP-1 have been associated with insulin resistance. Since insulin resistance is a pivotal factor in the development of metabolic syndrome, we aimed to test the association between the rs997509 ENPP-1 gene variant (C/T), previously associated with type 2 diabetes in adults. We screened 409 obese children (213 girls). All of them underwent a standard oral glucose tolerance test (OGTT); baseline measurements included blood pressure, plasma lipids and fasting insulin levels. The homeostasis model assessment of insulin resistance (HOMA-IR) and whole body insulin sensitivity index (WBISI) were calculated. A general linear model (GLM) was generated to assess differences between groups. Non-normally distributed values were log transformed before the analysis, but geometrical means are shown. A logistic regression equation was generated to predict the natural log of the odds for a subjects to show IGT or metabolic syndrome according to genotype. Thirty seven percent of obese subjects showed the metabolic syndrome (p<0.001), HOMA-IR (p<0.001) and lower WBISI values (0.04) (table 1). Moreover, subjects carrying the rare allele (T) showed higher insulin (p=0.001), HOMA-IR (p<0.001) and lower WBISI values (0.04) (table 1). We conclude that the rare allele of ENPP1 rs997509 variant can predispose obese children to develop the metabolic syndrome and IGT.

<table>
<thead>
<tr>
<th></th>
<th>CC (357)</th>
<th>CT (48) + TT (4)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.2 ± 2.7</td>
<td>11.2 ± 2.9</td>
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</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>32.2±5.4</td>
<td>33.0±6.7</td>
<td>0.2</td>
</tr>
<tr>
<td>z-score BMI</td>
<td>5.4±2.1</td>
<td>5.3±2.5</td>
<td>0.8</td>
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<tr>
<td>Glicemia (mg/dl)</td>
<td>79.8±8.9</td>
<td>81.5±9.1</td>
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</tr>
<tr>
<td>Insulin (μU/l)</td>
<td>31.1±19.2</td>
<td>41.6±30.0</td>
<td>0.001</td>
</tr>
<tr>
<td>HOMA</td>
<td>6.2±4.1</td>
<td>8.3±5.2</td>
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<tr>
<td>WBISI</td>
<td>2.5±2.0</td>
<td>2.0±1.5</td>
<td>0.04</td>
</tr>
</tbody>
</table>

**P1-d2-233** Obesity and Fat 2

**Metabolic syndrome and impaired glucose tolerance in obese children and adolescents modulated by the rs997509 ENPP1 gene variant**

Nicola Santoro; Laura Perrone; Grazia Cirillo; Maria Grazia Lepore; Alfonsina Palma; Pierluigi Marzullio; Nicoletta Cresta; Emanuele Miraglia del Giudice  
Second University of Naples, Pediatrics, Naples, Italy

**P1-d2-235** Reproductive Endocrinology 1

**A complex submicroscopic chromosomal imbalance in Xp 21.2 with microduplication and one microtriplication containing the DAX1 gene in a patient with 46,XY/47,XY,+mar karyotype with partial gonadal dysgenesis and gonadoblastoma**

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**Introduction:** Models with transgenic animals demonstrated that DAX1 gene located at Xp21.2 chromosome (Ddos locus) acts as an anti-testicular factor when extra copies were expressed.

**Objective:** To analyze the dosage effect of DAX1 gene in a patient with partial 46,XY gonadal dysgenesis and 46,XY[11]/47,XY,+mar karyotype.

**Patient:** A non-syndromic 14 yr old patient was referred with primary amenorrhea and absence of breast development. External genitalia showed clitoromegaly (4.6X1.0 cm), a single perineal opening and non palpable gonads. LH and FSH levels (33 and 72 U/L, respectively) and testosterone levels (479 ng/dL) were elevated. Pelvic ultrasound showed a 2 mL uterus and no gonads. She underwent laparoscopy and a gonadoblastoma in the right dysgenetic testis and ipsilateral epididymes and deferent duct and a left dysgenetic gonadal tissue with ipsilateral Fallopian tube were identified.

**Methods:** FISH was performed using the clone RP11-89L23 which binds to the Xp21.2-21.3 region containing DAX1 and contiguous MAGEB genes. MLPA (Kit P185 Intersex) was performed to screen DAX1, SOX9, WNT4 and SRY genes dosage. Array-CGH was carried out using BAC/PAC clones that provide tiling-path coverage of X chromosome with a 100-200 kb resolution.

**Results:** FISH identified duplication in Xp21 region in the marker chromosome. MLPA revealed the presence of extra copies of DAX1 gene. CGH-array revealed a duplication of 1.6 Mb (16 clones) in Xp21.2 and a triplication of CTD-2225F20 and RP11-662D2 clones. This last clone contains DAX1 and MAGEB genes but the absence of triplication of RP11297K22 clone which contains the MAGEB genes indicates that only DAX1 is triplicated.

**Conclusion:** We described a non-syndromic girl with a 46,XY/47,XY,+mar karyotype containing a duplication in Xp21.2 region causing partial gonadal dysgenesis. Our findings support that DAX1 gene overexpression and not MAGEB genes cause gonadal dysgenesis.
Female-to-male transsexual adolescents of the VUmc receive testosterone esters i.m. every 2 weeks in an increasing dose from the age of 16. The goal of the study was to investigate the influence of exogenous testosterone on the fundamental voice frequency (f0). 25 Female-to-male transsexual adolescents were included in the study. F0 was measured every 3 months from start of the androgen treatment by means of electroglottography during speech and reading. In addition serum testosterone concentrations were gathered at these time points. Fundamental frequencies were analyzed longitudinally and were related to testosterone levels and the testosterone dose given. During the first 6 months of the androgen treatment a maximal decrease in f0 was observed. A logarithmic relation was found between serum testosterone concentrations and the f0. The voice break occurs at the beginning of the induction of the male puberty, not at the end as is observed in biological males. This early change might be caused by the high serum testosterone levels within the first 72 hours after injection. There might be a certain threshold level at which the enlargement of the vocal tract and resulting lowering of the voice frequency takes place. Increasing doses of testosterone do not result in an additional effect on the f0. Another explanation might be the continuous presence of androgens in the blood during exogenous testosterone administration. In the onset phase of the biological male puberty testosterone levels fluctuate over the day, in this way the vocal tract is not exposed to continuous levels of testosterone and the subsequent change of the voice frequency will take longer than in biological male puberty.

**P1-d2-237 Reproductive Endocrinology 1**

46, XY partial gonadal dysgenesis: A cohort of 29 patients with clinical, biological, histological and genetic studies

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46, XY gonadal dysgenesis (GD) is an anomaly of testicular development which leads to a defect in the masculinisation of the foetus. The aims of this study were to describe patients with partial XY GD (circumstances of diagnosis, anatomy of external and internal genitals, biology, histology), their management (sex of rearing, attitude toward gonads, genitoplasty), their puberty and to evaluate mutation frequency of SF1, DAX1 and SOX9. Data from 29 patients born between 1966 and 2006 were analysed. Inclusion criteria were ambiguous genitals, karyotype 46, XY without mosaicism, strong arguments for GD (persistent Müllerian structures, gonadal histology and/or low serum testosterone or anti-mullerian hormone (AMH) level) and no evidence of renal or adrenal disease. Diagnosis was suspected at birth for 24 patients. External and internal genitals varied from very masculinised to very feminine with only clitoral enlargement. Sexual hormones (testosterone, AMH) could be normal in the first year of life but AMH was the more often disturbed. Testosterone response after hCG stimulation was variable (35% < 1 ng/ml but normal > 3 ng/ml in 39%). 11 patients were raised as males and 18 as females. The number of genitoplastic procedures was higher for males (2,8) than for females (2,2). Gonads were dysgenic testes with peripheral tubules like embryonic cords and a thin albuhinea, but few had only a reduced tubule density. 3 patients had a gonadoblastoma (aged 4 months, 1,5 and 7 years). Mutational screening is in progress but at least 4 patients without adrenal deficiency had a SF1 mutation and another a deletion of 9p. Phenotype of partial 46, XY GD is variable and diagnosis can be difficult when biochemistry is normal with no histological analysis (patients raised as males). SF1 mutations in isolated GD seem to be frequent.

**P1-d2-238 Reproductive Endocrinology 1**

A novel heterozygous GATA4 mutation in a patient with 46,XY DSD

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Gata4 is a transcription factor of the zinc-finger family, which plays a role in murine gonad and heart development. Gata4 is expressed in the urogenital rigde and the heart during embryonic development. The Gata4 knockout mouse presents with a severe heart defect and absence of the gonads. Recently, heterozygous GATA4 mutations were described in few patients with androgen insensitivity. The Gata4 mutation in 46,XY DSD patients with associated heart defect were found. We have sequenced the GATA4 gene in 87 patients (8 patients with associated heart defect) with 46,XY disorders of sex development (DSD). We have detected one heterozygous missense mutation p.A200P/WT (c.598 G>C, Exon 2) in a patient with severe hypospadias, bifid scrotum, palpable gonads, pulmonary artery stenosis and patent ductus arteriosus at birth. No Muellerian structures were visible by ultrasound. The patient was born small for gestational age (1600g) at 24 weeks of gestation. Testosterone increased normally from 2.0 to 7.1 ng/ml after HCG stimulation at 3 months. The mutation p.A200P/WT was absent in 100 healthy controls. Functional characterization of the mutation is in progress. Mutations of the androgen receptor and 5alpha-reductase gene were excluded in the patient. Mutations in GATA4 seem to be rather rare in patients with 46,XY DSD, but should be considered in patients with associated heart defects. However, the minor heart defect in the patient might also be an independent association.

**P1-d2-239 Reproductive Endocrinology 1**

A 46 XY Girl with fragile X syndrome and a novel nonsense mutation (Q116X) in the AR gene

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Background: Premature ovarian failure is well known to be associated with Fragile X Syndrome, but not sex reversal. Androgen insensitivity syndrome is the most common cause of sex reversal in a male and is caused by androgen receptor (AR) gene mutations. The AR gene is located on the X chromosome at Xq11-12 consisting of eight exons. There can be a wide variety of phenotypic abnormalities ranging from partial androgen insensitivity syndrome to complete androgen insensitivity syndrome based on the functional abnormality caused by the AR gene mutation.

Objective: To describe a novel mutation in AR gene that resulted in complete sex reversal in a 46XY girl with Fragile X Syndrome.

Methods: We describe a 15 month girl with Fragile X Syndrome (>200 CGG Repeats), of Bangladeshi origin who came to medical attention for developmental delay and macrocephaly at which time the karyotype was noted to be 46 XY. The child has normal female genitalia with no clitorormegaly, no palpable testes in the labia or inguinal canal. Neither uterus nor ovaries were seen on MRI of the pelvis. HCG stimulation test revealed a good testosterone response from 45 ng/dl to 699ng/dl. The AR gene was subsequently analyzed.

Results: Sequencing revealed the presence of a novel CAG to TAG nonsense mutation (Q116X) in exon 1 of the AR gene. This mutation results in a glutamine substitution which induces a stop codon.

Conclusions: To our knowledge this is the first report of this Q166X mutation in the AR gene. This is the first account of androgen insensitivity syndrome and Fragile X Syndrome to be reported in the same patient. The mutation in the AR gene causing the complete androgen insensitivity in our patient is thought to be unrelated to the Fragile X Syndrome however the FMR1 gene is located on Xq27.3.
The first and the rate-limiting step in the biosynthesis of hormones in all steroidogenic tissues, conversion of cholesterol to pregnenolone, is catalyzed by the cholesterol side-chain cleavage cytochrome P450 (P450scc) encoded by a single gene CYP11A1. Until now mutations in CYP11A1 gene were reported in 6 patients, all of whom presented with adrenal insufficiency within the first 4 years of life, gonadal failure in both sexes and severely under-androgenized external genitalia (Prader stages 1-2) in 46,XY individuals. Here we identified and functionally characterized a novel homozygous mutation in P450scc, which was associated with late-onset adrenal insufficiency and sexual ambiguity in a male. A 46, XY patient was born at term to consanguineous parents. At birth he presented with hypospadias and bilateral cryptorchidism and was assigned as male. At 5 yrs the hypospadias and cryptorchidism were surgically corrected without any complications. At 9.5 yr the patient presented with lethargy, hyperpigmentation, salt loss, undetectable adrenal steroids and elevated ACTH. Hormonal replacement with hydrocortisone and 9α-fluorocortisone was started. A defect at the early step of the biosynthesis of adrenocortical and gonadal steroids was suspected. Results of STAR gene sequencing were normal. Direct sequencing of CYP11A1 gene revealed a novel homozygous L222P mutation. To clarify effect of this mutation on P450scc function, HEK293 cells were transfected with human adrenodoxin, adrenodoxin reductase and STAR expression plasmids together with either wild-type (p450scc-WT), mutant P450scc (P450scc-L222P) or empty expression plasmids. P450scc activity was determined by measuring concentration of pregnenolone synthesized from cholesterol in the medium. P450scc activity of P450scc-L222P mutant was ~7% compared to P450scc-WT. The results confirm functional significance of L222P mutation and illustrate existence of a milder phenotype associated with P450scc deficiency.

The Mayer-Rokitansky -Küster-Hauser syndrome (MRKH) is defined by a normal development of the female phenotype with thelarche and pubarche, primary amenorrhea with Müllerian duct hypoplasia in XX adolescent patients. The molecular basis of the MRKH syndrome is currently unknown.

The finding of Wnt4 mutation in 3 patients with MRKH syndrome associated with hyperandrogenism prompted us to study this gene in a small series of 4 adolescents with MRKH and hyperandrogenism. The absence of mutation in 3 out of 4 adolescents with MRKH and hyperandrogenism does not exclude a potential abnormality within the Wnt4 transduction signal. We suggest that in adolescent girls with primary amenorrhea, Müllerian duct abnormality and hyperandrogenism, a Wnt4 mutation should be sought.

A new mutation within the Wnt4 gene was identified in 1 out of 4 patients. Our data confirm that Wnt4 is involved in the regulation of Müllerian duct development and ovarian androgen biosynthesis.

Context: PCOS is a common endocrine-metabolic disorder with strong familial aggregation. It has been demonstrated that an important proportion of male members of PCOS families exhibit insulin resistance and related metabolic defects. However, the reproductive phenotypes in firstdegree male relatives of PCOS women have been less described.

Objective: To evaluate the pituitary-testicular function and Sertoli cell function in sons of women with PCOS during different stages of life: early infancy, childhood and adulthood.

Design: Eighty four sons of women with PCOS (PCOSS) and 60 sons of control women without hyperandrogenism (CS), matched for age, were studied. In infants, children and adults of both groups, the pituitary gonadal axis was evaluated by a GnRH agonist test (leuprolide acetate, 10 μg/Kg s.c.). Serum anti-Müllerian hormone (AMH) and inhibin B were used as Sertoli cell markers. Serum concentrations of gonadotropins, steroid hormones, sex hormone binding globulin (SHBG), inhibin B and AMH were determined by specific assays.

Results: Basal concentrations of gonadotropins, sex steroids and inhibit B were similar between PCOSS and CS during early infancy, childhood and adulthood. Stimulated gonadotropin and sex steroid concentrations were also similar in both groups in the three study periods. However, AMH serum concentrations were significantly higher in PCOSS compared to CS during early infancy (925.0 (457.3 - 1401.7) pmol/l vs 685.6 (417.9 - 1313.2) pmol/l, p=0.039) and during childhood [616.3 (304.6 - 1136.9) pmol/l vs 416.5 (206.7 - 801.2) pmol/l, p=0.007].

Conclusions: We conclude that AMH concentrations are increased in prepubertal sons of women with PCOS, suggesting that these boys may show an increased Sertoli cell number or function during infancy and childhood. Supported by FONDECYT Grants 1050915 and 1030487.
togenesis over a time span similar to that of normal puberty. We aimed to compare past treatment outcomes, using gonadotrophins in adult males for fertility induction, with similar treatment regimens for induction of puberty in adolescent males. Five entirely pre-pubertal males aged 13-19 were treated with HCG 1500IU x 2 per week for 3 months with addition of FSH in 2/5. Linear growth, pubertal status and testicular volume were measured at 0.6 and 8 months with onset of spermatogenesis determined by spermaturia. Findings were compared with those of 4 men aged 27-33 given similar treatment for 2 years. The pre-pubertal group had a mean bilateral testicular volume of 4ml at baseline, 23ml at 6 months and 28ml at 8 months, compared with the adult group who achieved mean bilateral volume of 10ml by conception, with spermatogenesis occurring a mean 21.7 months from start of treatment. HCG and rFSH to treat hypogonadotrophic hypogonadism in pubertal boys results in normal linear growth, normal pubertal progress, testicular growth and normal spermatogenesis, over a time span similar to that of normal puberty. Delayed treatment results in impaired fertility and need for prolonged treatment to achieve fertility.

**P1-d2-244 Reproductive Endocrinology 1**

*Ascensus testis is a frequent cause of cryptorchidism in infancy and childhood*

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Accurate prevalence data for acquired cryptorchidism, also called ascensus testis, are currently sparse and systematic prospective studies have not yet been reported. In a prospective longitudinal population-based child cohort from Copenhagen, Denmark (1997 - 2007), testicular position was examined according to a standardized protocol in a total of 1071 boys, at birth (n=1051), at 3, 18 and 36 months of age, respectively. When including recurrent cryptorchidism the prevalence was 0.2%, 1.2% and 0.8%, respectively. Thus, ascensus testis accounts for 58% of all cases of cryptorchidism (congenital and acquired) at 18 months, 71% at 36 months and thereafter 69%. Ascensus testis accounts for more than half of cryptorchid testes seen in childhood and occurs in both previously scrotal and cryptorchid testes. Thus, regular examinations of testis position during childhood should be performed on all boys.

**P1-d2-245 Reproductive Endocrinology 1**

*Congenital cryptorchidism and dioxin levels in breast milk and placenta*

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Dioxins are persistent fat-soluble environmental toxins. They accumulate into human body especially via fatty foods. In animal studies, fetal and lactational exposure to TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) has been shown to affect the male reproductive system, including testicular descent. The aim of this study was to evaluate whether congenital cryptorchidism is associated with dioxin levels in placenta and breast milk samples reflecting fetal exposure. The study included breast milk and placenta samples representing cryptorchid or healthy boys attending the Danish-Finnish cohort study on the incidence and risk factors of cryptorchidism. The boys were born 1997-2001. They were examined for cryptorchidism at birth and at 3 months. Breast milk samples were collected as additive aliquots between the ages of 1 and 3 months. Altogether 280 placenta samples [112 Finnish (56 cases and 56 controls), 168 Danish (39 cases and 129 controls)] and 130 breast milk samples [65 Finnish (33 cases and 32 controls), 65 Danish (29 cases and 36 controls)] were analysed for the 17 toxic polychlorinated dibenzop-dioxin and dibenzofurans (PCDD/Fs).

**Placenta Samples:** In Danish boys, median (range) PCDD/F WHO-TEq* (pg/g fat) was 11.8 (7.4 - 27.4) in cryptorchid boys and 10.9 (4.2 -31.3) in healthy boys, p=0.25. In Finnish boys, the level was 9.8 (4.0 - 22.4) in cases and 8.5 (3.0 - 30.6) in controls, p=0.39.

**Breast Milk Samples:** The median (range) PCDD/F WHO-TEq (pg/g fat) in Danish cryptorchid boys was 15.5 (9.0 - 28.3) and 11.4 (4.7 - 26.0) in controls, p=0.02. In Finnish boys, there was no significant difference between cases and controls 11.0 (4.0 - 25.2) vs. 10.5 (3.9 - 31.9), p=0.75. In conclusion, in the Danish group, but not among Finnish boys, congenital cryptorchidism was associated with increased maternal breast milk levels of PCDD/Fs. No association was found between placental dioxins and congenital cryptorchidism.

*WHO-recommended 2,3,7,8-TCDD equivalent quantity for PCDD/Fs.

**P1-d2-246 Reproductive Endocrinology 1**

*Testicular histology related to fertility outcome and post pubertal hormone status in cryptorchidism*

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**Background:** Early surgical correction of an undescended testis is performed to prevent the development of male infertility. However, in boys with cryptorchidism early successful surgery cannot prevent infertility if they lack Ad spermatogonia. In this study, sperm concentrations and postpubertal hormone levels were correlated to bilateral testicular histology. The aim was to define the risk of future infertility via a testis biopsy program for boys with cryptorchidism.

**Methods:** Eighty-nine boys who had an orchidopexy were subjected to bilateral testicular biopsy. Histological analysis of 178 biopsies indicated three groups of high, intermediate, and low risk of infertility according to the presence of Ad spermatogonia. After puberty, sperm concentrations were analyzed and correlated with plasma gonadotropin and testosterone levels.

**Findings:** In patients with unilateral cryptorchidism 70% of scrotal testes had impaired transformation of Ad spermatogonia, indicating that cryptorchidism is a bilateral disease. Sperm concentrations correlated to the number of Ad spermatogonia found at the time of orchidopexy (p=0.0005). All males in the high risk of infertility group were oligospermic (mean: 8.9 x 106 sperm/ejaculate) and 20% were azoospermic. These patients had 25 times less sperm...
Sexual precocity in McCune-Albright syndrome (MAS) has been reported in
only 15 % of boys, and little is known on the long-term evolution of MAS
in males. In a boy with MAS we studied spermatogenesis, testis histology
and immunohistochemistry with the aim to shed light on seminiferous tubule
activity. A boy who presented at the age of 2.9 years with sexual precocity,
monolateral macroorchidism, increased testosterone levels and suppressed
gonadotropins, was followed up until the age of 18. Throughout follow-up
testicular asymmetry persisted and gonadotropin and testosterone parameter
did not change. At the age of 18, inhibin B was undetectable while alpha-immu-
noreactive inhibin was within normal range. Anti-Mullerian hormone level
was slightly subnormal. Sperm cells were 3 900 000 per ejaculate. Histology of
both testes showed spermatogenesis, spermatocytes, and, in some tubules,
mature spermatozoa. Sertoli cells were markedly stained with anti-inhibin alpha-
subunit antibody in both testes. There was no immunostaining of Sertoli,
Leydig or germ cells with anti-beta or anti-betaB antibody. MAS R201H
mutation was identified in both testes. Conclusion: The 15-year follow-up in
this boy with MAS demonstrated that autonomous testicular activation and
gonadotropin suppression persisted over time. This provides an interesting
model of active spermatogenesis despite long-term FSH suppression. It also
suggests that FSH is needed for full expression of the inhibin betaB-subunit
gene, expression previously reported in normal adult subjects in germ and
Leydig cells.

Results: IS was strongly correlated to breast stage (p = 0.01) in girls, and
with genital stage (p = 0.001) as well as testicular volume in boys (p = 0.001).
IS showed a curvilinear pattern with high pre-pubertal levels followed by a
decline towards mid-puberty, after which an increase was seen in late puberty.
In gender and puberty adjusted models IS was significantly correlated with
age (p = 0.007), TBFG (p = 0.002) and IGF-I levels (p = 0.004), respectively.
No correlation was found to VO2max (p = 0.597). In a multivariate analysis
(GLM) including all factors IS remained significantly associated with puber-
ty, age, TBFG and IGF-I, but not with VO2max or gender (adjusted R2 =
0.435). No correlations was found between GH-R genotypes and IS.
Conclusion: Adolescent insulin sensitivity was strongly dependent on pu-
bertal development, age, TBFG and IGF-I, but not on VO2max or GH-R
genotypes.

P1-d2-247 Reproductive Endocrinology 1

Regulation of spermatogenesis in McCune-Albright syndrome:
Lessons from fifteen year follow-up
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P1-d2-248 Reproductive Endocrinology 1

Changes in insulin sensitivity during puberty:
Relation to body composition, physical fitness and the GH-IGF-I axis
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Background: In adults, insulin sensitivity (IS) is dependent on body compo-
sition, physical fitness and the GH-IGF-I axis. Marked changes in IS occur
during puberty, but the primary determinants remain unclear.
Objective: The objective of the present study was to evaluate IS during puberty in relation to physical fitness (VO2max), total body fat percentage (TBFG) and IGF-I levels in healthy children and adolescents, and to evaluate the potential influence of a common polymorphism in growth hormone recep-
tor (GH-R), the exon 3 deletion.
Population and Methods: Hundred and thirty-two healthy Caucasian sub-
jects (70 girls) aged 8.5 - 16.1 years were recruited as a part of the COPEN-
HAGEN Puberty study. TBFG was evaluated with a whole body DEXA scan.
Physical fitness was determined by cycle ergometry with direct measurement
of maximal oxygen uptake. IS was calculated from a standard 2-hour oral
glucose tolerance test. The GH-R genotypes were determined by multiplex PCR.

P1-d2-249 Reproductive Endocrinology 1

Premature thelarche — Characteristics at presentation and clinical follow-up
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Premature thelarche (PT) is defined as isolated breast development without
any other signs of sexual maturation. The possible progression of PT towards
true precocious puberty (PP) is not well established. The study aimed at inves-
tigating the clinical course and growth pattern of girls with PT. The charts of
140 girls with PT followed in our clinic between 1995 and 2005 were review-
ed. Data on general and endocrine evaluation, course of growth and puberty,
and the outcome of PT were collected. Analysis was conducted by age at appearance of PT: birth (n=58), 1-24 months (n=62), and 2-8 years (n=20). PT was diagnosed at mean age of 2.1±1.8 years. It was bilateral in 108 girls (93
-Tanner stage 2; 15 -Tanner stage 3), and unilateral in 32 girls (all -Tanner stage
2), (p=0.02). The prevalence of unilateral PT was similar in all age groups.
Anthropometric, bone age and laboratory parameters on admission were comparable in the 3 age groups, except for a lower weight SDS (p<0.05) on admission, significant increase in height-SDS during follow-up, (0.06±0.75 to
0.36±0.87 p<0.04), and higher FSH (basal and GnRH stimulated) (p<0.05), in
girls with PT who presented before the age of 2 years. Mean duration of follow-
up was 3.0±2.5 years (0.2-9.3). PT regressed in 51 girls, persisted in 46,
grown to a severe pattern in 13. A progressive or cyclic course was significantly more prevalent among girls with PT presented after the age
of 2 year (55%) compared to girls who presented at birth (13.3%) or at age
1-2 months (5.9%), (p<0.001). Only 7 girls (5.9%) progressed to PP: three
with PT appearing at birth, one - at 6 months, and three between 4.4-6.08
years. Our data confirm the benign nature of PT. Late presentation (after age
2 years) is associated with a higher rate of either progressive or cyclic course.
Neither age at presentation nor course of thelarche appears to be associated
with different anthropometric, biochemical or bone age characteristics.

P1-d2-250 Reproductive Endocrinology 1

Validation of BoneXpert in children with precocious puberty
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BoneXpert performs fully automated analysis of hand radiographs and de-
termines Greulich Pyle bone age (BA). The aim of this study was to asses the
performance of BoneXpert in all X-rays performed in our patients with
precocious puberty (PP) between 1976 and 2007. A total of 752 X-rays from
13 boys and 103 girls with PP of various etiologies (age range 0.3 to 14.8 y;
mean BA acceleration 2.3 years) were rated by BoneXpert. The rating was
compared to the original Greulich Pyle rating (manBA, performed by one
of three experts in our hospital). BoneXpert analyzed all 752 images except
17 X-rays: 3 had poor quality and had not even been rated manually and 14
were rated by manBA. In gender and puberty adjusted models IS was signifi-
cantly associated with pubertal stage (p=0.012); no correlations was found between GH-R genotypes and IS.
These were re-rated by the authors not knowing the chronological age, man-BA or BoneXpert’s rating. All the new ratings (large circles in Figure) were within 1.5 years of the automatic BA values except 5. In 2 of these, man-BA placed large emphasis on the accelerated carpal bones, which BoneXpert ignores.

The accuracy (standard deviation) between manual and automatic rating is 0.69 years [0.65; 0.72] 95% CI; mean difference 0.08 years (n.s.), bias 0.08 y/y (n.s.).

Conclusion: BoneXpert’s ability to process virtually all image automatically, to avoid errors, and to obtain good agreement with an operator for BA range 2 - 16 years suggests that the method is efficient and reliable in children with precocious puberty.

**P1-d2-251 Reproductive Endocrinology 1**

**Extractive testosterone: Contribution to the diagnostic evaluation of female precocious pubertal development**

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Early modifications of testosterone (T) of ovarian origin occur during female pubertal development as a result of LH pulsatility onset (Mitamura R et al, JCEM 2000). To analyze the usefulness of T measurement, with extractive methodology (Text) in female pubertal pathology and to compare its diagnostic efficiency (Def) with other biochemical parameters, we have retrospectively studied 83 patients whose diagnoses were confirmed by clinical follow-up (3.7 years): Non-precocious Pubertal Telarche (PT) = 59, Central Precocious Puberty (CPP) = 24. In all patients serum Text was measured in a modified DPC Coat a Count assay. Both serum samples and Standard assay (Testosterone Sygma T1500) were measured after n-hexane and diethyl ether extraction (Sensitivity: 0.17 nmol/L). LH Spec, FSH and E2 were also measured. LHRR test was performed in 23 patients with PT and in 20 patients with CPP. The cut off for Text was obtained by ROC plot, in 83 healthy girls (CG): 38 prepubertal (pp) and 45 pubertal (p) girls. Text (nmol/L, median) was obtained in: PT: 0.17, CPP: 0.35, CG-pp: 0.21 y CG-p: 0.73. We found p < 0.05 in CG-p vs CG-pp and PT and in PT vs CPP (Kruskal-Wallis - Dunn test). ROC plot analysis showed a cut off for Text of 0.35 (S: 0.86, Sp: 0.87, Def: 87%). We have used a cut off 0.30 IU/L for LH, previously reported (Sequera et al, Ped Research, 1999). 49/59 patients had LH > 0.30. Of 10 PT with Text < 0.35, 2 had LH > 0.30. 14/24 CPP had Text > 0.35. Out of 10 CPP with Text < 0.35 only 1 had LH > 0.30. To differentiate PT from CPP the Text showed Def of 76%, LH spec: 79% and both of 81%. All tests performed in PT resulted in a prepubertal response. 5/20 LHRR tests in CPP showed a prepubertal response with Text > 0.35.

Conclusion: This study suggests that the Text measurement contributes to a wider characterization of female patients with precocious pubertal development. We propose the association of Text and LH spec measurement in the initial screening of these patients.

**P1-d2-252 Reproductive Endocrinology 1**

**Ovarian volume correlates with inhibin B levels in girls with central precocious puberty but not in girls with premature telarche**

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Precocious puberty (PP) in girls is defined as the appearance of secondary sex characteristics before the age of 8 years, mostly caused by premature idiopathic activation of the hypothalamic gonadotropin releasing hormone pulse generator. Like PP, premature thelarche (PT) is characterized by early breast development, but it is not associated with acceleration of growth or bone maturation and thus does not require therapy. GnRH stimulation test is considered the gold standard for diagnosis despite its accuracy remains controversial. Ovarian Volume (OV) is also used as a diagnostic tool, as well as Inhibin B levels. We evaluated the correlation between OV and inhibin B levels in girls with CPP and PT in order to identify a new combined diagnostic tool. Thirty-three girls were studied and separated into three groups: group A, CPP (n = 15), group B, PT (n = 8) and group C, prepubertal girls (n = 10). The mean age was different in the three groups. Patients with secondary precocious puberty were excluded from the study. OV was acquired by a GE730 Voluson machine equipped with a transabdominal volume transducer. All the stored volumes were off line analyzed by using the VOCAL® imaging program set to manual contour mode. No difference was found between the OV of girls of group A and B (2.52 vs 3.21 ml, p = n.s.), whereas the difference was evident with group C (1.2 ml p < 0.001) thus, the only OV didn’t differentiate the two conditions. As previously demonstrated, inhibin B levels were different between group A and B when the whole CPP cohort was considered, but the significance disappeared when only girls in Tanner stage 2 were considered. Analyzing together OV and inhibin B, a significant correlation was found only in girls with CPP (p < 0.01). The association of OV > 2 ml with inhibin B levels > 20 pg/ml could represent a reliable tool for diagnosis of CPP.

**P1-d2-253 Reproductive Endocrinology 1**

**Usefulness of GnRH infusion test in the diagnosis of boys with delayed puberty**

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In boys who present with delayed puberty, the differential diagnosis between hypogonadotropic hypogonadism (HH) and constitutional delay of puberty is difficult. Although different tests have been proposed to distinguish these two conditions, no consensus exists. The aim of this study was to evaluate the usefulness of the GnRH infusion test to predict complete or partial HH in boys presenting with delayed or arrested puberty. Thirty-five boys (16.4 ± 3.0 yr, 30 with absence of and 5 with arrested puberty) were submitted to IV GnRH infusion (0.83 ug/min for 120 min). LH and FSH were determined by IFMA generator. Like PP, premature thelarche (PT) is characterized by early breast development, but it is not associated with acceleration of growth or bone maturation and thus does not require therapy. GnRH stimulation test is considered the gold standard for diagnosis despite its accuracy remains controversial. Ovarian Volume (OV) is also used as a diagnostic tool, as well as Inhibin B levels. We evaluated the correlation between OV and inhibin B levels in girls with CPP and PT in order to identify a new combined diagnostic tool. Thirty-three girls were studied and separated into three groups: group A, CPP (n = 15), group B, PT (n = 8) and group C, prepubertal girls (n = 10). The mean age was different in the three groups. Patients with secondary precocious puberty were excluded from the study. OV was acquired by a GE730 Voluson machine equipped with a transabdominal volume transducer. All the stored volumes were off line analyzed by using the VOCAL® imaging program set to manual contour mode. No difference was found between the OV of girls of group A and B (2.52 vs 3.21 ml, p = n.s.), whereas the difference was evident with group C (1.2 ml p < 0.001) thus, the only OV didn’t differentiate the two conditions. As previously demonstrated, inhibin B levels were different between group A and B when the whole CPP cohort was considered, but the significance disappeared when only girls in Tanner stage 2 were considered. Analyzing together OV and inhibin B, a significant correlation was found only in girls with CPP (p < 0.01). The association of OV > 2 ml with inhibin B levels > 20 pg/ml could represent a reliable tool for diagnosis of CPP.

In boys who present with delayed puberty, the differential diagnosis between hypogonadotropic hypogonadism (HH) and constitutional delay of puberty is difficult. Although different tests have been proposed to distinguish these two conditions, no consensus exists. The aim of this study was to evaluate the usefulness of the GnRH infusion test to predict complete or partial HH in boys presenting with delayed or arrested puberty. Thirty-five boys (16.4 ± 3.0 yr, 30 with absence of and 5 with arrested puberty) were submitted to IV GnRH infusion (0.83 ug/min for 120 min). LH and FSH were determined by IFMA at 0, 15, 30, 45, 60 and 120 min. Final diagnosis of complete HH (n = 17) was done when testes were < 4 ml at 18 yr. Partial HH (n = 11) was diagnosed when testes enlargement started but remained arrested for a period of at least 1 yr and did not reach 15 ml. ROC curves were used to determine the optimal cut-off points. LH peak occurred variably between 15 and 120 min after infusion start, while FSH peak occurred at 120 min in 32 of 35 patients. LH peak value < 5.8 IU/L showed a 95.8 % positive predictive value (PPV) for HH, with 82% sensitivity and 85% specificity; the positive likelihood ratio (LR+) was 5.8. FSH peak value < 4.6 IU/L showed similar results. Interestingly, basal FSH < 1.15 IU/L had a 100% PPV for HH, with 64.3% sensitivity and 100% specificity. A peak LH > 5.8 IU/L and peak FSH > 4.6 IU/L in the same patient detected delayed puberty with 85.7% sensitivity and 89.2% specificity; the
Many studies report the occurrence of polycystic ovary syndrome (PCOS) following idiopathic central precocious puberty (ICPP) and it is unclear whether the association of PCOS with ICPP is any more frequent than PCOS would be expected by chance. The aims of this study were to find the prevalence of PCOS in a cohort of women who had ICPP as defined by the Rotterdam 2003 criteria, and to detect any predictive factors of PCOS at the time ICPP was diagnosed. Forty-six young women who had been treated with GnRH analogues during infancy, were observed at gynaecological age of 6.23 ±3.3 yrs post-menarche. We found that 15% of the women had oligomenorrhea, 28% had clinical hyperandrogenism, 48% biochemical hyperandrogenism and 50% poly cystic ovary morphology (PCOM). 41% had PCOS according to the Rotterdam 2003 criteria. The prevalent phenotype of PCOS was characterized by clinical and/or biochemical hyperandrogenism and PCOM. We did not find any predictive factors for PCOS at the time ICPP was diagnosed. In conclusion, patients with ICPP are prone to developing PCOS. The prominent phenotype in our cohort was due to PCOM associated with clinical and/or biochemical hyperandrogenism. Further follow-ups of these young adult patients will clarify whether this phenotype persists and if it will have important long-term implications in terms of increased risk of infertility or metabolic complications.

To study the neural substrate of EFs, we used the Tower of London planning task, adapted for the scanning environment. Until now, 35 healthy adolescents (mean age 16.33 years, range 11-20 years) were scanned, results of which show robust activity of the PFC.

In summary, our results demonstrate that fMRI is applicable even in young children, as we were able to acquire task-related data in an episodic memory paradigm, which can be used from the age of 4. Also, fMRI can be used to study the neural substrate of complex higher-order functions such as planning. fMRI therefore is a promising tool to study cognitive changes during brain development, including the effects of growth hormone therapy or puberty blocking medication in children and adolescents.

**P1-d2-255 Reproductive Endocrinology 1**

**Functional magnetic resonance imaging (fMRI) in children, promising preliminary data**

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Paediatric brain imaging studies have shown an increase of cortical grey and white matter volume across the age range of 4 to 20 years. In children with endocrine abnormalities, aberrant hormone activities may interfere with brain development. Especially thyroid hormone, corticosteroids and growth factors, receptors of which are found throughout the brain are suggested to be important in this respect. Functional MRI (fMRI) allows non-invasive assessment of brain functioning and is an exciting tool in neuroscience: It allows us to "watch the brain thinking". Especially in longitudinal designs, it may also contribute to better understanding of functional brain maturation. At present, one of the critical limitations is that fMRI is extremely sensitive to motion and therefore difficult to perform in young children. To address this issue, we developed an extensive subject preparation protocol including a mock MRI session in a dummy scanner. Using this protocol, we were able to collect fMRI data also from very young children (range, 4.5 - 20 years). In our research to date, we focused on episodic memory, associated with intact medial temporal lobe function, and executive functions (EF), associated with dorsolateral prefrontal cortex. Development of the PFC continues through adolescence. To investigate brain activity associated with declarative memory processes, we designed a visual encoding task, suitable to children aged 4 to 7 years. Neuropsychological tests of general intelligence and memory were also administered. We here report data from 20 healthy children (mean age 6 ± 1 years, range 4.5-7.2 years). Preliminary analyses revealed robust activity of the parahippocampal and hippocampal areas during encoding versus baseline (picture).

**P1-d2-256 Reproductive Endocrinology 1**

**Effect of prenatal and early postnatal androgens on adult hippocampal pyramidal neuron count and morphology of preoptic area in rats**

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1Pamukkale University, School of Medicine, Pediatric, Denizli, Turkey; 2Pamukkale University, School of Medicine, Pediatric Endocrinology, Denizli, Turkey; 3Pamukkale University, School of Medicine, Anatomy, Denizli, Turkey; 4Pamukkale University, School of Medicine, Histology, Denizli, Turkey

Hippocampus is implicated on functions related to learning, and memory and hippocampal morphology exhibits sexual differences. Mechanisms were not clearly exhibited. Hippocampus is developmentally sensitive to gonadal steroids especially in prenatal period. Preoptic Area (POA) is the critical neural substrate underlying gender specific behaviors and morphologically different in both sexes. It is known that androgens assessed in prenatal period enlarge
POA volume and contribute to male specific behaviors. Although previous studies demonstrated that administration of testosterone to newborn rats changes astrocyte morphology in POA, there is no information whether these differences continue in adulthood. In our study we investigated the effects of testosterone propionate (TP) administered in prenatal and postnatal periods on pyramidal cell count of hippocampus and morphology of astrocyte in rats. Wistar Albino rats were used in the study. Rats were assigned to one of 3 treatment regimes, including prenatal androgen group (maternal TP assessed in pregnancy), prenatal and postnatal androgen group (maternal TP assessed in pregnancy and TP assessed in postnatal period), and postnatal androgen group (TP assessed in postnatal period). Sesame oil was injected to male and female control group. When the rats were adult, they were sacrificed under general anesthesia after intracardiac perfusion. Acquired sections were exposed to cell count in hippocampal pyramidal layer with cresyl violet and astrocyte morphology in POA visualized by glial fibrillary acidic protein (GFAP) immunocytochemistry. hippocampal cell count was higher in the male controls than in the female controls (p=0.01). The cell count in both prenatal and postnatal androgen groups was higher than that in female controls (p<0.03).

In prenatal and postnatal androgen group, primary and secondary astrocyte branching count was higher than that in both postnatal androgen and male control groups. No statistical study was done because most sections couldn’t be visualized by GFAP immunocytochemistry.

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**P1-d3-257 GH Treatment 2**

**Effect of growth hormone treatment on insulin secretion and sensitivity in relation to growth of children born small for gestational age**

Burak Salgin
University of Cambridge, Paediatrics, Cambridge, United Kingdom

NESGAS is a randomised multi-centre trial to evaluate the safety and efficacy of GH treatment at varying doses in short SGA children without catch-up growth. The aim of this analysis was to explore the relationship between changes in insulin secretion and sensitivity, IGF-I levels and growth over one year of treatment. We studied 32 (22 males, 10 females) pre-pubertal children born SGA (aged 3.9-9.9 years) who had failed to show catch-up growth and were naïve to GH treatment. Fasting blood samples were taken to assess IGF-I, glucose and insulin levels. Subjects underwent a short intravenous glucose tolerance test to measure acute insulin secretion, and HOMA was used to calculate insulin sensitivity. The disposition index gave an estimate of insulin secretion for the degree of insulin sensitivity. These measurements were repeated after treatment with GH (67µg/kg/day) for 12 months. GH treatment resulted in increases in SDSs for height (-3.1±0.6 to -2.2±0.7, p<0.001), height velocity (-1.2±1.2 to 3.6±1.9, p<0.001) and IGF-I (44.0±24.2pmol/l, p<0.001) and acute insulin secretion (area under the curve: 1593.0±909.4 to 3146.8±2873.7pmol/l, p=0.001) increased so that the disposition index remained similar (31.7±17.8 vs 31.6±15.3, p=1.0). Backward stepwise regression identified sex, age at baseline, and the increases in basal insulin levels and acute insulin secretion as the most important determinants of the improvement in height velocity with GH therapy (R²=0.635, p=0.001). The increase in IGF-I levels adequately compensated for the fall in insulin sensitivity with GH therapy (R²=0.5, p=0.001). Backward stepwise regression identified sex, age at baseline, and the increases in basal insulin levels and acute insulin secretion as the most important determinants of the improvement in height velocity (p<0.05). In TS within the low IGF-I responder groups, expression was analysed within the upper or lower quartile in IGF-I SDS and between upper and lower quartiles at baseline for both experiments; only those results that were unlikely to occur by chance are presented.

Results: In GHD patients within the low IGF-I responder group, expression of 49 genes (notably JAK2) changed in response to GH in both experiments (p<0.05 for this to have occurred by chance). In TS within the low IGF-I responder group, the expression of only two genes changed (p<0.05). In GHD at baseline, 16 genes were differentially expressed between high and low IGF-I responders (p<2E-04), but none in TS. There were no genes common to both experiments in response to GH in the high IGF-I responder groups in GHD and TS.

Conclusions: Expression profiling has identified genes that are modulated by GH only in the low IGF-I responder groups (49 genes in GHD and only two in TS). Importantly, baseline gene expression in GHD was different between high and low IGF-I responders.

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**P1-d3-258 GH Treatment 2**

**Pangenomic gene expression profiles associated with changes in serum insulin-like growth factor-I (IGF-I) in prepubertal children with growth hormone deficiency (GHD) or Turner syndrome (TS) after one month of GH therapy: The PREDICT Study**

Peter Clayton¹; Pierre Chatelain²; Sei-Won Yang³; Roland Pfäffle⁴; Berndt Destrénæs⁵; Marc Lamanire⁵; Luciano Tato⁶

PREDICT Investigators
'St Mary’s Hospital, Children’s Outpatient Department, Manchester, United Kingdom; "Hôpital Debrousse – Université Claude Bernard, Faculté de Médecine – Pédiatrics, Cedex 05, France; "Seoul National University College of Medicine, Pediatrics, Seoul, Republic of Korea; "Kinderzentrum Endokrinologie Station KIK3, Kinderzentrum, Leipzig, Germany; "Serono International S.A., Research, Genes and Protein Sciences, Geneva, Switzerland; "Serono International S.A., Research and Pharmaceutical Development, Geneva, Switzerland; "Università degli Studi di Verona, Clinica di Pediatría, Verona, Italy

Background: The PREDICT study investigates the relationships between genomics and biomarkers before and during GH therapy in children with GHD or TS.

Objective: Analysis of mRNA expression in relation to changes in IGF-I SDS after one month of GH treatment in previously untreated children with GHD (n=169) or TS (n=149).

Methods: Blood samples were obtained before and after one month of GH treatment (Saizen®, Merck Serono) at approved doses. Serum IGF-I was measured centrally, corrected to age and gender-related standard deviation scores (SDS), and the change in IGF-I SDS after one month of GH therapy was categorized by quartiles. mRNA was extracted and hybridized (Affymetrix GeneChip® HG-U133 plus 2.0), followed by array analysis, with two experiments conducted on independent subgroups of subjects. Genes showing a ≥1.4-fold change in absolute value and significant (p<0.05) change in expression were analysed within the upper or lower change in IGF-I SDS quartiles and between upper and lower quartiles at baseline for both experiments; only those results that were unlikely to occur by chance are presented.

Results: In GHD patients within the low IGF-I responder group, expression of 49 genes (notably JAK2) changed in response to GH in both experiments (p<1E-05 for this to have occurred by chance). In TS within the low IGF-I responder group, the expression of only two genes changed (p<0.05). In GHD at baseline, 16 genes were differentially expressed between high and low IGF-I responders (p<2E-04), but none in TS. There were no genes common to both experiments in response to GH in the high IGF-I responder groups in GHD and TS.

Conclusions: Expression profiling has identified genes that are modulated by GH only in the low IGF-I responder groups (49 genes in GHD and only two in TS). Importantly, baseline gene expression in GHD was different between high and low IGF-I responders.

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**P1-d3-259 GH Treatment 2**

**Three years safety and efficacy of a novel once-a-week sustained release rhGH (LB03002) in treatment-naïve prepubertal children with GHD**

Ferenc Peter¹; Conrad Savoy²; Hyi-Jeong Ji³; Mihaly Juhasz⁴; Paul Saenger⁵

¹Buda Children’s Hospital, for Biopartners-LG Life Sciences GH Study Group, Budapest, Hungary; Biopartners GmbH, , Baar, Switzerland; ‘LG Life Sciences Ltd., , Seoul, Republic of Korea; ‘Accelsiors Ltd., , Budapest, Hungary; ‘Albert Einstein College of Medicine, , New York, United States

LB03002 is a novel once-a-week subcutaneous sustained release rhGH. Less frequent administration could provide a considerable improvement on patients’ compliance and convenience. Previously untreated children with GHD (N=51; boys/girls: 30/21; median age at baseline: 7.3 ± 2.19 years) were randomized into 4 groups in a parallel, assessor-blinded, phase II/III study. They were treated for the 1st year with either daily rhGH 0.03mg/kg/day or with LB03002 at any of the 3 different
The GH-dependent peptide IGF-I is low in patients with severe GH deficiency, and it has been suggested that growth response to GH may be optimized by adjusting GH dose to increase IGF-I into the upper-normal range. However, as the relative benefit/risk of this strategy has not been assessed we evaluated whether normalization of IGF-I is required for optimum growth during GH treatment. We evaluated growth and IGF-I after 1 year of GH treatment in GH-naive GHD children (peak GH<10 µg/L; IGF-I SDS<-2) who had IGF-I and height (ht) available at baseline and 1 year (n=193). Patients were divided into those whose IGF-I SDS at 1 year remained subnormal (<-2 SDS, n=118) or increased into the normal range (≥-2 SD, n=75). Analyses included changes from baseline. There were no obvious safety concerns, such as pubertal advancement or acceleration of bone age. Occasional injection site reactions were mostly mild and transient and resolved within 2 to 3 days post-dose without intervention. No patient discontinued the study over the 3 year period.

Table 1: Height SDS (mean ± SD)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>End of 1st year</th>
<th>End of 2nd year</th>
<th>End of 3rd year</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB03002: 0.2 mg/kg/week (N=15)</td>
<td>-5.00 ± 1.61</td>
<td>-3.97 ± 1.28</td>
<td>-3.10 ± 1.24</td>
<td>-2.56 ± 1.26</td>
</tr>
<tr>
<td>LB03001: 0.5 mg/kg/week (N=13)</td>
<td>-3.94 ± 0.81</td>
<td>-2.65 ± 0.61</td>
<td>-1.87 ± 0.56</td>
<td>-1.49 ± 0.75</td>
</tr>
<tr>
<td>LB03002: 0.7 mg/kg/week (N=13)</td>
<td>-4.64 ± 1.32</td>
<td>-3.03 ± 1.11</td>
<td>-2.22 ± 1.13</td>
<td>-1.86 ± 1.26</td>
</tr>
<tr>
<td>Daily rhGH: 0.03 mg/kg/day (N=12)</td>
<td>-4.52 ± 1.39</td>
<td>-3.06 ± 1.27</td>
<td>-2.17 ± 1.08</td>
<td>-1.89 ± 1.05</td>
</tr>
</tbody>
</table>

In children with GHD, prolonged administration of once-a-week LB03002 over 3 years was shown to be safe and well tolerated. Sustained growth over the entire 3 year period was observed, similar to the growth pattern known for daily rhGH. A pivotal phase III study in children with GHD at the dose of 0.5 mg/kg/week is well underway.

*In cooperation with Biopartners and LG Life Sciences' GH Study Group

**Background** The PREDICT study investigates the relationships between genomics and biomarkers before and during GH therapy in children with GHD or TS.

**Objective:** Analysis of genetic polymorphisms in relation to changes in IGF-I SDS after one month of GH treatment in previously untreated children with GHD (n=169) or TS (n=149).

**Methods:** Blood was taken for measurement of serum IGF-I and for DNA extraction at baseline, and after one month of GH treatment (Saizen®, Merck Serono) for a repeat IGF-I level. DNA was analysed for 1536 single nucleotide polymorphisms (SNPs) using Illumina® platform in 98 candidate genes. Baseline and one month IGF-I SDS were analysed centrally and corrected to age and gender-related standard deviation scores (SDS). Genotypes were compared with changes in IGF-I SDS by ANOVA, corrected for multiple testing based on the number of linkage disequilibrium blocks within each gene, with significance defined as p<0.05.

**Results:** In GHD, genotypes were correlated with change in IGF-I SDS for six of the 98 selected genes, including SH2B2 (involved in JAK2 activation) and two isoforms of PI3 kinase (A and G). In TS, genotypes were correlated with change in IGF-I SDS for 12 genes that were distinct from those identified in GHD, including PI3 kinase B, receptors for oestrogen, insulin and retinoic acid, the signalling molecules AKT2 and GRB10, and two genes related to the GH axis: GHRH and POU1F1.

**Conclusions:** Multiple genetic polymorphisms (different in GHD and TS) in pathways related to the control of growth are associated with an early response to GH, as defined by change in IGF-I. These genotypes should be tested for their effect on growth and metabolic responses to GH to enhance our ability to predict the diverse effects of GH.

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**P1-d3-261 GH Treatment 2**

**Genetic polymorphisms associated with changes in serum insulin-like growth factor-I (IGF-I) in prepubertal children with growth hormone deficiency (GHD) or Turner syndrome (TS) after one month of GH therapy:**

**The PREDICT Study**

**Pei F Teast,* Judith C Lee,* Charlotte S Vergnaud,* Alessandro Cicognani,* Juan Pedro Lopez-Sigueri,* Benoît Destaneses,* Jerôme Davuillier,* Pierre Chatelain,* NA PREDICT Investigators (1)*St Mary’s Hospital, Children’s Outpatient Department, Manchester, United Kingdom; (2)Université des Studi di Verona, Clinic di Pediatria, Verona, Italy; (3)Ospedale S. Orsola Malpighi, Unità Operativa di Pediatria, Bologna, Italy; (4)Hospital Materno Infantil, Servicio de Pediatria, Malaga, Spain; (5)Serono International S.A., Research, Genes and Protein Sciences, Geneva, Switzerland; (6)Research, Scientific Computing, Serono International S.A., Geneva, Switzerland; (7)Hospital Debrousse – Université Claude Bernard, Faculté de Medicine – Pediatrics, Cedex 05, France

**Background** The PREDICT study investigates the relationships between genomics and biomarkers before and during GH therapy in children with GHD or TS.

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**Conclusions:** Multiple genetic polymorphisms (different in GHD and TS) in pathways related to the control of growth are associated with an early response to GH, as defined by change in IGF-I. These genotypes should be tested for their effect on growth and metabolic responses to GH to enhance our ability to predict the diverse effects of GH.

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**P1-d3-262 GH Treatment 2**

**Lipolytic and anabolic effects of growth hormone are dissociated**

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**Context:** There is a broad variation in longitudinal growth response during GH treatment among prepubertal short children. Yet, growth is but one of the several effects of GH.

**Hypothesis:** The metabolic effects of GH vary, and GH sensitivity is unique to each.

**Objective:** To investigate whether diverse metabolic functions respond according to growth.

**Design:** A randomized, prospective, multicentre trial was performed for a 2 years period, with two treatment regimens a) individualized GH dose, six different dose groups ranging 17-100 µg/kg/d (n=87) and b) standard GH dose
of 43 μg/kg/d (n=41). Patients: 128 prepubertal short children, 75% of them diagnosed as GH deficient and 25% as idiopathic short stature.

Results: The anabolic and carcaille variables IGF-1, IGFBP-3, FFM, and insulin target in the same direction as height gain. All variables in this vector bundle show high mutual correlations. IGF-1 increment correlated with height gain (r=0.54, p<0.0001). Stepwise regression showed that 71% of the variation in IGF-1 was explained by height gain (49%), changes in insulin (9%), free fat mass (FFM) (5%), and biceps skinfold (4%), with only a small impact of the GH dose (4%). Changes in IGF-1 solely accounted for all explainable changes in FFM (31%). FFM, but not total fat mass, increased after 2 years of GH treatment in doses >40 μg/kg/d (p<0.001). The anabolic component is dose-dependent. Independent samples t-test shows dose-dependency of ΔIGF-1 (p<0.0001), ΔIGFBP-3 (p<0.001), AFFM (p<0.001), and Δinsulin (p<0.05) comparing low (17, 33, 40 μg/kg/vs high GH dose groups (50, 66, 100 μg/kg/d). Variables derived from adipose tissue (total fat mass, leptin, subcutaneous and internal visceral fat, triglycerides) and lipid metabolism (cholesterol, LDL, triglycerides, lipoprotein(a), apolipoprotein A-II) form up a lipolytic bundle. This lipolytic effect is unrelated to the anabolic component and dose-independent.

Conclusions: The lipolytic and anabolic effects of GH are dissociated. The threshold of GH’s lipolytic effects is much lower than its anabolic and growth effects.

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**P1-d3-263 GH Treatment 2**

Regional variation in growth hormone (GH) dose and response in GH-deficient (GHD) children: Does higher dose equal greater response or more adverse events?

Christopher J. Child; Alan G. Zimmermann; Elena F Shavvrikova; Jan Lebl; Charmian A. Quigley; Werner F Blum

1Ell Lilly and Company, Lilly Research Laboratories, Windlesham, United Kingdom; 2Ell Lilly and Company, Lilly Research Laboratories, Indianapolis, United States; 3Pharma Support Inc, Statistics, St Petersburg, Russian Federation; 4Charles University, 2nd Faculty of Medicine, Prague, Czech Republic; 5Ell Lilly and Company, Lilly Research Laboratories, Bad Homburg, Germany

The GH dose used in the USA for the treatment of GHD children is typically greater than in other countries. To evaluate if a higher dose leads to better growth response and/or more adverse events (AEs), prospective observational study data were compared for 5 countries [Czechia (CZ, N=30), France (FR, 286), Germany (GE, 764), Spain (SP, 169), USA (744)]. Patients (pts) included were GH naïve at baseline (BL) and had a 1st-yr height (HT) velocity (HV) value available.

Table: Demographics, GH dose and height response (mean±SD, unless otherwise stated).

<table>
<thead>
<tr>
<th>Country</th>
<th>BL age (yr)</th>
<th>Median Max BL GH peak (µg/L)</th>
<th>BL HT SDS</th>
<th>BL GH dose (mg/kg/wk)</th>
<th>1st-Yr Δ HT SDS, Lower–Upper 95% CI</th>
<th>1st-Yr Δ HV (cm/yr), Lower–Upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CZ</td>
<td>8.2±1.4</td>
<td>3.6 [2.0, 6.2]</td>
<td>-2.3±1.1</td>
<td>0.17±0.04</td>
<td>0.60±0.38, 0.46–0.74</td>
<td>9.0±1.8, 8.3–9.7</td>
</tr>
<tr>
<td>FR</td>
<td>10.5±3.5</td>
<td>6.6 [4.2, 9.0]</td>
<td>-2.3±0.7</td>
<td>0.24±0.05</td>
<td>0.60±0.41, 0.55–0.64</td>
<td>9.3±2.6, 9.0–9.6</td>
</tr>
<tr>
<td>GE</td>
<td>9.4±3.7</td>
<td>6.7 [4.6, 9.4]</td>
<td>-2.5±1.0</td>
<td>0.19±0.05</td>
<td>0.59±0.48, 0.56–0.63</td>
<td>8.8±2.5, 8.7–9.0</td>
</tr>
<tr>
<td>SP</td>
<td>10.7±3.4</td>
<td>5.9 [3.4, 8.2]</td>
<td>-2.6±0.9</td>
<td>0.21±0.05</td>
<td>0.62±0.50, 0.55–0.63</td>
<td>9.0±2.7, 8.6–9.4</td>
</tr>
<tr>
<td>USA</td>
<td>10.6±3.9</td>
<td>7.0 [4.0, 9.7]</td>
<td>-2.2±1.1</td>
<td>0.31±0.08</td>
<td>0.60±0.48, 0.56–0.63</td>
<td>9.4±2.9, 9.1–9.6</td>
</tr>
</tbody>
</table>

The BL age and maximum stimulated GH values were lower in CZ than in the other 4 countries, while mean GH dose at BL was 29% greater in USA than in FR and up to 82% greater than the other countries, but growth response (1st-Yr Δ HT SDS and HV) was similar in all countries.

Rates of the following potentially GH-related AEs were assessed in patients with ≥1 follow-up visit: arthralgia, diabetes mellitus, edema, gynecomastia, hypothyroidism, increase in nevi, pseudotumor cerebri, recurrent otitis media, scoliosis and slipped capital femoral epiphysis. The rates of patients with ≥1 AE varied only slightly (CZ 3.3%, FR 3.4%, GE 9.8%, SP 4.7%, US 6.9%) and that of the USA was lower than GE. Although BL age and severity of GH deficiency are among the important factors in growth response, the higher dose administered in the USA appears to elicit no better growth response, nor does it appear to cause additional AEs.

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**P1-d3-265 GH Treatment 2**

Serum proteomic profiles of response to growth hormone (GH) treatment in children with idiopathic short stature (ISS)

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The etiology of idiopathic short stature (ISS) is heterogeneous and affected children have a broad spectrum of responsiveness to growth hormone (GH) therapy. At present no markers of GH responsiveness are available to facilitate treatment decisions for this patient population. Therefore, this pilot study sought to identify and characterize as yet unknown serum proteins that might be predictive of, or associated with, response to GH in such children.

Ten children with ISS ages 7.0-14.8 yr at baseline were studied in a sub-study of the GenESIS observational research program. Serum samples were collected prospectively at baseline and following 6-14 months of GH treatment. Patients were classified on the basis of their annualized change in height SDS (AHISDS) during GH treatment as good responders (AHISDS ≥1.0) or poor responders (AHISDS <0.5). Sera were immuno-depleted for high abundance proteins and analyzed using fluorescence 2-dimensional differential in-gel electrophoresis (2D-DIGE) and Matrix Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) peptide profiling. Ge-
We hypothesise that GH treatment will have a differential cognitive and QoL effect on GHD and ISS children. Ninety-nine children between the ages of 3 and 11 years at start of treatment were followed for two years in a randomised control study. The population was grouped into ISS (n=67) and GHD (n=33). Child and parent completed measurements included IQ (WISC) and Child Behaviour Checklist (CBCL); “I think I am”; the Silhouette Apperception Test (SAT) and the Bifleson depression scale. Time points were at baseline 12 and 24 months. At baseline, the GHD group had significantly lower Performance IQ (p=0.021), lower Perceptual Organisation (p=0.020), and lower Performance Speed (p=0.004) than the ISS group. Following 24 months of GH treatment, improvements in full IQ for the GHD children (p=0.002) and Performance IQ (p=0.001) led to no significant difference between ISS and GHD. Verbal IQ, showed no difference between the groups at baseline and increased significantly in both groups (p<0.015 & p<0.041). Those children who increased Full IQ the most were those whose baseline F IQ was below 90 IQ points. Perceptual organization remained significantly lower in the GHD group. There was a significant reduction in childhood depression at 12 and 24 months (P<.011; P<.004). Improvement were found only in the ISS group (P<.020; P<.016). Behavioural problems (CBCL) reduced more in the ISS group (p >.001) in several areas. ISS children become less withdrawn, and less aggressive at 12 and 24 months (F=.002; F=.015 and P=.000; P=.001), and their tendency to externalise their problems reduced (P=.006 and P=.004). Similar findings were not found in the GHD group. We conclude that cognitive improvements could be found in the GHD group whereas QoL improvements were made in the ISS group.

**P1-d3-268 GH Treatment 2**

**Differential effects of GH in children with Growth Hormone Deficiency (GHD) or Idiopathic Short Stature (ISS): Evidence from an insulin-like growth factor-based GH-dosing study**

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We recently demonstrated that in IGF-based GH dosing in short children with low IGF-I levels, the IGF-I target chosen determines GH dose and growth response. To assess the response of children with ISS versus GHD to IGF-I-based GH dosing, we studied prepubertal short children with low IGF-I (<1 SDS) in a 2 yr-open label, randomized, IGF-I concentration-controlled clinical trial. The patient population (n=165) was subdivided, based on peak GH values in an arginine/L-Dopa stimulation test, into GHD (n=63; peak GH<7 ng/mL) and ISS (n=102; GH≥7 ng/mL) groups. In both groups the GH dose was targeted to either 0 SDS (G0T) or <2 SDS (G2T) and the targeted mean IGF-I levels were achieved within 6-9 months and did not differ between GHD and ISS. In both groups, G2T patients required significantly higher GH doses (median 119 and 65 mcg/kg/d for ISS and GHD) than G0T patients (median 26 and 33 mcg/kg/d for ISS and GHD). At 2 yrs, significantly greater changes in height SDS (AHSDS) were observed in G2T patients and AHSDS values were significantly greater for GHD than ISS in both IGF-I target groups: AHSDS of 2.04 for GH and 1.33 for ISS groups in G2T, and 1.41 for GHD and 0.84 for GHD in G0T. Patients with the lowest baseline GH and IGF-I levels had the greatest AHSDS during treatment, whereas patients with the highest baseline GH and IGF-I levels had the least AHSDS. Bone age changes during the 2-yr trial did not differ between groups. IGF-I-based GH dosing is clinically feasible in both GHD and ISS patients, although GH dose requirements and auxological outcomes are distinct among these groups. When targeted to an IGF-I SDS of 0, patients with ISS require slightly lower GH doses, but exhibit lower growth rates. However, when targeted to an IGF-I SDS of -2, patients with ISS require dramatically higher GH doses, and still display reduced growth rates compared to GHD. Our findings suggest a certain degree of both GH and IGF-I insensitivity in patients with ISS that requires specific management strategies to optimize growth during GH therapy.