Primary hyperparathyroidism is a rare endocrine disease in children. We report a 22 days old baby boy who presented with signs and symptoms of heart failure, presumptive diagnosis of dilated cardiomyopathy by echocardiography. Found to have hypocalcaemia, hyperphosphatemia, and inappropriately low parathyroid hormone. On examination: weight, length and head circumference on 50%, with mild dysmorphic features in form of micrognathia, low-set small posteriorly rotated ears, hypertelorism, down slanting short palpebral fissures, narrow alar nasi with broad base of the nose and short philtrum. His Flourescence In Situ Hybridization (FISH) study proved to be DiGeorge syndrome with chromosome 22q11.2 microdeletion. No apparent cause for dilated cardiomyopathy was found. He requires intravenous inotropes as part of his initial management. With restoration of normocalcemia, his left ventricular ejection fraction markedly improved and (his inotropes, lasix) and captopril were discontinued after 1 week and 6 weeks respectively. We believe that this is the first case of congenital hypoparathyroidism - confirmed DiGeorge syndrome presenting with dilated cardiomyopathy induced by hypocalcaemia. Physicians should consider hypocalcaemia as a possible reversible cause of congestive heart failure in children.

Hypoparathyroidism in childhood is considered to be rare. However the exact incidence is unknown and little is known regarding the etiology percentages.

Objectives: Hypoparathyroidism is considered to be rare. However the exact incidence is unknown and little is known regarding the etiology percentages.

Patients and Methods: This is a retrospective study collecting cases through the Seine-Maritime area (around 18 000 births a year) over a 30 year experience (1974-2004).

Results: 23 cases were collected ranging in age from neonatal period to 18 years. The hypoparathyroidism incidence is estimated at 0.7 case a year or 3.8 cases per 100 000 births. The main etiologies diagnosed were: DiGeorge syndrome 6, Kenny syndrome 1, Kearns-Sayre syndrome 1, Autoimmune (APECED) 2, Hypomagnesemia 1, IMAGE syndrome 1, CaSR mutations 3, polyendocrine autoimmune syndrome 6, Kenny syndrome 1, Kearn-Sayre syndrome 1, Autoimmune polyendocrine syndrome 1, Williams syndrome.

Conclusions: Hypoparathyroid state appears to be rare. Molecular biology tests constitutes new tools of clinical relevance.

Background: The diagnosis of nutritional rickets (NR) regarding with only clinical findings without checking serum 25 hydroxyvitamin D levels may cause a redundant treatment that leads to hypercalcemia. If hypercalcemia/hypercalciuria left untreated, it may result in problems such as dysrhythmia, dehydration, etc. which need to be treated. Although several therapeutic approaches including glucocorticoids, high intravenous saline intake, furosemid and calcitomin treatment have been used in the treatment of hypercalcemia, they may not result in a favorable reduction of hypercalcemia. We experienced the case of idiopathic hypercalcemia with persistent hypercalciuria in which hypercalciuria was not normalized after the correction of the serum calcium level. The mechanism of hypercalcemia and hypercalciuria of this case is still unknown.

Objective: to avoid this side effect, we report our experience with intragastric alendronate treatment in two infants with hypercalcemia secondary to vitamin D intoxication. Methods: Aledronate in doses of 5 and 10 mg/day was successfully administrated by feeding tube to treat hypercalcemia. Clinical and laboratory characteristics of the patients are listed in the Table.
A 10.5 year old Caucasian girl fainted suddenly, without convulsive movements. Consciousness returned spontaneously to normal. Clinical examination showed nothing but round face, short neck and brief 4th and 5th metacarpals. There were no signs of Albright hereditary osteodystrophy, no dental alterations.

Immediate biological study showed profound 1.09 mmol/l hypocalcemia and 3.5 mmol/l hyperphosphatemia. Blood glucose and ionogram were normal. Electrocardiogram showed QT prolongation. Calcium in urine was almost zero, phosphorus was elevated. Parathyroid hormone was undetectable. Normal calcium and phosphorus (Ca/P) balance was restored after one month of calcium supplementation (first IV, then oral), low phosphate diet, and 1alphahydroxyvitamin D3. There was no cataract or ectopic calcifications. Ultrasonography showed no renal calcifications. Karyotype was normal, 46,XX, ish22q11 (tulex12) (no 22q11 deletion). There were no parathyroid auto-antibodies or any other auto-antibodies after screening on standard Hap-2, 5-tissue blocks and endocrinopathy-associated specificities as antibodies against thyroglobulin and thyroid peroxidase. Thyroid function was normal, (TSH 1.14 μl/ml, free T4 17 pmol/l, free T3 5.2 pmol/l). There was no mutation of the calcium-sensing receptor gene and of the preproparathyroid hormone gene. Ca/P of both parents is normal. The severity of hypocalcemia leads us to conclude that it is secondary rather than primary. Other genetic investigations are still in progress, but it is concluded that this case of hypophosphatrosis is of unknown origin according to present knowledge.

**P1-323 Calcium, PTH and Vitamin D**

**Pseudohyoparathyroidism: treatment of short stature with GH**

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Pseudohyoparathyroidism type Ia (PHP Ia) is associated with a distinct phenotype, hypocalcemia, hyperphosphatemia, elevated parathyroid hormone (PTH). Resistance to PTH is due to Gs protein deficiency, which results in impaired adenylate cyclase response to PTH. Short stature has been attributed to early meet of puberty, or secondary to GHRH resistance. Because GHRH receptors, and not GH, are affected by the Gs protein, patients should respond to recombinant human GH (rhGH). In this report we describe two PHP Ia patients, one of them treated by rhGH. Patient 1, a girl, that at 15 years was evaluated due to hypocalcemia, hyperphosphatemia, short stature: 144cm (Z-score -2.47), weight: 37.3kg (-1.92), round face, short neck, 5th short metacarpal, stocky build, positive Trouseau and Chvostek signals. Intact PTH: 213pg/dl (12-65); serum calcium (sCa): 7.6mg/dl; 24 urine calcium (uCa): 37mg/vol; sP: 7.6mg/dl; TSH: 2.8mUI/ml; FT4: 0.81ng/ml; LH: 6.3mUI/ml; FSH: 2.4mUI/ml; E2: 95pg/ml; basal GH: 0.31ng/ml (stimulated by clonidine 0.10mg/m2) and insulin (0.1U/kg) 4.3 and 8.2ng/ml respectively. Bone age: 12yr. CT scan showed calcified basgal ganglia. Standard therapy with calcium and calcitriol did not normalize growth. On rhGH therapy at a dose of 2.5 U/day growth velocity was improved. In conclusion, GH responds to pharmacological stimulus, however serves during early childhood. At the average age of 35 months in boys their growth retardation, and global developmental delay.

The aims of our study were: 1. To summarize our long term (15 years) experience with a relatively large number of HDK patients in a single medical center. 2. To characterize their linear growth during infancy and early childhood by applying the ICP model. 3. To compare between HDK polymorphonuclear cells (PMNC) functions with the functions of PMNC from healthy control group. 23 (13F/10M) HDK patients were followed. Their average age was 3.8 years (0.25-14.5), 7 patients (30%) died. The main causes of the patients multiple hospitalizations were infections and convulsions. A special susceptibility to pneumococcal infections was documented. 9-pm-sulfur colloid scintigraphy of the spleen detected functional asplenia/hypoplasia in 9 out of 11 examined HDK patients. All children suffered from IUGR (BW SDS -3.0±0.6 for boys and -2.4±0.6 for girls). Further decrease of weight gain was observed during early childhood. At the average age of 35 months in boys their weight and height SDS were: -5.6±1.1 and -7.1±1.7, while at HRD girls, 50 months of age, weight and height SDS were -6.2±1 and -6.6±1 respectively. Growth analysis showed a markedly delayed appearance of the childhood component of growth: 5.6±1.76 months in boys and 6±1.97 months in girls (normal: 6-12). Chemotaxis of PMNC obtained from HRD patients was reduced by 42±17% (p<0.01) in comparison to controls. There were no differ-
ence in superoxide production and phagocytosis between the two groups. Growth retardation in young HRD patients is due to combined effects of IUGR and delayed appearance of the childhood component of growth. Splenic dysfunction and reduced PMNC chemotaxis contribute to the increased susceptibility of HRD patients to infections. Our findings are probably best explained by profound impaired microtubules synthesis.

**P1-326 Calcium, PTH and Vitamin D**

**Vitamin D resistant rickets, alopecia and autoimmune type 1 diabetes in a child with a double heterozygous mutation of the vitamin D receptor**

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Vitamin D lowers the risk of insulin-dependent diabetes in NOD mice and may reduce the susceptibility to this disease in humans. But the mechanism of its putatively protective role remains unknown: (i) transgenic VDR-deficient mice show lymph nodes hypertrophy and alteration of dendritic cell maturation, but no diabetes mellitus; (ii) no association between vitamin D resistant rickets and autoimmune diabetes has been reported so far. Here we describe a patient with severe hereditary vitamin D resistant rickets, total alopecia, who also developed early childhood-onset type 1 diabetes. The child was first seen at 2yr3mo with florid hypocalcemic rickets, growth delay (-2SD), and total alopecia. Correction of the clinical, radiological and biological signs of rickets was obtained after 5 months of treatment with calcium (1g/d) and 0.2 mg/d of 25-hydroxyvitamin D3. Subsequent daily supplementation with 0.1 mg/d maintained normal calcium metabolism and bone mineralization, and corrected growth retardation.

Two novel heterozygous mutations were identified in the ligand-binding domain of the patient’s VDR. Both mutations reduced the ligand-binding capacity of the mutant VDR and the induction of 25-OH(D)2D3-responsive activity, a classical 1,25-(OH)2D3-responsive gene, in patient’s fibroblasts and transfected COS-7 cells.

At the age of 5 years, the child presented with insulin-dependent diabetes. His genotype was HLA DRB1*03 and anti-islet antibodies were detected from the genotype was HLA DRB1*03 and anti-islet antibodies were detected from the patient with severe hypothyroidism causes precocious puberty, and it is usually seen in girls. It is worthwhile to report this case, because congenital hypothyroidism resulted in precocious puberty in a boy.

**Case Report:** A six-year and eight-months old male was admitted to the hospital with dwelling in the hands and feet. He had motor and mental retardation. On physical examination, height-SDS: -4; bone age and status was 5 and 4, respectively. He had a coarse face with low nasal bridge and hypertelorism Skin was pale and over dry with extensive nonpitting edema covering whole all body surfaces. His hair was sparse. Nails of both toes were dystrophic. Bilateral testes volumes were 5 ml, penis size 5x2.7 cm without the signs of virilization. Other clinical findings were normal. Laboratory investigations revealed the following data: T4:0.01 mcg/dl (4.5-10.9), T3:0.05ng/ml (0.6-1.8), FT4:0.04ng/dl (0.7-4.0), FT3:0.30g/ml (1.7-3.7), TSH: 170mcIU/ml (0.35-4.94), thyroglobulin: >0.5 ng/ml (1.6-60), PRL: 48 ng/ml (2.5-17). Thyroid antibodies were negative. Other biochemical studies were normal. Thyroid glands could not be visualized by Tc-99 scan. The thyroid volume was found to be hypoplastic for age (0.76 ml, normal: 3.9+/-0.8 ml) by ultrasound. The tests parenchyma was normal at scrotal ultrasound. In the LHRH test, obtained peak response for LH an FSH was 3.2 IU/ml and 3.0 IU/ml. He was diagnosed as congenital hypothyroidism associated with peripheral precocious puberty and L-thyroxin was started. At the third month of L-thyroxin therapy testicle volumes were normal for age and thyroid hormone levels returned to the normal.

**Conclusion:** We emphasized that, serum thyroid hormone levels should be controlled in children with precocious puberty who has short height and bone age retardation.

**P1-327 Thyroid**

**A novel Asparagine to Aspartate(N132D) mutation in the FOX1 gene in congenital hypothyroidism**

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Congenital hypothyroidism (CH) occurs in approximately 1 in 3000 - 4000 livebirths and 85% of cases are due to thyroid dysgenesis, which comprises of defects in the migration, differentiation or growth of the thyroid gland. To date, the molecular defects that are known to be associated with CH include mutations in the FOXE1 gene. In this study, we screened CH patients (n = 44; 8 with thyroid agenesis; 1 ectopic and 35 with thyroid gland) for mutation(s) and or polymorphism(s) in the FOXE1 gene by PCR-SSCP technique. Polymerase Chain Reaction (PCR) was carried out using 7 pairs of overlapping primers covering the entire coding region followed by single strand conformational polymorphism (SSCP) analysis. One patient of Malaysian-Indian origin with thyroid agenesis diagnosed through neonatal screening (cord TSH >100 mIU/ml, repeat serum TSH=149 mIU/ml, FT4=5pmol/L on day 5 of life, despite early treatment IQ at 5 years was 87+) demonstrated an electrophoretic mobility shift in SSCP analysis. Subsequently, with direct DNA sequencing and analysis this patient was found to carry a heterozygous A>G substitution that alter the amino acid asparagine (AAC) to aspartate (GAC) at codon 132 (N132D). The N132D mutation was not found in normal healthy individuals (n = 100). Since N132D mutation is localised in the conserved region of forkhead binding domain of FOXE1 protein, responsible for DNA binding, the mutation is functionally significant.

**P1-328 Thyroid**

**Peripheral precocious puberty caused by congenital hypothyroidism: a case report**

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**Background:** Children with hypothyroidism often present with delayed puberty and growth retardation. However, precocious puberty appears associated with hypothyroidism in some occasions. Generally severe acquired hypothyroidism causes precocious puberty, and it is usually seen in girls. It is worthwhile to report this case, because congenital hypothyroidism resulted in precocious puberty in a boy.

**Case Report:** A six-year and eight-months old male was admitted to the hospital with dwelling in the hands and feet. He had motor and mental retardation. On physical examination, height-SDS: -4; bone age and status was 5 and 4, respectively. He had a coarse face with low nasal bridge and hypertelorism Skin was pale and over dry with extensive nonpitting edema covering whole all body surfaces. His hair was sparse. Nails of both toes were dystrophic. Bilateral testes volumes were 5 ml, penis size 5x2.7 cm without the signs of virilization. Other clinical findings were normal. Laboratory investigations revealed the following data: T4:0.01 mcg/dl (4.5-10.9), T3:0.05ng/ml (0.6-1.8), FT4:0.04ng/dl (0.7-4.0), FT3:0.30g/ml (1.7-3.7), TSH: 170mcIU/ml (0.35-4.94), thyroglobulin: >0.5 ng/ml (1.6-60), PRL: 48 ng/ml (2.5-17). Thyroid antibodies were negative. Other biochemical studies were normal. Thyroid glands could not be visualized by Tc-99 scan. The thyroid volume was found to be hypoplastic for age (0.76 ml, normal: 3.9+/-0.8 ml) by ultrasound. The tests parenchyma was normal at scrotal ultrasound. In the LHRH test, obtained peak response for LH an FSH was 3.2 IU/ml and 3.0 IU/ml. He was diagnosed as congenital hypothyroidism associated with peripheral precocious puberty and L-thyroxin was started. At the third month of L-thyroxin therapy testicle volumes were normal for age and thyroid hormone levels returned to the normal.

**Conclusion:** We emphasized that, serum thyroid hormone levels should be controlled in children with precocious puberty who has short height and bone age retardation.

**P1-329 Thyroid**

**Papillary thyroid carcinomas have increased expression of LIM kinase-2**

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Papillary thyroid carcinoma (PTC) is the most common histological variant of thyroid cancer in children. Knowledge of differential protein expression by PTC compared to normal thyroid could be important in determining the molecular mechanisms involved in the development and progression of thyroid cancer. We designed this study to identify proteins differentially expressed by PTC compared to normal thyroid. We protein prepared lysates from paired bi-
opspecimens of PTC and corresponding normal thyroid tissue from 4 adult patients (4 PTC and 4 paired normal specimens). The PTC extracts, normal thyroid extracts, and a pooled internal standard were labeled with different CyDye™ DIGE fluoros. A mixture of protein-labeled PTC, normal thyroid, and standard for each patient sample was then resolved by 2-dimensional gel electrophoresis. The spectrally resolvable CyDye fluoros were detected by the Typhoon™ variable mode imager, and the averaged PTC proteins and normal thyroid proteins were analyzed by DeCyder™ differential analysis software. Differentially expressed proteins were punched from the gels, digested, and identified by MALDI-TOF™ tandem mass spectrophotometry. Compared to normal thyroid, 18 of 1847 proteins (1%) were differentially expressed by PTC. One protein with increased expression in PTC was identified as LIM kinase-2 (LIMK-2). LIMK-2 expression was increased 2.4-fold in PTC compared to normal thyroid with a p-value of 0.0012. The Rho-ROCKII-LIMK-2 signaling pathway is involved in the regulation of the actin cytoskeleton. This pathway plays a role tumor metastasis. Furthermore, this pathway is a mechanism of BRAF signaling. Activating mutations in BRAF are the most common mutation found in adult PTC. For these reasons, this signal pathway could have a major role in the tumorigenesis of PTC.

Morphological changes in masseter motor pools during suckling to weaning period in hypothyroid neonates

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It has been known that under thyroid hormone influence, the morphological properties of masseter muscle alter from sucking to biting around 3 weeks after birth. In order to define the development of masseter innervation pattern in hypothyroid neonates, horseradish peroxidase (HRP) (40%, 0.5-µl) was injected into the masseter muscle of normal and prenatal PTU-treated pups at 1, 7, 15 and 23 postnatal days. After 24h - 48h, the 50 µm brain stems sections were processed for TMB histochemical procedure. Labeled motoneurons in trigeminal motor nucleus (Mo5) were counted, measured and classified into heavy (H) and light (L) groups on the basis of HRP labeling intensity. In each group motoneurons were divided into small (<500µm2) and large (>500µm2) populations. No significant difference was observed in the total number of motoneurons between normal and hypothyroid pups from day 1 up to day 23. However, at the time of weaning (day 23), while the number of small H labeled neurons stayed high (P<0.01) in hypothyroid pups, the number of large H neurons raised up about two times more in normal pups Mo5 (P<0.001).

The same results were obtained for small and large L neurons with a level of significance P<0.05. It should be noted that the appearance of large motoneurons starts from day 15 and will be completed at day 23 to 25 when feeding behavior of rat needs to chewing and biting force. It can be concluded that prenatal thyroid hormone dysfunction severely delay the neuromuscular mechanism of shifting from suckling to biting profile.

Iodine intake in schoolchildren under iodine prophylaxis in Belarus

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The study aimed to estimate iodine intake in schoolchildren living in Belarus where active measures on elimination of iodine deficiency have been carried out since 1999, when the State Program on Iodine Deficiency Elimination by mandatory iodization of domestically produced salt with 40±15 mg of potassium iodate per kilogram of NaCl was accepted along with intensive information campaign in mass media for the benefits from the use of iodized salt.

In the year 2004, 898 schoolchildren have been examined, including 278 children from Minsk city (120 girls and 158 boys, mean age 13.8±2.78 years) and 620 children from Minsk region (325 girls and 295 boys, aged 11.6±3.40 years). The schoolchildren were interviewed by the method of questionnaires and underwent urine testing for iodine excretion measured by cerium-arsenite method. 89.6% schoolchildren from Minsk city received iodized salt regularly and 10.4% periodically. 88.8% schoolchildren from Minsk region received iodized salt regularly and 11.2% periodically, while in 1999 only 35% of schoolchildren from both studied sites received iodized salt regularly and 21% periodically.

The median urinary iodine excretion was 200.6 µg/l in girls and 213.5 µg/l in boys from Minsk city and only 6.8% schoolchildren had urinary iodine excretion levels of less than 100 µg/l compared to median urinary iodine excretion of 38.1 µg/l in Minsk city in 1999 before salt iodization was widely introduced. In Minsk region the median urinary iodine excretion was 168.7 µg/l and 158.7 µg/l in girls and boys respectively, 19.1% children had urinary iodine excretion levels of less than 100 µg/l and only 3.8% - less than 50 µg/l compared to median urinary iodine excretion of 57.6 µg/l in Minsk region in 1999 before salt iodization became mandatory. All these data indicate that the measures on iodine deficiency elimination being carried out in Belarus proved to be effective.

Thyroid Screening program in Saudi Aramco Medical Services Organization (SAMSO) Hospitals in the Eastern Province of Saudi Arabia, use of Cord blood Total T4(CB-TT4) is sensitive and specific modality of screening Mohammad Abduljabbar1, Ashraf Alaffi2, Ahmed Al Shahrani2, Jamal Jubeh3

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Background: Neonatal thyroid screening programs went through many phases of development to improve their sensitivities and specificities. The implementation of postnatal early discharge policy urged many hospitals worldwide to re-engineer their screening programs to meet this policy yet to be cost effective. Objective: To review the results of congenital hypothyroid (CH) screening program in our center and whether it is comparable to the international standards. Methods: The study covers the period 1990-2003. All infants born in SAMSO facilities were screened by measuring their CB-TT4, if the result is below a pre-determined cut off value, then cord blood TSH (CB-TSH) will be done and if it is above pre-determined cut off value, then the patient is called to do venous FT4&TSH. The cut off values for CB-TT4 &TSH were reviewed and adjusted every 2 years to improve program’s sensitivity and specificity. From year 2001 onwards, venous FT4&TSH were done at 4 weeks of age for all preterm infants born at <32 weeks of gestation. All flagged cases were referred to Pediatric Endocrine service to confirm diagnosis and start treatment. Results: 84,894 babies were screened by CB-TT4. 8337 cases were referred to Pediatric Endocrine service to confirm diagnosis and treat 24 primary CH cases were confirmed, 3 of which were preterm. The incidence rate was 1:3537 live births with a female to male ratio of 2.1. The main causes were ectopic thyroid (48%), athyreogenesis (24%), and dyshormonogenesis (14%). No seasonal variation was noted. The mean age of starting treatment was 8.9 days (±3.5 days). The mean starting thyroxine dose was 41.1 microgram/day. There were 2 cases of central hypothyroidism with an incidence of 1:42447 live births. The recall rate was double the accepted figure (1.98% versus 1.1%). There were no missed cases during this period.

Conclusion: The use of CB-TT4 as the neonatal thyroid screening modality at SAMSO hospitals captured all CH cases with 100% sensitivity and 98% specificity. Doing CB-TT4 with subsequent CB-TSH meet the early discharge policy, guarantee screening all the cases delivered in the hospital and proved to be sensitive and specific, yet with higher recall rate.
**P1-334 Thyroid**

**Evaluation of functioning of the muscular system in children with hyperthyroidism**

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**Background:** Thyroid hormones affect systemic metabolism, including that of muscular system. The assessment of creatine kinase (CK) activity enables insight into metabolic processes taking place in muscle.

The aim of the study was to assess the functioning of the muscular system in children with hyperthyroidism on the basis of biochemical and physiological parameters. Material and methods: The study comprised 20 children aged 11 to 17 years suffering from hyperthyroidism and 54 healthy peers. Observed parameters were assessed twice: 1) the moment hyperthyroidism was recognized, prior to the commencement of the treatment, 2) following the commencement of the treatment, after the clinical improvement and the normalization of thyroid hormones. CK was assessed according to the kinetic method while CK-MB by means of the immunochemical UV test. Muscle strength measurement was taken by means of a handgrip dynamometer.

**Results:** CK activity in patients with hyperthyroidism equalled 61.55 ±U/L prior to the commencement of the treatment, whereas after attaining hormone normalization it increased significantly and amounted to 128±7.99 U/L exceeding the norm. Respectively, in the first study CK-MB activity was 22.75±10.38 u/L, and then increased to 23.30±10.14 U/L, but still remained significantly below the norm. The maximum muscle strength (Smax) and muscle strength after fatigue increased significantly after euthyroidism, while fatigability index decreased.

**Conclusions:** Results accomplished in the course of the study demonstrate a decrease in the muscular system capacity of children with hyperthyroidism and point out tendency to abnormalities of energetic processes in muscular cells. The hormonal normalization accomplished in a result of the applied treatment is reflected in better parameters of functioning of the muscular system in the children studied. The steady decrease in CK-MB activity below the norm, despite the accomplished clinical and hormonal normalization, may indicate unfavourable influence of hyperthyroidism on the heart muscle.

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**P1-335 Thyroid**

**Thyroid hypoplasia in children with Williams syndrome**

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**Background:** Patients with Williams syndrome (WS) are described as showing thyroid disorders, so far only a few cases concerning alterations in thyroid function or morphology have been reported.

**Objective:** to evaluate the prevalence of abnormalities of thyroid function and morphology in a cohort of patients with WS.

**Patients and Methods:** From January 2003 to September 2004, serum concentration of free-T3, free-T4, TSH, anti-thyroid antibodies, as well as ultrasonographic data of twenty-two consecutive patients with WS (14 females and 8 males, aged 1.7-34.9 years) were collected. All subjects lived in Tuscany, in areas of long-term iodine sufficiency. In all WS patients typical microdeletion at 7q11.23 was detected. In these patients, thyroid volume was compared to an age- and sex-matched control groups (1 to 14 years of age, and adult age control groups) living in iodine-sufficient areas.

**Results:** three cases (3/20, 15%) of subclinical hypothyroidism was identified. Only five (25%) patients presented a thyroid gland with normal volume; in one patient with normal total thyroid volume, a left thyroid lobe hypoplasia was recognized, prior to the commencement of the treatment, whereas after attaining hormonal normalization it increased significantly and amounted to 128±7.99 U/L exceeding the norm. Respectively, in the first study CK-MB activity was 22.75±10.38 u/L, and then increased to 23.30±10.14 U/L, but still remained significantly below the norm. The maximum muscle strength (Smax) and muscle strength after fatigue increased significantly after euthyroidism, while fatigability index decreased.

**Conclusions:** thyroid hypoplasia is commonly found in children with WS. Left lobe was prevalently involved. Further studies are needed to define the cause of this abnormal development of the thyroid gland in these children.
deficiency can occur in patients who receive these iodine—deficient enteral formulas for a long period. In 18 of the 20 enteral formulas, the content of iodine was less than the recommended daily allowance (RDA) of 80 µg/100 kcal. Moderate iodine deficiency was found in 13 formulas and severe iodine deficiency in 7 formulations. In the formula with the lowest iodine content, which is the RDA for iodine in adults. In 18 of the 20 enteral formulas, the iodine intake was calculated to be 16 µg/day, which was lower than the recommended daily allowance (RDA) of 80 µg/day for 4 year olds. The goiter and hypothyroidism disappeared by replacing the enteral formula with another one. Material and Methods: The iodine concentration was measured by using ICP-MS in 20 different popular nutritional formulas available in Japan.

Results and Discussion: When the calorie intake in an adult patient receiving an enteral formula is 2,000 kcal/day, the iodine concentration in the enteral formula should be 8.5 µg/100 kcal in order to provide 150 µg of iodine, which is the RDA for iodine in adults. In 18 of the 20 enteral formulas, the iodine concentration was less than 8.5 µg/100 kcal. The iodine concentration was less than 5 µg/100 kcal in 13 formulas. These results suggest that iodine deficiency can occur in patients who receive these iodine—deficient enteral nutritional formulas for a long period.

P1-338 Thyroid
The effect of long-term low dose lead exposure on thyroid function in adolescents
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Lead has been one of the most common heavy metals with wide applications for many centuries. Occupational and environmental lead exposure continues to be one of the significant public health problems.

Purpose: To investigate blood lead (Pb-B) levels and Pb-B effects on thyroid functions in long-term low level lead-exposed male adolescents who work as auto-repairers.

Methods: Pb-B and ALAD index (logarithm of activated o-aminolaevulinic acid dehydratase / non-activated o-aminolaevulinic acid dehydratase) were measured as indicators of exposure to lead. Thyroid function tests including free thyroxin (FT4), free triiodothyronine (FT3) and thyroid stimulating hormone (TSH) were studied and thyroid ultrasounds were performed in 42 lead-exposed adolescents and 55 healthy control subjects.

Results: Mean Pb-B levels and ALAD index were found significantly higher in the study group than normal control group (7.3 ±2.92 vs 2.08 ± 1.24 µg/dl, p<0.001 and 0.44 ± 0.26 vs 0.29 ± 0.23, p<0.05, respectively). Free-T4 levels were found significantly lower in the study group (1.02 ± 0.18 and 1.12 ± 0.14 µIU/ml, p<0.05). No subject in the control group had abnormal FT4 level, but FT4 levels were found under normal limits in 11 cases (26%) in the study group. FT3 and TSH levels in study and control groups did not differ (p>0.05). Thyroid volumes in study and control groups did not exhibit any significant difference (p>0.05). Pb-B was found to be negatively correlated to FT4 levels (r = -0.26, p = 0.009).

Conclusions: This study revealed that long-term low level lead exposure may lead to reduced FT4 level without significant changes in TSH and T3 levels in adolescents even at low Pb-B levels.

P1-339 Thyroid
Subclinical hypothyroidism in children with acute lymphoblastic leukemia after intensive chemotherapy
Fani Athanassiadiou1, IsraeL Roussou1, Athanassios Triannidis3, Maria Kourt1, Ioannis Karamouzis2, Michael Karamouzis2, Theodotis Papageorgiou2, Anastasios Vassiliadis2, Athanasios Triannidis3
1Aristotle University of Thessaloniki, 2nd Pediatric Dpt, AHEPA Hospital, Thessaloniki, Greece; 2Aristotle University of Thessaloniki, Biochemistry Dpt, Thessaloniki, Greece

Aim of our prospective study was to evaluate the effect of intensive chemotherapy on serum thyroid hormone concentrations in children with acute lymphoblastic leukemia (ALL). Material and methods: Serum levels of triiodothyronine (FT3), thyroxine (FT4) and thyroid-stimulating hormone (TSH) were determined in 30 children with ALL (aged 2 to 13 years, mean age=SD=± 6.8±3.5 years) at diagnosis and after intensive chemotherapy treatment (at 6 months after diagnosis and before prophylactic cranial irradiation) with ALL BFM'95 protocol. Patient’s values were compared with those of 30 healthy controls. None of 30 children had a known condition affecting thyroid function before diagnosis of ALL. A statistical significance was established at p<0.05.

Results: among the 30 children 20 (66.6%) were grouped as standard, 4 (13.3%) as median and 6 (20%) as high risk respectively. Mean serum levels of TSH differ significantly at diagnosis (2.67±0.9) and 6 months after intensive chemotherapy (4.16±1.85) (p<0.05). Compensated hypothyroidism at 6 months (elevated TSH with a normal FT3 and FT4 value) occurred in 3 (10%) children and received thyroid hormone replacement therapy. A statistical significance was also found between patients’ mean TSH value at 6 months after initiation of chemotherapy and controls’ TSH mean value (p<0.05). Conclusions: our results demonstrate that intensive chemotherapy for childhood ALL can cause subclinical hypothyroidism and this endocrine complication is unrelated to prophylactic radiotherapy. This suggests the need for a continuous and careful monitoring of thyroid hormone concentrations in children undergoing chemotherapy for hematological malignancies.
Congenital hypothyroidism is known to significantly impact on cardiovascular function and its treatment requires careful biochemical monitoring. To this aim, 30 young adults with congenital hypothyroidism (ageSEM, 18.5±2 yrs) and 30 age- and sex matched normal controls (19.0±5 yrs) underwent symptom-limited cardiopulmonary exercise testing. Hypothyroidism was diagnosed by neonatal screening and levothyroxine treatment was initiated within the first month of life. Levothyroxine dosage was carefully adjusted every 3-6 months in order to maintain TSH levels in the normal range and FT4 in the high-normal range, and at the time of the study was 2.1±0.1 micrograms/kg/day. Exercise capacity was significantly impaired in the hypothyroid patients compared with controls as shown by reduced peak VO2 (30±1.7 vs. 37±2 ml/kg/min), peak power output (219±17 vs. 272±17 watt) and anaerobic threshold (1.6±1 vs. 2.4±2 ml/kg/min), all p<0.01. Despite careful monitoring of serum TSH and adjustment of levothyroxine dosage, long-term levothyroxine treatment is associated with impaired exercise capacity in young adults with congenital hypothyroidism.

TSH receptor is a member of the leucine-rich repeat-containing G protein-coupled receptors. Inactivating mutations of the TSH receptor have been described (ref) and a new missense mutation in exon 2 was found in the proband of a new family. Parents’ DNA analysis showed that the Asp410Asn mutation and the Met527Thr variant were carried by the maternal and the paternal allele, respectively. The variant Met527Thr was found in a female with isolated hyperthyrotropinemia (serum TSH range: 5-24 MCIU/mL), normal free thyroid hormone levels, negative thyroid antibodies, and normal thyroid ultrasound.

Inactivating mutations of the TSH receptor have been detected in several cases of resistance to TSH both partial and complete. In the present study we observed 11 unrelated patients with hyperthyrotropinemia (serum TSH range: 5-24 MCIU/mL), normal free thyroid hormone levels, negative thyroid antibodies, and normal thyroid ultrasound. By direct sequencing of the TSHR gene coding regions we detected 2 different nucleotide substitutions in two families: The G>A substitution at codon 410 that results in the known inactivating mutation Asp410Asn and the T>C substitution at codon 527 that results in the variant Met527Thr, affecting a residue located in the second intracellular loop, that as far as we know has never been described. Parents’ DNA analysis showed that the Asp410Asn mutation and the Met527Thr variant were carried by the maternal and the paternal allele, respectively. The variant Met527Thr was found in a female with idiopathic central precocious puberty and serum TSH levels of 12 MCIU/mL. She started therapy with L-Thyroxine and the dose was increased during the follow-up. Her father (serum TSH levels 5.12 MCIU/mL) was clinically euthyroid and never needed therapy.

In conclusion we found the new variant Met527Thr located in the second intracellular loop that could be important for the adenylate cyclase activation in receptor signalling. The variability of the phenotypical expression leads to a long term follow-up in these patients to decide the correct therapeutical approach.

Thyroid insufficiency in congenital hypothyroid patients with eutopic gland may be caused by altered iodine organification due to defects of the thyroid peroxidase (TPO) and less frequently in dual oxidase 1 (DUOX1) genes. To identify TPO and DUOX2 gene mutations we studied 15 of 50 patients with CH and goitre, detected most of them by newborn screening, selected if they had had at diagnosis or revvaluation high TSH levels and thyroglobulin values, low T4 and percholate discharge test ≥ 45 %. All 17 exons of TPO gene and DNA coding sequences and the flanking regions of the N-terminal and central regions of DUOX 2 gene were studied by SSCP and DNA sequencing of fragments with abnormal migration.

5 different mutations for the TPO gene were found in heterozygocity in 6 patients in exons 5, 8, 9 and 14: a frameshift mutation a C deletion nt 477 in exon 5, a missense mutation in exon 8 nt1010 A>C, a missense mutation in exon 9 nt1586 C>T, a missense mutation in exon14 nt2512 T>C and a frameshift mutation in exon 8 GCCC duplication nt1276-1277. Only 1 patient was a double heterozygote. Mutations of the DUOX2 gene were found in two families: In the first 2 affected siblings, the father and two healthy brothers were heterozygous for a single base change of a adenine to cytosine transversion at position ‘C2 of the splice acceptor site in the intron 19 (2652 (-2) A>C). A second heterozygous mutation in exon 11 was identified in the mother and the 2 affected children. A del GTTC at position 2895 in exon 21 (mutation already described) and a new missense mutation in exon 2 was found in the proband of the second family.

Our findings confirm the prevalence and genetic heterogeneity of TPO and DUOX2 defects in patients with CH and severe organification disorders.

TSH deficiency is not related to osteoporosis in childhood

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Osteoporosis has been related to human hyperthyroidism as a result of the effect of increased thyroid hormone levels, endogenous or exogenous, on bone. However, it was reported recently that TSH receptor (TSHR) null mice, rendered euthyroid by thyroid extract administration, are severely osteoporotic and that the bone loss is independent of thyroid hormone levels suggesting that it is the low TSH that induces osteoporosis (Cell 2003;115:151-162). To examine whether low TSH results in osteoporosis in the human, we determined bone mineral density (BMD) and markers of bone metabolism in two male siblings with isolated TSH deficiency. This rare disorder results from mutations of the gene encoding for the TSH receptor and the patients have very low TSH levels from fetal life. Our patients (1 and 2) are of Greek origin, aged 9.8 and 6.8 years old, respectively. The clinical and molecular data of the patients have already been reported (Ped Res 2002;52:935-41). They had always had very low to undetectable TSH levels, except patient 2 that, only at diagnosis, had TSH in the low normal range. BMD of both low thyroid hormone levels. BMD of both patients was within the normal range for age and sex. Patient 1 had a BMD Z-score -0.55 and patient 2 had a BMD Z-score -0.23. Serum Ca and P were normal. Our data show that chronic very low TSH in the face of normal thyroid hormone levels is not related to bone loss during childhood. Since TSHR is expressed in the bone, it is possible that ligands, other than TSH, may bind to the TSHR and exert their effect in bone formation.

Other investigations carried out are shown in Table.
The incidence of childhood thyrotoxicosis in the UK and Ireland is unknown. The commonest cause worldwide is Graves’ disease (60-90% of cases), with reported incidences varying from 0.79 (Denmark) to 6.5 (Hong Kong) per 100,000 pop./yr. Mean age at diagnosis is reported as 11.3 years with a F:M ratio of 5.5:1. Some countries have recently reported an increasing incidence. To ascertain the UK and Ireland incidence of childhood thyrotoxicosis and to describe the presenting features, we have established a national prospective surveillance study from September 2004 to September 2005, coordinated by The British Paediatric Surveillance Unit (BPSU). All paediatricians across the UK and Ireland are requested monthly by the BPSU to report new cases <16yrs of age. Presenting features of each case are then obtained by questionnaire. In the first 4 months there were 34 confirmed cases of childhood thyrotoxicosis in the UK and 1 confirmed case in Ireland. Confirmation of a further 18 reports from the UK and 4 from Ireland is awaited. Currently this estimates the annual incidence at 1.35 per 100,000 (0-15 yr olds) for UK and Ireland. The underlying causes are: Graves’ disease 74%; Toxic phase of Hashimoto’s thyroiditis 11%; Congenital (Maternal Graves’ disease) 3%. In Graves’ disease the mean age at diagnosis is 12.4yrs. The were 5 prepuberal cases (F:M ratio of 1.5:1) and 18 post-pubarche cases (F:M ratio of 5:1).

A variety of presenting symptoms were reported with the commonest being “change in behaviour” (40%) and weight loss (37%). The commonest signs were goitre(57%) and tremor(43%). These preliminary findings show a similar incidence of Graves’ disease to other countries in Europe, and confirm a sharp increase in the incidence in girls after the onset of puberty.

### P1-346 Thyroid

**Higher BMI values and altered BMI pattern in patients with congenital hypothyroidism (CH) in early childhood, compared to controls**

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1Athens University, Medical School, First Dept of Pediatrics, Aghia Sophia Children’s Hospital, Athens, Greece; 2Institute of Child Health, Department of Biochemical Laboratories, Athens, Greece; 3Athens University, Medical School, Endocrine Unit, First Dept of Pediatrics, Athens, Greece

Obesity in children and adolescents has reached epidemic proportions and this phenomenon is more evident in groups with specific nosology. Certain reports have shown an increased prevalence of obesity in children with CH and a recent report (2004) on 53 children indicated that the adipocity rebound (AR) occurs earlier in this group of children. Since earlier AR has been considered a risk factor for the later development of obesity, this observation, if confirmed, is of both theoretical and practical interest.

We analyzed the BMI values of 136 CH children (males: 58, females: 78) aged 0.1 to 6 years diagnosed and followed-up in our center. The data were compared to BMI values recently collected in normal Greek children and are shown in the table. BMI was expressed as mean ± SD. Comparisons of BMI values between the two groups were analyzed using two-sample t-test at each time measurement.

Data analysis was performed using SPSS ver 10.00 and statistical significance was taken at p<0.05.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>CH (Mean ±SD)</th>
<th>Controls (Mean ±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>1 year</td>
<td>16.83 ±2.08*</td>
<td>14.77 ±2.70</td>
</tr>
<tr>
<td>2 years</td>
<td>17.06 ±1.46</td>
<td>16.86 ±1.65</td>
</tr>
<tr>
<td>3 years</td>
<td>16.7 ±1.56*</td>
<td>15.75 ±2.29</td>
</tr>
<tr>
<td>4 years</td>
<td>16.77 ±2.05*</td>
<td>15.72 ±1.73</td>
</tr>
<tr>
<td>5 years</td>
<td>17.09 ±2.32</td>
<td>15.83 ±1.87</td>
</tr>
<tr>
<td>6 years</td>
<td>16.83 ±2.30*</td>
<td>16.29 ±2.21</td>
</tr>
</tbody>
</table>

* p<0.005

The data indicated that: a) in general BMI values are higher in children with CH compared to controls b) the rise and fall and subsequently rebound of BMI observed in normal children does not seem to occur in children with CH.

### P1-347 Thyroid

**The role of T cells costimulatory molecules in autoimmune thyroid disease**

Qin Zhang1, Fan Zhang2

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**Objective:** To investigate the expression of T cell costimulatory molecules for CD54, CD40 and B7.1 in the thyroid tissue of autoimmune thyroid disease (AITD) and its contribute to the pathogenesis.

**Method:** 48 cases of Hashimoto’s thyroiditis (HT), 51 cases of Graves’ disease (GD) and 50 nontoxic goiter(NTG) thyroid specimens were selected. The expression of CD54, CD40 and B7.1 were detected by immunohisto-
Thyroperoxidase (TPO) gene mutations are the most frequent cause of congenital hypothyroidism. Impaired thyroid hormone production is due to the defect in iodid organification. The inheritance is autosomal recessive. However in 17% of patients with severe clinical presentation only one mutation in the coding region is demonstrated, and in some cases single allele mutations cause the disease with milder hormonal deficit. Thirteen patients with thyroid dysmorphogenesis were screened for mutations in TPO gene. Polymerase chain reaction and direct sequencing were used for evaluation of all 17 exons including splicing regions. Sequences were compared to GenBank accession number AH003466. Three different mutations were identified. The common GGCC duplication in exon 8 (c.1277_1278insGGCC;p.R396fsX472) was detected in six patients, three of whom were homozygous. In two patients novel heterozygous missense substitution in exon 11 c.2086G>A (p.G667S) was found and in two other patients a novel deletion of 21 bp in exon 9 (c.1518_1538del) was detected. This mutation caused a deletion of 7 amino acids (p.477_483delANPTVSN) in the conserved region of the protein. The majority of patients with identified mutations had severe thyroid dysfunction, and only one with heterozygous G667S mutation was associated with a milder form of the disease. In two patients with severe disease only a single heterozygous mutation was detected in the entire reading frame. Two novel TPO gene mutations associated with thyroid dyshormonogenesis were detected. The majority of mutation carriers had severe form of CH. Further analysis is needed to obtain more information about the causative relationship between TPO gene defects and milder form of the disease.

**P1-348 Thyroid**

**Two novel TPO gene mutations in congenital hypothyroidism**

Magdalena Avbelj, Katarina Trebusak Podkrjašek, Nevenka Bratanic, Ciril Krzišnik, Tadej Battelino

University Children's Hospital, Dept. of Endocrinology, Diabetes and Metabolism, Ljubljana, Slovenia

Thyroperoxidase (TPO) gene mutations are the most frequent cause of congenital hypothyroidism (CH) due to dyshormonogenesis. Impaired thyroid hormone production is due to the defect in iodid organification. The inheritance is autosomal recessive. However in 17% of patients with severe clinical presentation only one mutation in the coding region is demonstrated, and in some cases single allele mutations cause the disease with milder hormonal deficit. Thirteen patients with thyroid dysmorphogenesis were screened for mutations in TPO gene. Polymerase chain reaction and direct sequencing were used for evaluation of all 17 exons including splicing regions. Sequences were compared to GenBank accession number AH003466. Three different mutations were identified. The common GGCC duplication in exon 8 (c.1277_1278insGGCC;p.R396fsX472) was detected in six patients, three of whom were homozygous. In two patients novel heterozygous missense substitution in exon 11 c.2086G>A (p.G667S) was found and in two other patients a novel deletion of 21 bp in exon 9 (c.1518_1538del) was detected. This mutation caused a deletion of 7 amino acids (p.477_483delANPTVSN) in the conserved region of the protein. The majority of patients with identified mutations had severe thyroid dysfunction, and only one with heterozygous G667S mutation was associated with a milder form of the disease. In two patients with severe disease only a single heterozygous mutation was detected in the entire reading frame. Two novel TPO gene mutations associated with thyroid dyshormonogenesis were detected. The majority of mutation carriers had severe form of CH. Further analysis is needed to obtain more information about the causative relationship between TPO gene defects and milder form of the disease.

**P1-350 Thyroid**

**Biochemical severity of thyroid ectopia in congenital hypothyroidism demonstrates sexual dimorphism**

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A recent study suggests that sexual dimorphism differs according to aetiology and should therefore be considered as a modulator of the mechanisms underlying the function of ectopic thyroid cells. The aim of the study was to determine if there is sexual dimorphism of thyroid function in congenital hypothyroidism and its aetiology in our population. The charts of 140 infants with congenital hypothyroidism diagnosed at the Regional Neonatal Screening Laboratory at Alder Hey Children’s Hospital, Liverpool, born between 1982 and 1999 were reviewed. Tc-precintehinate radiodine scans were performed at diagnosis on all infants to establish the aetiology of CH prior to commencement of treatment. Patients were classified into athyreosis, ectopia and presumed dyshormogenesis. A comparison of males and females were made within the 3 aetiological groups for gestational age, birth weight, initial dose of levothyroxine (LT-4), screening thyroid stimulating hormone (TSH), confirmatory plasma thyroxine (T4), confirmatory plasma TSH and age of TSH normalisation. In thyroid ectopia, screening TSH and confirmatory plasma TSH were significantly lower in males compared with females, while confirmatory plasma T4 was significantly higher in males (Table 1). There was no significant difference between sexes for gestation, birth weight, initial treatment dose and age of TSH normalisation. Sexual dimorphism exist for biochemical parameters at diagnosis of thyroid ectopia in congenital hypothyroidism in our population. This effect was not apparent in patients with athyreosis or dyshormogenesis. Further advances in the molecular genetics of congenital hypothyroidism is essential to evaluate this phenomena.
Table 1: Thyroid function according to sex and aetiology.

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Thyroid function</th>
<th>males n=15</th>
<th>females n=24</th>
<th>males n=54</th>
<th>females n=11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Screening</strong></td>
<td>TSH (mU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(8-265)</td>
<td>(10-7)</td>
<td>(26-316)</td>
<td>(15-230)</td>
<td>(27-244)</td>
</tr>
<tr>
<td><strong>Confirma-</strong></td>
<td>TSH (mU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>tory</strong></td>
<td>(6-880)</td>
<td>(17-760)</td>
<td>(12-400)</td>
<td>(35-420)</td>
<td></td>
</tr>
<tr>
<td><strong>Age of</strong></td>
<td>TSH normalisation</td>
<td>(12.0)</td>
<td>(12.0)</td>
<td>(12.0)</td>
<td>(12.0)</td>
</tr>
<tr>
<td>(<strong>months</strong>)</td>
<td>(6-122)</td>
<td>(6-122)</td>
<td>(11-123)</td>
<td>(8-139)</td>
<td>(9-80)</td>
</tr>
<tr>
<td><strong>Incidence</strong></td>
<td>Percent (%)</td>
<td>420</td>
<td>277</td>
<td>329</td>
<td>118*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>% patients</strong></td>
<td>evaluated &gt;10 days</td>
<td>10.7</td>
<td>7</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td><strong>% patients</strong></td>
<td>notified &gt;15 days</td>
<td>19.6</td>
<td>8.75</td>
<td>13.5</td>
<td>11</td>
</tr>
<tr>
<td><strong>% patients</strong></td>
<td>started treatment</td>
<td>19</td>
<td>7</td>
<td>19</td>
<td>7</td>
</tr>
</tbody>
</table>

*P<0.01 (males vs females) Results were expressed as median (range)

We have demonstrated a definite improvement in the performance of the CH screening programme and diagnosis of CH in Period 2 compared with Period 1. However, late sampling, which is never clinically justified, remains a problem.

P1-352 Thyroid
Late rise of TSH in ill newborns

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Controversy continues from database screening studies about the need for repeat screening or testing of newborns to identify late rise in TSH (LRT). We previously recommended continued thyroid monitoring in very low birth weight (VLBW) and other sick newborns. This study aims to determine from case reviews the frequency of LRT, and describe the characteristics of affected infants. Data were analyzed from infants evaluated for abnormal thyroid tests over a 13 month period at one hospital. Repeat thyroid tests were performed by filter paper screen if hospital care > 4 wks or by serum if thyroid dysfunction was clinically suspected. LRT was defined as a serum TSH >10 uU/ml after a normal TSH on initial newborn screen. Serum TSH, T4, T3 and urine iodine were measured by standard assays. LRT was identified in a total of 14 infants. Of 736 admissions to the NICU, 10/15 consultations with normal initial TSH screens had LRT (1.4 %). Four additional cases of LRT occurred in other ICU settings. TSH elevation resolved in 6/14 (group A); TSH elevation persisted in 8/14 and were treated (group B) (Table). Concurrent medical features included surgery 10 (A4, B6); dopamine use 6 (A1, B5); gastrointestinal disease 6 (A4,B2); congenital heart disease (CHD) 4 (A2, B2). Urine iodine was elevated (> 350 mcg/L) in 6/11 measured (A 2/4, B 4/7). Group B included 3 with TSH >40 uU/ml; all had elevated urine iodine (1 Down Syndrome, CHD, surgery, 1 CHD, multigorgan disease, surgery; 1 VLBW, surgery).

<table>
<thead>
<tr>
<th>Age evaluated (day); range</th>
<th>(mean)</th>
<th>Age evaluated (day); range</th>
<th>(mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 (10-30)</td>
<td>(14.5)</td>
<td>21 (10-30)</td>
<td>(14.5)</td>
</tr>
</tbody>
</table>

A (No Rx); N=6 B (Rx); N=8
Birth wt (g); range (#<1500g) 910-1300 (3) 489-3750 (4)
Gest. age (wk); range (#=30 wk) 25-40 (2) 24-40 (4)
Age evaluated (day); range (mean) 6-85 (40) 7-99 (41)
Initial TSH uU/ml; range (mean) 10.6-20.6 (12.9) 10.5-1326 (234.8)
Age resolved/Rx (day); range (mean) 10-105 (62) 11-104 (52)

1. LRT was demonstrated in 14 infants. In 10 infants this comprised 1.4% of NICU admissions. Thyroxine therapy was used in 8/14 (57%); TSH >40 uU/ml occurred in 3/14 (21%). 2. Of 14 cases of LRT half were not VLBW. 3. Groups A and B had wide overlap in clinical features. Dopamine use was greater in Group B (A 1/6; B 5/8). 4. We recommend ongoing thyroid evaluation of ill newborns regardless of birth weight.
**P1-353 Thyroid**

**Persistently Raised TSH in adequately treated Congenital Hypothyroidism followed-up for a long time**

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Medical School of Ankara University, Pediatric Endocrinology, Ankara, Turkey

Suppression of thyroid stimulating hormone (TSH) has been used as an index of adequate therapy in congenital hypothyroidism (CH). However, the presence of persistently raised TSH has been reported despite adequate treatment and normal thyroxine levels. The aims of this study were to determine the percentage of patients with inappropriate secretion of TSH (IITH) in a large cohort of CH, and describe the clinical features of these patients.

We examined retrospectively the records of 500 children diagnosed with CH during last 30 years. Inclusion criteria of IITH were appropriate doses of L-T4, improvement of clinical findings, normalization of serum TT4 levels (> 8 ng/dl) and persistently high TSH concentrations. A group of patients who demonstrated adequate suppression of TSH (<6 mIU/l) with therapy among 500 CH patients were chosen randomly as control group. The mean values of TSH and TT4 concentrations were calculated during the follow-up period. Both groups were compared with regard to auxological data and TFT at baseline and during treatment period. Of the 500 patients with CH, 27 (5.4%) had IITH. Four out of 27 patients with IITH had organic lesions (3 empty sella, 1 corpus callosum agenesis). All the patients with and without IITH showed a good response to therapy at the basis of auxological and clinical improvement. There was no significant difference for etiological classification in both groups. The patients’ hormonal characteristics and TSH normalization periods are given at the table. The mean normalization period of TSH was 4.3 ± 2.83 years in IITH group. Nine out of 27 (33%) patients with IITHS did not showed TSH normalization during follow-up period.

In conclusion, a group of patients with CH (5.4%), TSH levels might not be able to suppress without undertreatment. TSH levels should not be used as the only criterion of adequacy in therapy. Pre-treatment TSH and TT4 levels and the etiological classification no longer appear to influence the suppression of TSH in CH.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CH patients with IITH (n=27)</th>
<th>CH patients without IITH (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow-up duration (yr)</td>
<td>8.66 ± 4.20</td>
<td>8.90 ± 4.42</td>
</tr>
<tr>
<td>Mean values of TFT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.63 ± 1.22a</td>
<td>10.04 ± 1.43c</td>
</tr>
<tr>
<td>During treatment</td>
<td>3.32 ± 1.60b</td>
<td>9.75 ± 1.71d</td>
</tr>
<tr>
<td>TT4 (mcg/dl)</td>
<td>57.8 ± 23.3e</td>
<td>22.87 ± 11.52g</td>
</tr>
<tr>
<td>TSH (mIU/ml)</td>
<td>52.3 ± 30.1f</td>
<td>3.13 ± 2.1h</td>
</tr>
<tr>
<td>Duration of TSH normalization (yr)</td>
<td>4.30 ± 2.83i</td>
<td>0.22 ± 0.03j</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SD.

**P1-354 Thyroid**

**Subclinical thyroid disorders and Cognitive Performance among Adolescents in the United States**

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Whether sub-clinical thyroid disorders have significant impact on cognitive function in adolescents were not well studied. Our study was to examine the relationships between acquired sub-clinical thyroid disorders and cognitive function among adolescents in the United States. The data used in this study were from The Third National Health and Nutrition Examination Survey (NHANES III). Subjects: We analyzed 1,327 adolescents, 610 M and 717 F, aged ranged from 13-16 years old. The racial distribution was 26.1% white, 34.5% African American, 33.8% Mexican American, and 5.6% other.

**Methods:** All subjects had T4, TSH and Cognitive assessment by using parts of the Wide Range Achievement Test-Revised (WRAT-R). Standardized scores for arithmetic, reading, block design, and digit span were derived. euthyroid state was defined when TSH and T4 were both normal. Sub-clinical hypothyroidism was defined when TSH was elevated and T4 was normal. Sub-clinical hyperthyroidism was defined when TSH was suppressed and T4 was normal. Hyperthyroidism was defined as clinically significant if TSH was <0.1 mIU/L and T4 was elevated. Hyperthyroidism is defined as clinically significant if TSH was >10 mIU/L and T4 was low.

**Results:** Sub-clinical hypothyroidism was found in 1.7% (22 subjects) and sub-clinical hyperthyroidism was found in 2.3% (30 subjects). There was no hyperthyroidism or hypothyroidism identified by our criteria. Using ANOVA and SUDAAN statistical software, mean cognitive assessment scores in sub-clinical hypothyroid subjects were significantly higher than those subjects with euthyroid in block design and reading. However, there was no difference in cognitive function between sub-clinical hyperthyroid and euthyroid subjects.

**Conclusions:** These data suggest that sub-clinical hypothyroidism and sub-clinical hyperthyroidism have no significant detrimental effect in cognitive function. Sub-clinical hypothyroid subjects tend to have better block design and reading ability.

**P1-355 Thyroid**

**Incidence and geographical distribution of childhood & adolescent thyrotoxicosis from a well-defined region of eastern England**

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Recent evidence suggests the incidence of thyrotoxicosis in adults may be increasing yet there are few epidemiological data to support these observations in the paediatric age group. We aimed to determine the incidence of thyrotoxicosis presenting in children and adolescents (<16 years) from a well-defined geographical area of Eastern England over a 10 year period. A retrospective observational case study was performed. All cases of acquired autoimmune thyrotoxicosis for the period 1994 to 2004 were identified from the paediatric endocrinology clinic records from 5 separate clinic centres, located in the Cambridgeshire, Suffolk and Norfolk regions. All children aged < 16 years with thyrotoxicosis were referred for management to these clinics.

Case ascertainment was validated against other data sources. Local population data were obtained from published hospital data and from the UK Office of National Statistics Census 2001. The postcode of residence at diagnosis was used to assess geographical clustering. Twenty-six cases of thyrotoxicosis presented between 1994 and 2004 (20F:6M; median (range) age 12.5 (3 to 15) years). The average annual incidence rate was 0.9/100,000, but increased from 0.54/100,000 (1994–1999) to 1.2/100,000 (1999-2004). Individual clinic centre data are shown in the table. Despite differences in referral pattern to each clinic centre, post-code plotting revealed no evidence of geographical clustering. Incidence of thyrotoxicosis 1994 - 2004

<table>
<thead>
<tr>
<th>Clinic Centre</th>
<th>No. Cases</th>
<th>Local population aged &lt;16 years</th>
<th>Incidence per 100,000 population over 10 year period</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5</td>
<td>79,200</td>
<td>6.3</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>54,450</td>
<td>18.4</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>97,000</td>
<td>7.2</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>29,100</td>
<td>10.3</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>38,640</td>
<td>2.5</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>298,390</td>
<td>8.0</td>
</tr>
</tbody>
</table>

In conclusion, the incidence rate of thyrotoxicosis is lower than contemporary data published for adults (0.9 vs. 25.8 per 100,000/yr). The increasing incidence rate with time is in keeping with reported trends and supports the hypotheses that significant changes in environmental – gene interactions may be responsible for these observations.
P1-356 Thyroid
A family with autonomous thyroid nodules: do the hoof beats suggest the zebra of thyrotropin receptor mutations

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The TSH receptor is a G protein coupled receptor which controls the function of thyroid cells through stimulation of adenyl cyclase. Activating mutations of this receptor cause TSH independent activation leading to hyperthyroidism. Somatic mutation of the TSH receptor has been reported in solitary thyroid adenomas and similar mutations have been reported in multinodular goiters. This is a report on a family with autosomal dominant pattern of inheritance, where three family members in two generations had solitary actively secreting adenomas. The index case was an eight year old girl who presented with a solitary hot nodule confirmed on 131I uptake and scan. Further evaluation revealed an elevated total T3 293ng/dl (94-241) and normal T4 (11.1ug/dl, normal 5.6-14.9). She had a subtotal thyroidectomy and was euthyroid four years later. Her mother had a similar history and was diagnosed with a benign adenoma; she was euthyroid after excision. The next family member was her 16 year old son who presented with an elevated T3 of 193ng/dl (45-127ng/dl), normal free T4 and suppressed TSH. He had a solitary hot nodule on the thyroid scan like his sister. The TSH receptor was analyzed in all three subjects. The coding sequence of the gene was studied in peripheral leucocytes by direct sequencing of the genomic DNA. The sequence was amplified by PCR and then sequenced. No mutation was identified in the TSHR gene. It is possible that this family may have another, as yet unidentified mutation at another location or a promoter gene. Such mutations should be looked for in all cases of solitary adenomas with a dominant pattern of inheritance. This will help with genetic counseling and management.

P1-357 Thyroid
Growth in childhood thyrotoxicosis: Longitudinal follow-up to final height

Somchit Jaruratanasirikul, Hutcha Sripung
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Objective: to study the growth pattern of children affected with thyrotoxicosis.

Patients and methods: A retrospective study of growth data of 40 patients with thyrotoxicosis diagnosed at prepuberty or at early puberty was conducted. All patients were evaluated for height and weight every 3-6 months. Height and weight were transformed to standard deviation score (SDS) to account for difference of age and sex.

Results: At the time of diagnosis, the patients were slightly underweight for height (weight SDS -0.27 ± 1.24, height SDS -0.06 ± 1.26). After 1 year of treatment, the average weight gain of the patients was 4.9 ± 3.1 kg resulting in relatively overweight for height (weight SDS +0.32 ± 1.42, height SDS +0.02 ± 1.32). At the time of reaching final height, the patients were averagely appropriate weight for height (weight SDS +0.06 ± 0.21, height SDS -0.04 ± 1.01). The average final height SDS of the patients was -0.04 ± 1.01 which was at the average of general population, but was +0.57 ± 0.48 SDS or +2.7 ± 1.0 cm. greater than their target height (p =0.01)

Conclusion: Growth of patients with thyrotoxicosis showed the same pattern as in general population. The final height of thyrotoxicosis patients was averagely +0.57 SDS or +2.7 cm. greater than their genetic potential which could be from the result of secular trend in general population rather than being the effect of thyrotoxicosis.

P1-358 Thyroid
Signs of congenital infection mimicking neonatal thyrotoxicosis due to a TSH receptor defect

Wicke Basker-van Waarde1, Bert Timmer2, Hester Kroes3, Catmensus Rouwe1, Roelof Odkin1, Klasien Bergman4
1University Medical Center Groningen, Pediatric Endocrinology, Groningen, Netherlands; 2University Medical Center Groningen, Pathology, Groningen, Netherlands; 3University Medical Center Groningen, Medical Genetics, Groningen, Netherlands; 4University Medical Center Groningen, Neonatology, Groningen, Netherlands

Neonatal thyrotoxicosis is rare and commonly caused by transplacental transport of maternal TSH receptor-stimulating antibodies, although activating mutations of the TSH receptor gene have also been described. A male infant was born at 30-1/7 weeks, birthweight 1355 gram (P10), height 45 cm (P90), head circumference 27.5 cm (P10). Polyhydramnios, fetal growth retardation, preterm rupture of membranes and meconium stained amniotic fluid complicated the pregnancy. He was admitted to the neonatal unit with tachycardia (250/min), hypertension (mean arterial pressure 75 mmHg), fever (39 °C) and respiratory distress syndrome needing artificial ventilation. On examination the infant was agitated with petechiae, blue berry muffin spots, hepatosplenomegaly and a small fontanel. Thyrotoxopoenia (20 * 10^11/l), CRP 36 mg/l and disseminated intravascular coagulation (APTT 87 seconds, PT > 120 seconds, fibrinogen 0.4 g/l) were present. Cranial ultrasound showed left sided periventricular haemorrhage. On the presumption of congenital infection antibiotics were started. Tests for sepsis and congenital infections remained negative. The infant showed failure to thrive and developed hypokalaemia (2.4 mmol/l) and severe intra-hepatic cholestasis (direct bilirubin 829 µmol/l, ASAT 390 U/l, ALAT 129 U/l, γGT 53 U/l). Hyperthyroidism was diagnosed (FT4 75 µmol/l, TSH 0.007 mU/l). Treatment with propylthiouracil (10 mg/kg/day) and atenolol (8 mg/kg/day) was started. The child died at the age of three weeks due to persistent bradycardia after accidental extubation. Family history was negative for Graves disease, TSI < 5 U/l. Sequencing of the TSH receptor gene revealed substitution of methionine (ATG) in position 453 by threonine (ACG) confirming the diagnosis congenital hyperthyroidism due to an activating TSH receptor mutation. In conclusion, hepatosplenomegaly, thrombocytopenia, disseminated intravascular coagulation, hypokalaemia and severe cholestasis can be signs of neonatal thyrotoxicosis. When there are no signs of neonatal Graves' disease, an activating TSH receptor mutation has to be considered.

P1-359 Thyroid
Diagnosis and treatment of juvenile Graves’ disease

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1University Children’s Hospital, Clinic of Pediatric Endocrinology, Sofia, Bulgaria

The therapy of Graves’ disease in children is difficult and controversial. Objective: The aim of this study was to evaluate clinical features of Graves’ disease in children and adolescents and the efficacy of antithyroid drugs therapy.

Material and methods: The study group included 28 boys and 111 girls aged from 2 years to 18 years. The patients were treated between 1990 and 2004 in our Clinic of Pediatric Endocrinology. The diagnosis was based on clinical symptoms, high levels of T3 (FT3), T4 (FT4) with suppressed TSH. The therapy was primarily conservative with methimazole or propylthioracil and L-Thyroxine was added when TSH became detectable. Surgery was considered after second relapse.

Results: Family history of Graves’ disease was positive in 10 % of the patients. We observed goiter in 100 % and tachycardia in 92 % of the patients, systolic hypertension in 46.8 %, palpitation in 63.3 %, nervousness in 69 %, weight loss in 53 %, fatigue in 36.7 %, hyperdefecation in 43.3 %, hyperhidrosis in 50 %, increased appetite in 25.9 %, restless sleep in 37.4 %, enuresis in 7.9 %, headache in 10.1 %, menstrual disturbances in 5 %, polydipsia and polyuria in 6.5 %. Psychological assessment was performed in 18 patients. The patients with Graves’ disease had significantly higher anxiety scores in comparison with healthy volunteers. We investigated ocular manifestation of Graves disease. Propothesis was found in 21 % of patients. Only 3 patients required therapy with glucocorticoids. 18 % of the patients developed side effects (maculopapular rash, urticaria, angioedema, leukenopia, arthritis or...
Selenium deficiency is a common accompaniment to iodine deficiency in Belarus. It is common knowledge, that other trace elements have an impact on the development thyroid disorders including selenium (selenium is the part of deiodinase type 1, 2, 3 glutathione peroxidase).

The aim of the study is to estimate possible selenium deficiency impact on thyroid hormones metabolism.

Two groups of adolescents were included in research. Groups were similar on age and gender. Group 1 consists of 51 persons (32 girls, 19 boys). Their diets were corrected by addition of Natrium selenate 20 µg within 12 weeks.

The group 2 consists of 27 persons (17 girls, 10 boys) who received a usual diet. The investigation included determination of the thyroid gland volume by ultrasonography, thyroid hormones (TSH, FT4, FT3, rT3), urinary iodine excretion, serum selenium (Se) (normal >120 µg/l).

Before research the median urinary iodine excretion was 170µg/l and 202µg/l in the two groups respectively. These data prove the absence of iodine deficiency at the moment of investigation. At the same time 15 children (29.4%) of the group 1, and 9 (33.3%) of the group 2 showed an increase in thyroid. 3 persons of the group 1 had thyroid function decrease. The school children of the group 1 and 2 had subnormal Se levels basal, (Median[25%, 75%]) 53,5 [44,4; 72,8]µg/l and 89,0 [79,5; 97,4]µg/l respectively. Se level statistically significant rising in group 1 in 12 weeks was marked, values 112,8 [90,2; 123,2]µg/l (p<0.01).

We revealed statistically significant rT3 fall in group 1 (p<0.01). It is important to note, that cases of subclinical hypothyroidism weren’t revealed after research. Statistically significant FT3 fall in group 2 after research was marked (p=0.012). All of the findings (TSH, FT4, FT3, rT3) in two groups were within the reference limits. We revealed statistically significant correlation between Se levels and FT3 levels (R Spearman=0.22; p=0.034).

Rising of selenium level resulted in rT3 fall, probably, due to deiodinase type 1 activation.

It is possible the finding of low Se levels suggests that this could be a factor in the thyroid pathology, reduce iodine prophylaxis efficiency.

**P1-360 Thyroid**

**Selenium deficiency impact on thyroid function in adolescents**

*Alena Mokhort1, Elena Kholodova2, Dmitry Garmen2*

1Minsk City Endocrinology Centre, Endocrinology, Minsk, Belarus; 2Belarus Medical Academy of Postgraduate Education, Endocrinology, Minsk, Belarus; 3State Endocrinology Centre, Endocrinology, Minsk, Belarus

We report a case of a 15 year old boy with human chorionic gonadotropin (hCG)-induced hyperthyroidism due to a testicular germ cell tumour. He presented with rapid weight loss (10kg in 10 weeks), abdominal pain and symptoms and signs of hyperthyroidism. Thyroid function tests were consistent with hyperthyroidism: TSH 0.03mU/L (0.3-5.0), Free T4 31.8 pmol/l (7.5-21). Initial thyroid autoantibody screen was negative. Thyroid scan showed asymmetric enlargement and increased uptake. Treatment was commenced with Carbimazole 10mg tds; however there was little clinical response despite normalisation of FT4 and TSH. Three weeks after commencement of carbimazole he remained unwell with tachycardia, further weight loss and abdominal and back pain. He was markedly hirsute, requiring daily shaving of his body hair. Examination showed a 10cm right testicular mass, a very large midline abdominal mass and enlarged left supraclavicular nodes. Further biochemical investigations showed an extremely elevated (hCG level of 396,917 U/L (0-2), and an alpha-fetoprotein level of 2240 µg/l (0-10)). Biopsy of the testes showed a malignant mixed germ cell tumour. CT chest and abdomen demonstrated extensive tumour involving retroperitoneal nodes with liver and lung metastases. Anti-tumour therapy including surgery (orchidectomy) and chemotherapy (cisplatin, etoposide and bleomycin) resulted in clinical improvement, normalisation of hCG and cessation of anti-thyroid therapy. Hyperthyroidism secondary to excess hCG secretion has been previously documented in women with trophoblastic disease; however it is less well recognised in men. TSH and hCG share the same (hCG)-induced hyperthyroidism due to a testicular germ cell tumour. He

**P1-361 Thyroid**

**Neuropsychological and behavioral outcome in 5 year old children with Congenital Hypothyroidism treated early and sufficiently**

*Royel Jeanne1, Bhavna Dagnani2, Esther Perlman3*

1The Hospital for Sick Children, Brain and Behavior Program, Toronto, Canada; 2The Hospital for Sick Children, Pediatrics, Toronto, Canada

**Conclusion:** Despite normal-range abilities, early and adequately treated children with CH still have mildly reduced cognitive abilities and increased behavior problems relative to controls. While some findings are related to disease and treatment factors, we found no evidence of increased behavior problems from a high starting dose and a dose above 10.8 mg/kg is recommended.

**P1-362 Thyroid**

**BhCG-Induced hyperthyroidism in a 15 year old boy with a testicular germ cell tumour**

*Frances Mouni1, Philip Bergman1, Chris Pappas2, Peter Downie2*

1Monash Medical Centre, Diabetes Ambulatory Care Unit, Melbourne, Australia; 2Monash Medical Centre, Paediatrics, Melbourne, Australia

We presented with rapid weight loss (10kg in 10 weeks), abdominal pain and symptoms and signs of hyperthyroidism. Thyroid function tests were consistent with hyperthyroidism: TSH 0.03mU/L (0.3-5.0), Free T4 31.8 pmol/l (7.5-21). Initial thyroid autoantibody screen was negative. Thyroid scan showed asymmetric enlargement and increased uptake. Treatment was commenced with Carbimazole 10mg tds; however there was little clinical response despite normalisation of FT4 and TSH. Three weeks after commencement of carbimazole he remained unwell with tachycardia, further weight loss and abdominal and back pain. He was markedly hirsute, requiring daily shaving of his body hair. Examination showed a 10cm right testicular mass, a very large midline abdominal mass and enlarged left supraclavicular nodes. Further biochemical investigations showed an extremely elevated (hCG level of 396,917 U/L (0-2), and an alpha-fetoprotein level of 2240 µg/l (0-10)). Biopsy of the testes showed a malignant mixed germ cell tumour. CT chest and abdomen demonstrated extensive tumour involving retroperitoneal nodes with liver and lung metastases. Anti-tumour therapy including surgery (orchidectomy) and chemotherapy (cisplatin, etoposide and bleomycin) resulted in clinical improvement, normalisation of hCG and cessation of anti-thyroid therapy. Hyperthyroidism secondary to excess hCG secretion has been previously documented in women with trophoblastic disease; however it is less well recognised in men. TSH and hCG share the same (hCG)-induced hyperthyroidism due to a testicular germ cell tumour. He

**P1-363 Thyroid**

**Nodular thyroid changes four years after prenatal exposure to Radioactive Iodine therapy; the importance of persistent mild TSH elevation**

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1Royal Hospital for Sick Children, Department of Child Health, Yorkhill, G3 8SJ, Glasgow, United Kingdom; 2Victoria Hospital, Department of Paediatrics, Kirkcaldy, United Kingdom; 3Royal Hospital for Sick Children, Department of Child Health, Glasgow, United Kingdom

A 42y old woman presented with nausea, weight loss, palpitations and irritability. She was thyrotoxic (total T4: 1786(60-160)nmol/l; TSH:0.1mu/l) with increased uptake (16%) on isotope scan and subsequently received 460MBq of Iodine-131. She presented 3wks later with advanced pregnancy and it was realised that she had received radioactive iodine (RI) while at 17wks of intelligence (108.7 vs 114, p=0.02), language (p=0.4), and verbal and spatial working memory (p=0.05) but not other abilities. Parent and teacher questionnaires revealed increased working memory, attention, and cognitive problems in CH. More severe disease initially was associated with weaker spatial ability, memory, and attention (p<0.05), later treatment onset with weaker attention abilities and behavior problems, and a longer time to TSH normalisation with more attention, executive processing, and anxiety problems. A median split analysis comparing CH by starting dose level (+10.8 mg/kg) showed better attention but not more behavior problems in children started at the higher dose.

**Conclusion:** Despite normal-range abilities, early and adequately treated children with CH still have mildly reduced cognitive abilities and increased behavior problems relative to controls. While some findings are related to disease and treatment factors, we found no evidence of increased behavior problems from a high starting dose and a dose above 10.8 mg/kg is recommended.
gestation. A pregnancy test was not performed prior to RL. Cordocentesis was performed at 27 and 30 wks gestation (see table). Intra-amiotic thyroxine was not administered because despite TSH elevation, T4 levels were normal. A healthy boy was born at term weighing 3520g. TSH was initially suppressed, then mildly elevated, and he was not treated with thyroxine. At 2.5y he remained well and, in view of what was felt to be minimal TSH elevation he was discharged. When the case was independently reviewed for a legal report it was felt that he merited reassessment given the mild but significant TSH elevation at time of discharge. On review at 4y he had normal neurodevelopment and no clinical goitre. TFT showed free T4:9.6pmol/L, TSH:15.4mIU/L rising to 67.9 at 30y and falling to 49.8 at 60y after TRH and a high Tg:117ug/L (N=48). TPO antibodies were negative. Ultrasound showed a normal sized thyroid (1.5mls) but several hypoechoic nodules within the left lobe; the largest measured 9 by 6mm. After thyroxine a repeat ultrasound at 6.8y showed resolution of the nodules and a normal Tg (16ug/L).

This case illustrates the need for caution when administering RI to women of childbearing age. As she was 8wks pregnant at first consultation, routine pregnancy testing would have sufficed. However, an early pregnancy (1-2wks) may not have been detected due to the diagnostic gaps of pregnancy tests. Any infant inadvertently exposed to RI should have regular surveillance of thyroid function. TSH elevation alone is a signal to start thyroxine at a suppressive dose (100mcg/m²) to help prevent nodular formation and reduce the theoretical risk of thyroid cancer. Despite the low risk the thyroid should be protected from excessive TSH stimulation with lifelong thyroxine and regular surveillance.

## P1-364 Thyroid

### Serum measurements of Thyroglobulin in the etiological diagnosis of congenital hypothyroidism

**Adriana Boyanova**,1 **Graciela Testa**,2 **Silvia Martin**,2 **Gabriela Sobredo**,3 **Gonzalo Pérez**,3 **Mariana Jarovsky**,3 **Liliana Muñoz**,2 **Mirta Mira2**

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The etiological diagnosis of congenital primary hypothyroidism (CH) plays an important role in determining disease severity, outcome, genetic investigation and therefore its treatment schedule. The ultrasound and 99mTc thyroid scintigraphy are the most currently imagine procedures used to establish the etiology of hypothyroidism. There are not enough reports about real contribution of serum thyroglobulin (TG), considering enhancement of TG techniques allows to count with quick and reasonable new sensitive assays to improve the diagnosis orientation. Aims: to assess the utility of TG determination regarding CH etiologic diagnosis performed by imaging techniques.

### Patients and Methods:

64 newborns with CH diagnosis were retrospectively analysed using thyroid Tc 99 scintigraphy, ultrasound (General electric Voluson 730 expert) and Tg(ng/ml), FT4(ng/dl) values by EQLIA (elecsys®2010). ANOVA and ROC curves were applied.

### Results:

 disclosed the data according to imaging diagnosis were classified in: Agenesis/Hypoplasia (AH) n: 13, Ectopy (ECT) n:23, Eutopy without hyperplasia (EU) n:6 and Eutopy with hyperplasia (EUH) n:22. The TG levels (median and range) in AH were significantly lower [1.0 (1-85)] than ECT [250 (28-709.8)], EU [177.8 (33-1260)] or EUH [709 (1.3-7400)], p< 0.001. According to growing TG levels four categories were established: 1) TG<2 2) TG 2-8.5 3) TG 8.5-730 4) TG >730, allowing the concordant classification of 73.4% of the patients (p=0.001).

### Conclusion:

The initial TG measurement was specific and sensitive for patient with agenesis and extreme hypoplasia. The scintigraphy could be reserved, after TG determinations for patients with eutopic or ectopic thyroid tissue.

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## P1-365 Thyroid

### Differentiated Thyroid Carcinoma (DTC), Clinical presentation and follow up in 27 Chilean children

**Claudia Godoy**,1 **Andrea Cattani**,1 **Isabel Torrealba**,1 **Hernán García1,2**, **Rossana Romaní1,2, Ricardo Silva1,2, María Loreto Reyes1,2, **Alejandro Martínez-Aguayo**,1 **Edna Mancilla1**

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DTC is rare in childhood and usually presents as an advanced disease, but with good long term prognosis.

### Objective:

Report the clinical characteristics, treatment and follow up of DTC in Chilean children.

### Study Design:

Retrospectively, clinical charts of patients <19 yrs, with DTC diagnosed between 6/1993 and 2/2005 in 3 Endocrinology Units in Santiago, Chile were reviewed.

### Results:

Age at diagnosis was 11±3.8 y (range 4.1-18), 16/27 were females. Follow-up time was 4.4±4y. 14 patients presented with thyroid mass, 4 with palpable cervical lymph nodes (CLN), and 9 with both. Goiter was in 13 uninnodular, 7 multinodular, and 3 diffuse. One patient presented as a critical laringeal obstruction. 19 patients presented metastases (CLN=8; lung=1; both= 7; other=4). Tumor’s diameter from 24 biopsies was <1cm:2, 1-4 cm:19 and >4cm:3. 16 were papillary carcinomas, 9 papillary-follicular, 1 follicular and 1 sclerosing. 74.1% had extracapsular extension. All patients underwent total thyroidectomy and 84% CLN dissection; 3 suffered recurrent laryngeal nerve damage and other 3 permanent hypoparathyroidism. All received postoperative thyroid hormone suppressive therapy. All children with papilar and follicular carcinoma received (131)I after initial surgery. The mean dose was 142±43 mCi (range 50-200); 19/25 patients showed a positive whole body scan after I131, 14 regional, and 5 pulmonary metastases; 44% received more than one dose (max 1000 mCi). 40.7% of patients recurred in 6 in CLN, 2 in lung and 3 in both. The time to first local recurrence was 2.0±1.6 y and pulmonary 1.4±0.7y. Local recurrence in <10y old vs>10y was 66.7% vs 20%(p=0.03) and pulmonary was 40% vs 5.9%(p=0.04). No deaths were reported.

### Discussion:

Thyroid mass was the most frequent manifestation of childhood DTC. Therapy of choice was total thyroidectomy, CLN dissection, (131)I and thyroid hormone suppressive therapy. Most patients presented with advanced stage disease, however at a mean of 4.4 y of follow up 59.3% are free of local and distant recurrence of tumor and no death reported. Children <10y had a greater recurrence risk than older.
P1-367 Thyroid
Multinodular goiter in children
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There was analyzed the treatment of 71 children with isolated (41) and bilateral (30) lesion of anatomic lobes of thyroid gland (TG). The examination included ultrasonic examination (USE), color Doppler mapping (CDM), selective flowmetry (SF), radio-isotope scintigraphy (99mTc-pertechnitnate) (RIS) and aspiration puncture biopsy under US control (APB-USE). Isolated lesion was identified in 16 patients. Cancer of TG was registered in 6 children: papillary - 4 (26,6%), follicular - 2 (13,3%). Irrespective of lesion character all nodes in one patient had identical US-characteristics. During CDM in 66,7% of patients with CTG an intra-nodular blood flow was revealed. During SF in 83,4% of observations of CTG it was recorded vascularization intensification at the side of lesion. During RIS all nodes had equivalent characteristics. In the result of APB-USE, CTG was diagnosed in 4 patients (66,7%). In 86% of patients homogenous lesion was recorded. Bilateral lesion was identified in 30 children, CTG was registered in 11 patients: papillary - 10 (33,3%), follicular - 1 (3,3%). US-characteristics of each node in one patient in 81,8% of observations were different. During CDM in case of CTG 63,6% of nodes had intra-nodular blood flow. During SF while localizing CTG foci in one anatomic lobe intensification of the blood flow at the side of lesion was registered. During RIS no equivalent isoetoine accumulation was recorded. USE-APB made it possible to verify CTG in 8 children. In the majority of patients heterogeneity of lesion was confirmed. Thus, in case of isolated lesion of a TG lobe, homogenity of MNG was registered, in case of bilateral lesion - heterogeneity of MNG. The proposed algorithm of diagnosis made it possible to define the character of lesion prior to operation in 91% of patients.

P1-368 Thyroid
BRAF Mutation in Papillary Thyroid Carcinoma Associated with Hashimoto’s thyroiditis
J.B. Quintero1, K Suphaphiphat2, J Dong3,5, Castells4
1SUNY Downstate Medical Center, Pediatrics, Brooklyn, NY, United States; 2Mount Sinai School of Medicine, Pathology, NY, NY, United States

We report a 19 year old female who initially presented at age 14 years with euthyroid goiter secondary to Hashimoto’s thyroiditis. After careful follow up of two and half years, she developed a right thyroid nodule. Thyroid ultrasound confirmed an echogenic right thyroid nodule in the middle of the right thyroid gland which measures 1.9 x 1.5 x 1.7 cms. Total thyroidectomy was subsequently performed. Analysis of the thyroid tissues revealed papillary thyroid carcinoma (PTC) with lymphocytic thyroiditis. Since a somatic point mutation in the BRAF gene has recently been considered to be a feature of sporadic, non-radiation-associated PTC, PCR and direct sequencing techniques were used to screen for the BRAF V600E mutation. Interestingly, this mutation was present in PTC, but absent in the adjacent thyroid tissue. Here we report the first case of Hashimoto’s thyroiditis associated with PTC harboring the BRAF V600E mutation. Further studies are required to address the significance of BRAF mutation in association between Hashimoto’s thyroiditis and PTC.

P1-369 Thyroid
Cardiac function after long-term thyroxine treatment in patients with Congenital Hypothyroidism (CH)
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The long-term effects of levothyroxine treatment on cardiac function in children with CH are incompletely known. In order to accomplish the desired biochemical response, the L-T4 dose given may suppress TSH concentrations. The aim of this study was to determine the impact of suppressed TSH levels on cardiac function in a group of 28 patients with CH, diagnosed by neonatal screening aged 17.8±3.07 years. All were clinically euthyroid. Their mean daily thyroxine dose was 2.6±mg/kg. Left ventricular dimensions and function were investigated by means of Echocardiography (M-mode, two-dimension- al, Doppler, color Doppler). Cardiac parameters were correlated to the T4, T3 and TSH levels at the day of echocardiography as well as to the mean T3, T4, and TSH values in the last 3 years. The T4 levels on the day of examination and in the last 3 years were 9.9±±2.34 and 9.40±1.46, and those of TSH were 3.16±3.14 and 3.19±3.02. In 14 of our patients TSH levels were suppressed at examination and in 5 during the last 3 years. Cardiac parameters were within the normal range for the age of the patients. However there was a statistically significant positive correlation between TSH (at examination as well as in the last 3years) and left ventricular end-diastolic diameter, LV end-systolic diameter, end-diastolic volume, end-systolic volume and ejection time and a significant negative correlation between TSH and interventricular septum diastolic diameter, shortening fraction, left ventricular mass, LV mass index, deceleration time, stroke volume, cardiac index and heart rate. T4 values had a opposite significant correlation with the above parameters. Cardiac parameters of patients with suppressed TSH levels differed significantly to those of patients with normal TSH values. We conclude that patients with CH may have suppressed TSH values under customary doses of thyroxine and this may affect their cardiac function.
P1-370 Thyroid

Gain in body mass index following treatment of childhood thyrotoxicosis is significant, however overweight and obesity is rare

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Weight loss is common at presentation of thyrotoxicosis. In adult patients treatment is associated with significant gain in BMI and obesity, however patterns of weight gain in childhood are poorly documented. It was the aim of this study to investigate influences on weight gain following initiation of treatment. A retrospective study of 48 patients with thyrotoxicosis was performed. 3 patients with coexisting pathology affecting auxology were excluded. 45 patients (9M, median age 13.2 years, range 3.6 to 16.0, 13 prepubertal) were studied during a follow up period of 32 months (7 - 108 months). All patients were treated by thionamides ± thyroxine. BMI at diagnosis was -0.21 SDS (-2.1 to 2.95) at 1 year (n=37) 0.70 SDS (-1.9 to 3.08), and 3 yrs (n=22) 0.28 SDS (-1.56 to 2.72). Gain in BMI was significant at each time point (p<0.001 and p = 0.001 respectively). The incidence of overweight (BMI >2 SD) and obesity (BMI >3 SD) was 5.4% and 2.7% at 1 year and 4.5% and 0% 3 years. BMI SDS at presentation correlated positively with age (p = 0.01) however gain in BMI SDS was unaffected by age or pubertal status. Gain in BMI SDS 3 and 6 months following initiation of treatment correlated negatively with time to normalisation of T4 (p = 0.001 and p=0.01 respectively). There was no relationship between the number of relapses, development of hypothyroidism, treatment and timing of treatment with T4 and BMI SDS. There was no difference in gain in BMI SDS between the dose titration and blockade regime. Weight gain is significant during treatment of thyrotoxicosis in childhood and is related to the timing of normalization of T4. However, the prevalence of overweight and obesity is low.

P1-371 Thyroid

Serum total thyroxine is related to body mass index in non-obese pubertal girls

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The relationship between thyroid function and body adiposity is not well defined in children. In obese children, serum thyrotropin (TSH) and thyroxine (T4) have been noted to be elevated in some studies, with levels normalising after weight loss. In malnourished children, T4 but not TSH, is reduced. The influence of body mass index (BMI) within the normal range of thyroid function has not been explored. We aimed to assess if serum total T4 and/or TSH concentrations are related to BMI in healthy, euthyroid pubertal children. Healthy school-going pubertal boys (n=160) and girls (n=206) between the ages of 9 and 16 years were recruited to an ongoing observational study investigating lead contamination in Kolkata, India. As part of the study, these children had measurements of BMI, serum total T4 (50-155 mmol/l) and TSH (0.3-5.5 mU/l) concentrations. Boys of mean (SD) age 13.2 (1.8) years had mean (SD) BMI of 17.1 (2.4) kg/m2 (Z-score –0.8 (0.8)) whereas girls of age 13.7 (1.6) years had BMI of 18.1 (1.1) kg/m2 (Z-score –0.5 (0.8)). In our group, girls were older (p=0.003) and heavier (p<0.001) than boys. The mean (SD) total T4 (nmol/l) and TSH (mU/l) concentrations were 104 (27.1) and 1.4 (1.0) in boys and 113.5 (24.3) and 1.9 (1.0) in girls. Serum T4 (r=0.2, p=0.003) but not TSH (p=0.4) correlated with BMI in girls and the correlation remained significant after controlling for age (r=0.15, p=0.04). In boys, T4 was inversely correlated to age (r=-0.2, p=0.009) when controlling for BMI, but not to BMI (p=0.3) when controlling for age. In multiple linear regression in the whole group, T4 was influenced by gender (p=0.001) but not by TSH (p=0.4), age (p=0.6) or BMI (p=0.3). We conclude that serum total T4 was positively correlated with BMI in euthyroid, normal-weight, pubertal Indian girls but not boys.

P1-372 Thyroid

Urinary iodine concentration and goiter prevalence in Slovenian adolescents

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More than half of European school-age children are still considered to be iodine deficient. In Slovenian school-age children median urinary iodine concentration, prime indicator of individual's nutritional iodine status, is currently estimated to be below 100 µg/l indicating iodine deficiency. Objective of our study was to determine median urinary iodine concentration and goiter prevalence in population of Slovenian adolescents with the goal of determining success of increased supplementation of salt in 1999 from 10 to 25 mg of KI per kg of salt. 2464 adolescents (1264 girls vs. 1200 boys, age 15.7 ± 0.6 years in girls vs. 15.8 ± 0.8 years in boys) representing 10% of 15 year-olds from all Slovenian regions were studied. A random urine sample was collected from all examinees. Urinary iodine concentration was measured by the method based on the Sandell-Kolthoff reaction. Thyroid size was estimated by neck palpation in all examinees. When enlarged thyroid was suspected thyroid volume was determined using real-time sonography according to Brunn. Volume of one lobe in milliliters was calculated by the formula: 0.479 x anteroposterior diameter (cm) x mediolateral diameter (cm) x craniocaudal diameter (cm). Thyroid volume was the sum of volumes of both lobes. Median urinary iodine concentration was 140 µg/l (equally 140 µg/l in girls and boys). In all regions it was equal or over 100 µg/l (range 100 to 190 µg/l). Values less than 50 µg/l were determined in 2.5 % of examinees (2.9 % of girls and 2.0 % of boys). Enlarged thyroid was determined by ultrasound in 0.9 % of all examinees (19 girls and 5 boys). 9 (all girls) were diagnosed with autoimmune thyroiditis. Slovenian adolescents are iodine sufficient and the prevalence of goiter is low indicating that increased KI supplementation of salt in 1999 was successful.

P1-373 Thyroid

A new mutation in the TR-b gene in a patient with RTH and ADHD

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Introduction: Resistance to thyroid hormone (RTH) is an inherited defect manifesting as variable tissue hyporesponsiveness to thyroid hormone, caused by mutations in the thyroid hormone receptor beta (TR-beta) gene. Up to now 122 mutations in this gene have been identified, mostly clustered in two regions located in exons 7 - 10. Case: A 18 year old boy presented with weight loss, sleeping disorder, and attention deficit hyperactivity disorder (ADHD). Laboratory analysis showed elevated serum levels of T4, free T4, T3, free T3, and a normal TSH value. This led to the diagnosis of RTH. Ultrasound showed a total thyroid volume of 14.5ml, homogeneous thyroid texture and an increased echointensity. Molecular analysis revealed a heterozygous mutation in exon 7 of the TR-beta gene (codon 302, ATC to TTC) leading to an amino acid substitution from isoleucine (I) to phenylalanine (F). RTH due to this novel point mutation was confirmed in our patient by a validated delta-TRH test. The patient was first treated with carbimazole, however the weight loss, sleeping disorder and ADHD symptoms persisted. After several failed attempts to control the symptoms, and after a thorough genetic and clinical work-up, a mutation at the TR-beta gene was identified. Conclusion: A novel de novo mutation (I302F) in the thyroid hormone receptor beta gene (TR-b) was found in a 18 year old boy who presented with the syndrome of RTH and ADHD.
Hyperthyroidism is rare in early childhood with potential effects on growth, body composition, activity and behaviour. It is most commonly caused by Graves' disease.

We report 14 children aged 3.4-7.5 yrs (4 boys, 10 girls). At diagnosis, all patients had weight loss, hyperkinesis, tachycardia, difficulty sleeping and poor concentration. There was a positive family history in 7 cases. All had clinically enlarged thyroid glands (3 large, 6 medium, 4 small glands, 1 size not specified) and 11 presented with protrusion but none had ophthalmoplegia. At diagnosis, height was advanced compared to the population standard and target height, and BMI SDS was low for height SDS (Table 1). Bone age was advanced in 3 of the 5 children in which it was recorded. Total T4 and free T4 levels were elevated (Table 1) and TSH was undetectable in all cases. Ninety percent of patients had positive thyroid autoantibodies. Initial treatment was with antithyroid medication (13 carbimazole, median dose 0.75 mg/kg/day, 1 PTU 15 mg/kg/day). Thyroxine was added later in 6 patients. Two patients had adverse drug reactions. Normalisation of T4 levels occurred at a median of 4 months (range 1-9) and normalisation of TSH took longer; median 7 months (3-24). Relapse was recorded in 9 out of 11 patients treated for longer than one year. Median duration of therapy in those treated for 18 months or longer was 58 months (range 18-132). Only four have had definitive therapy (2 surgical, 2 radioiodine). Of note, four patients have long-term problems with concentration and behaviour. In conclusion, young children with Graves' disease, response to antithyroid drugs was satisfactory, but relatively slow. Continuous medical therapy throughout childhood was usually required. Spontaneous remission was rare. Long term neuropsychological deficit was not uncommon.

Features at Presentation | median | min | max | n
---|---|---|---|---
Height (HT) SDS | 1.25 | 0.58 | 5.24 | 12
Target HT (TH) SDS | -0.80 | -2.13 | 1.34 | 10
DIFF SDS (HT SDS-TH SDS) | 1.63 | 0.81 | 9.38 | 9
BMI SDS | -0.48 | -1.65 | 1.26 | 12
Total T4 | 239 | 171 | 377 | 7
free T4 | 54.7 | 25.2 | 63.1 | 4

56.7±8.1 vs 49.4±9.6 p=0.05). In scales: emotionally reactive (55.8±6.2 vs 52±3.1 p=0.05), withdrawn (58.2±8.5 vs 53±3.3 p=0.05), sleep problems (55±5.2 vs 50.8±1.3 p=0.01), affective problems (58±6.7 vs 53±3.8 p=0.05) and pervasive developmental problems (57.8±6.9 vs 53.4±4.2 p=0.05) CH subjects of group 1 showed significantly higher scores than controls. No significant differences were found between CH subjects of group 2 and controls. There was a significant positive correlation only in CH subjects of group 1 between L-thyroxine dose during the first year of therapy and affective scale (r=0.65 p<0.01). We did not find significant influences of IQ and any hormonal parameters at diagnosis and during the follow up on behavioural and DSM-oriented scales.

Conclusions: the successful follow up seems to resolve anxious approach of parents in the first period of therapy. Further behavioural follow up is needed in infancy and adolescence age.

**Bone mineral density and vitamin D receptor genotype in children with congenital hypothyroidism**

**Introduction:** Bone mineral density (BMD) is modulated by genetic and environmental factors or certain diseases. In several conditions, an influence of vitamin D receptor (VDR) polymorphisms on BMD has been suggested, as in patients with hyperthyroidism the VDR genotype might influence the risk of low BMD. Objective: To study the effect of long-term L-thyroxine (LT4) replacement therapy on bone mineral density in children with congenital hypothyroidism (CH).

**Patients and Methods:** Fifteen children and adolescents (mean age 13.3 ± 4.8 years) with CH who started treatment at a mean age of 3.5 ± 3.0 years for a mean period of 9.7 ± 6.0 years were studied. BMD was measured at the lumbar spine by dual X-ray absorptiometry (DXA). Serum alkaline phosphatase was measured to exclude rickets and osteomalacia.

Polymerase chain reaction (PCR) and VDR Fok I genotyping were performed.

Results: BMD SDS ranged from -0.2 to -3.2 (-1.58 ± 1.0). Mean TSH on treatment was 11.18 ± 11.2 mU/L, [range (0.78 - 31.93 mU/L), median 5.43 mU/L], mean T4 on treatment was 11.94 ± 2.3 µg/dl, [range (8.2 - 15.3 µg/dl), median 12.2 µg/dl], duration of treatment ranget from 1.25 - 18.9 years. Mean LT4/m2/day was 147.2 ± 29.36 µg, [range (102 - 196 µg), median 146.43 µg]. There was a significant positive correlation between BMD SDS and mean TSH on treatment, r=0.55, p<0.05. The prevalence of the VDR Fok I polymorphisms in the patients was Ff heterozygote 38.5%, ff homozygote 15.4% and FF homozygote 46.2%. Percentage of the ff phenotype in children with osteoporosis was 50%, the Ff phenotype was 25% and the FF phenotype was 25%, p=0.07.

Conclusion: BMD SDS in children with CH on LT4 replacement therapy is influenced by treatment with detrimental effects of excessive doses of LT4 causing TSH suppression. The VDR phenotype does not appear to have an adverse effect on bone mineralization.

**Critical laboratory aspects in evaluating thyroid function and hypotalamic-pituitary-thyroid axis in paediatric population**

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The thyroid axis undergoes progressive maturation and modulation until after the puberty. Higher TSH concentrations are typically seen in children. The
maturation process dictates the use of age-specific range limits, related to the method of assay. This study was performed to investigate thyroid function in healthy and normal weight children, related to the methods employed for TSH, T3 and T4 measurement and to evaluate the influence of rhGH therapy on thyroid function in GH deficient population. In healthy population 65 children (M=31.1, F=34) were pre-pubertal and 85 children were in puberty (M=53.7, TV=44; F=32.0, B=2). The GH deficient group recruited 37 clinically euthyroid patients (M=17.7, F=20). TSH, T3 and T4 were assayed using chemiluminescent methods (IMMULITE 2000, DPC). A number of discrepancies was observed concerning out-of-range levels of free hormones despite normal TSH values, particularly related to T3, likely due to inadequacy of normal reference limits. Only the distribution of T3 (p=0.57; W=0.98), calculated with a Shapiro-Wilk’s test, was normal during puberty; in pre-pubertal population, only the distribution of T4 (p=0.6; W=0.98) and log TSH (P=0.45; W=0.97) were normal. The correlation T4/log TSH was not significant in both groups. In 95% of pre-pubertal healthy children T3 (4.37-4.72 pg/ml) was higher than that found during puberty (4.02-4.37 pg/ml). The same was observed for T4. In 95% of the healthy children of the two populations TSH levels were similar (1.8-2.2U/ml) but higher than those found in the adult population (1.4-1.6U/ml). In 95% of the healthy children T3 levels were higher (4.2-4.45 pg/ml) than those found in the adult population (3.56-3.8 pg/ml). In the evaluation of thyroid function in GH-deficient patients no significant difference was found between TSH level before (1.71-2.19U/ml) and after 6 months (1.83-2.37U/ml) and 12 months (1.68-2.5U/ml) of GH therapy. These data suggest that specific reference ranges must be identified for pre-, peri- and post-pubertal children as concerning serum levels of T3, T4 and TSH. TSH, however, remains, as for adults, the most suitable single test for the evaluation of thyroid function.

P1-378 Thyroid
Congenital primary hypothyroidism presenting as pituitary macroadenoma and hyperprolactinaemia, galactorrhea that resolved shortly after introduction of levothyroxine therapy: A report of two cases
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Pituitary pseudo tumor (pituitary thyrotroph/ lactotroph hyperplasia) caused by unrecognized hypothyroidism has been described as a rare condition in adults. There are only few reports in children with variable clinical presentation. Two cases of congenital thyroid dysgenesis showing pituitary enlargement mimicking a pituitary macroadenoma which resolved completely after T3 therapy, are described.

First case: male child 10½ years, presenting with short stature -3.5 SDS, normal IQ and school performance, no symptoms and signs of hypothyroidism, no goiter. GH by ITT and clonidine: peak of 2.7 ng/ml and 10.4 ng/ml. MRI revealed a macroadenoma 1.4x1.2x1.4 cm with suprasellar extension. Field of vision was affected. Prolactin: 91.2 ng/ml (4.6 - 37). Although relatively asymptomatic, he was found to have profound primary hypothyroidism. TSH > 75 mIU/ml; FT3, 0.25 ng/dl (0.8 - 1.9); US thyroid volume: -2.4 SDS. Anti-thyroid antibodies negative. Bone age: 5 years. Two months after the start of T3, prolactin normalized. One month after, MRI showed complete disappearance of the adenoma and showed an empty sella. GH stimulation was retested and found normal. The child gained 1 SDS in one year.

Second case: 5 years old girl presenting with galactorrhea. Height 91.7 cm (-3.5 SDS), bone age: 2 years; IQ: 69; Prolactin 64.8 ng/ml; CT scan showed a pituitary macroadenule 3cm encroaching upon suprasellar cistern superiorly. FT3: 0.4 ng/ml; TSH: > 75mIU/ml; Thyroid U/S: atrophy. Two months after T3 therapy, macroadenoma disappeared and prolactin normalizes. Primary hypothyroidism can present only with hyperprolactinaemia, galactorrhea and pituitary mass mimicking a pituitary macroadenoma, and should therefore be considered in the differential diagnosis of hyperprolactinaemia and pituitary enlargement. TSH levels should be measured in all patients presenting with a suspected pituitary mass in order to avoid misdiagnosis for pituitary tumor, obviating unnecessary surgery.

P1-379 Thyroid
Iodine deficiency status in Bulgaria based on the Neonatal Thyroid Screening Data 1997-2004
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The proportion newborns with TSH levels above 5 mU/l whole blood represent a core indicator for monitoring national progress towards the goal of virtual elimination of iodine deficiency disorders. Current WHO guidelines consider that under an adequate iodine intake less than 3% of the newborns should have TSH levels of > 5 mU/l when screening is performed. Data of 305 895 newborns screened from their 3rd - 5th day of life by TSH Delfia in dried blood spots from 28 Bulgarian districts were processed from 1997-2004. The proportion of newborns in the screening increased from 83 - 96%. Initially (1997/98) four districts were found to be free of iodine deficiency, but their number gradually decreased to two and one (1999 - 2002). In 2003 the number of iodine repleted districts sharply increased (n=7) and in 2004 the procedure continued further (n=11). The overall picture of the country revealed two different patterns: increase of the proportion of newborns with TSH=5 mU/l from 7.8% (1997) to 9.6% (2000), followed thereafter by a permanent decrease. Only 3.6% of the newborns in 2004 showed TSH concentrations > 5 mU/l. Ten years after the reintroduction and fortification of the iodine supplementation program by universal salt iodization for the whole country our results showed the substantial progress towards elimination of iodine deficiency disorders as a public health problem, especially in the most vulnerable population for development of mental deficits - the newborns. The neonatal thyroid screening represent an universal comprehensive epidemiological indicator, which, if age-adjusted allows a reliable monitoring of the extent of iodine deficiency, its disappearance or reemergence.
and mostly related to the degree of hormone deficiency, little is known about the association of hypothyroidism and muscular hypertrophy. In our patients KDS was caused by autoimmunthyroiditis due to TRAK. The question arises if this is only a phenomenon of metabolic myopathy or an additional underlying muscular disorder.

**P1-381 Thyroid**

**Changes in attention patterns of school-aged children with Congenital Hypothyroidism (CH)**

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Children with Congenital Hypothyroidism, detected by neonatal screening and early-treated, despite a normal cognitive development, may show subtle attention problems.

The aim of this study was to investigate in CH affected children changes in attention abilities in different school grades and the influence of severity of CH at diagnosis, thyroid hormones levels at time of testing on attention performance. 43 children (30 F), aged 8 to 11 years, with early-treated CH and regularly followed-up, attending grades from 2nd to 5th were compared with an age-sex-IQ-and grade matched control group.

The neuropsychological assessment included WISC-R subtests, test MF, behaviour rating scales completed by parents and teachers. Scores were retrospectively correlated to prognostic factors (TSH, T4) at diagnosis and thyroid hormone level at the time of testing. T4 level at diagnosis (>50nmol/l), time of starting replacement therapy (<20days), dose of starting therapy (>8mcg/kg/die), thyroid hormones level at the time of performance were taken into consideration and analyzed with ANOVA/MANOVA.

Data showed these results:
- CH subjects were significantly more impulsive (p=0.015) and less accurate (p=0.018).
- CH was associated with mild attention difficulties in grade 2nd and 3rd, but not in grade 4th and 5th (MF 2nd-3rd latency: CH=18.4 vs 13.7 p=0.009) and more inattentive by teachers (p=0.04).
- An earlier starting therapy (<20days) with a dose>8mcg/kg/die was correlated with less accuracy and more inattentive by teachers (p=0.04). An earlier starting therapy (<20days) with a dose>8mcg/kg/die was correlated with less accuracy and more inattentive by teachers (p=0.04).
- CH subjects were significantly more impulsive (p=0.015) and less accurate (p=0.018).
- CH was associated with mild attention difficulties in grade 2nd and 3rd, but not in grade 4th and 5th (MF 2nd-3rd latency: CH=18.4 vs 13.7 p=0.009) and more inattentive by teachers (p=0.04).

Our findings confirmed problems of attention connected to the disease in school-aged children. The improvement of attention patterns in children at school-aged was planned.

**P1-383 Thyroid**

**Hashimoto thyroiditis in children and adolescents - long-term follow up**

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Hashimoto thyroiditis (HT) is the most common cause of goiter and hypothyroidism in children older than 6 years. Spontaneous remission may occur in 30-50% of adolescent patients, though long-term reports on HT in children and adolescents are scarce.

We investigated the clinical manifestations at presentation, clinical course, and long-term outcome of HT in children.

We reviewed charts of 93 patients (F=77, M=16, 41 prepubertal, 52 pubertal) with HT. Mean age at presentation was 12.2 years, and mean follow-up duration was 5.6 years.

The common complaints leading to referral were, either isolated (30%) or associated with other complaints (7.6%), growth retardation (11.8%), fatigue (7.6%), irregular menses (6.5%), weight gain (6.5%) and increased appetite (3.3%). Of the 71 patients who had goiter at presentation, only 35 noticed thyroid enlargement before admission. Although the prevalence of goiter was similar in males and females, it accounted for significantly more referrals in females (46.3% vs 16.7%). At referral, more males complained of growth retardation (37.5%) than females (6.5%), although height-SDS was actually similar. Hypothyroid patients had lower levels of alkaline phosphatase and higher levels of total cholesterol than euthyroid patients (136±76 vs. 205±96.6 U/L and 196±51.6 vs 162.7±36.4, respectively). Euthyroid patients with family history of thyroid disease had a lower likelihood of remaining euthyroid than those without 9% vs. 26%, p<0.04.

Fourty-four patients were treated by LT4 for hypothyroidism and 40 were treated for other indications. Nine patients remained euthyroid without treatment. Therapy was discontinued in 10 patients: In 5 hypothyroidism recurred and 5 (5.3%)remained euthyroid for at least 18 months.

Although goiter is the most common complaint at presentation in HT, it goes unnoticed in half the children, especially in males. Growth retardation is more common in males than in females. A positive family history of thyroid disease is associated with a low likelihood of remaining euthyroid. Spontaneous remission may occur in a lower percentage of children and adolescents than previously reported.

**P1-382 Thyroid**

**Thyroid function in patients with Hodgkin’s disease 6-16 years after remission**

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The aim of this study was to estimate thyroid function in patients with Hodgkin’s disease. In examined group of children (20 females, 9 males), aged 5-14 years, being in disease remission the thyroid function (T3, T4, TSH, TG, anti-thyroid antibodies, anti-thyroid peroxidase antibodies) and ultrasonographic picture of thyroid gland were estimated twice: 6 and 16 years after treatment cessation. All children were treated by MVPP and B-DOPA chemotherapy and additionally by neck or mediastinal radiotherapy (15-40 Gy).

In all patients, except 1 subject, examined hormone levels were within normal range and no significant changes were observed during the follow-up period. Only in one patient clinical hypothyroidism was diagnosed 6 years after chemotherapy was finished. In 9 patients ultrasonography revealed slightly diminished volume of thyroid gland. In 6 patients single hyperechogenic nodule and in 1 subject multiple hypoechogenic nodules were seen during the second examination 16 years after chemotherapy cessation. Fine needle aspiration biopsy confirmed papillary carcinoma in one patient previously treated by neck irradiation and benign colloid nodules in 3 patients.

We conclude that examination of the thyroid should be performed during the follow-up of Hodgkin’s disease patients and we speculate that thyroid carcinoma may be radio- and chemotherapy treatment complication.

**P1-384 Thyroid**

**Thyroid function in children born small for gestation age**

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Intrauterine growth restriction is frequently associated with short stature and with numerous endocrine changes later in life. Recently alteration in thyroid function has been found in children born small for gestation age,what additionally might influence on their growth.

The aim of this study was to evaluate thyroid function in short prepubertal children born small for gestation age (SGA) compared with short born appropriate for gestation age (AGA).

Methods: 84 prepubertal SGA children (birth weight ≤ -2SDS) (43 female, 41 male) in mean age 6,19±1.2 yr and 71 short -AGA (28 female, 43 male) in mean age 6,33 ±1,37 yr were studied. TSH, free T4(T4), free T3 (T3) and antithyroid antibodies were determined in all of them. All patients lived in iodine sufficient areas.

Results: Height SDS was -3,44±0,81 in SGA and -3,15±0,84 in AGA chil-
Graves disease (GD) is an autoimmune thyroid disease. Recent findings suggest a role for viral and bacterial agents in the pathogenesis of GD.

We present a case with GD following Epstein Barr virus (EBV) infection.

A 11 6/12 year old girl first presented with fever and hepatosplenomegaly and was diagnosed as infectious mononucleosis confirmed by EBV specific antibodies testing which were positive for viral capsid antigen (VCA) IgM and IgG. After 4 months, she developed tachycardia and tremor. On physical examination, bilateral exophthalmos and goiter at stage 3 were present. Her height SDS was –0.9 and weight SDS –2.1. Laboratory findings showed suppressed TSH (0.022mIU/L), increased thyroid hormones (FT4 36.3pmol/L, normal 12-22) and high antithyroid antibodies levels (AntiTg 4000IU/L, AntiTPO 173 IU/L). Antithyroid receptor antibody (TRAC) was positive (36.1 IU/L, normal; 9-14). The diagnosis of GD was established and antithyroid (methimazole) therapy was started. This is the first report of GD following EBV infection, which suggest that EBV may play a role in the pathogenesis of GD.

First described in 1966, Hashimoto encephalopathy is a rare steroid responsive disorder associated with Hashimoto thyroiditis resulting in myocloni, generalized or focal seizures, pyramydal tract dysfunction, cerebellar signs, psychosis or coma. We report on a 6-year-old girl with Hashimoto encephalopathy.

Case Report: At the age of 6 years the first generalized seizure occurred. Behavioral changes towards aggressive behavior were observed. In the following months several seizures and neurololgic deficits including hemiparesis, ataxia, tremor and psychotic episodes with hallucinations were present. Different anticonvulsives drugs such as oxcarbazepine, sulthiam, valproate, phenobarbital and phenytoin were ineffective. Finally the patient developped coma with tremor and psychotic episodes with hallucinations and delirium. Anti-TG and anti-TPO antibodies were positive (2974IU/ml and 7058IU/ml, respectively), and anti-Thy 1-Enolase antibody in cerebrospinal fluid (CSF) was positive. A five-day high-dose steroid therapy was initiated. The patient was kept on a maintenance dose of prednisone 30 mg/kg and had no relapse.

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P1-398 Thyroid

High prevalence of thyroid developmental anomalies in the first degree relatives of children with thyroid dysgenesis
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Congenital hypothyroidism (CH) secondary to developmental anomalies of the thyroid gland accounts for 85% of congenital hypothyroidism. Its pathogenesis is at yet unknown. It has recently been seen to have a familial component. In some families, asymptomatic members were found to have thyroid developmental abnormalities. The aim of this study was to investigate the presence of thyroid developmental anomalies (TDA) in asymptomatic first degree relatives of children with thyroid dysgenesis. The study included 44 first degree relatives of 19 children with CH due to thyroid dysgenesis (athyreosis (n = 11) or hypoplasia (n = 8)), 9 males and 10 females. Relatives group included parents (25) and siblings (19) of the CH children. Thyroid ultrasonography and function were evaluated among the first degree relatives and were compared with those of a control population (n = 51). We found that seven relatives (15.9% of cases) belonging to 7 families (36.8%), had thyroid developmental anomalies; four of them had thyroid hypoplasia (3 females and 1 male) and 3 females had abnormalities of the isthmus (2 had agenesis of the isthmus and one had a very thin isthmus, 2 mm in thickness). They were all asymptomatic with normal TSH and FT3 levels. In controls, the thyroid gland was normal in all of them. The high proportion observed of asymptomatic TDA among first degree relatives of patients with CH due to thyroid dysgenesis supports the hypothesis of a common genetic origin of the disorder with heterogeneous phenotypes. Family data analysis favored an autosomal dominant mode of inheritance for TDA with a low penetrance (21%) for asymptomatic TDA.

P1-399 Thyroid

The relationship of maternal iodine status in the first trimester to neonatal thyrotropin concentrations: a study in southern Thailand
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Objective: to examine the correlation between maternal urinary iodine in the first trimester and neonatal TSH concentrations in southern Thailand.

Study design: cross-sectional observation study.

Material and Methods: The urine specimens of 185 pregnant women were collected. The mean maternal age was 28.0 ± 5.3 years with mean gestational age of 9.7 ± 2.7 weeks. Urinary iodine concentration was measured by ceric ammonium method. Neonatal TSH data were collected from the national TSH screening program.

Results: The mean urinary iodine concentration was 140 ± 42 µg/L. Of the total 185 women, 50 (28.2%) had their urinary iodine concentration <100 µg/L. The mean TSH of their offsprings was 4.06 ± 2.7 weeks. Urinary iodine concentration was measured by ceric

Conclusion: Iodine deficiency is still prevalent in southern Thailand. However, there was no correlation between maternal iodine status and neonatal TSH concentrations.

P1-391 Thyroid

Definitive diagnosis in children with Congenital Hypothyroidism detected by the Neonatal Screening Programme of Buenos Aires Province between 1995 and 2001
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Congenital hypothyroidism (CH) occurs in 1:2545 newborns in Buenos Aires Province. Some children originally diagnosed with CH will have a transient form (TCH). Initially it represents a condition that resembles definitive CH. The aims of this study were to investigate the definitive diagnosis, underlying causes and to compare permanent and transient forms in 225 children with CH (detected among 868010 evaluated newborns). Thyroid ultrasound and technetium scans were performed at start of treatment. In children ≥ 3 years old without an initial identified permanent cause, therapy was discontinued for 4 weeks and thyroid function tests and imaging studies were repeated. If no abnormalities were diagnosed as having TCH, TSH and T4 levels at start and end of treatment were compared among all etiologic groups. The percentage of children with T4 level ≤3 and >3 µg/dL at start of treatment and L-thyroxine dose (LTD) at each 6 month visit, were compared between dysmorphogenesis and TCH children. Mann Whitney and Chi-square tests were used for comparisons. The etiologies found were athyreosis (19.9%), ectopic (57.5%) and euthyroid (2.8%) dysgenesis, dysmorphogenesis (14.3%), and TCH (5.6%). The incidence of TCH was 1:64144 newborns. Female to male ratio was: 4.5:1 in athyreosis, 2.2:1 in dysmorphogenesis, 0.7:1 in dysmorphogenesis and 1:1 in TCH. TSH and T4 levels showed differences only between athyreosis and the other groups (p<0.01). 48% of children with dysmorphogenesis and 78% with TCH had initial T4 >3 µg/dL, but this did not reached significant differences. At follow-up, however, differences were found in LTD from 6 to 36 month visit (p<0.05) between these 2 groups. In summary, the incidence of TCH was 5.6% in children diagnosed CH. At the original diagnostic no data allowed to distinguish between transient and permanent CH. However, from 6 month visit both groups showed differences in the L-thyroxine requirements.

P1-392 Thyroid

Growth in children with congenital hypothyroidism(CH): A longitudinal study
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In the absence of neonatal screening for CH and socioeconomic constraints delayed diagnosis is common. To assess the influence of age at onset of treatment on subsequent growth, records of 134 (52M, 82F) children with CH diagnosed before 3 years of age over the last decade and followed up periodically up to 10 years of age were studied.

Based on age at onset of treatment these children were divided into 4 groups. Group 1: <3 month (n=45); Group 2:3-6months (n=23); Group 3: 6mo-1yr (n=35); Group 4: 1yr-3yr (n=31). Detailed history, anthropometry, hormonal evaluation and thyroid scan(46)sonography (n=37)at diagnosis and follow up records of height SDS(Ht SDS),bone age SDS (BA SDS),serum T4-TSH levels and the thyroxine supplementation dose to maintain euthyroid state at 6months,1,3,5and10 years age were compared by unpaired 't' test between the groups.

At presentation, the mean Ht SDS,BA SDS in Group1(-2.26±1.7;1.21±1.4) and Group 2(-4.25±1.7;1.82±2.5) were significantly(p<0.01)less affected than Group3(-3.51±1.59; 3.22±2.7)and Group4(-4.71±1.83; 3.99±2.44). The mean level of T4- TSH in Group 1&2 showed significantly(p<0.05) more severe deprivation than Group 3 & 4.The mean dose of L-thyroxine supplementation in groups 1&2 (1.95 µgm/kg/d)was significantly(p<0.01)less than Group 3&4. Detailed history, anthropometry, hormonal evaluation and thyroid scan at diagnosis and follow up records of height SDS, bone age SDS, serum T4-TSH levels and the thyroxine supplementation dose to maintain euthyroid state at 6months, 1, 3, 5 and 10 years age were compared by unpaired ‘t’ test between the groups.
those treated at or before 1 year of age. Difference in BA SDS was insignificant by 5 years of age. The mean dose of thyroxine in group 1 & 2 was significantly (p<0.05) higher than group 3 & 4 till 3 years of age. At presentation the mean 

HtSDS and BASDS in athyrotics (n=42) was more affected than hypoplastic (n=9), ectopic (n=10) or the dysmormogenesis (n=22) cases. By 10 years of age follow up difference was insignificant (p>0.05).

To conclude, the onset of therapy after 1 year of age has significant adverse effect on growth achieved by 10 years of age in children with CH irrespective of the underlying cause.

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**P1-394 Thyroid**

**Neonatal screening for congenital hypothyroidism in Chiang Mai University Hospital (from 1997-2003)**

Kewalee Unachak, Prapai Dejkhamron

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**Objectives:** To determine the incidence of congenital hypothyroidism in the newborn infants born at Chiang Mai University Hospital during July 1997-December 2003.

**Study design:** Dried blood spots were collected from newborn infants aged 48 hour or older, and analyzed for TSH by immunoradiometric assay. Infants with the screening TSH higher than the cut off level (25 mU/L) were recalled for re-evaluation which consisted of complete physical examination and blood test for T3 or FT3 and TSH. Bone age determination and 99Tc thyroid scan were performed in those with suggestive clinical symptoms or abnormal thyroid functions.

**Results:** 21,000 infants (98.35 % of total births) were enrolled in the study. Forty-nine infants were recalled for re-evaluation established a recall rate of 0.23 percent, and the response rate was 93.7 percent. Eleven infants with congenital hypothyroidism were found, two of these were transient, and the rest were permanent hypothyroidism. The etiology of permanent hypothyroidism were identified in eight patients, namely athyreosis(1), ectopic thyroid(2), thyroid dysmormogenesis(4), and concomitant single lobe and ectopic thyroid(1).

**Conclusions:** The incidence of congenital hypothyroidism is 1:1909 live births, higher than those reported in the literature.

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**P1-393 Thyroid**

**Symptomatic destructive thyroiditis in a patient treated with interferon alfa-2b**

Louise G. Vanasse1, Cynthia E. Herzog2, Steven G. Waguespack3

1University of Texas Medical School at Houston, Division of Pediatric Endocrinology, Houston, United States; 2University of Texas M. D. Anderson Cancer Center, Division of Pediatrics, Houston, United States; 3University of Texas M. D. Anderson Cancer Center, Dept of Endocrine Neoplasia and Hormonal Disorders, Houston, United States

Interferon-related autoimmune thyroid disease is an uncommon but not unexpected cause of clinical thyroid dysfunction. We present the case of a patient who developed symptomatic destructive thyroiditis while receiving interferon alfa-2b (IFN) therapy. A 14-year-old Caucasian female on adjuvant therapy with IFN for melanoma was referred for hyperthyroidism. Prior to IFN therapy, there was no clinical evidence of thyroid dysfunction, although family history was positive for hyperthyroidism in her mother. After five months of IFN therapy, she began to experience fatigue, weight loss, decreased appetite, hot flashes, sweating, irregular menses, increased pulse, anxiety, and hand tremors. The patient had no complaints of thyroid tenderness. Physical examination was pertinent for no Graves’ ophthalmopathy and a prominent firm thyroid gland without an associated bruit. Laboratory evaluation showed a T3-predominant thyrotoxicosis and autoimmune thyroid disease: free T3 103.4 IU/ml (<2.0), Thyroid stimulating immunoglobulins were normal. A 85pg/dl (287-455), free T4 2.0 ng/dl (0.9-1.8), TSH <0.01 MCU/ml (0.50-5.50), T3-predominant thyrotoxicosis and autoimmune thyroid disease: free T3 853 pg/dl (600-1700), free T4 4.3 ng/dl (1.1-2.4), TSH <0.01 MCU/ml (0.50-5.50). The patient’s IFN was stopped due to elevated transaminase levels, and hyperthyroidism was treated symptomatically with a β-blocker. Two months after presentation, she became overtly hyperthyroid and has since maintained on thyroid hormone replacement. A genetic predisposition to autoimmune thyroid disease is probably necessary for the development of clinical thyroid disease associated with IFN therapy. Although not specifically tested for at the outset of IFN therapy, it is likely that pre-existing thyroid autoimmunity was present in our patient. To our knowledge, this is the first report of IFN-induced thyrotoxicosis/destructive thyroiditis in a pediatric patient. Our case illustrates the importance of closely monitoring thyroid function during treatment with IFN, particularly in those patients with a positive family history or elevated thyroid antibody titers at baseline.

**P1-395 Thyroid**

**Papillary thyroid carcinoma: report of a girl with the rare variant diffuse follicular**

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The aim of this study is to report the outcome of a rare variant of papillary thyroid carcinoma in a five years old girl, already in the eighth year of follow-up.

Patient report: JPC, female, five years old, evaluated in the Pediatric Endocrine Unit due to a solitary, firm, 4 x 3cm nodule. The child had no cervical compressive symptoms, height and weight Z-score of +2.18 and +1.4 respectively. Thyroid function tests were normal, the nodule was “cold” on isotope scan and cytology by FNAB exhibited features of follicular tumor. Total thyroidectomy was proceeded. In the right lobe, several nodules associated 0.5x0.5cm were found, there was one dominant, encapsulated, measuring 2.5x2.0cm. Histologie features indicated a variant follicular of papillary carcinoma in several nodules, giving the character “diffuse”, more aggressive than the solid variant. In addition, two limonodes had metastases. Postoperative surgical radioidine ablation of residual thyroid was performed with 90mcCi of 131I followed by suppressive therapy with L-thyroxine. Seric thyroglobulin (Tg) was determined each 6 month (<2ng/ml). In the third year, Tg increased to 35 ng/dl and a firm, 0.8cm limonode was suggestive at FNAB for metastases. A neck dissection followed by administration of 30mcCi of 131I was performed and Tg decreased to 0.8ng/ml. Patient is now thirteen, 162cm (Z-score +3.37), 40.7kg (Z-score +0.87), has no metastases evidence and Tg < 0.5ng/ml. Despite more aggressive than classical papillary carcinoma, this variant diffuse follicular can outcome favorably. However, follow-up must be caution and suspicious elevation of Tg (>1ng/ml- Irma) during suppressive therapy with L-thyroxine, indicate body scan and if necessary an ablation dose of 131I. So far, the total dose administered, 120mcCi, did not result in undesirable effects.

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ESPE/LWPES 7th Joint Meeting Paediatric Endocrinology in collaboration with APEG, APPES, JSPE and SLEP
The most common cause of thyrotoxicosis in children and adolescents is Graves’ disease. We report the results of a retrospective study conducted on 623 patients suffering from Grave’s disease. Forty-six patients (7.4 %) were less than 20 years old: 45 of them were per pubertal (10-19 years old) and one patient was 6 years old at diagnosis. Family history of Grave’s disease was found in 6 cases (13%). Type 1 diabetes mellitus was associated in two cases (4.3%). Grave’s ophthalmopathy was present in 28 cases (60.8%) of cases aged 1 to 4 according to ATA classification. Antithyroid drugs were given initially in 41 cases (21.9%). Six patients were loss of follow-up, and 6 patients are currently receiving medical treatment. Two patients developed definitive hypothyroidism (4.9 %) during medical therapy. Thyroidectomy was performed in 16 cases (34.7%), initially in three cases and because of relapse after prolonged medical therapy in 13 cases. It achieved remission in 11 cases (63.2%). Hypoparathyroidism was observed in 3 cases, transient in one case and definitive in two cases. Radioiodine therapy was administered initially in 4 cases, and because of relapse after thyroidectomy in one case.

Clinical characteristics and diagnosis investigations are examined. Schedules of antithyroid drugs and complications of medical therapy and surgery are discussed.

Acquired hypothyroidism is a common endocrine disease of childhood. Failure of catch up growth in treated patients has been reported although the reasons for this remain obscure. It was the aim of this study to investigate whether antibody status (ABS) influences thyroid hormone levels, and height and BMI SDS at diagnosis and catch up growth following treatment. A retrospective study of 75 patients was performed. 3 patients having coexisting pathology affecting growth were excluded. ABS (thyroid peroxidase, anti microsomal antibody, anti thyroglobulin), thyroid stimulating hormone (TSH) and thyroxine (T4) were measured at diagnosis. Age, duration of symptoms, height SDS and BMI SDS at diagnosis, 1 and 3 yrs were compared in Ab positive and negative patients.

47/140 were Ab positive, median age at diagnosis was 11.2 yrs (range 3.9 - 15.9) and duration of symptoms 9 months (1 - 48), not significantly different to Ab negative patients (11.8 yrs, 6.2 - 15.4 and 12 months, 0.5 - 36 respectively). T4 was significantly higher in the Ab positive group (73 nmol/L, 1 - 137 vs 20 nmol/L, 1 - 120) and TSH significantly lower (29 mu/L, 4 - 940 vs 90mu/L, 11.4 - 1400), p = 0.01 for both analyses. BMI SDS at presentation was lower in Ab positive patients (0.60, ±0.9 to 3.69 vs 1.69, ±1.01 to 2.93), p = 0.009 and correlated negatively with initial T4, (p = 0.001), however BMI SDS at 1 and 3 years did not differ between groups. Height SDS, and gain in height SDS did not differ between groups.

In conclusion, biochemical abnormalities are less profound at presentation in Ab positive patients which may account for the lower BMI. Differences in height may not be apparent until hypothyroidism has existed for a number of years. These data may reflect a less aggressive disease process in Ab positive patients.

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Clinical characteristics and diagnosis investigations are examined. Schedules of antithyroid drugs and complications of medical therapy and surgery are discussed.
Iodine deficiency is the leading cause of potentially preventable mental retardation in childhood in the world. The prevalence of goiter and median urinary iodine concentration are the most important indicators for assessing iodine deficiency disorders (IDD). Scant information is available regarding iodine sufficiency in Israel. Local clinics reported excessive cases of thyroid dysfunction in Arava region of Israel to the Southern District of the Israeli Ministry of Health. Low iodine levels in well water in the region have been demonstrated and drinking water in the region is commonly supplied by desalinated well water or bottled mineral water.

The objective of this study was to determine whether IDD exist in this region. A school based survey of ninety-nine Jewish school children was performed in two sub-regions in the Negev. First to third graders were studied from a school in the Arava and from an additional school in another sub-region of the Negev. Children’s thyroid volume was appraised by an experienced pediatric endocrinologist and spot urine samples were collected and assayed for urine concentration.

We found no iodine deficiency in this school population with normal iodine intake measured by spot urinary excretion (median urinary iodine concentration for the whole group was 172 mcg/L). 8.08% of the children had a palpable thyroid gland (type Ia in PAHO classification). 6.06% had a palpable and visible goiter only when the neck is fully extended (IIb). 4.04% had a goiter visible with the neck in normal position (type II). None have a goiter type III.

None of the children had an abnormal thyroid function. Percentage of goiter 18.18% is much greater then 5%, as expected in an iodine sufficient region. The causes of this finding should be verified in further studies.

**Reference:**

Abnormal thyroid function after cardiac surgery: Should we be routinely re-screening these infants? A case report and literature review
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Background: Although the newborn screen is the standard of care for identifying infants with congenital hypothyroidism, there are no specific recommendations for routine re-screening of infants at risk for later development of thyroid dysfunction. We describe an interesting case of delayed TSH elevation, review the relevant literature and propose a policy for routine retesting of infants at risk.

Case: JS is a 5-week-old infant status post hypoplastic left heart repair on day of life 2. The baby had normal weight gain, vital signs and no goiter. Because newborn screen results were not immediately available, a repeat screen was sent. It revealed a T4 of 4.5ug/dL and a TSH of greater than 100 uIU/mL. A knee film showed normal ossification of the distal femoral epiphysis. Baby was started on thyroxine with normalization of TSH within 2 weeks. Previous newborn screens sent at 2 days and 20 days were subsequently reported as normal. When we requested that the sample be sent at 20 days be assayed for TSH, it was, in fact, high (50 uIU/mL).

Discussion: The most common risk factors associated with a delayed elevation of TSH after a normal newborn screen are very low birth weight and congenital cardiac abnormalities. There are multiple case reports of transient hypothyroidism associated with exposure to iodine. Following cardiac catheterization or surgery infants have been noted to have extremely elevated urinary iodine excretion and transient hypothyroidism has been reported to occur in 25-29% of these. The number is increased to 35% when there is delayed sternal closure with continued exposure to iodine antigens. Other factors implicated in thyroid dysfunction in these infants include: cardiac bypass resulting in decreases in serum T3 and T4; chest tube drainage, with loss of TBG; recovery from sick euthyroid syndrome; and suppression of TSH release by dopamine.

Conclusion: Because infants with cardiac disease and surgery are exposed to multiple factors that impact the thyroid it would seem advisable to routinely re-screen them to avoid undiagnosed transient hypothyroidism during a critical stage of thyroxine-dependent neurodevelopment.

Assessment of skeletal maturity in children with perinatal thyroid disorders born of West Pomeranian Region in Poland
Adela Nureczynski1, Elzbieta Petriczko2, Janusz Fydryk3
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The aim of the study was to assess the incidence of bone maturity disturbances in children with perinatal thyroid disorders. Additionally evaluation of bone age retardation as a parameter of severity of fetal hypothyroidism was analyzed.

Material and Methods: The study group consisted of 49 patients of West-Pomeranian Region in Poland; limits of age 28-182 day of life; with perinatal thyroid disorders (neonatal TSH below 20 mIU/L), which were subjected of neonatal TSH, FT3, FT4 collected at first visit, patients were qualified into 3 subgroups: congenital hypothyroidism (CH n=42), transient hypothyroidism (TH n=31), hyperthyreotropinaemia (hiperTSH n=1).

Results: In the whole study group we detected 5 children with Down’s syndrome. Two with typical trisomy 21 and one with mosaicism. The higher incidence of CA occurred in 3 patients with TH, in 2 children bone age was adequate for chronological age, and 1 child was shown bone age acceleration for chronological age. Significant negative correlation was found between bone age retardation and TSH level at the second visit in children with CH (p=0.0029; r=-0.53)

Conclusion: Bone age assessment is a very good method evaluating severity of fetal hypothyroidism. It could influence on therapeutic doses of L-thyroxin in children with CH.

Machroorquidism in child with acquired hypothyroidism
Vali Dichtchekian1, Vanessa Baudichon Domingues2, Flavia Camila Rezende Mosquera3, Leandra Steinmetz2, Hamilton Cabral Menezes-Filho1, Thais Delia-Marina1, Hilton Kuperman1, Durval Damiani1, Nuvarte Setian2
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Background: An uncommon finding in acquired hypothyroidism is machroorquidism without other sexual features. A possible mechanism is interaction of TSH with the FSH receptor on Sertoli cells, where levels of testosterone and gonadotrophins are low.

Objective: case report of acquired hypothyroidism with machroorquidism.

Case: Male, 5 years-old, evaluated for loss of counsciousness (syncope). At physical examination, we found weight Z-score= -2.39 and height Z-score = -3.56, paled mucosas, mixedema, hypophyotic heart sounds with sinus bradi- cardy, penis 4,5cm and testicles 6cm (G2P1) and no abnormal findings on the neck. Previous neurologic development was normal.

Laboratory: Total Cholesterol: 354 mg/dL, LDL 264 mg/dL, HDL 63 mg/dL, VLDL 27 mg/dL. ECG with sinus bradycardia, echocardiography showed laminar pericardial effusion. TSH 581,67 uIU/mL, FT4<0,2 ng/dL, T4<1,5 µg/dL, T3<33 ng/dL, Anti-TPO>3000 U/mL (<35), Anti-TG 309 U/mL (<35), TRAb 12% (<12%), IGF-I 15 ng/mL. Testosterone <10 ng/dL, LH<0,05 U/L, FSH 1,68 U/L, Prokletin 28,5 ng/mL.BA 3y6mA(5y1m).

Ultrasonography of the neck compatible with thyroiditis.

Conclusion: In this case uncommon interesting features were syncope as first symptom, presence of enlarged testicles, and early age of onset. Cardiac alterations were shown in ECG and Echocardiography. The most prominent metabolic disturbance was hypercholesterolemia. All findings were related to hypothyroidism, confirmed by high levels of TSH and low levels of thy- roidal hormones. Hashimoto’s thyroiditis was the etiological cause, as seen by high levels of anti-thyroidal antibodies. The testicular growth was due to high levels of TSH secondary to Hashimoto’s thyroiditis, with stimulated the receptors of FSH in Sertoli’s cells and led to macrorquidism with low testosterone and gonadotrophins levels.

Incidence of congenital anomalies in children with perinatal thyroid disorders born of West Pomeranian region in Poland
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The aim of the study is to assess the type and incidence of congenital anomalies [CA] in children with perinatal thyroid disorders born in West Pomeranian Region from 01.01.1992 to 01.02.2001.

Material and Methods: The study group consisted of 127 patients whose TSH level was below 20 mIU/L and determines 0.64% of all children that were screened (196 267 screening tests). It comprised 67 girls (52.8%) and 60 boys (47.2%). Based on neonTSH, FT3 an FT4 collected at 1. visit, patients were qualified into 3 groups: congenital hypothyroidism (CH n=43), transient hypothyroidism (TH n=31), hyperthyreotropinaemia (hiperTSH n=53).

Results: In the study group we detected 5 children with Down’s syndrome. Two with typical trisomy 21 and one with mosaicism. The higher incidence of CA occurred in a group of CH (10 children, 25.6%) was found. In a group TH CA were detected in 5 children (20%). It’s worth to emphasize the absence of CA in children from hiperTSH group. We noticed statistically important correlation...
between incidence of CA in children and the type of their thyroid disturbance (p = 0.007). The incidence of CA in general population is described as 3.2 %. Incidence of CA among children with CH is established from 5.4% to 23%. In a study group as the most frequent CA we determined congenital heart anomalies (8 patients). In a CH group congenital heart anomalies were diagnosed in 5 children. These were: ventricular septal defect, atrial septal defect, complete atrio-ventricular septal defect (diagnosed in a child with Down’s syndrome), persistent foramen ovale, complex congenital heart anomaly (CVC, PDA, hypoplasia arteriosus pulmonalis). Among our patients we detected presence of additional non cardiac defects: small skeletal anomalies (syndactyly, polydactyly), genito-urinary tract anomalies (cryptorchidism and hypospadias), that facies hemangiomas and imperforate anus.

Conclusions: Higher incidence of CA is detected not only among children with CH but also those with TH. Higher incidence of CA, including cardiac defects indicates the need of careful diagnosis in purpose of detection the concurrent malformations.

Methods: Transversal clinical study of frequency that included children that sought ambulatory pediatrics consultation from the Caracas University Hospital between January of 1996 to December 1998 and were diagnosed with short stature. 167 preschooler and school age children with low stature were included. Determinations of free T4, blood TSH, bone age and TRH stimulation test were carried out.

Results: 62 patients (31.7%) showed normal free T4 and TSH in the upper limit. These patients were then tested with TRH stimulation in order to determine TSH, taken at 0, 20 and 60 minutes. Data showed that in 52 (19.2%) patients the TSH was greater than 20 mU/l at 20 minutes (normal cut point at 20 minutes 19 mU/l). There was a statistically significant correlation between subclinical hypothyroidism and primary hypothyroidism, although exact predictive indexes did not show strength in this association.

Conclusion: Subclinical hypothyroidism was observed in 19.2% of patients with short stature, so we consider important to include in the evaluation of growth and development of a short stature child bone maturation and thyroid function studies, besides the anthropometric parameters.

Hypothyroidism may be defined as the clinical and biochemical syndrome that result from decreased hormone production by the thyroid gland or, rarely, from defects in the integration of thyroid hormone receptors. Hypothyroidism in infant and children results in marked slowing of growth and development, with serious permanent consequences including mental retardation. The aim of this paper is to report a case of congenital hypothyroidism. H, 5 months old was referred to our hospital with growth and developmental delayed. His body weight was 4.4 kg, body length was 60 cm. He looked like 2 months baby with fluffy face, lack of interest, somnolence, fear poorly, large tongue, hypotonic muscles, rough and dry skin. From anamnesis, he suffered from prolonged jaundice in early days of live. Test revealed a low serum level of T3 and T4 (0.08 ng/ml and 0.49 ng/ml), TSH were elevated (>40 uI/ml). Osseous development was that newborn. Two months after treatment with sodium-t-thyroxin given orally, we noticed the decreased fullness of the face, the decreased hirsutism of the forehead and the alert appearance. His neck muscles begin to tighten to steady the head. His whole arms wave when excited by stimuli, kicking gradually becomes stronger and he has rolled over. It would be useful to know the clinical features and the laboratory value for thyroid function in congenital hypothyroidism, because it is preventable cause of growth and developmental delay and mental retardation. Routine screening tests is necessary to early detection of this disorder but in general it is too expensive for our population in Indonesia.
Flutamide was added together with testosterone (10-8M), in order to identify the pathway mediating the testosterone effect. Androgen receptors (AR) in ASM cells were detected by indirect immunofluorescence with an anti-human androgen receptor monoclonal antibody. Testosterone induced a statistically significant increase in the ASM cells proliferation ranging from 15.87% to 26.17% for the concentrations of 10-12 to 10-6 M (p<0.01). Initial experiments show that the androgen receptor inhibitor flutamide reduced the cell proliferation evoked by testosterone to control levels. In addition, indirect immunofluorescence reveals that serum starved ASM cells express AR, which are localized in the cell nucleus. Treatment of cells with 10-8 M testosterone resulted in the reduction of the AR signal. In conclusion, testosterone in concentrations of 10-12, 10-8 and 10-6 M caused a statistically significant increase on ASM cells proliferation that was inhibited by flutamide. This fact together with the detection of androgen receptors in ASM cell nuclei suggests that testosterone influences ASM cell proliferation, possibly via a genomic pathway.

**P1-412 Testes and Androgens**

**Atypical presentation of McCune-Albright Syndrome in a prepubertal male: Macrocordism, Hyperphosphatasia but, short stature**

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McCune-Albright Syndrome (MAS) is caused by a mosaic mutation in the GNAS1 gene and characterized by café-au-lait spots, polyostotic fibrous dysplasia and multiple hormonal abnormalities. We report an 8 yr old Russian boy, of nonconsanguineous parents, with a complex presentation. Physical examination revealed large café-au-lait spots on right side of face, encompassing buttocks and several small spots on back and abdomen. Left testis = 8ml, right = 6ml, penile length = 2cm, with no pubic or axillary hair. Height was -3SDS and weight -1.28 SDS. Skeletal radiography: Numerous cystic areas in femoral bones. Testicular sonogram: several scattered echogenic foci consistent with microlithiasis. Normal pituitary MRI. Initial evaluation showed prepuberal testosterone = 5ng/dl (n=10), LH = <0.1mIU/ml (n=0.3), FSH = <0.1mIU/ml (n=3.0), Serum inhibin B =218pg/ml (n=180) and AMH=157ng/ml (n=60.2), indicating Sertoli cell activation. Serum hCG, thyroid function, prolactin, adrenal function, Vit D 25, Vit D 1,25 and serum calcium = normal while serum phosphorus = low, with elevated alkaline phosphatase and PTH. Renal tubular reabsorption of phosphate=normal.

Peripheral blood leukocyte DNA=non-confirmatory for activating Gs α mutation. PCR-based DNA analysis of bone=positive activating mutation of arginine 201 in the Gsα protein, a substitution for cysteine.

Growth hormone (GH) stimulation test peaked at 13ng/ml (n=10). Bone age: 7½ years at chronological age of 8½ years, with predicted height =164cm and target height =179cm. GH therapy at 0.04mg/kg/d, increased growth velocity from 2.8 cm/yr to 10.2 cm/yr.

At 2 yr follow up, physical exam was unchanged. Prepubertal testicular enlargement and no sexual precocity were described by Coutant et al. 2001. Additional features in our patient are short stature, elevated PTH and ALKP, but no evidence of testicular enlargement. This points out the complexitiy of the clinical and biological presentation of MAS in boys.

**P1-413 Testes and Androgens**

**McCune-Albright syndrome in a boy may present with a monolateral macroorchidism as early and isolated clinical manifestation**

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Here we describe for the first time an unreported clinical expression of McCune-Albright syndrome (MAS) in a 4.6-yr-old boy presenting with monolateral testicular enlargement, no other signs of sexual precocity and no evidence of other clinical manifestations of MAS at the time of macroorchidism presentation. Such a clinical presentation is absolutely atypical due to the following reasons:

a) macroorchidism has not been frequently reported as an early clinical finding in this syndrome;

b) in the few cases presenting with testicular enlargement other clinical evidences of sexual precocity have been observed;

c) in all the previous reports macroorchidism was always bilateral. There is only one literature’s report that demonstrates many similarities with the ours (Coutant et al. 2001). Also the histological findings in our case were similar to the ones described by Coutant regarding Sertoli cell hyperplasia and the absence of maturation to Leydig cells: the only difference in our case is represented by the variability in germ cell number and the occasional evidence of incomplete spermatogenesis. In that boy, however, testicular enlargement was bilateral. Diagnosis of MAS in the present case was confirmed by molecular analysis from both leukocytes and frozen histological section of right testis biopsy (R201C mutation of GNAS1 gene). The present report must alert the paediatricians to the fact that MAS may also present with a monolateral tests enlargement, as early and isolated clinical manifestation.
In conclusion, these first data on INSL3 in prepubertal boys indicate that 1) INSL3 is very low in normal prepubertal boys, reflecting the immature state of the Leydig cells, and 2) INSL3 serum levels are strongly responsive to hCG, reflecting the LH-induced Leydig cell differentiation occurring throughout puberty.

P1-415 Testes and Androgens
Interaction of GH and IL-1α increases the expression of STAR protein in immature Leydig cells and is directly related with the expression of STAT5b
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Growth hormone (GH) plays a role in gonadal function and GH deficiency has been implicated as a cause of subfertility and testicular dysfunction in humans and experimental animal models. The cytokine interleukin-1α(IL-1α) is produced constitutively in large amounts by the intact testis. Its expression is parallel with pubertal development in the rat but the function in the testis remains largely unknown. Recently we have found that IL-1α interacts with GH to enhance steroidogenesis by Leydig cells from pubertal (40-day-old) rats in vitro. Here we have examined the mechanism of action and the signaling pathways involved in this interaction, employing primary cultures of Leydig cells from rats of different developmental ages (10, 20, 40, 80 postnatal days).

We examined the effect of GH and IL-1α on STAR expression by using PAGE/Western analysis and RT-PCR. The production of androgens was measured by RIA. GH or IL-1α alone stimulated steroidogenesis and expression of STAR protein in Leydig cells from 40-day-old rats. Cells co-stimulated by IL-1α and GH exhibited a marked increase in the level of STAR protein. This was parallel with the induction of phosphorylation of the transcriptional factor STAT5b, with a maximal phosphorylation level at 15 min. of incubation. GH alone was able to enhance the transcription of SOCS-3. In contrast, co-stimulation with GH and IL-1α decreased the expression of SOCS-3 in immature Leydig cells.

STAT5b is known to be involved in the GH regulated somatic growth pathway but its role in other GH regulated processes is largely unknown. From the present findings we suggest that STAT5b is involved in the regulation of steroidogenesis in immature Leydig cells. Our findings also confirm the previous results that IL-1α potentiates the stimulation of steroidogenesis in Leydig cells by GH. The expression pattern of IL-1α indicates that it plays a physiological role in the postnatal differentiation of Leydig cells. We suggest that increasing levels of GH at puberty interact with locally produced IL-1α to modulate Leydig cell steroidogenesis at the onset of puberty in boys.

P1-416 Testes and Androgens
Androgen receptor (AR) in the human prepubertal testis: high expression in interstitial and peripheral cells and very low expression in Sertoli and germ cells
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We have described a strong immunoeexpression of aromatase in interstitial cells (IC) and in germ cells (GC), gonocytes and spermatogonia, in the neonatal and post natal human testicular activation periods, as well as during early puberty for GC. Strong expression of ERβ in neonatal IC and in neonatal and prepubertal SC and GC was also observed. However, no data on AR immunoeexpression in human prepubertal testis is available. In this study, 1) AR immunoeexpression (cell localization and quantification of positive cells) in human prepubertal testicular tissue (n=20) was analyzed in different age groups. Human testicular tissue was collected at necropsy. As previously reported (Berensztein et al. J Clin Endocrinol Metab 87:5113, 2002), three age groups were defined: Gr1, neonatal (1- to 21-day-old newborns, n=7), Gr2, postnatal testicular activation (1- to 7-month-old infants, n=7) and Gr3, early prepubertal (12- to 36-month-old children, n=6) group. In IC, as well as in peritubular cells (PeC), a strong signal of AR immunoeexpression (36±14.7, 34.8±19.8 and 20.5±9.23 % stained cells in IC, 63.5±14.7, 47.1±21.4 and 23.3±14.1 % in PeC) was found in Gr1, Gr2 and Gr3, respectively. In PeC, % of Gr1 was significantly higher than that of Gr3, p=0.004. In the 3 Grs, SC immunoeexpression of AR was weak or absent (less than 5%), in contrast to one adult SC control, 75.8%. Immunoeexpression was completely absent in GC of the 3 Grs. We conclude that in human prepubertal testis, IC and PeC might be targets for androgen action, while SC might be targets for estrogens rather than androgens. In IC and PeC androgens might modulate its own AR. The absence of AR in immature SC prevents androgen action, mainly the stimulation of spermatogenesis during prepuberty.

P1-417 Testes and Androgens
Role of IGFs in human prepubertal testis: differentiation of steroidogenic cells and maintenance of a pool of germ cell during the first three years of life
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The IGF system might be involved in the differentiation of Leydig cell precursors and in the proliferation of cells of the seminiferous cords, during human puberty. Three groups (Gr) were defined: Gr1, neonatal (1- to 21-day-old newborns), Gr2, postnatal testicular activation (1- to 7-month-old infants) and Gr3, early prepubertal group (12- to 36-month-old children). We analyzed 1) IGF1, IGFL2 and IGFLR1 immunohistochemistry (IH) in human prepubertal testicular tissue collected at necropsy (n=22) at different prepubertal ages, and 2) the effect 4-day rhIGF1 (50 ng/ml) stimulation on testosterone (T) secretion in cultures of human somatic testicular cells of Gr1 and Gr2 (n=11). Results were as follows: 1) Interstitial cell (IC) IH, very low expression of IGF1 and IGFL2 in the 3 Grs. IGFLR1 IH, proportion of + tissues in Gr1 and Gr2 was significantly higher than in Gr3 (7/7, 4/5 and 0/6, p=0.018 Fisher exact test), % of + cell was 11.7±4.5, 7.2±2.9 and <5 in Gr1, Gr2 and Gr3, respectively (p=0.046, Gr1 vs Gr3, Kruskall-Wallist test). 2) Sertoli cell (SC), no expression of IGF1, IGFL2 or IGFLR1 was found. 3) Germ cell (GC), IGFL1, proportion of + tissues was 4/6, 5/7, and 4/7, % + cells, 10.1±4.5, 12.5±9.8 and 7.1±4.1, IGFL2, 4/4, 7/7, and 5/5, 25.8±3.1, 29.2±7.1 and 28.3±9.6 %, IGFLR1, 4/7, 5/5, and 6/6, 7.7±5.6, 21.5±12.7 and 17.1±6.0 % (Gr1, Gr2 and Gr3, respectively). 4) In testicular cell cultures of somatic cells, rhIGF1 stimulation increased T secretion by 330±243 % over basal conditions, x±SD, p= 0.004 paired-test. The expression of IGFLR1 in IC, in Gr1 and Gr2 and the increment of T under rhIGF1, in the absence of LH, suggest that IGFs might modulate testicular steroidogenesis, in the first months of life. The absence of local IGFL1 and IGFL2 expression suggests that IGFs are provided by the blood supply. The lack of IGFL1 expression in SC suggests that IGFs do not have a role in immature SC. Finally, the high expression of IGFL1 and its ligands in GC, particularly IGFL2, in the 3 age periods, suggests that the IGF system has an important role in maintaining an adequate pool of GC (gonocytes and spermatogonia) during prepuberty.

P1-418 Testes and Androgens
Large rearrangements of the HSD17B3 gene: A new type of mutation responsible for 17β-HSD deficiency
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17β-Hydroxysteroid dehydrogenase (17HSD) deficiency, a rare genetic disease, inherited in an autosomal recessive fashion, is responsible for male pseudohermaphroditism (MPH) : typically, 46XY patients present with normal Wolffian duct derivatives but female external genitalia (blind-ending vagi...
Children's Hospital, Δ4A) to testosterone (T). Among our large cohort of about 300 46,XY patients with female or ambiguous genitalia and normal testis differentiation (normal AMH levels), when the stimulated Δ4/T ratio (at birth, miniptuberty or after ICG) was superior to 1, homozygous or compound heterozygous mutations of the Δ4A17B3 gene were found. Nevertheless, in 2 unrelated patients out of 44 with 17βHSD deficiency, only one heterozygous mutation has been found. For one of them, gonadectomy was performed and mRNA was therefore available for analysis. After KF-PCR and sequencing, a heterozygous duplication of exon 2 was suspected. Since mRNA was not often available, we decided to develop detection of large rearrangements on exon 2. Using fluorescent dosage analysis, the 11 exons of the Δ4A17B3 gene were co-amplified and run on a 3100 ABI sequencer after 18 cycles of PCR. A double-spotter comparing the peak height of all exons of patient and of control, was calculated. The duplication of exon 2 was confirmed in our patient and in his father. Using this strategy in our other patient with only one maternal mutation identified, an other heterozygous duplication encompassing exons 3 to 10 was detected in patient and his father. This is the first time large rearrangements of the Δ4A17B3 gene are reported. Their detection should be considered in all patients carrying only one mutation or having a typical hormonal profile of 17βHSD deficiency.

**P1-419 Testes and Androgens**

**Testicular growth from birth to two years of age, and the effect of orchidopexy at age 9 months. A randomized controlled study**

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**Background:** The optimal age for surgical treatment of undescended testes remains controversial. Based on histopathological studies, some clinical scientists advocate early surgical correction of undescended testes-preferably within the first two years of life—since at that age and onwards the number of spermatogonia per tubular crosssection has been shown to be decreased in undescended testes. However, to our knowledge there are no studies published that have randomized boys with undescended testes to surgery during infancy and later in childhood, and then followed to see whether spermatogenesis is better if the testes are placed in the scrotum at an earlier age.

**Aim:** To study whether surgical treatment at age 9 months in boys with congenital unilaterally palpable undescended testes (cryptorchidism) is followed by improved growth of the previously retained testes compared to non-treatment.

**Methods:** In this prospective randomized study we recruited a total of 156 boys at ages 0-3 weeks and 6 months. Ultrasonography was used to determine testicular volume. At the age of 6 months 70 boys were randomized to surgical treatment at 9 months and 79 boys to treatment at 3 years of age, this group served as a control for those with early surgery. 7 boys had not yet been randomized at the time of this publication. The boys were then followed at 12 and 24 months.

**Results:** After orchidopexy at age 9 months, the previously retained testes resumed growth and were significantly larger than the non-operated control testes at 2 years (p<0.001). The improved testicular growth after orchidopexy was also demonstrated by an increased ratio between the previously retained and the scrotal testes of the individual boys at 2 years (p<0.001), which was not the case for the untreated group.

**Conclusion:** Surgical treatment at 9 months resulted in a significant catch-up growth at 2 years compared to the non-treated group, clearly indicating that early surgery has a beneficial effect on testicular growth. Since testicular volume is an approximate measure of spermatogenic activity, these results suggest that orchidopexy at early age may improve future fertility.

**P1-420 Testes and Androgens**

**X-linked Adrenal Hypoplasia Congenita: New Mutations and Testicular Histology In a Prepubertal Patient**

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X-linked adrenal hypoplasia congenita (AHC) is a developmental disorder of the human adrenal gland caused by DAX-1 gene (NR0B1) mutations. Affected boys typically display adrenal insufficiency in early infancy or childhood and hypogonadotropic hypogonadism (HHG) at the time of puberty. Although administration of exogenous human chorionic gonadotropin has been shown to induce a normal testosterone response in most AHC patients, spermatogenesis has not been induced with only rare spermatogonia in testicular biopsies. We analyzed the DAX-1 gene in 6 patients with AHC and identified 4 novel mutations (c.1533delT, c.815-1054del, c.849insCTGGTG, c.341delG). In the AHC patient due to the c.1533delT, a 9-year-old boy, a physical examination and MRI study revealed that left and right testes were supracrotal and in the inguinal canal, respectively. A GnRH loading test (100μg) did not induce appropriate responses of LH and FSH, 0.4 IU/L and 4.0 IU/L at peak response, respectively, suggesting the absence of HHG. A 3-day loadings of hCG induced a positive response of testosterone, 461 nmol/L (adult normal, 1,214-3,571 nmol/L). To evaluate the influence of testicular undescended, biopsy samples were obtained from the bilateral testes when orchidopexy was performed after his parents’ consent. Small testicular sections were taken toward the peripheral pole of the bilateral testes. Histologically, seminiferous tubules were well defined and contained mixed Sertoli cells and many germ cells. Sertoli cells were positively stained for vimentin and distinguished from negative germ cells. The seminiferous tubules were surrounded by the interstitium containing Leydig cells. Leydig cell hyperplasia, which has been observed in the microscopic findings of the tests from patients with AHC, was not observed in this patient. This observation suggests that such episodes, which cause the loss of germ cells in the seminiferous tubules and Leydig cell hyperplasia, may take place after the prepubertal period in this disease.

**P1-421 Testes and Androgens**

**Dissociated tubular-interstitial testicular dysfunction in a patient with McCune-Albright syndrome (MAS): Pathogenic mechanism**

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MAS is classically characterized by polyostotic fibrous dysplasia, café-au-lait skin lesions, and gonadotropin-independent gonadal activation (though less frequently in boys). Somatic gain of function mutations in the Gsα protein gene have been found in affected tissues. In the testis, Gsα is normally involved in signal transduction of gonadotropins: LH in Leydig cells and FSH in Sertoli cells. We previously reported a 3 year-old boy presenting with macroorchidism, high serum levels of Sertoli cell markers anti-Müllerian hormone (AMH) and inhibin B, but low testosterone. The patient’s parents had given informed consent for the study. Testicular biopsy showed Sertoli cell hyperplasia with otherwise prepubertal features. A somatic R201H mutation was detected in the Gsα protein gene in DNA extracted from testis. We aimed at understanding why only Sertoli cells showed hyperactivity, whereas Leydig cells remained quiescent. We performed laser capture microdissection of testicular paraffin-embedded tissue in order to selectively isolate seminiferous tubules from interstitial tissue. DNA extracted from the different compartments was screened for R201H mutation by direct sequencing of PCR products obtained by nested PCR. DNA from seminiferous tubules, but not interstitial tissue, displayed the mutation. Further, we compared the trans-ac-
Physical examination and determination of FSH and LH (IFMA), T (RIA) and AMH, InhB and Pro-αC (ELISA) were performed in 20 patients with Klinefelter syndrome (ages 2-23 yr).

Gonadotropins and T were normal in all patients at Tanner stages I-II (n=9). AMH and InhB levels were normal in 8 out of 9 patients. Pro-αC levels were low in 4 cases (44-68 pg/ml). In patients at Tanner stages IV-V (n=11) FSH was elevated in all of them (14.2-4.82 mIU/ml) and LH in 9 (8.4-27.3 mIU/ml). The majority of these patients showed extremely low or undetectable AMH (<10 pmol/L) and InhB (<16-48 pg/ml) levels; whereas low Pro-αC levels were observed in 2 of them (33 and 41 pg/ml). T levels fell within the normal range in 7 patients (324-557 ng/dl) and were slightly decreased in 4 (210-281 ng/dl).

Our results suggest, that in patients with Klinefelter Syndrome, Sertoli cell basal activity is preserved during childhood and early puberty as shown by AMH and InhB levels; abnormal Pro-αC levels may reflect an early impairment of Leydig cell function. In advanced stages of pubertal development, the increase in FSH and the extremely low InhB and AMH levels reflect a primary alteration of Sertoli cell function. Normal/low plasma T and Pro-αC concomitantly with elevated LH levels may indicate a compensated Leydig cell dysfunction.

**P1-422 Testes and Androgens**

The prognostic value of serum inhibin B in relation to the fertility potential in bilateral cryptorchid boys expressed by histopathological changes in testicular biopsies

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**Background:** Inhibin B is a heterodimeric glycoprotein that consists of an α-subunit and either a αB-subunit or a αA-subunit giving the inhibin-A and inhibin-B forms respectively. Inhibin-B is the predominant form in the male and is produced by the testes, where the Sertoli cells are believed to be the major site of production. In bilateral cryptorchidism the mean number of germ cells in the biopsies taken at orchiopexy correlates to the future fertility potential (the sperm concentration and the FSH-values).

**Objective and hypothesis:** The aim of the study was to investigate if the inhibin-B levels correlate to the histopathology of the testes in bilateral cryptorchid boys. Methods: 24 boys with bilateral cryptorchidism median aged median 2 years and 4 months (range 9 months to 5.0 years) were included. All had blood samples collected for inhibin-B analysis. The boys underwent surgery for cryptorchidism with simultaneous testicular biopsy. The number of spermatogonia and gonocytes per tubule cross section was assessed.

**Results:** The patients with low serum inhibin-B levels had significantly lower number of germ cells per tubular cross section (median: 0.035 (range: 0-0.50)) compared to those patient with normal inhibin-B levels (median: 0.407 (range: 0.012-1.221)), (Mann-Whitney test, p<0.05). The ages of the patients in the 2 groups were equal (Mann-Whitney test, p=0.834). The predictive value of a subnormal inhibin-B level for infertility in bilateral cryptorchidism was high. In contrary, the predictive value of a normal inhibin-B level for a normal fertility potential in bilateral cryptorchidism was only 50%. In the total material 3/24 (12%) lacked germ cells in both testicular biopsies at time of orchiopexy.

**Conclusion:** Serum values of inhibin-B were a useful supplementary prognostic tool to predict future infertility in bilateral cryptorchid boys.

**P1-423 Testes and Androgens**

Klinefelter syndrome: Assessment of Sertoli and Leydig endocrine function in childhood and adolescence

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Secretion of anti-Müllerian hormone (AMH), inhibin B (InhB) and inhibin α-subunit (Pro-αC) by Sertoli cells and testosterone (T) and Pro-αC by Leydig cells are regulated by gonadotropins and a paracrine interaction between Leydig, Sertoli and germ cells. Testicular function is impaired after puberty in patients with Klinefelter syndrome, but scarce information is available at earlier stages of life. Our objective was to assess Leydig and Sertoli cell function in children and adolescents with Klinefelter syndrome.

**P1-424 Testes and Androgens**

Re-evaluation of adult patients with the clinical diagnosis of androgen insensitivity

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Patients who presented with primary amenorrhoea were often given the diagnosis of complete or partial androgen insensitivity syndrome (AIS) on clinical grounds and the finding of 46XY genotype until full endocrine tests and mutation screening of genes were available. In an adult intersex clinic many patients with presumed AIS had undergone gonadectomy, to prevent malignancy, making biochemical re-investigation difficult. We have studied 32 women with 46XY genotype of mean age 38 years in order to confirm the presumptive diagnosis of complete AIS (CAIS), partial AIS (PAIS), 5α-reductase defect (5AR) or 17-ketosteroidreductase (17KSR) and to examine long term outcomes for these patients. We hypothesised that high serum androstenedione to testosterone ratios by immunoassay and urine 16-hydroxy-DHA to androstenediol metabolite ratios may reveal undiagnosed 17 ketosteroid reductase deficiency and that the 5α:5α ratio of urinary steroids may reveal undiagnosed 5α reductase deficiency. Androsterone (Andro), aetiocholanolone (Aetio), Tetrahydrocortisol (THF) and alloandrosterone (aTHF) in urine were examined using gas chromatography and mass spectrometry after enzyme hydrolysis of conjugates and formation of methyloxime-trimethylsilyl ether derivatives. RESULTS (Range or mean)

<table>
<thead>
<tr>
<th>CONTROLS</th>
<th>CAIS</th>
<th>PAIS</th>
<th>5AR</th>
<th>17KSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>5</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>A4/Testo</td>
<td>2.5-8</td>
<td>5.5</td>
<td>3.02</td>
<td>&lt;8</td>
</tr>
<tr>
<td>Aetio/andro</td>
<td>0.91-2.9</td>
<td>1.71</td>
<td>1.29</td>
<td>4.83</td>
</tr>
<tr>
<td>THF/αTHF</td>
<td>0.58-2.2</td>
<td>2.59</td>
<td>2.15</td>
<td>18.9</td>
</tr>
</tbody>
</table>

Only one patient showed a raised 16-hydroxy-DHA to androstenediol ratio (3) that might be the result of 17KSR deficiency. Conclusion: A diagnosis of 5α-reductase deficiency was made on the basis of raised 5α to 5α reduced metabolites of DHA and cortisol in urine in four patients. Hence fourteen percent of patients in this cohort with presumed AIS had biochemical evidence for defects in 5αR that need genetic verification.

**P1-425 Testes and Androgens**

Sexual maturation in Egyptian boys

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**Background:** Descriptive data on pubertal stages in Egyptian boys depending on testicular volume by palpation have not been published to our knowledge.
To determine at what ages boys in Egypt reach each of the sexual maturity stages for genital, pubic hair growth as well as axillary hair and to compare our data with previously published data in Egypt

Methods: A sample of 1563 boys aged 6.5 to 18.5 years were included as a part of a much larger cross-sectional study to establish standard growth curves for Egyptian infants, children and adolescents. Pubertal evaluation of the secondary sexual characteristics of boys was done according to Tanner (1975) and Marshal and Tanner (1970). Maturity stages were assigned to pubic and axillary hair in males. In addition, left and right testicular size was recorded. Pubertal events were studied separately: Pubic hair by inspection (range PH2 to PH5), axillary hair (A1 to A3), and testicular volume by palpation of the testes and was compared to the models of Prader orchidometer.

Results: The mean age at stage 2 for pubic hair development of Egyptian boys was 11.86 ± 1.45 years, and at stage 2 for axillary hair was 13.55 ± 1.52 yr. For testicular volume, the mean age of children with (T4) was 15.06 ± 1.4 years (T3) and 3.11 years for (T2) gonadal stage 4; it was 15.30 ± 1.09 years and (T15) stage 5: 14.27 ± 1.2 yr as the last pubertal event reached by all normal boys. All boys at age of 17 showed axillary, pubic hair as well as genital stage 5 development.

Conclusions: The mean ages at the onset of genital and pubic hair growth were younger than in past studies done in Egypt.

P1-426 Testes and Androgens

Hypothalamic-pituitary-testicular axis evaluation in boys presenting with nonpalpable gonads during infancy, childhood or puberty

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The assessment of the presence and functional capacity of the testes is essential in the evaluation of boys with virilization and nonpalpable gonads. Surgical procedures are invasive and imaging techniques may not be accurate. Hormones evaluation is therefore indispensable. Anti-Müllerian hormone (AMH) and testosterone (T) measurement is used to assess the functional capacity of the tubular compartment, regulated by FSH, and the interstitial tissue, regulated by LH, respectively. An integral analysis of the whole pituitary-testicular axis might be more informative in the diagnosis of boys with nonpalpable gonads. We evaluated the levels of FSH (IFMA), AMH (ELISA), LH (IFMA) and T (RIA) in samples of 6 boys 0-6 months-old, 39 prepubertal (>6 months-old) and 7 pubertal, with normal virilization but nonpalpable testes. Clinical charts of all patients included were thoroughly reviewed. Absence of functional testicular tissue was diagnosed when AMH was non detectable and T was not >50 ng/dl (post-hCG or basally in patients with elevated LH). Four anorchid patients >6 months-old had elevated FSH (40—199 IU/L) and LH (40-92 IU/L) and low T (<10-17 ng/dl). The other 2 patients, with present testes, had low FSH, AMH, LH and basal T with normal response to hCG test. Twelve of the 39 prepubertal patients were anorchid: in 9 both FSH (3.4-130 IU/L) and LH (0.5-25 IU/L) were elevated; in 2, FSH but not LH was elevated, and in 1 both gonadotropins were normal. The remaining 27 prepubertal boys had testicular tissue. In 18 cases, testes were hypofunctional, resulting in low AMH and/or poor T response to hCG. Gonadotropins were elevated in and normal in 11. In 9 cases, testes were functionally normal. Five of the 7 pubertal patients were anorchid: in 4 both FSH (24-99 IU/L) and LH (15.6-28.6 IU/L) were high and in 1 only FSH was elevated. The remaining 2 patients had testicular tissue: T was normal but gonadotropins were elevated. We conclude that absence of gonads can be diagnosed by measuring either basal FSH (high) or AMH (ND) or T post-hCG (low) in 95% of cases. Conversely, for the diagnosis of presence and functional capacity of testes, AMH and T are more helpful.

P1-427 Testes and Androgens

Atypical testicular adrenal rest in male with congenital adrenal dysfunction (CAD)

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Wilkins first described the presence of testicular lesion, so called adrenal rest tumours, in male patients with uncontrolled CAD due to 21-hydroxylase deficiency. We reported here 2 atypical cases. Case 1 was 18 yrs and case 2 was 8 yrs old. They both presented an uncontrolled CAH during a long period due to a poor treatment observance for patient 1 and a poor biological survey for patient 2. Both were asymptomatic and no testicular lesion was palpable. Patient 1 presented with adrenal insufficiency diagnosed at 15 months with recurrent hypoglycaemic episodes and hyper pigmentation. Tests for achalasia and alacrina were negative, and fatty acids were normal. No abnormality in the coding exon of the ACTH receptor gene was detected leading to conclusion of familial glucocorticoid deficiency type 2 (FGD) based on the presence of an affected sister. At 16 yrs, he complained for an acute testicular pain. Tumorous markers were negative.

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormone dose (mg/m2) (1)</td>
<td>13 (15)</td>
<td>9.4 (15)</td>
<td>15 (15)</td>
</tr>
<tr>
<td>ACTH 1 (pg/ml)/(17)</td>
<td>17</td>
<td>OHP (mg/ml)</td>
<td>28x15x17</td>
</tr>
<tr>
<td>Testis ultrasonography 1</td>
<td>hyperechohy</td>
<td>hyperechohy</td>
<td>hyperechohy</td>
</tr>
<tr>
<td>L : 36x12x21 mm</td>
<td>pervasc</td>
<td>pervasc</td>
<td>pervasc</td>
</tr>
<tr>
<td>R : 28x13x17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testis ACTH 2 (pg/ml)/(17)</td>
<td>11</td>
<td>OHP (mg/ml)</td>
<td>28x15x17</td>
</tr>
<tr>
<td>Testis ultrasonography 1</td>
<td>no modification</td>
<td>stable</td>
<td>stable</td>
</tr>
<tr>
<td>L : 49x17</td>
<td>R : 26x18</td>
<td>R : 18x2</td>
<td></td>
</tr>
<tr>
<td>HC Dose 2 (mg/m2)</td>
<td>15+</td>
<td>OHP (mg/ml)</td>
<td>17</td>
</tr>
<tr>
<td>Testis ACTH 3 (pg/ml)/(17)</td>
<td>15+</td>
<td>OHP (mg/ml)</td>
<td>17</td>
</tr>
<tr>
<td>Testis ultrasonography 3</td>
<td>L : 14x14x14 mm</td>
<td>L : lesion 3</td>
<td>L : lesion 3</td>
</tr>
<tr>
<td>R : 14x14x14 mm</td>
<td>R : lesion 3</td>
<td>R : lesion 3</td>
<td></td>
</tr>
<tr>
<td>Testis ACTH 4 (pg/ml)/(17)</td>
<td>152</td>
<td>OHP (mg/ml)</td>
<td>17</td>
</tr>
<tr>
<td>Testis ultrasonography 3</td>
<td>76x15x15 mm</td>
<td>L : lesion 3</td>
<td>L : lesion 3</td>
</tr>
<tr>
<td>L : 38x24 R : 28x19 mm</td>
<td>no lesion</td>
<td>no lesion</td>
<td>no lesion</td>
</tr>
</tbody>
</table>

Testicular adrenal rest tumours could appear early in childhood period. It is important to keep in mind these cases in order to check testicular sonography, to explain the absolute necessity of a strict hormonal control and to propose sperm conservation in late pubertal period. To our knowledge it is the first case described in an adolescent with adrenal insufficiency.

P1-428 Testes and Androgens

Cryptorchidism in the structure of indifferent form of Connective Tissue Dysplasia

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The aim of our work is clinicobiological and histochemical diagnosis of indifferent form of connective-tissue displasia (CTD) of cryptorchism. 70 children at the age of 2 up to 13 were examined and surgery treated. According to the clinical forms of cryptorchism they were divided into groups with unilateral, bilateral and recurrent cryptorchism. To explore phenotypic characteristics of CTD the results were registered in phenotypic cards, which included „the main” markers of CTD and “secondary” markers of disembodygenic stigmas. Being biochemical markers of CTD daily renal excretion of oxyproline and glucosaminoglicans were examined. Biopsy material of inguinal channel was taken intraperforatively for the further histochemical analysis about the connective-tissue pathology. Analyzing the results, it was found that
the majority of children (65) had phenotypical characteristics of CTD with the little predominance in all groups. The majority of children had asthenic body (54), and only some of them had normosthenic body (7). The account of weight-and-height index showed the lack of weight of 60 children. Some patients had the deformation of the chest: funnel (2), keeled chest (1) and sciotic chest (45). Some children (13) had deformation of lower extremities. Some children had hypermobility of joints, such as: physiological norm (23), moderate hypermobility (34), and pathological hypermobility (2). Epicanthus, ocular hypertelorism, “gothic” palate and others were met more often among disembsiotics stigmas. The majority of children (64) had higher level of renal excretion of oxyproline. The increase of total quantity of uronic acids and hexose and disbalance between their sulfatic fractions was registered (60). By histochemical exploring disorganization and lyses collagenous fibres, the changing of composition and spatial structure were detected. The great number of metachromasia granules with the accommo-
dation of glycosaminoglycans was found. The results of clinico-biochemical and morphological investigation showed that the anomaly of development of testicule is the result of the plastical processes pathology in the structure of indifferent form of CTD.

**P1-429 Testes and Androgens**

**Correction of nopalpable testicular depending on aetiology and risk factors of pathology developing**

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**Introduction:** Nopalpable testicular - (NT) is a state which is aetologically heterogenic. Correction methods of NT depending on aetiology and risk factors are being not considered, yet.

**Materials and methods:** Medical and genetically study, making a list of questionnaires for clearing out the possible risk factors was performed, the hormone therapy-HT was carried out by 1000 units on m² on the body-surface twice a week with the interval during 1.5 month.

**Results:** There were examined 319 patients with grain forms of NT at the age from one to twelve years old. The risk factors, the testimony to HT and its efficiency were studied: there were 87 patients with NT and 64 pseudo revelation of testicular in the 1-st group. They are supposed to have the genetically determined passivity of groin circle of components: the relatives of the 1-st and 2-d extent of kinship had grain scrotal hernia and NT. The patients (120) who had indications for teratogene influence on embryonic and hametogenesis (settlements in unfavorable ecological places or parents professions were included in the 2-d group. There were 47 patients with the heredity syndromes and cryptorchidism in the 3-d group (Noonan, Prader-Willy, Rubinstein-Taybi syndromes). The efficiency of HT was observe red in 78% of patients of the 1-st group and 6% in patients of the 2-d group, the effect of HT was absent in the 3-d group and orchiopexy was needed for NT correction.

**Conclusion:** polyaetiologie of NT obliges the physician, to examine the children thoroughly with such pathology for individual choice of correction method.

**P1-430 Testes and Androgens**

**Intracytoplasmic sperm injection conceived children aged >5 years: pilot study on neuropsychological and somatic development**

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To date, very few studies have been focused on neurodevelopmental status and somatic development in children conceived after intracytoplasmic sperm injection (ICSI). Data on “ICSI children” aged >5 years are still missing. Aim of study was the analysis of somatic and neuropsychological development in children aged >5 years.

Open cross-sectional pilot study started on September 2004. To date twenty-six children, 14 girls and 12 boys (20 singletons, 6 twins) aged 6.6±0.6 yrs (mean±SD) (range 5.6-7.8 yrs) were enrolled. Auxological parameters, cognitive and motor development (using McCarthy Scales of Children’s Abilities, MSCA cognitive, memory and motor scales) were compared with controls (reference standards). All children were seen by a neurologist. Mean gestational age was 39±2 wks (range 34-42) (in all twins less or equal 37 wks), birth weight 3122±595 g (range 1770-3950) and birth length 49±3 (range 42-54) cm. At the time of study, body height SDS (0.1±0.9) and body weight SDS (0.2±0.9) were in normal range in majority of children, nevertheless more borderline findings were found, e. g. 8/26 children had skinfolds thickness <10th percentile. In all “ICSI children” a prominent hypotonia of upper girdle muscles tested by scarf sign were found. In our group mean IQ was 104±12 (reference standards corrected to date of study 110±16). Normal memory scale 45±9 (reference standards 50±10; p=0.022). No difference was found between singletons and twins in IQ (singletons 105±13; twins 102±10) or in memory scale (singletons 45.0±10; twins 41±6). In majority of ICSI conceived children aged >5 years somatic development was normal. There was a tendency to slightly worse nutritional status. All children in our group have some neurodevelopmental changes in the muscle tone maturation. Results of our pilot study suggest that future research needs to address the psychosomatic development, neurodevelopmental status and maturation in ICSI conceived children older than 5 years. Supported by Grant IGA MII CR 8118-3.

**P1-431/MP Endocrine Disruptors**

**Explosive rise in malformations of newborn male genitalia in Northeastern Brazil:**

**Consequences of a polluted environment?**

Dianece Sampaio1, J Brandão Neto2, Françoise Audran2, Charles Sultan2

1Hospital Alcides Carneiro, UFCG, Campina Grande and Pós-Graduacao em Ciencias da Saude, University Of Rio Grande Do Norte, Service de Pédiatrie, Parabia, Brazil; 2Hôpital Lapeyronie, CHU Montpellier, Service d’Hormonologie, Montpellier, France;

The aim of this study was to determine the incidence of microepispadias and cryptorchidism in male newborns in the state of Paraiba and the etiology of these malformations.

In Northeastern Brazil, one of the poorest regions where 50% of the population lives in favellas, mosquito and rodent proliferation is rampant. DDT and other insecticides are widely used by the inhabitants, in addition to broad agricultural pesticide application.

In the study of a polluted environment, one of the poorest regions where 50% of the population lives in favellas, mosquito and rodent proliferation is rampant. DDT and other insecticides are widely used by the inhabitants, in addition to broad agricultural pesticide application.
During 2002-2004, 6000 boys were born, of which 56 presented with genital malformations. They all underwent an HCG stimulation test to evaluate the Leydig cell function. All parents responded to a detailed questionnaire concerning professional activity, the work environment and the family life style. Blood DNA was obtained and analysed for 5α-reductase gene and androgen-receptor gene mutation. This study was approved by the Ethics Institutional Committee of the Hospital Alcodes Carveiro of Campina Grande, Paraiba.

The incidence of genital malformations in male newborns was microgenis (n = 18), hypospadias (n = 15), unilateral cryptorchidism (n = 16), bilateral cryptorchidism (n = 2), and associated malformations (n = 5), for a total of 56/6000 or 9.4/1000.

This incidence was dramatically higher that expected. Of these 56 male newborns, 2 had testicular dysgenesis (T after HCG < 2 ng/ml), 3 exhibited a mutation within the 5α reductase gene (5αRD) and 2 DNAs were not obtained. Of the remaining 49 newborns, all showed normal T values after HCG (> 3 ng/ml) and no mutation of either 5α reductase or androgen receptor gene. Of these 49 males, 44 were from families either living in favelas (n = 23 with DDT used 2-3 / wk) or involved in heavy agriculture labor (n = 21, extensive use of pesticides, herbicides and insecticides).

Since DDT and other pesticides are well known to exhibit significant anti-androgenic activity, it is likely that they are involved in the disruption of normal fetal male sex differentiation and in the explosive rise in genital malformations of newborn males from Northeastern Brazil. The long term consequence of prenatal contamination by these environmental pollutants should be evaluated, as well.

### Table 1 Pesticides (ng/g lipid)

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Denmark median min/max</th>
<th>Finland median min/max</th>
<th>Mann-Whitney U-test p</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDE</td>
<td>133.76 24.60-427.60</td>
<td>59.05 19.00-102.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DDT</td>
<td>5.68 1.62-37.88</td>
<td>3.43 12.90-5.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>β-Hexachlorocyclohexane (β-HCH)</td>
<td>16.85 5.97-66.23</td>
<td>10.93 2.74-30.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hexachlorobenzene (HCB)</td>
<td>12.40 6.00-25.00</td>
<td>7.95 3.00-19.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>4.88 1.74-35.50</td>
<td>2.37 0.77-7.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oxychlordane</td>
<td>4.74 2.26-12.01</td>
<td>3.56 0.91-9.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>cis-Heptachloropoxdie (c-HE)</td>
<td>2.87 1.25-10.82</td>
<td>2.04 0.63-17.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>α-Endosulfan</td>
<td>7.43 1.92-18.05</td>
<td>6.40 1.19-22.66</td>
<td>0.068</td>
</tr>
</tbody>
</table>

Four compounds were not found in any sample (aldrin, β-endosulfan, trans-Heptachloropoxdie and β-Hexachlorocyclohexane).

For the remaining 15 compounds the detection rate varied from 2.3%-98.5% (listed in ascending order: Heptachlor, β-Hexachlorocyclohexane (β-HCH), cis-chlordane, Methoxychlor, Pentaichloronaisole, γ-HCH, op-DDD, transchlordane, α-HCH, op-DDD, Oxachlorostyrene, Pentaichlorobenzene, pp-DDD, op-DDT and mirex).

The lipid content in the Finnish samples was significantly higher than in the Danish (4.26 vs 2.84 p<0.001).

A regional difference in lipidbased breast milk concentrations of pesticides was demonstrated, suggesting Danish children to be more exposed than Finnish children. Whether this difference contributes to the previously reported regional difference in birth prevalence of cryptorchidism and hypospadias remains to be seen.

### The phytoestrogen resveratrol suppresses adrenal steroidogenesis by inhibiting P450 c21 hydroxylase

**Vichet Supornsimilai**, Konstantin Svechnikov, D Seidlova Wuttke, Wolfgang Wuttke, Olle Soder

Karloinska University Hospital, Woman and Child Health, Stockholm, Sweden; Georg-Universität, Abteilung fur Klinische und Experimentelle Endokri, Göttingen, Germany

The phytoestrogen resveratrol is found in grapes, mulberries and peanuts.
Phytoestrogens (PE’s) are compounds found in soy that are chemically similar to 17β-estradiol, allowing them to bind to estrogen receptors and thereby act as endocrine disrupters. For years it has been postulated that PE’s can exert estrogenic effects, but very recent literature refutes this postulate. The question remains important in pediatrics because of the prevalent use of soy formula. A 16 mo white female had an incidental finding of Tanner 2 breasts. Her infantile breast buds had resolved and she had never been exposed to any hormone containing medicines, creams or hair products. Birth, dental and past medical history were unremarkable. She had no other signs or symptoms on physical and review of systems to suggest puberty onset or tumor. She was Tanner 1 on genital exam; her growth chart showed normal growth velocity. Lab evaluation revealed an estradiol level of 23 pg/ml (normal <10 pg/ml). At follow-up, a dietary history revealed that her diet was based entirely on soy. From 3 months old until present she drank soy formula or soy milk. She started on solids at 6 mo, and all of her solids (from that point on) consisted of soy based foods. We decided to remove all soy from her diet. A repeat estradiol level three weeks later was 8pg/ml. Further follow up noted her estradiol level to be stable and that her Tanner 2 breasts had decreased in size.

The apparent causal relationship in this case stands in opposition to the current position that PE’s cannot function as endocrine disruptors. The liability of an endocrine disruptor to exert estrogenic effects depends on its potency, its concentration and the concentration of endogenous estrogens. In vitro studies have proven that PE’s have an estrogenic potency ranging from 10-2 to 10-3 relative to that of 17β-estradiol but note their concentrations may be 100-fold higher in vivo. In lieu of the above data, it seems plausible that soy can function as an endocrine disruptor in children despite the arguments of current literature. This information, coupled with our case report, warrants that pediatricians consider soy in the differential when evaluating premature thelarche.

**P1-437/MP Endocrine Disruptors**

**Botanical Extracts (BE) with Selective Estrogen Receptor Beta Modulators (SERM-β) Accelerate Bone Growth and Maturation**

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2Centre Hospitalier Universitaire, Service d’Hormonologie, Montpellier, France

BEs are prescribed by “naturopaths” to stimulate children’s growth. They are often regarded lightly, with little concern for their activity and safety. We describe a BE, its clinical effects and mechanism of action. A 5 year-old boy with MPH (GH-, TSH-, ACTH-, gonadotropins-deficiency) received a repeat therapy of hydrocortisone and L-thyroxin. For his growth retardation, he was treated for 18 months by a “naturopath” with a mixture of BEs. He responded with growth acceleration from -2 SD to -0.5 SD, and bone-age advancement of 2 ’y’/year; he had no signs of sex-hormone activity on his breast or genitalia. The BE mixture was tested for its selective estrogen receptor (ER) activity, using HE-la cell lines that were stably transfected with one or the other of the ERs, and was found to have selective ERβ activity. Eighteen components of the BE mixture were then reconstituted as prescribed the naturopath, and tested for their estrogenicity by analyzing estrogen transactivation of luciferase. 50% transactivation was reached with 2x10-11 M E2 for the ERα cell line and 5x10-11 M for the ERβ cell line. Four BE components were found to have greater SERM-β than SERM-α activity.

**P1-435/MP Endocrine Disruptors**

**Precocious puberty: Prevalence and symptoms upon presentation in Bogota Colombia**

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It has been postulated that there is an increased incidence of precocious puberty in girls adopted from developing countries. Colombia is one of the leading countries in number of children given for adoption per year. Our goal was to evaluate the prevalence of precocious puberty in our pediatric endocrinology referral center, characterizing the initial symptoms upon presentation, and to correlate those findings with BMI and age of parental puberty. We evaluated 1549 patients referred to our clinic during the year 2004. Most of the patients (90%) were female. We found a prevalence of precocious puberty of 13%. The initial clinical presentation was consistent with adrenarche in 9% and thelarche in 18.5%. Our data suggest a high prevalence of precocious puberty in Colombia. Our data correlates those findings with BMI and age of parental puberty. We evaluated 1549 patients referred to our clinic during the year 2004. Most of the patients (90%) were female. We found a prevalence of precocious puberty of 13%.

**P1-436/MP Endocrine Disruptors**

**Phytoestrogens as an exogenous etiology of premature thelarche**

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Phytoestrogens (PE’s) are compounds found in soy that are chemically similar to 17β-estradiol, allowing them to bind to estrogen receptors and thereby act as endocrine disrupters. For years it has been postulated that PE’s can exert estrogenic effects, but very recent literature refutes this postulate. The question remains important in pediatrics because of the prevalent use of soy formula. A 16 mo white female had an incidental finding of Tanner 2 breasts. Her infantile breast buds had resolved and she had never been exposed to any hormone containing medicines, creams or hair products. Birth, dental and past medical history were unremarkable. She had no other signs or symptoms on physical and review of systems to suggest puberty onset or tumor. She was Tanner 1 on genital exam; her growth chart showed normal growth velocity. Lab evaluation revealed an estradiol level of 23 pg/ml (normal <10 pg/ml). At follow-up, a dietary history revealed that her diet was based entirely on soy. From 3 months old until present she drank soy formula or soy milk. She started on solids at 6 mo, and all of her solids (from that point on) consisted of soy based foods. We decided to remove all soy from her diet. A repeat estradiol level three weeks later was 8pg/ml. Further follow up noted her estradiol level to be stable and that her Tanner 2 breasts had decreased in size.

The apparent causal relationship in this case stands in opposition to the current position that PE’s cannot function as endocrine disrupters. The liability of an endocrine disruptor to exert estrogenic effects depends on its potency, its concentration and the concentration of endogenous estrogens. In vitro studies have proven that PE’s have an estrogenic potency ranging from 10-2 to 10-3 relative to that of 17β-estradiol but note their concentrations may be 100-fold higher in vivo. In lieu of the above data, it seems plausible that soy can function as an endocrine disruptor in children despite the arguments of current literature. This information, coupled with our case report, warrants that pediatricians consider soy in the differential when evaluating premature thelarche.

**P1-438 Ovaries and Estrogens**

**Serum Ghrelin and Resistin levels in adolescent girls with Polycyctic Ovary Syndrome**

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Gazi University Medical Faculty, Pediatric Endocrinology, Ankara, Turkey

The hormonal environment and cause-effect relationship between hormones in polycystic ovary syndrome (PCOS) is still not clearly documented. This study is planned to investigate serum ghrelin and resistin levels and their interaction with insulin sensitivity, obesity and lipid profile in subjects with PCOS. The study group consisted of sixteen obese with PCOS (OPCOS) [age;
Polycystic ovary syndrome (PCOS), characterized by hyperandrogenism and chronic anovulation, is the most common reproductive disorder of pre-menopausal women. The high prevalence of Ins resistance in PCOS suggests a fundamental role for Ins in the androgen over-production. LH-driven thecal androgenesis is enhanced by Ins in vitro. We previously showed that Ins augmentation of human theca CYP17 activity proceeds via PI3K. The purpose of the current study was to evaluate those more distal elements that transduce insulin’s signal to modulate androgen synthesis. Serum-starved third passage human theca cells were incubated with test agents for 72 hours. CYP17 activity and mRNA expression were measured. PKC activity was determined as incorporation of p-JP into specific PKC substrate. In the presence of Forskolin (Fsk) (stimulating the requisite LH activation of adenylate cyclase), Ins stimulates thecal CYP17 activity and transcription. Use of recombinant Adenovirus (Adv) to over-express PI3K catalytic subunit (p110) alone did not alter CYP17 activity. Fsk-Adv+p110 enhanced CYP17 activity comparable to that achieved with Fsk+Ins. Adv expressing the constitutively active form of distal element Akt augmented Fsk-stimulated CYP17 activity. As Ins stimulation also enhanced thecal PKC activity, we over-expressed specific PKC isoforms, to test their capacities to mediate Ins up-regulation of CYP17. Immunoblotting confirmed PKC isoform α, β and ε expression in cultured human theca. Neither PKC-α nor β promoted the activation of CYP17 by Fsk. Although ineffective alone, PKC-ε augmented Fsk-stimulated CYP17 activity comparable to the maximal levels achieved by Fsk+Ins. Ins augmentation of CYP17 activity in human theca proceeds through PI3K. Akt and PKC, elements distal to PI3K, mediate the downstream effects of Ins on CYP17 activity. Ins stimulation of atypical PI3K-ε uniquely promotes human thecal androgen production. These results extend our knowledge of functional signals linking Ins and steroidogenesis, providing possible molecular mechanisms for the androgen excess observed in PCOS.

**P1-440 Ovaries and Estrogens**

Elements distal to PI3 Kinase (PI3K) transduce Insulin (Ins) signaling to promote steroidogenesis in cultured human theca cells

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Polycystic ovary syndrome (PCOS), characterized by hyperandrogenism and chronic anovulation, is the most common reproductive disorder of pre-menopausal women. The high prevalence of Ins resistance in PCOS suggests a fundamental role for Ins in the androgen over-production. LH-driven thecal androgenesis is enhanced by Ins in vitro. We previously showed that Ins augmentation of human theca CYP17 activity proceeds via PI3K. The purpose of the current study was to evaluate those more distal elements that transduce insulin’s signal to modulate androgen synthesis. Serum-starved third passage human theca cells were incubated with test agents for 72 hours. CYP17 activity and mRNA expression were measured. PKC activity was determined as incorporation of p-JP into specific PKC substrate. In the presence of Forskolin (Fsk) (stimulating the requisite LH activation of adenylate cyclase), Ins stimulates thecal CYP17 activity and transcription. Use of recombinant Adenovirus (Adv) to over-express PI3K catalytic subunit (p110) alone did not alter CYP17 activity. Fsk-Adv+p110 enhanced CYP17 activity comparable to that achieved with Fsk+Ins. Adv expressing the constitutively active form of distal element Akt augmented Fsk-stimulated CYP17 activity. As Ins stimulation also enhanced thecal PKC activity, we over-expressed specific PKC isoforms, to test their capacities to mediate Ins up-regulation of CYP17. Immunoblotting confirmed PKC isoform α, β and ε expression in cultured human theca. Neither PKC-α nor β promoted the activation of CYP17 by Fsk. Although ineffective alone, PKC-ε augmented Fsk-stimulated CYP17 activity comparable to the maximal levels achieved by Fsk+Ins. Ins augmentation of CYP17 activity in human theca proceeds through PI3K. Akt and PKC, elements distal to PI3K, mediate the downstream effects of Ins on CYP17 activity. Ins stimulation of atypical PI3K-ε uniquely promotes human thecal androgen production. These results extend our knowledge of functional signals linking Ins and steroidogenesis, providing possible molecular mechanisms for the androgen excess observed in PCOS.

**P1-441 Ovaries and Estrogens**

Predictive value of serum anti-mullerian hormone concentration in polycystic ovary syndrome

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Polycystic Ovary Syndrome (PCOS) is characterized by at least two out of three Rotterdam Consensus Criteria (Hum Reprod 2004): oligoovulation or chronic anovulation, clinical or hormonal androgen excess, polycystic ovaries on ultrasound. The high prevalence of PCOS within the normal adolescent population lead us to look for markers of morphological and ovarian dysfunction of PCOS. AMH, a member of the TGFβ superfamily, is important in the recruitment of ovarian primordial follicles and in selection of a dominant follicle, at least in mice. In women, the AMH expression pattern suggests its role in human follicular maturation. In this study, 52 patients referred for hirsutism, were prospectively studied from January 2004 to March 2005. Clinical examination including height, weight, body mass index (BMI), hirsutism scoring, according to Ferriman and Gallway (score > 8 to be considered) and menstrual cycle, in fasting conditions, blood samples were obtained, and androgens (non-SHBG-bound testosterone, androstenedione), gonadotropins (LH, FSH), ovarian factors (inhibin B, AMH immunoreactive), metabolic parameters (lipid profile, glucose, insulin and oral glucose tolerance test) were measured. Statistical analysis was performed using t test to compare PCOS (n=36) and non-PCOS hirsute patients (n=16). The results show that inhibin B was not significantly different between the two groups of patients. In contrast, AMH level was significantly (p=0.04) higher in PCOS (80.8 ± 48.9

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**P1-439 Ovaries and Estrogens**

A randomized controlled trial of OCP vs. OCP with metformin in adolescents with PCOS

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Polycystic ovary syndrome is an extremely common problem in women and frequently presents in adolescence. The mainstay of hormonal therapy has been use of oral contraceptives to suppress ovarian androgen synthesis with or without androgen receptor blockade. The association of PCOS with obesity, dyslipidemia, and insulin resistance is well-known and, over the past 10 years, agents which improve insulin sensitivity such as metformin or TZDs have demonstrated effectiveness in treatment of women with PCOS with reduction of testosterone levels. The purpose of this study was to evaluate efficacy of OCP vs. OCP with metformin in adolescent girls with PCOS.

**Methods:** 20 girls aged 12 to 21 with hyperandrogenism and oligomenorrhea were randomized to receive a 6 month course of OCPs or OCPs with metformin 1g po bid. PE and labwork was done at 3 and 6 months. The primary outcome was free testosterone level.

**Results:** 17 girls completed the 6 month study, 10 on OCP alone and 7 on OCP with metformin. There was a significant reduction in total (p=.02, .04 months) and free testosterone (p=.001) in the metformin treated group but did not change in the OCP group. As expected the insulin levels declined in the metformin treated group but did not change in the OCP alone group. Lipid levels were similar in both group with an increase in HDL in the OCP group.

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**Conclusion:** Adding metformin to OCP in adolescents with PCOS results in similar clinical responses. It seems to attenuate the increase in SHBG with estrogen therapy potentially blunting the decrease in free testosterone - this is an area which requires further investigation.
Ovarian cysts with R201 mutation of the GNAS1 gene

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A gain-of-function mutation at codon 201 (R201) in the GNAS1 gene, encoding for Gs-alpha protein, has been described in DNA samples from ovarian cysts in girls with peripheral precocious puberty, isolated or included in the McCune Albright syndrome (MAS).

We report the clinical/hormonal, genitopelvic US and ovarian histological features of 6 patients with ovarian cysts, in which the GNAS1 analysis in DNA samples from ovarian cyst after cystectomy allowed to identify the R201 mutation. All 6 patients began with thearche, menarche and external genitalia hyperestrogenism at 4-43 months of age. Cushing syndrome and liver dysfunction was also present in 1 patient, skin dysplasia in 2 others. Hormonal investigations showed in all patients gonadotropin-independent peripheral precocious puberty, with low LH/FSH and high 17-beta-estradiol values. At genitopelvic US examination, uterus had adult-like morphology and thin stripes of endometrial echos in all patient, the ovaries were multifollicular with 1-3 monolateral anechogenic cysts, even reaching diameters of 50x42 mm.

The macroscopic appearance of the cysts was consistent with the follicular type. Microscopic evaluation showed granulosa cells stratified upon a theca layer; signs of luteinization were occasionally recognizable, as well as the presence of smaller cysts in the wall.

In a follow-up period ranging 5-8 years, bone and skin dysplasia were disclosed in the patient with Cushing syndrome, and bone dysplasia in the 2 patients with skin dysplasia, thus depicting a classical MAS.

Notwithstanding antiestrogen treatment, these 3 patients alternated periods of progression and regression of pubertal development and hyperestrogenism for the appearance of new cysts, even bilaterally. In a follow-up period ranging 5 months-3 years in the 3 subjects with isolated precocious puberty no other MAS signs appeared and pubertal development and hyperestrogenism regressed after cystectomy; in 1 of them an estrogen-secreting cyst recurred monolaterally and was responsible for a relapse of precocious puberty.

Gonadal function during early infancy in female infants of women with polycystic ovary syndrome

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It has been proposed that prenatal exposure to elevated androgen levels may have deleterious consequences in reproductive function later in life. Recently, we have demonstrated that women with polycystic ovarian syndrome (PCOS) have a significant increase in androgen concentrations during pregnancy, which might modify the gonadal function of the female fetus. We evaluated the pituitary-gonadal axis during the first three months of life in female infants born to PCOS mothers, by means of a GnRH agonist test. Ten female infants born to PCOS mothers (PCOSI) and 14 infants born to mothers with normal menses and without hyperandrogenism (CI) were recruited. PCOSI and CI had normal birth weight. During the first 2-3 months of life an GnRH analogue test was performed (leuprolide 10 µg/Kg s.c.), with measurements of circulating concentrations of gonadotrophins, testosterone (T), androstenedione (A4A), estradiol (E2), 17 OH Progesterone (17OH), sex hormone binding globulin (SHBG) and inhibin B. Data are expressed as mean ± SEM. Similar basal gonadotropins, gonadal steroids and SHBG were found in both groups. However, in PCOSI basal inhibin B concentrations were significantly higher than in CI (61.6 ± 12.7 vs 31.5 ± 3.4 pg/ml, p=0.047). Moreover, stimulated testosterone and estradiol levels were higher in PCOSI compared to CI (0.31 ± 0.04 vs 0.18 ± 0.02 ng/ml; p<0.02 and 70.7 ± 32.89 vs 29.8 ± 9.8 pg/ml; p<0.01, respectively). These data suggest that female infants born to PCOS mothers may have abnormalities in gonadal steroidogenesis and/or alterations in ovarian development, as shown by their higher testosterone and estradiol response to GnRH agonist and by elevated basal levels of inhibin B.

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Absence of mutations in the follicle-stimulating hormone receptor gene in children with primary hypothyroidism and gonadal hyperstimulation

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Severe primary hypothyroidism in children can be associated with gonadal hyperstimulation. A potential pathophysiological mechanism for this condition could be a decreased number of functional cAMP producing follicle-stimulating hormone (FSH) receptors (FSHR) or an increased number of non-functional FSHR. To test this hypothesis, we screened 36 Brazilian children with severe primary hypothyroidism for deleterious mutations in the FSHR gene. We sequenced the exons of the human FSHR gene in 36 children with severe primary hypothyroidism and confirmed the absence of mutations in the FSHR gene.
tion may be the paradoxical activation of the FSH receptor by elevated TSH levels. Recently, three mutations in the transmembrane region of the human FSH receptor were identified in women with a rare isolated form of spontaneous ovarian hyperstimulation syndrome, leading to the promiscuous activation of FSH receptor by hCG. Two of these mutations (D567N and T449A) also caused concomitant increase of FSH receptor activity by TSH. In the present study, we investigated the entire coding region of FSH receptor gene for TSH-sensitive mutations in 4 children with severe primary hyperthyroidism (TSH: 446-1053 mIU/L). Three girls (age 12-16 yrs) had significantly enlarged ovaries (10-59 cc), while one boy (age 9) presented with early testicular enlargement (5 cc). Genomic DNA was extracted from peripheral blood and all exons (1 to 10) of the FSH receptor gene were amplified by polymerase chain reaction using specific intronic primers. The amplified products were submitted to enzymatic purification and automatic sequencing. No TSH-sensitive activating mutation of the FSH receptor was found in all patients studied. We found two known polymorphisms (A3697T and c678A/T) in linkage disequilibrium located in the transmembrane domain of the FSH receptor in three patients. We conclude that TSH-sensitive mutations of the FSH receptor are not the cause of gonadal hyperstimulation in these four children with severe primary hyperthyroidism.

P1-446 Ovaries and Estrogens
Contrast enhanced sonographic imaging in the evaluation ovarian tumors in children
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1Medical University, Dept of Endocrinology and Diabetology for children, Wroclaw, Poland; 2Medical University, Dept of Surgery for children, Wroclaw, Poland; 3Medical University, Dept of Radiology, Wroclaw, Poland

Fifty four girls were treated. They ranged in age from 2 months to 16 years (mean 10.6 years). Diagnostic assessment included in all cases conventional US, Color Doppler (CD) and Power Doppler (PD) before and after the administration of contrast agent. The echo-enhancing effect of an ultrasound contrast medium in Color Doppler of vessels in ovarian tumors was studied. A vascular morphological score was devised, based on ovarian tumoral vessels location, complexity and density. Germinal tumors were most common group of patients. There were 28(51,9%) benign and 26(48,1%) malignant tumors. In 63% patients we observed cystic tumors and in 37% solid or mixed masses. In 43% of cases color flow mapping showed increased vascularity either in the cystic wall or in solid structures within the tumor. In all girls with a suspicion of primary adnexal neoplasms we observed enhancement of Doppler signal intensity. Mean pulsatility and resistant index were significantly higher in benign than in malignant masses. In the group of GCT the largest number of lesions were enhanced for 2-3 points, carcinoma embryonale for 4-5 points, teratoma immaturn for over 4 points. Malignant germ cell tumors and carcinoma embryonale presented peak enhancement in the time under than 120 sec., malignant teratomas presented peak enhancement in the time of under than 180 sec. Transit time of the contrast agent were prolonged in malignant lesions compared to benign masses. Observed vessel number and tortuosity were increased in all malignant adnexal masses. All children received chemotherapy depending on character of tumors. Our results show that Color Doppler ultrasound is more important to achieve the correct diagnosis and better results of treatment compared with conventional ultrasound. Ultrasound contrast agent-Leovist is very helpful in precise visualization of pathological vessels inside the tumor mass and demonstrated improved characterization of the vessels as well as exhibiting vessels not previously visualized, therefore enhancing diagnostic accuracy and can be considered useful in initial differentiation between benign and malignant ovarian tumors.

P1-447 Ovaries and Estrogens
Polycystic Ovary Syndrome in early post-menarcheal adolescents: Experiences with MR Imaging
Paola Cambiaso1, Cinzia Orazi2, Valeria Fiaschetti2, Marco Cappa2, Daniela Galeazzi3, Cristina Geremiew3, Elisabetta Del Duca4, Anna Maria Pasquini5, Azzurra Colasanati5, Brunetto Boscherini1
1Bambino Gesù Children’s Hospital, Endocrinology Unit, Piza S. Onofrio n. 4 00165, Rome, Italy; 2Bambino Gesù Children’s Hospital, Radiology Department, Rome, Italy; 3Tor Vergata University, Pediatrics, Rome, Italy; 4Bambino Gesù Children’s Hospital, Endocrinology Unit, Rome, Italy; 5La Sapienza University, Pediatrics, Rome, Italy

Background and Objective: Polycystic Ovary Syndrome (PCOS) is characterized by 2 out of 3 of the following criteria: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism, and/or obesity. We suggest to use MRI in adolescents, whenever pelvic US results are not according to clinical and biochemical data.

Conclusion: MRI can represent a more reliable diagnostic technique in comparison with US in recognizing ovarian changes typical of PCOS in adolescents.

Subjects and Methods: We examined, by means of MRI, 10 patients aged 12 to 16 years no more than 2 years after menarche, in whom pelvic US was negative or doubt, in the presence of clinical and biochemical signs of hyperandrogenism and in whom other disorders had been ruled out. All our patients underwent complete hormonal evaluation demonstrating high levels of testosterone (mean ± SD 100.14 ± 32.72 ng/dl), low levels of SHBG (mean ± SD 24.9 ± 13.69 mmol/L), and high levels of DHEAS (mean ± SD 2400.25 ± 280.7 ng/ml). Ten age-matched normal girls were submitted to the same imaging study, after obtaining informed written consent.

Results: MRI turned out to be positive in all our patients, with demonstration of enlarged, rounded ovaries, with multiple, small follicles, mainly located at the periphery.

Conclusions: MRI can represent a more reliable technique, with respect to US, in the diagnosis of PCOS in early post-menarcheal period, in that it is not affected by the technical limits of US, like obesity. We suggest to use MRI in adolescents, whenever pelvic US results are not according to clinical and biochemical data.

P1-448 Ovaries and Estrogens
Metabolic syndrome and glucose intolerance in Indian adolescents and women with polycystic ovary syndrome
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Ethnic differences exist in the manifestations of polycystic ovary syndrome (PCO). Glucose intolerance (GI) and metabolic syndrome (MS) are important long term implications. Indians are an ethnic group at high risk for these conditions. Therefore we studied the prevalence of diabetes mellitus (DM), impaired glucose tolerance (IGT) and MS in consecutive subjects with PCO of age 15 to 40 years. PCO was defined as the presence of oligomenorrhea and/or hyperandrogenism, in the absence of secondary causes of the same. Age matched controls were selected from a community survey for glucose intolerance. A standard oral glucose tolerance test was performed. WHO criteria were used for diagnostic categories. Ninety nine subjects with PCO (mean age 23.2 ±6.7 years) and 74 controls (mean age 24.2 ± 6.9 years, p = NS) have been studied so far. DM and IGT were more frequent in PCO (6% and 14% respectively) than in control subjects (0 % and 4 %, p=0.04). Abnormal glucose tolerance (DM, IGT or impaired fasting glucose was present in 22 % of PCO vs 6.8 % of control subjects (p=0.003). Family history of DM was present in an equal number in both groups (41.4 and 43.2 %). Acanthosis was more frequent in PCO subjects (62.6 % vs 12.2 % in controls, p=0.001).
Salutary effects of combining early low-dose systemic estradiol (LDE2) with growth hormone (GH) therapy in girls with Turner syndrome (TS)

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We tested the hypotheses in girls with TS that physiologic estradiol replacement administered early in addition to GH will preserve height potential as much as if administered late and that it will bring about a greater height gain than standard oral estrogen therapy given with GH.

After obtaining a baseline height velocity, 14 girls with TS receiving recombinant GH (Genentech) were randomized to start either LDE2 treatment at 12-12.9 (early group) or 14-14.9 (late group) yrs. GH dosage was 0.05 mg/kg/daily. Depot estradiol was started at 0.2 mg/mo. IM, and the dose was gradually increased at successive 6 month intervals. Three girls randomized to the late LDE2 treatment group dropped out of the study before achieving near-adult height. Six of 7 patients in the early group were followed until adult height, as were 3 of the 4 subjects remaining in the late treatment group; the others were followed to near-adult height. We also compared the growth of these groups to matched groups in the National Cooperative Growth Study (NCGS) registry whose treatment had been similar except for the use of oral conjugated estrogens.

As a whole, the subjects treated with LDE2 reached adult or near-adult heights significantly taller than predicted at 12 yrs (p < 0.02). They gained all this height potential by 14-14.9 yrs, whether they received GH plus LDE2 or GH alone. During the first two years of the study the early LDE2 group grew 3.5 cm more than the group on GH alone: 12.7 ± 0.4, SEM, cm vs 9.2 ± 0.6 cm (p < 0.01). The early LDE2 group also gained 5.9 cm more height after starting estrogen than did the NCGS group treated early with conjugated estrogen (p < 0.05). A pubertal growth spurt did not occur in the group treated late with LDE2. Although breast development proceeded slowly on the lowest dose of LDE2, it advanced at a normal pace thereafter. Seventy-five percent menstruated between 1.5 and 3.0 years of LDE2 treatment.

These results suggest that the use of very low-dose systemic estradiol permits relatively age-appropriate feminization without interfering with the effect of GH on the enhancement of height potential.

Specific features of ultrasound performance for ovaries and uterus in hyperprolactinemia of girls

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Background: Pathologic hyperprolactinemia (HPL) develops due to anatomic or functional disorders of hypothalamic-pituitary system. The pathol...
We have analyzed 104 girls aged 11 to 12 years, with menarche around the age of 9 years and 123 girls (control group) aged 13 to 16 years with menarche between 11 and 14 years.

We have assessed the features of the menstrual cycle, the development of the secondary sexual characteristics and the serum level of estradiol and progesterone, assayed in the luteal phase for the menstruated patients or in the same, random day for the patients with secondary amenorrhea. The majority of patients with precipitate puberty presented irregular menses: oligomenorrhea (71.2% vs. 28.8% in control group) and secondary amenorrhea (85.7% vs. 14.3%) and delays in development of secondary sexual characteristics (75.2% presented stage 5S Tanner of breast development vs. 24.7% in the control group). We found that the majority of patients with precipitate puberty have had low levels of estradiol and progesterone (81.4% vs. 18.6%) and at ultrasonography a higher incidence of ovarian cysts (64% vs. 36%). In conclusion, girls with precipitate onset of puberty should be investigated in order to detect the pathologic disturbances related to their early sexual development.

P1-454 Ovaries and Estrogens

Precocious puberty and hirsutism due to ovarian steroid cell tumor: a case report

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Ovarian steroid cell tumors are extremely rare in the pediatric age group. Majority of these tumors derive from germ cells and present with precocious puberty. Case: A 5.6 years old girl who had been on tamoxifen with the diagnosis of precocious pseudo puberty was referred. She had no breast development, pubic and axillary hair development were appropriate for Tanner stages 3 and 2, respectively. She also had hirsutism. Serum 17 OH progesterone, androstenedione, cortisol, DHEAS, total and free testosterone levels were normal. Her bone age was appropriate with 8.9 years. ACTH stimulation test revealed a normal 17 hydroxypregesterone response. Tamoxifen treatment was stopped. One month later, vaginal bleeding was observed when breast development was found to be appropriate for Tanner stage 2. On LHRH stimulation test, LH and FSH levels were suppressed (all values < 0.1mIU). On pelvic ultrasonography, uterus and ovaries were of pubertal size. The right ovary was significantly larger than the left ovary, however no tumoral mass was observed. Medroxyprogesterone (20 mg/day) treatment was started and bleeding stopped. However three months later, the patient presented with recurrence of bleeding. Hirsutism (Ferriman-Gallwey score12) and further development of pubertal characteristics were observed. Serum estradiol and androstenedione levels were high (466.4 pg/ml and 10.2 ng/dl, respectively). Pelvic ultrasonography revealed a right ovary of 15.6 cc and a left ovary of 6.3 cc. An unusual solid appearance of the right ovary was observed on magnetic resonance imaging. This finding was interpreted as an indicator of a tumor. Right ovariectomy was performed. Pathological diagnosis was steroid cell tumor of ovary. Three months later, body hair had significantly decreased, she had no breast development, no bleeding, no axillary hair, LH,E2, androstenedione, total and free testosterone levels were all normal.

P1-453 Ovaries and Estrogens

Unusual presentation of primary hypothyroidism — spontaneous ovarian hyperstimulation syndrome

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We report case of spontaneous ovarian hyperstimulation syndrome (OHSS) as presentation of primary hypothyroidism without pregnancy. This is unusual presentation of primary hypothyroidism. There are few case reports of OHSS with primary hypothyroidism has been reported. But in all cases natural pregnancy was associated with it. Sixteen year old girl presented with pain in abdomen & bilateral ovarian cyst. The pain was aggravated a week before coming to our centre. She has attained menarche 6 months back & later amenorrhea. A week later she came with severe pain in abdomen, vomiting & abdominal distension. She was puffy pale, dehydrated with ascites. She was short in height & was looking hypothyroid. Her pulse was 80 minute & BP was 100/70 mm of Hg. Her skin was dry, rough. She was uncomfortable. We did investigation (Table No 1).

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<thead>
<tr>
<th>Test</th>
<th>ON ADMISSION</th>
<th>AT DISCHARGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT3</td>
<td>2.00 pg/ml</td>
<td>-----</td>
</tr>
<tr>
<td>FT4</td>
<td>0.08 ng/dl</td>
<td>-----</td>
</tr>
<tr>
<td>TSH</td>
<td>125.48 mIU/ml</td>
<td>-----</td>
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<tr>
<td>Estradiol</td>
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<tr>
<td>CA 125</td>
<td>83.90 IU/ml</td>
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<tr>
<td>Sr Creatine</td>
<td>4.0 mg/dl</td>
<td>1.44 mg/dl</td>
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<td>TLC</td>
<td>18,900 per cc mm</td>
<td>7,600 per cc mm</td>
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<tr>
<td>Hemoglobin</td>
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<td>8.31 gm%</td>
</tr>
<tr>
<td>HCT</td>
<td>19%</td>
<td>24.5%</td>
</tr>
<tr>
<td>Proteins</td>
<td>5.2 gm%</td>
<td>6.71 gm%</td>
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Repeat USG at our centre showed ascitis with hydrothorax with huge ovaries & are multicystic in nature. The diagnosis of spontaneous OHSS with untreated hypothyroidism was made. We treated this patient with correction of dehydration, whole blood transfusion, letrozole (2.5 mg/ day) & levothyroxine (100ug/day). She responded nicely. Repeat serum Estradiol on 8th day was 9.0 pg/ml. Her USG showed reduction of ovarian cysts to 50%. She got menses on 14th day. At the time of discharge her ovaries reduced to size with in pelvis. Her hemoglobin was improved with normalization of renal parameters. We found that letrozole has helped in rapid recovery in clinical & biochemical parameters. Clinically ascitis disappeared in eight days. Letrozole was discontinued after fifteen days. Letrozole is useful when pregnancy is not associated in OHSS.

Follow-up ultrasound revealed resolution of ovarian cysts in nine girls and cysts in six. Bilateral ovarian cysts were present in seven girls. Treatment with letrozole (2.5 mg / day) & levothyroxine (100ug/day) was started. She responded nicely. Repeat serum Estradiol on 8th day was 9.0 pg/ml. Her USG showed reduction of ovarian cysts to 50%. She got menses on 14th day. At the time of discharge her ovaries reduced to size with in pelvis. Her hemoglobin was improved with normalization of renal parameters. We found that letrozole has helped in rapid recovery in clinical & biochemical parameters. Clinically ascitis disappeared in eight days. Letrozole was discontinued after fifteen days. Letrozole is useful when pregnancy is not associated in OHSS.

Poster Presentations
Permament Neonatal Diabetes Mellitus (PNDM) is a rare form of diabetes characterized by insulin-requiring hyperglycemia that is diagnosed within the first 3 months of life. In most cases, the cause is not known. Recently, mutations in the gene KCNJ11 encoding the Kir6.2 subunit of the ATP-sensitive K⁺ channel (KATP channel) have been described in patients with PNDM. Tolbutamide has been shown to stimulate insulin secretion in some patients. We describe an infant with PNDM due to an arginine-to-histidine substitution at position 201 (R201H) of the gene encoding Kir6.2.

Diagnosed at 15 weeks of age and initially treated with subcutaneous insulin, our patient transitioned to oral Tolbutamide therapy at a daily dose of 250 mg 4 times a day (80 mg/kg/day) at age 3 1/2 years. Before Tolbutamide treatment, c-peptide was unmeasurable and hemoglobin A1c 7.1% (normal less than 6%). After 2 days of treatment, her c-peptide level was 1.5 ng/ml (normal 0.8-3.1).

After 3 months of treatment, the hemoglobin A1c, insulin and c-peptide levels were in the normal range without any episodes of hypoglycemia. Continuous Glucose Monitoring (CGMS) demonstrated a marked improvement in glycemic control and confirmed the lack of hypoglycemic episodes. Growth and weight gain continued normally. To our knowledge, we describe the first reported case of an infant with PNDM due to an identified mutation in the KCNJ11 gene who is successfully transitioned from subcutaneous insulin to oral Tolbutamide. This case demonstrates that oral sulfonylurea (in this case Tolbutamide) may be the treatment of choice in PNDM patients with KCNJ11 mutations even at a young age.

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Permanent neonatal diabetes mellitus (PNDM) may be caused by heterozygous, activating mutations in the KCNJ11 gene coding for the KATP-channel subunit Kir6.2, or homozgyous inactivating mutations in the glucokinase gene.

We report on the first child with PNDM with a mutation in the ABCC8 gene, coding for the sulfonylurea receptor 1 (SUR1), the other subunit of the KATP-channel.

The girl was born at term, birth weight 2540 g, birth length 49 cm, of unrelated Caucasian parents. She was hospitalized 5 weeks old with dyspnoea, tachypnoea, severe dehydration and convulsions. Blood glucose was 60 mmol/l, pH 7.07, p-sodium 158 mmol/l. EEG showed epileptic activity. By MRI, wide-spread thomoboses of the venous sinuses and intracranial hemorrhage were found. GAD ICA antibodies negative. She was put on ventilator, re-hydrated, pH-balanced, heparinized and treated with phenobarbiturate and insulin with good response. Six months old, blood glucose was well-controlled on a total insulin dose of 0.4 IU/kg/day.

By DHPLC screening and sequencing of rare DNA variants, no mutations were found in the glucokinase gene or the KCNJ11 gene. In the ABCC8 gene, however, we found a novel, highly conserved mutation in exon 28, 3533A>G, leading to a change Q1178R. The mutation was found in the mother too. She had no history of diabetes, but is now undergoing clinical evaluation.

The affected codon 1178 is situated at the border between the SUR1 transmembrane spanning region 15 and the cytosolic loop 8 linking to the transmembrane spanning region 16. This area is shown to be a binding site for the type 2 diabetes drug glibenclamide, which selectively inhibits KATP-channel opening and facilitates insulin secretion.

We propose that the ABCC8 mutation Q1178R, unlike glibenclamide, results in increased KATP-channel activity and this suppress glucose-induced insulin secretion leading to PNDM. The activating mutation may alter nucleotide-dependent modulation of KATP channels and/or increase basal levels of channel activity. This is the first report of ABCC8 mutations and PNDM.
Neonatal diabetes mellitus (NDM), which occurs within the first three months of life and requires insulin therapy, is a rare disorder and classified into transient form (TNDM) resolving by 18 months and permanent form (PNDM). Abnormalities in chromosome 6q24 were previously identified to be responsible for PNDM. Recently heterozygous activating mutations in the KCNJ11 gene encoding the Kir 6.2 subunit of the pancreatic ß-cell ATP-sensitive K+ (KATP) channel have been identified to be the cause of PNDM and also a part of TNDM. The patients with KCNJ11 mutations are reported to manifest heterogeneous symptoms from mild to severe, which include neurological features in addition to diabetes. In the present study, we screened KCNJ11 mutation in 11 Japanese sporadic patients with NDM (including 1 TNDM and 10 PNDM) in whom abnormalities of 6q24 region was excluded. Sequence analysis identified heterozygous missense mutations in four patients: three of them (R50Q, C166Y and A174G) are novel mutations and one of them (R201H) was already reported. Age at diagnosis was between week 1 and 12. The patient with R50Q recovered his insulin secretion in later of life, thus presenting transient form. The patients with the mutation of R50Q, A174G or R201H in the intracellular domain of Kir6.2 manifested diabetes only. The patient with the mutation of C166Y located in one end of the inner helix in transmembrane domain manifested marked developmental delay, epilepsy and dysmorphic features. This position was previously shown to affect intrinsic gating kinetics. In conclusion, prevalence of KCNJ11 mutations in Japanese NDM is high (36%). Genotype-phenotype correlation was not apparent. However, the new mutation C166Y, in which the location is important for the gating of the channel pore, resulted in severe phenotype.

**KCNJ11 gene Activating mutations in Japanese patients with Neonatal Diabetes Mellitus**

Shigeru Suzuki, Tokuo Mukai, Kunihiro Matsuo, Osamu Ueda, Yoshiya Ito, Yoshio Makita, Kenji Fujieda

Asahikawa Medical College, Pediatrics, Asahikawa, Japan

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**P1-460/MP Neonatal Diabetes/Hyperinsulinism**

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**P1-462/MP Neonatal Diabetes/Hyperinsulinism**

**Sulfonylurea Therapy in 2 patients with Insulin-Treated Permanent Neonatal Diabetes due to Mutations in Kir6.2**

Sylvie Nivot-Adamiak1, Jean Pierre Brossier2, Sabine Baron2, Jean Jacques Robert3, Christine Belliané-Chantelot4, Regis Coutant4

1University Hospital, Pediatrics, Rennes, France; 2General Hospital, Pediatrics, La Roche sur Yon, France; 3University Hospital, Pediatrics, Nantes, France; 4Hospital Necker Enfants Malades, Pediatric Diabetology, Paris, France; 5Saint Antoine Hospital, Molecular Biology, Paris, France; 6University Hospital, Pediatrics, Angers, France

Neonatal diabetes is defined as insulin-requiring hyperglycemia diagnosed within the first 3 months of life. Heterozygous mutations in the KCNJ11 gene, which encodes the Kir 6.2 subunit of the pancreatic KATP channels, were recently showed to be the most frequent cause of permanent neonatal diabetes (PNDM). Since sulfonylureas stimulate insulin secretion by binding to SUR1 and closing KATP channels, they may be useful in patients with Kir 6.2 mutations and apparent insulin-dependent diabetes. We describe sulfonylurea introduction in 2 insulin-treated patients with PND due to Kir 6.2 mutations: a 5-month-old boy with a V59M substitution and a 7-month-old girl with a C166F substitution. Case 1. A 1.5-month-old boy presented diabetic ketoacidosis. Insulin therapy by subcutaneous infusion (CSII) was initiated. The insulin requirement was 0.5-0.6 UI/kg/d; this allowed normal growth but suboptimal blood glucose control: mean preprandial capillary blood glucose was 16.9 ± 6.2 mmol/L. Molecular studies showed a heterozygous de novo V59M substitution in the gene encoding Kir 6.2. Oral glibenclamide was introduced in addition to CSII at 4.7 months. Capillary blood glucose then lowered steadily, so insulin therapy could be decreased and then stopped 12 days after sulfonylurea introduction (Figure 1). Case 2. Diabetes was diagnosed in a 3-month-old girl because of dehydration. Insulin therapy with CSII was initiated. The insulin requirement was 0.8 UI/kg/d with mean preprandial capillary blood glucose of 9.4 ± 3.3 mmol/L. Infantile spasms then occurred, which were intractable despite phenobarbital, vigabatrin, phenytoin, and hydrocortisone use. Molecular studies showed a heterozygous de novo C166F substitution in the gene encoding Kir 6.2. Oral glibenclamide was introduced in addition to CSII at 7 months. However, the insulin dose was not decreased, and the development delay and epilepsy gradually worsened. At 9 months, the girl died from cardiac arrest. Autopsy showed hypertrophic cardiomyopathy, presumably due to glucocorticoid treatment. Our report confirms that sulfonylurea treatment was effective in 1 out of 2 affected patients.
Congenital hyperinsulinism (CHI) is a profound hypoglycaemia resulting from excess insulin secretion. CHI is associated with two distinct histological anomalies: diffuse pancreatic insulin hypersecretion and focal adenomatous hyperplasia, a solitary small lesion (2.5 to 7.5 mm in diameter). Diffuse CHI is an autosomal recessive or, less frequently, an autosomal dominant disorder, whereas focal CHI is a sporadic disorder. Hyperplasia results from a somatic loss of the maternal 11p15 allele. This event is responsible for a reduction in the activity of the glucose transporter 1 (GLUT1) gene, resulting in decreased glucose transport and increased insulin secretion.

In the third patient, transepithelial pancreatic venous sampling showed an excess of insulin secretion located in the head of the pancreas. Peroperative histology revealed a focal adenomatous hyperplasia. However, both patients had a second distinct macroscopic endocrine lesion. In the third patient, transepithelial pancreatic venous sampling showed a diffuse insulin secretion, but peroperative histology showed typical criteria of focal islet cell hyperplasia. Interestingly, the hyperplastic lesion of patient 3 was very large, involving the major part of the pancreas. Loss of the 11p15 maternal allele in the pancreatic lesions and paternally inherited mutations of the ABCC8 gene were detected in all three patients. A singel molecular event may occur during the pancreatic embryogenesis, very early for patient 3 and later on for patients 1 and 2. The hypothesis of two distinct somatic molecular events (patients 1 and 2) is discussed.

Consistent with findings from studies in individuals born at term, in the males and females born before 32 weeks of gestation of the multicenter prospective Project on Premature and Small-for-gestational-age infants (POPS) we have shown that low birth weight (SDS) and rapid early postnatal weight gain (delta-SDS) following low birth weight (SDS) were both associated with lower insulin sensitivity at age 19 years. With this study in the same cohort (N = 215) we aimed to investigate whether the relation between early growth and later insulin sensitivity could be extended to include other factors that may influence the development of IR. In the early postnatal life, factors such as nutritional status, infections, and the use of insulin replacement therapy, were prospectively followed with annual assessments.

To estimate the frequency of microvascular complications in young adults who underwent a near total pancreatectomy for CHI within the first 2 month of life. Method: 6 subjects with CHI who underwent a near total pancreatectomy (median age 30 years [26-33]), were examined for insulin replacement therapy, were prospectively followed with annual assessments consisting of: measurement of HBAlc, blood pressure, lipids and from puberty onwards: urine albumin excretion and retinal fundoscopy/photography. Three patients were followed up with a 3 hour oral glucose tolerance test at 2-5 yearly intervals. Data are expressed as median [range]. By a median age 18.5 years [17-30] and duration of diabetes 18.4 years [16.9-29.8]; despite poor glycaemic control (median HBAlc 9.1% [7.8-10.9%]) and insulin requirements of 0.5u/kg/day [0.5-6.6], in all 6 subjects there was no evidence of microalbuminuria (urine albumin:creatinine ratio <0.3mg/mmol), background retinopathy, hypertension (BP 105/67 mmHg [115-65]) or deranged lipid profile. Serial 3 hour oral glucose tolerance tests showed detectable endogenous serum insulin concentrations. At 3 hours there was a tendency toward hyperinsulinaemic hypoglycaemia (glucose <3mmol/L) with detectable serum insulin indicating continued dysregulation of insulin secretion. After nearly 20 years of DM, despite poor glycaemic control, there was no evidence of microvascular disease in post pancreatectomy patients. This may relate to persisting production of dysregulated endogenous pancreatic insulin/c-peptide which may impact upon complication risk by directly influencing the growth hormone/insulin-like growth factor axis and endothelial function. Data to test this hypothesis are currently lacking and further studies are required to understand this novel observation.

The polymorphism of the INS VNTR locus, also known as the IDDM2 locus, is associated with Type 1 (T1D) and Type 2 diabetes (T2D) susceptibility. Homozygosity for Class I alleles (short VNTR) is associated with increased insulin, predisposition to T1D and resistance to T2D versus Class III alleles (long VNTR). There are in fact many types of Class I alleles and we suspected they could have different functional effects upon insulin secretion and disease risk. We designed a new method to sequence Class I alleles after cloning of each DNA strand. OC were subclassified according to the presence of 2, 1 or 0 [AAAAAACAAAAABAAAA] motif within the minisatellite part close to the insulin gene, a motif known to bind MAZi transcription factor and to increase insulin gene expression. Insulin response to glucose was quantified with the insulinogenic index (IGI). IGI was 115 ± 134 (SD) in OC with VNTR Class I alleles carrying 2 copies of the said sequence (N=26), vs 72 ± 57 (p=0.003) in those with only one copy (N=143), and 75 ± 57 (p=0.006)
in those without the motif (N=202). Homozygotes for Class I alleles carrying [AAAFACAAAAAABAAA] in their proximal part have higher insulin responses than other Class I genotypes. IGI was 73 ± 50 and 64 ± 58 in OC with Class I/II or III/III genotypes, respectively. The precise molecular structure of the VNTR, not only its length, appears associated with major variations in insulin secretion in OC. The functional role of these specific Class I alleles will prompt us to re-investigate T1D and T2D predisposition at this locus.

Population-based studies in humans have shown that the offspring of diabetic mothers have an increased risk for obesity, insulin resistance, Type 2 diabetes, and hypertension (collectively termed the Metabolic Syndrome) in later life, but the mechanism is unknown. Previously we have hypothesised that metabolic syndrome might be induced by the overexposure to glucocorticoid in the fetal period and this is also supported by studies in rats. In the present study in rats, we have investigated the relationship between the higher incidence of metabolic diseases in offspring of diabetic mothers and tissue-specific glucocorticoid regulation programmed in utero. Offspring of diabetic mothers (ODMs) had significantly increased in body weight at birth and remained heavier than into adulthood. ODMs were glucose intolerant and insulin resistant at 10 weeks. Moreover, at 12 weeks of age prior to development of obesity ODMs had significantly higher levels of 11beta-hydroxysteroid dehydrogenase type I (11beta-HSD1) mRNA expression in adipose tissue and in liver than offspring of control mothers. This study provides new information on the molecular basis of dysregulation of local glucocorticoid metabolism in offspring of diabetic mothers. Furthermore, elevated 11beta-HSD1 expression in adipose and liver tissues preceding the appearance of obesity suggests a causal role in the development of visceral obesity and insulin-independent diabetic mellitus in offspring of diabetic pregnancy.

**P1-467** Type II Diabetes/Insulin Resistance

**Diabetic pregnancy leads to impaired glucose metabolism in offspring involving tissue-specific amplification of glucocorticoid by 11beta-hydroxysteroid dehydrogenase type 1**

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1Hamamatsu University School of Medicine, Department of Pediatrics, 1-20-1 Handayama, Hamamatsu-shi312, Hamamatsu, Japan; 2Hamamatsu University School of Medicine, Department of Pediatrics, Hamamatsu, Japan; 3University of Edinburgh, Endocrinology Unit, Edinburgh, United Kingdom

Assessment of impaired glucose tolerance in non-diabetic children with Cystic Fibrosis. A comparison of HbA1c, oral glucose tolerance testing and Continuous Glucose Monitoring System

Tiziana Gozzi1, Udo Meinhardt1, Jean-Marc Vuilsoz2, Margrit Walther2, Carmen Casalta2, Primus E Mullis2

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Cystic Fibrosis Related Diabetes is an increasingly recognised complication in patients with Cystic Fibrosis (CF), and is associated with adverse somatic development and lung function. The aim was a) to compare results of oral glucose tolerance testing (OGTT) in non-diabetic children with CF to healthy children and b) to correlate measurements of carbohydrate metabolism with clinical data and lung function. OGTT, 72-hours Continuous Glucose Monitoring System (CGMS, measuring subcutaneous glucose levels every 5 minutes), HbA1c and lung function measurements (FEV1, MEF50, FRC) were performed in 11 non-diabetic CF patients (mean age ± SD 11.7 ± 3.9, weight -0.9 SD ± 1.1, height -1.1 SD ± 1.3 and growth velocity -0.3 SD ± 2.9). OGTT reference ranges were obtained from 36 healthy children. CGMS data were analysed as overall mean glucose, mean early morning (4-6 am) glucose and mean out of 5 postprandial peak glucose values. During OGTT, fasting, 30 (G30), 60 and 90 minutes glucose values were significantly higher in CF than in normal children (p < 0.0001). There was no difference in HOMA-R, insulin sensitivity index and insulin at 30 minutes between the groups. In children with CF Fasting glucose correlated negatively with growth velocity (absolute: $r = -0.73$, p = 0.011; SD: $r = 0.61$, p = 0.045); G30 correlated negatively with BMI (absolute: $r = 0.70$, p = 0.016; SD: $r = 0.67$, p = 0.025). HbA1c and CGMS results did not correlate with any of the clinical data. No correlation was found between lung function and any of the measurements of carbohydrate metabolism. In summary, non-diabetic children with CF had higher fasting and early postprandial glucose than controls, however estimates of insulin secretion and sensitivity were not significantly different. Higher fasting and 30 minutes glucose values were associated with impaired growth and weight gain but did not correlate with lung function. We conclude, OGTT is abnormal in otherwise asymptomatic children with CF and might be associated with diabetes complications in this group. OGTT and CGMS monitoring provide information on glucose disposal in the prepubertal period and this is also supported by studies in rats. In the present study -0.9 SD ± 1.1, height -1.1 SD ± 1.3 and growth velocity -0.3 SD ± 2.9). OGTT reference ranges were obtained from 36 healthy children. CGMS data were analysed as overall mean glucose, mean early morning (4-6 am) glucose and mean out of 5 postprandial peak glucose values. During OGTT, fasting, 30 (G30), 60 and 90 minutes glucose values were significantly higher in CF than in normal children (p < 0.0001). There was no difference in HOMA-R, insulin sensitivity index and insulin at 30 minutes between the groups. 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Which minority youth should undergo an oral glucose tolerance test (OGTT)? Use of the Pre-Diabetes Score (PDS)

Mireya Garcia1, Katherine Freeman1, Hadassa Nussbaum2, Joan DiMarchi-Nardi3
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As obesity and T2DM in children are on the rise, the ADA recommends screening at risk youth for T2DM with a fasting blood sugar (FBS) every two years. Identification of impaired glucose tolerance (IGT) is important to prevent progression to T2DM. However, as 67% of our minority youth with IGT had a normal FBS <100 mg/dl, a FBS alone may not be reliable in screening.

The purpose of this study is to develop a scoring system to identify those obese youth most at risk for having an abnormal OGTT (IGT/T2DM). 198 minority pubertal youth (52 African American, 146 Caribbean Hispanic), 89 males and 109 females with a mean age of 14±2.3 years, BMI of 35.3±9.7 kg/m², and BMI z-score of 4.4±2.9, who met all the ADA criteria for T2DM screening underwent an OGTT. 10.6% of children had an abnormal OGTT (IGT/T2DM).

Stepwise logistic regressions were performed for all subjects. Initial models included age, z-score for BMI, HOMA, cholesterol, waist circumference, waist to hip ratio, glucose to insulin ratio, and HbA1C. Variables used in the final model were those significant at p<0.05. Interval weights of 0, 1, and 2 were assigned for variables with 3 intervals and SPIS. Such data are not available in the literature.

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Stepwise logistic regressions were performed for all subjects. Initial models included age, z-score for BMI, HOMA, cholesterol, waist circumference, waist to hip ratio, glucose to insulin ratio, and HbA1C. Variables used in the final model were those significant at p<0.05. Interval weights of 0, 1, and 2 were assigned for variables with 3 intervals and SPIS. Such data are not available in the literature.

The purpose of this study is to develop a scoring system to identify those obese youth who should undergo an OGTT.
at 0, 30, 60, 90 and 120 min. for glucose, insulin and IGFBP-1 measurement. Lipid profile, IGF-1 and IGFBP-2 were determined in the basal sample. Insulin sensitivity index (ISI) was calculated, based on insulin and glucose levels obtained from OGTT and taken as “gold standard”.

**Results:** We observed a negative correlation between fasting IGFBP-1 and both insulin (r=-0.67; P<0.01) and peak insulin concentration during OGTT (r=-0.48; P<0.01) beside a positive correlation between ISI and IGFBP-2 (r=0.57; P<0.01). A negative correlation was also found between peak insulin during OGTT and IGFBP-2 levels (r=-0.35; P<0.05). No correlation was found between ISI and IGFBP-2 levels or IGF-I SDS. No correlation was observed between BMI-SDS and IGFBP-1, IGFBP-2 or IGF-I SDS. Negative correlation was found between triglyceride levels and ISI but no correlation was observed between IGFBP-1 and lipid profile. When a ROC curve was performed IGFBP-1≥17 µg/L showed a sensitivity=100% and specificity=70% to detect IR. When considering IGFBP-1 ≤ 10.5 µg/L, sensitivity and specificity were 75% and 93%, respectively, and 5 patients were diagnosed as resistant.

**Summary:** IGFBP-1 concentration that is determined by portal insulin concentrations seems to detect early alterations in insulin secretion, even before changes in fasting insulin. In conclusion, we propose the use of fasting IGFBP-1 concentration as a screening method. While values between 10.5 and 17 µg/L strongly suggest insulin resistance, values equal or less than 10.5µg/L are present in insulin resistant individuals.

**P1-474** Type II Diabetes/Insulin Resistance

**Correlations of insulin and glucose indices derived from fasting samples and oral glucose tolerance test in obese children**

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Dynamic tests using oral glucose load or clamp studies have been used to assess insulin resistance, which is proposed to be the main pathomechanism in metabolic syndrome. Different surrogates of insulin resistance are used as simpler screening methods. The aim of the study was to determine relationship between anthropometric parameters and insulin and glucose concentrations in obese children in our practice, as well as to determine whether indices of insulin and glucose derived from fasting samples correlate with values after glucose load. Examinet group consisted of 47 obese children (BMI>97 percentile), 16 boys and 31 girls, aged 11.75±2.92 years, 10 subjects being pre-pubertal. Fasting blood samples were taken to assay insulin, glucose and sex hormone binding globulin (SHBG) and glucose tolerance test (OGTT 0', 30', 60', 90', 120'). A value of p <0.05 was considered statistically significant. Mean BMI-SDS of the group was 2.99±0.87 and mean duration of obesity was 6.87±3.69 years. Thirty eight (80.85%) children were found to be hyperinsulinaemic on the basis of OGTT results, including 11 (23.40%) subjects with impaired glucose tolerance. BMI-SDS correlated positively with concentration of fasting insulin (r=0.302; P=0.049) and peak glucose (r=0.315; P=0.040); no correlation was found in terms of duration of obesity and insulin or glucose parameters. Among evaluated indices, only HOMA and QUICKI correlated with peak insulin level in OGTT (p=0.006 and p=0.005 relatively). On the grounds of these data indices HOMA and QUICKI derived from fasting samples can substitute OGTT to select hyperinsulinaemic subjects, however further studies on larger groups should be performed to observe possible gender and pubertal differences.

**P1-475** Type II Diabetes/Insulin Resistance

**Atherosclerotic risk in children with Steatohepatitis and normal glucose tolerance is similar to risk in patients with Type 2 DM**

**Amit Shangoo**, Natalia Maksym, Zoya Isakov, Irene Lyttin, Henry Aihatt, Enrico Ascher, Svetlana Ten

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Endothelial dysfunction and Carotid artery intimal-medial thickness (IMT) are proposed as an early marker of atherosclerosis in children with diabetes mellitus type 2 (DM2). Steatohepatitis SH and DM2 often occur together. It is unknown at this point whether children with SH (S) are at risk of early Atherosclerosis (AS).

To compare endothelium-dependent vasodilation (EDV) and carotid IMT in children with DM2 and those with SH, Lipid profile, HbA1c, blood pressure (BP), and BMI were measured in 3 groups of children: (1) 8 obese children with biopsy-proven SH (S)[age 14.2±3.8, 2 girls, BMI 32.8±4.1 kg/m2], (2) 6 obese children with DM2 (DM2) [age 15.6±3.7, 3 girls, BMI 34.9±8.5 kg/m2] (3) 16 obese insulin sensitive children (NIS)[age 13.1±2.3, 7 girls, BMI 37.5±13.1 kg/m2]. IMT and EDV measurements were done in SH and DM2 groups. Echocardiogram was done in SH group. Oral glucose tolerance tests were done in SH and NIS groups. 7/8 children in the SH group had normal glucose tolerance. There were no differences between the 3 groups in age, BMI, BP, ALT and AST were higher in SH compared to DM2 and NIS groups (p<0.01). HDL was lower in SH group and DM2 groups compared to NIS (≤ 0.01). TG and TG/HDL ratio were higher in the SH and DM2 groups compared to the NIS group (p<0.01). Resistance and Pulsatility indices were higher in children in the DM2 group compared to SH group (p<0.05). IMT, ejection fraction were normal in both SH and DM2 groups. Both SH (5.8±8% ) and DM2 (4.4±7% ) groups had equally impaired EDV.

Children with SH and DM2 have increased risk of AS determined by EDV and dyslipidemia. Impaired EDV in children with DM2 and SH precedes an increase in carotid IMT.

**P1-476** Type II Diabetes/Insulin Resistance

**Effects of metformin therapy in children and adolescents with insulin resistance**

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**Objective:** The present generation has witnessed a surge in the number of children with type 2 diabetes.

The aim of this study was to evaluate the effects of preventive approach with metformin therapy in obese children with insulin resistance (IR)

**Methods:** In a prospective study we evaluate 90 obese children (BMI>85 percentile). The mean age was 9.1 years (sd=2.8 SD) with the range of 1 to 16 years. 55 were female and 35 were male. They were classified as overweight (BMI > 85th to 95 percentile), moderately obese (BMI > 95th to 97 percentile) and severely obese (BMI > 97 percentile). They were analyzed the variable: age, sex, basal insulin/glucose level, degree of obesity and side effects of metformin.

**Results:** Of the 90 patients, 68 (75%) showed IR. In two index : Homa >3 and glucose/insulin ratio < 7 and were submitted for therapy with metformin (250 to 1000mg) for a mean period of 4.6 months (+/- 1.99 SD). The age was higher in the group of IR (10.02 years +/- 2.4 SD) than in the second group without IR (7.02 years +/- 2.93SD) P=0.000. The frequency of sex distribution was not different between the two groups P=0.353)

The basal insulin level was higher in the group of IR (mean=18.98 micro U/mL +/- 10.2 SD) than in the group (6.04 micro U/mL +/- 2.8SD), P=0.000. When we compare the basal insulin level before and after metformin therapy we found a significant reduction after therapy (P=0.004). There were higher prevalence of severely obese children in the group with IR. We observed slight and transitory dipeptic symptoms in 11% of children with received metformin.

**Conclusions:** The prevalence of IR is high among severely obese children and adolescents. The metformin therapy was safe in this group of patients

Poster Presentations
and reduced the basal insulin level in our patients with IR. We concluded that metformin therapy may contribute for type 2 diabetes prevention in children and adolescents with insulin resistance.

**P1-477 Type II Diabetes/Insulin Resistance**

**Metabolic repercussions in children of mothers with Gestational Diabetes**

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Decreased maternal insulin sensitivity in women with Gestational Diabetes (GD) increases nutrient availability to the fetus, leading to pancreatic hyper trophy and hyperplasia that might be associated with alterations in carbohydrate and lipoprotein metabolism.

We evaluated insulin sensitivity and lipoprotein metabolism in 4-6 years old children of mothers with GD.

A longitudinal study including two groups of 30 children of 4-6 years old of mothers with GD and 30 controls of non diabetic mothers was performed.

We evaluated characteristics at birth and at 4-6 years of age. We measured fasting glucose (G) and insulin (I), triglycerides, total cholesterol (TC) and HDL and LDL cholesterol levels and obtained the glucose/insulin ratio (G/I) and HOMA-IR. Student’s t-test was used to compare I, G, HOMA-IR and lipoprotein profiles in both groups according to gender and size at birth, large for gestational age (LGA), appropriate (AGA) and small (SGA).

Average gestation for children of diabetic mothers was 38.5 (1.3)* weeks, birth weight (BW) 3680(400)* g, age 4.9(0.6)* years and body mass index (BMI) 17.62(1.4)*. The values for control were 38.1(1.6)* (p=0.6) weeks gestation, BW 3249(594)* (p=0.003), age 5.01(0.5)* (p=0.4) and BMI 17.7(2.7)* (p=0.9). We compared metabolic parameters from both groups and the only differences seen were in TC: 166(28)* vs 150(23)* (p=0.05) and LDL 113(21)* vs 77(14)* (p=0.05). LGA offspring of diabetic mothers had significantly higher levels of TC and LDL than the LGA offspring of the control group: TC 168(26)* vs 150(23)* (p=0.02) and LDL 113(21)* vs 77(14)* (p=0.008), respectively. Both groups had similar BMI, insulin, G/I ratio and HOMA-IR. However, we found significantly higher levels of TC and LDL in the offspring of diabetic mother especially in those LGA. These findings support the need for an earlier nutritional intervention in this group in order to avoid major cardiovascular risk later on.

**P1-478 Type II Diabetes/Insulin Resistance**

**Measures of β-cell function during Oral Glucose Tolerance Test (OGTT) and Liquid Mixed Meal (Boost) (NGT): How well do they correlate with the Gold Standard of the hyperglycemic clamp?**

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Clinically useful measures of β-cell function are desirable to assess insulin secretion in health and disease. Even though the hyperglycemic clamp (HC) is accepted as the gold standard, it is a cumbersome research tool. On the other hand, the OGTT, a WHO and ADA recommended diagnostic test for glucose homeostasis abnormalities, and the Boost are widely used clinically and well tolerated by youth.

We hypothesized that OGTT/Boost derived parameters are useful estimates of β-cell function and correlate well with insulin secretion measured during the HC. We assessed the correlation between the ratio of the incremental insulin/glucose responses at 15 and 30 min (ΔI/G15, ΔI/G30) of the OGTT and Boost, with first phase insulin(1stPI) during the HC (225 mg/dl).

The same indices were evaluated using C-peptide(C). Twenty-six(14M/12F) children (9.9±0.2yrs,BMI:22.1±1.2kg/m²) underwent a 2hr HC and 3hr OGTT and Boost with measurements of glucose (G), I and C. 1stPI and 1st phase C-peptide (1stPC) were calculated as the mean of 5 determinations from 2.5 to 10.2min of the HC. Glucose disposition index (GDI=GΔI/ΔG), a measure of insulin secretion adjusted for insulin sensitivity (IS), was calculated as IS*ΔPI/GDI x(10000-fasting G fasting I*Mean G*Mean I). Correlations (r and P values) are shown below; ∆I/∆G in µu/ml per mg/dl; ∆C/∆G in ng/ml per mg/dl.

<table>
<thead>
<tr>
<th>OGTT</th>
<th>Boost</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔI/ΔG</td>
<td>ΔC/ΔG</td>
</tr>
<tr>
<td>1stPC</td>
<td>(0.1)</td>
</tr>
<tr>
<td>1stPI</td>
<td>(0.3)</td>
</tr>
</tbody>
</table>

Correlations were stronger for 15 min values than for 30 min and for C-peptide than insulin. The latter may be due to differences in insulin clearance. The glucose homeostasis index, GDI correlated with GDI (r=0.53,p=0.008).

In conclusion, in youth with NGT, C-peptide at 15 min of OGTT and/or Boost provides a reliable estimate of β-cell function. Moreover, an index of glucose homeostasis, i.e. β-cell function relative to IS, can be derived from the OGTT reflecting GDI from the clamp.

**P1-479 Type II Diabetes/Insulin Resistance**

**Insulin resistance as a risk factor of diabetes type 2 in obese children and adolescents**

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Obesity is considered to be the prediabetes type 2 state. Its characteristic features are insulin resistance and deteriorated reaction of the beta cells to hyperglycemia.

**Aims:** 1. To evaluate the age, sexual maturation and gender influence on the severity of obesity in children and adolescents. 2. To find the relation between the obesity and the insulin secretion in comparison to age, gender and sexual maturation.

**Patients and methods:** Study comprises 160 children of 4 up to 18 years old. Weight, height, BMI, BMI-SD and Tanner stage were estimated in patients. OGTT (1,75g/kg, max.75g) with insulin levels and evaluation of HOMA-IR and QUICKI index was performed in each case. The children were divided into the groups according to the degree of obesity: 1) BMI-SD <1 (control group), 2) BMI-SD (1-2); 3) BMI-SD (2-4); 4) BMI-SD >4. ANOVA test and Pearson’s correlations were applied to evaluate the influence of age, Tanner stage and gender on the investigated parameters.

**Results:** Fasting glycaemia was found as dependent on age (p=0.02) and Tanner’s stages (p=0.013) without gender and severity of obesity influence. Fasting insulinaemia depended on age (p=0.004), puberty (p=0.009) and the worsening of obesity (p=0.006) but not on sex. HOMA-IR and QUICKI were depending on age (p=0.002 and p<0.001), on puberty (p=0.009 and p=0.001) and on the severity of obesity (p=0.009 and p=0.001) respectively. HOMA-IR in Pearson’s test showed the correlation with BMI-z score (r=0.284;p<0.001), fasting glycaemia (r=0.50;p<0.001), fasting insulin level (r=0.988;p<0.001) and QUICKI (r=0.834;p<0.001). 

**Conclusions:** Insulin resistance in obese children and adolescents increases together with the severity of the disease. Age and progress of puberty have the influence on and positive correlation to increasing BMI and insulin resistance, while gender has not influence on investigated parameters. It suggests, that prophylaxis and therapeutic intervention against diabetes type 2 should be started in both sexes at the earliest obesity stage and before sexual maturation if only possible.
## P1-481 Type II Diabetes/Insulin Resistance

### Fanconi anemia and diabetes

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Fanconi Anemia (FA) is an autosomal recessive multi-system disorder associated with excessive chromosomal breakage and bone marrow failure. Abnormalities of glucose homeostasis have been documented in the FA literature but the mechanism remains unclear. The life extending treatment of FA is bone marrow transplantation. Hyperglycemia during BMT can either be due to FA itself or to the chemotherapy and may contribute to poor outcome. 39 patients (24 boys) with FA were evaluated at Cincinnati Children’s Hospital Medical Center. 26 (15 boys) of the 39 had OGTT done. The age range was 0.5-16.5 yrs with mean age at the first visit 8.4yrs. Subjects were divided into 3 groups according to their insulin level at 2 hrs after glucose load.

<table>
<thead>
<tr>
<th>Index</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Mean Age (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-1</td>
<td>12</td>
<td>1</td>
<td>13</td>
<td>12.2 (1.3)</td>
</tr>
<tr>
<td>n-2</td>
<td>9</td>
<td>10</td>
<td>19</td>
<td>7.8 (1.5)</td>
</tr>
<tr>
<td>n-3</td>
<td>8</td>
<td>1</td>
<td>9</td>
<td>6.9 (1.6)</td>
</tr>
</tbody>
</table>

**Table:**

<table>
<thead>
<tr>
<th>Insulin at 2hrs of OGTT (15.0-53.0 uU/ml)</th>
<th>Glucose at 2hrs of OGTT (&lt;140 mg/dl)</th>
<th>Fasting Insulin (2.0-5.0 uU/ml)</th>
<th>Peak Glucose Insulin (2.0-5.0 uU/ml)</th>
<th>Peak Glucose 30 min Glucose (2.0-5.0 uU/ml)</th>
<th>Peak Insulin 30 min Insulin (2.0-5.0 uU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 29 ± 8</td>
<td>143 ± 44</td>
<td>7.9 ± 7.8</td>
<td>177 ± 39</td>
<td>65 ± 63</td>
<td>159 ± 29</td>
</tr>
<tr>
<td>n-13, Normal Insulin</td>
<td></td>
<td></td>
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<tr>
<td>2. 9 ± 5.4</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>n-6, Low Insulin</td>
<td>127 ± 36</td>
<td>5.7 ± 3.8</td>
<td>178 ± 65</td>
<td>20 ± 13</td>
<td>152 ± 60</td>
</tr>
<tr>
<td>n-7, High Insulin</td>
<td>3. 77 ± 33</td>
<td>169 ± 63</td>
<td>210 ± 49</td>
<td>141 ± 74</td>
<td>172 ± 30</td>
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</table>

**Group 2 showed insulinopenia and Group 3 showed hyperinsulinism.** Fast- ing glucose were normal in all groups. Group 1 and 3 showed glucose intolerance (glucose > 140 mg/dl) at 2hrs of OGTT while all groups had glucose intolerance by glucoses at 30 min and peak. The results of OGTT in Fanconi anemia patients showed 25% were insulinopenic and 25% were hyperinsulinemic. Those with higher insulin production were not as short. We conclude that hyperglycemia results from a combination of relative insulin resistance and insufficiency in children with FA.

## P1-482 Type II Diabetes/Insulin Resistance

### Evaluation of leptin levels and insulin resistance in Berardinelli-Seip Syndrome

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Berardinelli-Seip Syndrome (BSS) or congenital generalized lipodystrophy is a rare autosomal recessive disorder, mainly characterized by nearly complete absence of adipose tissue. The affected patients are predisposed to marked insulin resistance, specially after the onset of puberty. Some studies have correlated low leptin levels in these patients with insulin resistance, searching for new therapeutically approaches.

The aim of our study was to describe the clinical features of the syndrome, evaluate the leptin levels and estimate the insulin resistance of our patients through the mathematical model Homeostasis Model Assemente (HOMA). We studied 6 subjects (4 males and 2 females) with BSS, mean age: 6.6±4.0 years.

High stature, muscular hypertrophy and fibromegaly were present in all patients. Hepatomegaly and anacrosis nigricans were also frequent findings. Consanguinity was observed in four patient’s families. All patients presented low leptin levels, 83.3% high insulin levels and 66.6% insulin resistance.

## P1-483 Type II Diabetes/Insulin Resistance

### The effect of birth size, adult size and body composition on glucose homeostasis: the PROGRAM study

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Low birth weight is associated with a 35% increase in type 2 diabetes risk and, in addition, postnatal catch up in growth and short adult height per se increase this risk. Due to conflicting and heterogeneous studies, it is not clear if these indices are really the effect of birth size and adult size and not just due to body composition. These questions need answering before starting intervention or preventive measures. The PROGRAM study tested one hundred and forty nine 21 year old subjects in four predefined unique groups matched for birth length and adult height: 1. Small for Gestational Age (SGA)-Short in adulthood 2. SGA-with catch up growth 3. Appropriate for Gestational Age-Short in adulthood (Idiopathic Short Stature) 4. AGA-Controls (normal birth and adult height). Insulin sensitivity and insulin secretion indices were determined from a frequently sampled tolbutamide modified intravenous glucose tolerance test. Antropometry and DXA-scanning were used to assess body composition. Within the groups, disposition indices (insulin sensitivity/insulin resistance) were similar (p=0.4)-meaning that insulin resistance was well compensated for by insulin secretion. There were no differences in adult BMI, fat mass, lean body mass or waist circumference corrected for height. Across the groups, insulin sensitivity and also insulin secretion, was strongest predicted by waist circumference (R2=0.30, p=0.001). Waist circumference itself was strongest predicted by adult weight (R2=0.66, p=0.001). Birth size and Adult height had no independent effect on insulin sensitivity. In conclusion, our data show that SGA and ISS have no increased risk of insulin resistance at the age of 21 years. At this age Adult weight and Waist circumference predicted reduced insulin sensitivity and measures should primarily focus on preventing this weight gain in all groups, rather than focus on increasing fetal growth or adult height.
Prevalence and clinical features of MODY: Retrospective study on a cohort of 630 diabetic children and adolescents

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MODY is a distinct form of diabetes mellitus characterized by a strong family history of diabetes and a tendency to present at a young age. The possibility of identifying mutations in a single gene (usually GCK or HNF1 alpha) can allow a more accurate classification of diabetes. We conducted a retrospective study on the Pediatric Endocrinology Department of the Lyon University Hospital from January 1, 1991 to January 1, 2004. From these data we identified 630 cases of diabetes, of which 603 were from the IWK and 27 from other centers in the province. Of these 630 cases, 20% were from an at-risk ethnic group and 28% had a chromosomal or single gene disorder associated with diabetes. There is a high proportion of Type 2 Diabetes (T2D) in our study population (92% had documented positive c-peptide and/or negative autoantibodies). One case classified as T2D in the registry could not be confirmed by reanalysis of the medical charts. The incidence of T2D in Nova Scotia (DCPNS) registry through review of cases from the only tertiary care centre in the province (IWK) and 2) determine the incidence of T2D to: 1) validate the classification of diabetes in the Diabetes Care Program of NS, Pediatrics, Halifax, Canada; 2) determine the incidence of T2D from 1992 to 2002. The DCPNS registry includes all new diabetes diagnoses from IWK; and 2) determine the incidence of T2D. 92% had documented positive c-peptide and/or negative autoantibodies. One case classified as T2D in the registry could not be confirmed by reanalysis of the medical charts. The incidence of T2D in Nova Scotia (DCPNS) registry through review of cases from the only tertiary care centre in the province (IWK); and 2) determine the incidence of T2D.

The data were analyzed using PCR-SSCP or PCR followed by direct sequencing. BR-It was only possible to study 16 patients (belonging to 15 different families) by these molecular biology methods. 5 mutations of the GCK gene and 2 mutations of the HNF-1alpha were identified; a mutation in the non-coding region of the HNF1beta gene was also found in one patient (MODY 4). In comparison with MODY 2, MODY 3 patients are proportionally less frequent and their diabetes is both clinically and biologically more severe. Identifying molecular defects in patients with a clinical suspicion of MODY is important in terms of their management, prognosis and for genetic counseling. Our clinical and epidemiological data on MODY supports the data published in other pediatric populations in France, Spain, Italy and Greece. The relatively high yield of the GCK and HNF1alpha mutations screening in this study (8 mutations identified in 15 families tested) further supports the proposed diagnosis approach.

Incidence of Type 2 Diabetes in youth <19 years of age in Nova Scotia Canada: 1992-2002

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The incidence of Type 2 Diabetes (T2D) in youth, particularly in populations of mostly European background. We aimed to: 1) validate the classification of diabetes in the Diabetes Care Program of Nova Scotia (DCPNS) registry through review of cases from the only tertiary care centre in the province (IWK); and 2) determine the incidence of T2D from 1992 to 2002. The DCPNS registry includes all new diabetes diagnoses < 19 years seen in 37 Diabetes Centres in NS. Race is not coded, however, only a small proportion of the population is aboriginal (1.9%) or African Canadian (2.2%). All cases were screened for face validity. Charts from IWK cases were reviewed to confirm the diagnosis. T2D cases from the IWK clinic list were reviewed to ensure capture in the registry. Of 25 IWK cases, 8 did not appear as T2D in the registry (type missing (3) or misclassified (5)), apparently due to uncertainty as to diabetes type at the time of diagnosis. One case classified as T2D in the registry could not be confirmed as T2D. 92% had documented positive c-peptide and/or negative autoantibodies or a syndrome associated with T2D. 6 cases from other centres lacking face validity were removed after errors were confirmed. The number of cases increased with time: 0 (1992-93); 17 (1994-96); 17 (1997-99) & 35 (2000-02). The incidence in 2000-2002 for age <19 years was 5.43 per 100,000 (95%CI 5.12-5.74) or 10.5 per 100,000 aged 10.0-18.99 (95%CI 9.91-11.09). Mean age at diagnosis was 15.3 ± 2.3 years. Of all new diabetes diagnoses in the 10-18.99 year age group for 2000-02, 26% were T2D. 40% (n=25) of cases were diagnosed at the IWK. Of these, 20% were from an at risk ethnic group and 28% had a chromosomal or single gene disorder associated with increased risk of T2D. The trend of increased incidence of T2D in youth is mirrored in NS’s population of predominantly European descent. These numbers reinforce the need for screening at risk youth of all ethnic groups. Registries of cases of DM in youth must allow for correction of or delay in classification of diabetes type since the clinical diagnosis may not be clear at initial presentation.

The development of metabolic syndrome in large-for-gestational-age infants

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Objective: Both large-for-gestational-age (LGA) and small-for-gestational-age (SGA) size at birth and intrauterine exposure to diabetes may be risk factors for development of metabolic syndrome X (MSX) and type 2 diabetes. We believe that the primary pathogenesis of MSX is related to early insulin resistance and that it begins prior to birth. We hypothesize that the LGA group and the LGA Infants of Diabetic Mothers (IDM) group will demonstrate early insulin resistance when studied at birth. Method: 30 LGA- IDM infants, 30 LGA infants not born to diabetic mothers, 30 poorly-grown 5-10th% infants, and 30 healthy appropriate-for-gestational-age (AGA) controls are being recruited for longitudinal study at birth, 6 months of age, and 12 months of age. Only Hispanic infants are being studied. Maternal records are being reviewed for influence of confounding variables on infants' size. Insulin sensitivity and secretion are being assessed by modified, maternal records are being reviewed for influence of confounding variables on infants' size. Insulin sensitivity and secretion are being assessed by modified, maternal records are being reviewed for influence of confounding variables on infants' size. Insulin sensitivity and secretion are being assessed by modified, maternal records are being reviewed for influence of confounding variables on infants' size. Insulin sensitivity and secretion are being assessed by modified, maternal records are being reviewed for influence of confounding variables on infants' size. Insulin sensitivity and secretion are being assessed by modified, maternal records are being reviewed for influence of confounding variables on infants' size. Insulin sensitivity and secretion are being assessed by modified, maternal records are being reviewed for influence of confounding variables on infants' size. Insulin sensitivity and secretion are being assessed by modified, maternal records are being reviewed for influence of confounding variables on infants' size. Insulin sensitivity and secretion are being assessed by modified.
outcome variables include assessment of lipid levels, leptin, and inflammatory markers.

Results: Preliminary data from the first 6 patients shows higher mean fasting insulin levels in the LGA-IDM cohort: 14.5 pmol/L (LGA-IDM) vs. 10.6 pmol/L (5-10th%) vs. 1.6 pmol/L (AGA controls) vs. 0.8 pmol/L (LGA). Euglycemic glucose levels were noted in all groups. The LGA-IDM group shows the largest maternal weight gain during the first 6 months of pregnancy. Further data will be collected and analyzed by the time of the meeting.

Conclusions: Higher fasting insulin levels found in LGA-IDM group at birth suggest early insulin resistance and the early development of MSX.

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**P1-489** Type II Diabetes/Insulin Resistance

**Does the fasting or the OGTT 2 hour plasma glucose correlate better with cardiovascular disease (CVD) risk factors in overweight, non-diabetic youth?**

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It has been suggested that, in adults, an elevated 2 hour plasma glucose (2-hr PG) during an oral glucose tolerance test (OGTT) is more strongly related to CVD risk than an elevated fasting plasma glucose (FPG). The purpose of this study was to evaluate if the 2-hr PG correlates better than the FPG with traditional (lipid profile) and non-traditional (C-reactive protein (CRP), fibrinogen, tumor necrosis factor α (TNF-α), and interleukin-6 (IL-6)) CVD risk factors in an overweight, non-diabetic pediatric population. As part of an ongoing study we evaluated 66 overweight children (BMI ≥ 58th for age and sex; 34 Whites, 32 Blacks; 23 males, 43 females; age 12.5±2.2 years; BMI-SDS 2.31±0.41). Evaluation included OGTT’s, proinsulin, traditional and non traditional CVD risk factors as mentioned above. DEXA scans were done for assessment of % body fat. Statistical analysis included univariate and multivariate regression analysis. The FPG was positively correlated in univariate analysis with only proinsulin (r=0.472, p=0.003). There was no correlation with triglyceride/HDL ratio, CRP, fibrinogen, TNF-α or IL-6. The 2-hr PG was positively correlated, with proinsulin (r=0.345, p=0.027), CRP (r=0.396, p=0.025) and IL-6 (r=0.325, p=0.056). In the multivariate analysis, after adjusting for age, sex, race and % body fat, there was no relationship between the FPG and any of the CVD risk factors evaluated. However, the triglyceride/HDL ratio remained significantly associated with the 2-hr PG (β=0.470, p=0.050). These results suggest that, in obese youth, the OGTT 2-hr PG may reflect better the presence of traditional CVD risk factors than the FPG. Therefore, the 2-hr PG may be a better surrogate marker for early identification of CVD risk in overweight youth, and an OGTT, instead of just a FPG, may be needed to identify high-risk children.

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**P1-490** Type I Diabetes - Clinical Aspects and Complication

**Some epidemiological data of diabetes mellitus type-1 in children admitted in U.H.C. of Tirana(1990-2005)**

**Nioleta Grimlić**, Petrit Hoxhe, Zamira Ylli, Agron Ylli

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Background: Diabetes mellitus type-1 is a disease developing new expressivity these last years. It’s having higher prevalence even for very young ages, changing seriously their lives and their families life too. Objective: To evaluate the morbidity of D.M.Type-1 in children admitted in our hospital in a period of 14 years of time.

Material & methods: We studied cases admitted in our hospital from 1990-2005, specifying the following parameters: Personal information from the clinical chart, the exact age at diagnosis, the season, expression of D.K.A. The information about the total number of population is taken from Official Statistical Annals of our Ministry of Health.

Results: There are 201 new cases in this period of time, from 8mno.-14yrs. old, with the mean age 7.51±/-4.37yrs., without any preference of sex. The rapport between males and females is 1.2:1, respectively. There is slightly elevated preference for winter and summer, but without any significant difference with the other seasons. There is also nearly the same number of children in group ages 5-9yrs. old and 10-15 yrs. (1:1.3), but we see an increased number of children in group ages 0-4yrs. with 17.9% of the total number(36/201 cases) comparing with our earlier studies where it was only 11%. Starting from 1999, we had new patients younger than 2 yrs. of age(11 months), so we can say in this study, goes from 0.75x10^5 to 2.1x10^5 in 2004.

Conclusion: The admission rate of new diabetic children in our hospital is higher in the last 4 years and what impresses us, are very young ages of patients, under 2 yrs. old, who make the mean age of 0-4 yrs. group, lower. It looked an interesting item, to be someone’s subject of study in the future.

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**P1-491** Type I Diabetes - Clinical Aspects and Complication

**The specific HLA-DRB, HLA-DQA, and HLA-DQB alleles and haplotypes in Japanese children with type 1 diabetes mellitus**

**Noriyuki Takubo, Yukifumi Yokota, Mariko Shimohama, Shigeyuki Otsu, Mayumi Kazahari, Keiko Nomoto, Nobuo Matsuura**

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Backgrounds: The incidence of type 1 diabetes mellitus (T1DM) varies significantly depending on an geographical and ethnic or racial background. The selective distribution of susceptible (and protective) HLA-DR and HLA-DQ alleles is accounted for the lower incidence of T1DM in Asian children compared with that of Caucasian children. It is known that the onset of T1DM is associated with a familial accumulation, but the genetic predictor of the onset is not still proven.

Aim: The purposes of our study are to identify the susceptible genes for childhood-onset T1DM and to investigate the association of the HLA-DRB1-DQA1-DQB1 haplotypes with the onset of T1DM.
Methods: We investigated the HLA class II genes in 30 unrelated childhood-onset Japanese type 1 diabetics, 19 unaffected their siblings and 97 healthy controls using case-control study. The HLA-DRB1, HLA-DQA1, HLA-DQB1 alleles and haplotypes were determined by the PCR-sequencing-based typing method.

Results: The frequencies of DRB1*0901-DQA1*0301-DQB1*0303 haplotype in childhood-onset type 1 diabetics (53.3%) were significantly higher compared with control subjects (23.7%). Furthermore, high odds’ ratio of the homozygotes of DRB1*0901-DQA1*0301-DQB1*0303 genotype (OR=19.2) and the heterozygotes of DRB1*0901-DQA1*0301-DQB1*0303/DRB1*0405-DQA1*0301-DQB1*0401 genotype (OR=7.08) were revealed in Japanese type 1 diabetic children. However, the odds’ ratio of heterozygotes DRB1*0901/non-*0901 genotype did not show a significant difference between type 1 diabetics and control subjects. The mean age at onset of T1DM was significantly younger in children with DR9/DR9 homozygotes (6.8±4.7 years old) than in children with DR4/DR4 homozygotes (11.5±5.1 years old) (p = 0.02).

Conclusions: Our observations confirmed the association of specific HLA-DQ and HLA-DR regions with childhood-onset Japanese T1DM. A high penetration rate of a susceptibility haplotype may be responsible for the familial accumulation of T1DM.

Incidence of type 1 DM among Saudi children in the Eastern province of Saudi Arabia over 14 years, alarming figures
Mohammed Abdullah, Jamal Jubeh
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Background: The geographical incidence of Type 1 Diabetes Mellitus (T1DM) varies widely and has increased worldwide over the past three decades. Incidence data from Middle East in general and Saudi Arabia in particular are scarce. Epidemiological studies are essential to understand the behavior of this devastating disease.

Objective: To investigate the patterns in incidence rate (IR) of childhood T1DM by studying a population in the Eastern province of Saudi Arabia, a group that represents all parts of the country.

Methods: Dates of admission, diagnosis of T1DM, age, and gender were collected for the state of Victoria only. In the most recent census (2001) the state population of Victoria was 4,644,950 with 943,713 aged 0-14 years. In 1999 the incidence of type 1 diabetes was 17.2/100,000. This had increased steadily to 21.7/100,000 in 2002. Increases were seen across all age groups with a greater increase in females than in males. Both metropolitan and rural regions were affected by the increase in incidence. The marked increase (26%) in incidence of type 1 diabetes in Victoria over a four-year time period has major implications for diabetes health care providers.

The incidence of type 1 diabetes has been increasing worldwide. In Victoria, Australia there has been a marked increase in newly diagnosed patients seen at the tertiary hospital level.

The purpose of this study was to ascertain whether these observed increases were occurring on a state-wide basis. Data was obtained from the Australian Institute of Health and Welfare National Diabetes Database. This database collects information on newly diagnosed patients with type 1 diabetes aged 0-14 years from two independent sources- Australasian Paediatric Endocrine Group members and the National Diabetes Service Scheme database. Data was collected for the state of Victoria only. In the most recent census (2001) the state population of Victoria was 4,644,950 with 943,713 aged 0-14 years. In 1999 the incidence of type 1 diabetes was 17.2/100,000. This had increased steadily to 21.7/100,000 in 2002. Increases were seen across all age groups with a greater increase in females than in males. Both metropolitan and rural regions were affected by the increase in incidence. The marked increase (26%) in incidence of type 1 diabetes in Victoria over a four-year time period has major implications for diabetes health care providers.

During the last years an increase in the incidence of DM1 in very young children has been observed. With the purpose of comparing clinical and laboratory features of DM1 onset in infants and older children (n=137), we compared the clinical and laboratory characteristics in preschoolers aged 0-4 years (group I; n=27) and older children (group II: 5-9 years, n=60 and group III: 10-14 years, n=50) diagnosed from 1998 to 2003 in Santiago, Chile and admitted to 3 public hospitals and one private clinic. Birth weight and height, age at the onset, anthropometric data, and duration of symptoms before diagnosis, blood glucose level, blood gases and HbA1c levels at admission; conscious level and concomitant infectious diseases were evaluated in each child.

A shorter duration of symptoms was observed in younger children; 18.4±23.7, 26.4±27.4 and 40.1±60 days in groups I, II and III, respectively (p<0.0001). Hba1c levels were lowest in group I; 10.1±7.7 vs. 11.8±3.4% in the II (p<0.0001) and 12.4±2.6% in the III (p=0.028). Glucose levels were at diagnosis; 523.7±219.7, 514.9±200.1 and 482.3±198.8 mg/dl, in groups I, II, and III respectively (p<0.005). Metabolic acidosis was more severe in the first group too: pH 7.14±0.1 vs. 7.19±0.2 and 7.26±0.1 respectively (p<0.0001). A concomitant infectious disease was observed in: 33%, 20 and 28% of the patients in group I, II and III, respectively (p<0.05).

We conclude that infants and toddlers at diagnosis of DM1 have more severe acidosis, higher glucose levels and lower levels of alertness, with lower Hba1c levels and duration of symptoms. This data suggests an acute clinical presentation at younger age. This clinical picture can be confused with an infectious diseases at onset of DM1.
The incidence of Type 1 Diabetes Mellitus (TIDM) in childhood is increasing. The accelerator hypothesis suggests that age at onset of TIDM and body mass index (BMI) are inversely related. Studies testing this hypothesis have shown differing results. The aim of the study was to assess the risk factors which influence age at onset of TIDM in childhood. A retrospective case note study of 110 children with TIDM was performed. The risk factors assessed were sex, presence of family history, birth weight (BW) SDS, BMI SDS at 1 year, BMI SDS at diagnosis, using the BMI at the first clinic visit following diagnosis and the rate of weight gain SDS [defined as weight SDS at diagnosis - birth weight SDS / age (years)]. Relationships between age at onset of diabetes and BW SDS, BMI SDS at 1 year, BMI SDS at diagnosis and rate of postnatal weight gain were studied using Pearson’s correlation. Independent factors affecting age at onset of diabetes were studied by multiple linear regression. Results were expressed as mean ± SD. Rate of postnatal weight gain SDS (mean 0.3 ± 0.5) correlated negatively with age at onset of TIDM (mean 7.3 ± 3.8 yrs), (r = -0.4, p<0.0001). There was no significant relationship between BW SDS (mean -0.2 ± 1.0), BMI SDS at 1 year (mean 0.6 ± 0.4) and BMI SDS at diagnosis (mean 0.6 ± 0.9) with age at onset of TIDM. Multiple linear regression analysis showed that rate of postnatal weight gain was an independent factor affecting age at onset of TIDM in children (p<0.0001).

Our data shows that the rate of post-natal weight gain is an important factor which affects the onset of Type 1 Diabetes Mellitus in childhood. An improved lifestyle of healthy eating and reduced body fat from birth may be important in reversing this trend.

Induction of remission in type 1 diabetes
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Induction of remission in type 1 diabetes - Cresto JC, Bruno LF de, Pastorale C, Confalonieri N, Olives L, Cao G, Camberos MC, Basabe JC. Aim: This paper describes a successful, non aggressive treatment in a model of autoimmune type 1 diabetes in mice which can be used in prediabetic patients.

Methods: Male C57BL/6N inbred mice were daily injected IP during 5 days with streptozotocin subdoses. Four groups were studied: 1) Normal controls (C), 2) normal controls treated (CT), diabetic mice (D) and diabetic mice treated (DT). Treated mice were injected IP from day 6 to 110 with 50 mg/Kg of acetyl-L-carnitine and 25 mg/Kg of nicotinamide. Not treated mice were injected IP with saline. In all groups the weight and the glycemia were controlled. Mice were sacrificed at days "0", "12", "30", "93" and "110" during the study to determine: a) plasma insulin, b) immune aggression and c) insulin release from perifused pancreas slices. Statistical significance was analyzed by "t" test.

Results: The treatment did not induce differences in CT and C. The glycemia in D and DT increases until day "53" and then diverge, D continuous increasing and DT decrease (D [mg%]: days 0 190; 53 407; 86 355; 110 291). The plasma insulin was: CT [µU/ml] basal: 36. D [µU/ml] days 12" 8; 86" 8. DT [µU/ml] days 12" 18; 86" 23; 110" 44 (P<0.0005). Immune aggression: Controls [µU/min/5000 cells] basal: 40 D [µU/min/5000 cells] days 12" 8; 75" 8. DT [µU/min/5000 cells] days 12" 18; 75" 23; 110" 44 (P<0.0005). Insulin area from pancreas slices was: CT [µU ins] basal: 2397. D [µU ins] days 12" 462; 30" 709; 93" 627. DT [µU ins] days 12" 1326; 30" 1237; 110" 1696 (P>0.0005). Diabetic mice have a mortality of 4/11and treated mice have not mortality. The experiment was stopped at day "86" in D group to keep mice for the studies.

Conclusions: The treatment induces remission of streptozotocin induced I autoimmune diabetes in C57BL/6N mice and normalize all altered metabolic parameters. The know tolerance of acetyl-L-carnitine and nicotinamide suggest this treatment can be used in prediabetic patients.

Resistin and adiponectin, two novel adipokines with antagonistic influence on insulin sensitivity and insulin resistance, may play a great role not only in obesity and/or type 2 diabetes but also in type 1 diabetes. The aim of the study was the evaluation of resistin and adiponectin in pre-pubertal children with IDDM and estimates of the influence of different kinds of therapy.

Subjects and Methods: 67 patients and 15 age-matched, healthy children were included into the study. All children were pre-pubertal, diagnosed with IDDM for more than two years, and without any coexisting diseases. All patients were divided into groups according to the kind of therapy: 22 were treated with conventional insulin therapy (CTT-two injections daily), 21 received multiple insulin injection (MI) and 24 were treated with continuous subcutaneous insulin infusion (CSII). Blood samples were obtained between 7:30 a.m. and 8:30 a.m. from children in normoglycemia (after a night between episodes of hyperglycemia or hypoglycemia). All analyses were made by radioimmunoassay (RIA-adiponectin) or enzyme-linked immunosorbent assay (ELISA-resistin) commercial kits.

Results: resistin levels were higher in the children with IDDM than in healthy patients (the highest in CSII, lower in MI and the lowest in CTT in the lowest in MI and the highest in CSII, lower in CT, the lowest in MI- even lower in control group). Adiponectin levels were also higher in patients with IDDM than in healthy patients. Adiponectin levels were higher in patients with IDDM than in healthy patients. The kind of therapy appears to have had an influence on the resistin and adiponectin levels. It is necessary to estimate the influence of age, sex, weight, height, BMI, duration of illness, quantity of insulin and correlations. Supported by research grant KBN378/PO5/2002/23

Poster Presentations
One of the main features of type 1 diabetes mellitus (DM1) is the metabolic stress that affects adipose tissue due to its disability to use carbohydrates as an energy source. Because of their relationship with insulin sensitivity and body fat content, adipokines could experience changes in DM1. Furthermore, ghrelin, and especially its acylated form, is included among the factors involved in glucose homeostasis that could be altered in DM1. Our aim was to determine changes in adiponectin, resistin, leptin, TNF-α and acylated ghrelin serum levels in children with newly diagnosed DM1, as well as during the first four months of insulin therapy. Twenty-two children (Tanner I; 11 boys and 11 girls) with DM1 were studied at diagnosis and after 1 and 4 months of an intensive split-mix (NPH and Lispro analogue) subcutaneous insulin regimen. Body mass index (BMI) was determined. Fasting adiponectin, leptin, total and acylated ghrelin levels were quantified by radioimmunoassay. Enzymoimmunoassay was used for resistin (Linco® USA) and TNF-α (R&D Systems®, USA). Intra- and interassay coefficients of variation were below 10%. BMI significantly increased after insulin therapy. Adiponectin and resistin levels were normal at diagnosis. Circulating leptin and total and acylated ghrelin were significantly decreased, while TNF-α was elevated at diagnosis. No changes were observed in total ghrelin or resistin after insulin therapy. In contrast, adiponectin and acylated ghrelin significantly increased after one month of therapy, returning to normal values at 4 months. Leptin and TNF-α normalised after the first month (Table 1; *p<0.05 vs control; **p<0.01 vs diagnosis).

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Diagnosis</th>
<th>One month</th>
<th>Four months</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (SDS)</td>
<td>0.08±0.11</td>
<td>0.05±0.17</td>
<td>-0.16±0.86</td>
<td>-0.14±0.99</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>10.3±3.23</td>
<td>9.94±3.57</td>
<td>24.31±5.37</td>
<td>9.89±3.92</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>8.0±3.9</td>
<td>7.2±4.2</td>
<td>7.7±4.3</td>
<td>6.5±3.2</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>5.1±2.3</td>
<td>2.6±1.4</td>
<td>5.2±3.2</td>
<td>6.1±3.4</td>
</tr>
<tr>
<td>Total ghrelin (pg/ml)</td>
<td>1350±51</td>
<td>912±396</td>
<td>888±238</td>
<td>905±484</td>
</tr>
<tr>
<td>Acylated ghrelin (pg/ml)</td>
<td>80±31</td>
<td>50.2±17.5</td>
<td>92.4±75.6</td>
<td>64.4±32.4</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.4±1.2</td>
<td>2.3±3.8</td>
<td>1.2±0.8</td>
<td>1.04±0.68</td>
</tr>
</tbody>
</table>

Conclusions: 1. Changes in adiponectin levels are independent from BMI modifications in DM1. 2. Variations in acylated ghrelin concentrations indicate its precarious sensitivity to glycaemic changes in these patients.

**P1-499** Type I Diabetes - Clinical Aspects and Complication

**Changes in adipocytokines and acylated ghrelin in newly diagnosed type 1 diabetic children after insulin therapy**

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P1-501 Type I Diabetes - Clinical Aspects and Complication

**Benefits of immediate hemoglobin A1c results in pediatric type 1 diabetes patients**

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Immediate feedback of hemoglobin A1c (A1c) results to adults with diabetes mellitus improves glycemic control. We hypothesized that using a rapid fingerstick test to provide immediate feedback of A1c levels to children would have a similar benefit while being less painful. We conducted a 12-month prospective randomized controlled trial in children and adolescents with type 1 diabetes mellitus for ≥2 years at Children’s Hospital Boston. 214 subjects, ages 3-17 years, were randomized either to conventional feedback (CFB) where A1c was determined in the hospital laboratory (Roche-Hitachi 917) on a venipuncture blood sample, or immediate feedback (IFB) where A1c was determined (Bayer DCA 2000+) at the beginning of the clinic visit on a fingerstick blood sample. Clinicians were able to use the IFB A1c result to guide counseling and alterations in therapy. Baseline CFB and IFB A1c results were similar (CFB 7.90 ± 1.25% and IFB 7.90 ± 1.12%, P=0.52). Using paired analyses, CFB subjects remained at baseline at 3 and 6 months (3 mos: -0.08 ± 0.72%, P=0.27; 6 mos: +0.02 ± 0.83%, P=0.85) but increased at 9 and 12 months (9 mos: +0.27 ± 1.09%, P=0.01; 12 mos: +0.20 ± 1.01%, P=0.048). IFB subjects, however, improved from baseline A1c at 3 months (-0.20 ± 0.63%, P=0.065), and returned to baseline by 6, 9, and 12 months. IFB subjects rated the fingerstick less painful than venipuncture (0 to 10 Likert scale rating for fingerstick 0.3 ± 0.7 vs. venipuncture 3.9 ± 2.6; P=0.001). Members of the diabetes team communicated with IFB subjects about visits more often than with IFB subjects (38% vs 29%; P=0.04).

Immediate feedback of A1c is a more acceptable method of A1c determination in children with type 1 diabetes mellitus. It resulted in modest improvement in A1c at 3, 9, and 12 months as compared with conventional feedback, and less frequent need for communication between diabetes team members and patients/families in the intervals between visits.

**P1-500** Type I Diabetes - Clinical Aspects and Complication

**Inherited non-type 1 diabetes in childhood: a distinct pathology or variants of common diabetes?**

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Dominated inherited non-type 1 diabetes in children is often assumed to be due to maturity onset diabetes of the young (MODY), however some families have no known MODY gene defect, and children with type 2 diabetes often have family history.

We hypothesised that studying children with autosomal dominant inherited non-type 1 diabetes would define a homogeneous cohort suitable for finding new MODY genes. We aimed to characterise families with autosomal dominant childhood onset non-type 1 diabetes, in order to identify new diagnostic categories and genetic defects.

Recruitment was from 4 UK sources: A survey of childhood diabetes; MODY registry, Birmingham, UK; referrals from local endocrinologists; and all available members examined, and fasting bloods taken for metabolic profile and DNA extraction.

26 families have been visited. Index case median age at diagnosis of diabetes was 11 years (range 0-15). 12 were insulin treated, 5 with oral agents alone and 9 with diet. The families form 5 phenotypic groups; autoimmune diabetes (n=5; median BMI-SDS - 0.7, c-peptide 344 pmol/l, GAD positive); likely or confirmed MODY (n=8; median BMI-SDS 0.32, c-peptide 754 pmol/l); presumed type 2 diabetes (n=9; median BMI-SDS +2.2, c-peptide 1127 pmol/l); possible syndromic diabetes (n=2; median BMI-SDS + 2.7, c-peptide 1479 pmol/l); and neonatal diabetes (n=1). Our initial hypothesis has been disproved; inherited childhood non-type 1 diabetes shows phenotypically distinct categories which are likely to have differing genetic causes. BMI-SDS, GAD antibody status and c-peptide may be valuable pointers to diagnosis in childhood non-type 1 diabetes. Both Type 1 and Type 2 diabetes may mimic MODY pedigrees. MODY is not restricted to those of European ancestry and should be considered in any non-insulin dependent child with autosomal dominant family history of diabetes. Families with insulin resistant diabetes, hyperlipidaemia, obesity and moderate learning difficulties may represent a distinct syndrome.

**P1-502** Type I Diabetes - Clinical Aspects and Complication

**Osteoprotegerin serum levels in children with type 1 diabetes: relation with bone status and glycemic control**

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Background: Patients with type 1 diabetes mellitus (T1DM) have accelerated atherosclerosis and are at risk for decreased bone mass. Nuclear factor-kB ligand (RANKL) and osteoprotegerin (OPG) are a key agonist/antagonist cytokine system regulating important aspects of osteoclast biology; OPG is
expressed also in heart and vascular wall, and has been implicated in atherogenesis.

**Aim:** To evaluate OPG levels and skeletal status in prepubertal T1DM children and to investigate the possible relation between OPG levels, bone status and glycemic control.

**Patients and Methods:** 26 prepubertal children (median age 9.9 years, range 4.1 - 13.1 years) without diabetic microvascular complications were studied. In all patients serum OPG levels, HbA1c, and broadband ultrasound attenuation (BUA) were evaluated. Clinical (current medications, family and personal medical history, fracture history, age of diabetes onset, insulin regimen, calcium intake, and physical activity), and laboratory data (glucose, serum creatinine concentration) were also evaluated. Forty-five age-, sex- and body-size-matched healthy children (median age 9.6 ± 3.3 years; range 6.3 to 12.8), were recruited as control group.

**Results:** Z-score BUA appeared considerably reduced in children with T1DM in comparison with the control group (p < 0.001). Plasma OPG levels were significantly higher in diabetics than in controls (p < 0.0001). Spearman’s rank correlation test showed that in T1DM children z-score BUA values displayed a significant correlation with OPG (r = -0.62; p = 0.001), and HbA1c (r = -0.59; p = 0.007). Furthermore, OPG levels were significantly correlated with HbA1c (r = 0.56; p = 0.008). In a multiple regression analysis, with age, duration of diabetes, physical activity, calcium intake, mean HbA1c, z-score BUA, only HbA1c significantly predicted increase of serum OPG (Beta 0.67; p = 0.003).

**Conclusions:** Prepubertal children with T1DM have a significant increase of OPG levels and reduced bone mass and quality. A significant correlation between OPG, bone mass, bone quality and HbA1c value has been demonstrated. OPG could be a new marker of reduced bone mass in children with T1DM.

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**P1-503** Type I Diabetes - Clinical Aspects and Complication

**Left ventricular mass index, cardiovascular risk factors and fat distribution in children with type 1 DM**

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**Objective:** To investigate left ventricular mass index (LVMI) and its relationship with some cardiovascular risk factors in children with type 1 diabetes.

**Methods:** Children with diabetes (n = 22), aged 7-17 years and healthy subjects (n=20), aged 7-17 years were enrolled to the study. The blood pressure, body weight, height, triceps skinfold thickness, waist and hip circumferences were measured and LVMI (g/m2) was calculated. Fasting glucose, serum lipids and lipoproteins, leptin, Vitamin B12, folic acid and homocysteine concentrations were determined in groups, also % HbA1C and C-peptide levels in diabetics. Body fat distribution was assessed by DEXA.

**Results:** Diabetic duration was 5.5 ± 2.2 years for girls, 6.2 ± 2.7 years for boys (p > 0.05) and %HbA1C 11.5 ± 2.3 for girls and % 10.4 ± 2.1 for boys (p > 0.05). Means of weight, height, BMI, waist/ hip ratio, systolic and diastolic blood pressures and LVMI were similar in diabetic and control groups, but mean triceps skinfold thickness of diabetics was higher than those of controls. Mean blood glucose, HDL-C, Apo-A-I, vitamin B12 and folic acid concentrations were significantly higher in diabetics than in controls; but serum TG, T-C, LDL-C, Apo-B, Lp(a), leptin, and HDL concentrations were similar in both groups. Mean diabetic duration and blood glucose levels were higher in diabetic girls than boys. There were not any differences between diabetic boys and girls for CVR factors. In diabetics, LVMI was correlated positively with peripheral, central and total fat-free mass, and total body mass. Percent of central, peripheral and total fat mass were higher in diabetics than those of controls, but peripheral fat-free mass was higher in controls than those of diabetics.

**Conclusion:** In diabetic children, the CVR factors in early diabetic period were not significant, and LVMI was correlated with central, peripheral and total fat free mass and body mass.

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**P1-504** Type I Diabetes - Clinical Aspects and Complication

**Novel aspects of effect of α-lipoic acid on micro-circulation in type 1 diabetes mellitus**

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**Aim:** to study effect of Berliion 300 on micro-circulation (MC) and parameters of hemocoagulation (HG) in patients with type 1 diabetes mellitus (DM).

**Methods:** Examined 26 patients (10 boys, 16 girls, aged 12 - 17 years). HbA1c > 7.6% in 16 and < 7.6% in 10 patients with duration DM of more 5 years. The control group (without ALC) included 12 patients of matching age, sex and compensation. The patients received α-lipoic acid (ALC) in the dose of 300mg i/v for 10 days, then in tablets in the dose of 600 mg/d for one month. MC was assessed by conjunctival biromicroscopy (CB) with calculation of conjunctival index (CI) by scoring system. HG was assessed by prothrombin index (PI), thrombotest (TT), fibrinogen (F) and blood fibrinolytic activity (BFA).

**Results:** CI in the general group was 25.9 ± 1.8 and reducing to 20.6 ± 1.4 (P<0.05) after therapy. In the compensation group the reduction of CI was observed but it was unconfident. Improvement in MC accounted for improvement in walls of venules and capillaries. Pre- and post-therapy PI in the general group was 93.1 ±1.0% and 88.7 ± 0.9%, TT was 5.63 ± 0.13 and 5.52 ± 0.12*, F was 379.9 ± 22.9 mg%, and 208.7 ± 32.2 mg% (*P<0.05) respectively. BFA elevated from 14.6 ± 0.13% to 15.9 ± 1.1%, but unconfidently. In the control group significant improvements in MC and HG were unexposed.

**Conclusions:** for the first time ALC in the dose of 300-600 mg was found to confidently improve MC of eye conjunctiva and reduce blood hyper-coagulation in patients with type 1 DM.

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**P1-505** Type I Diabetes - Clinical Aspects and Complication

**Renal handling of zinc in insulin-dependent diabetes mellitus patients**

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**Objective:** Hyperzincuria is a common feature in diabetic patients, which is still not totally understood. As zinc takes part of a great number of enzymes, a hypozincemic status may lead to important respiratory and metabolic errors. Based on the above consideration, the aim of the present study was to investigate the renal handling of zinc in insulin-dependent diabetes mellitus (IDDM) patients. The glomerular filtration rate, urinary zinc excretion, zinc clearance, zinc clearance/creatinine clearance ratio, zinc tubular reabsorption, glycosuria, plasma glucose, C-peptide, glucagon, and cortisol were investigated in 10 normal individuals (Group C1 and Group C2, respectively) and 10 IDDM patients (Group E1: hyperglycemic and glycosuric and Group E2: normoglycemic and aglycosuric) during placebo or venous zinc tolerance test. The results showed that the urinary zinc excretion and the renal zinc clearance were increased after zinc injection in normal individuals (Group C2) and IDDM patients (Groups E1 and E2) when compared with normal individuals-placebo (Group C1). However, these renal parameters were statistically more significant in the hyperglycemic and glycosuric diabetes (Group E1). Since patients in Group E1 had the lowest plasma C-peptide levels and showed a strong negative correlation between Czat-Ccr ratio and this hormone, we suggest that in this setting insulin inhibits urinary zinc excretion.