The contiguous gene disorders that will be discussed today are very complex compared to the reassuringly familiar situations in which a single gene of known function is mutated causing either dominant or recessive inheritance of hormone deficiency. I will begin with a simple example in which various deletions of adjacent and evolutionarily related hormone genes result in phenotypes that are readily explained in terms of gene dosage. The discussion will then be extended to address the additional concepts and disciplines that have contributed to the understanding of the DiGeorge, Prader-Willi and Williams-Beuren syndromes.

The human growth hormone gene cluster consists of 5 very similar genes aligned over a distance of about 50 kb and located on 17q22-q24. Dr. Perez-Jurado made major contributions to the understanding of the positions and functions of these genes. They all lie in the same transcriptional orientation and 4 of the 5 encode structurally similar hormones. There is greater than 95% sequence homology among the individual coding and 5' regions of these genes. The GH-1 gene encodes pituitary GH, while the CS-P gene is a disabled pseudogene, CS-1 encodes chorionic somatomammotropin, GH-2 encodes a placental GH, and CS-2 also encodes chorionic somatomammotropin. Non-homologous crossing over between flanking sequences led to evolutionary expansion to 5 genes from a single ancestral GH gene. The same mechanism produces deletions that remove 1 or more of the genes. Homozygosity for deletion of GH-1 produces isolated GH deficiency, type IA. Combinations of larger deletions in the fetal genome removing genes from the 3' end of the cluster do not produce disease. They result in reductions in levels of placental GH and CS in the maternal circulation. For example, homozygosity for deletion of the CS-1, GH-2 and CS-2 genes results in total deficiency of the corresponding hormones. Compound heterozygosity for deletion of these 3 genes and a smaller deletion of the CS-1 gene results in a 75% reduction in maternal CS levels. There are similarities and differences between this simple example and the more complex conditions that will be discussed today. Similar features include non-homologous meiotic recombination as a mechanism for gene deletion and variable phenotypes that are dependent on the extent of the deletion. Differences include the formidable challenge of identifying the contiguous but otherwise unrelated genes that contribute to the various dimensions of the syndromes. A second major difference involves understanding the detailed mechanisms underlying parent of origin effects in conditions such as the DiGeorge and Angelman syndromes. The Digeorge, PWS and William-Beuren syndromes were all described in the 1950's and 1960's. The next two decades brought more refined descriptions of phenotypes and assembly of large numbers of well-characterized patient populations. Convergence of cytogenetic and molecular techniques in the 1980's and 1990's led to identification of the critical regions on chromosomes 22q11, 15q11 and 7q11.2. In each case, studies of large numbers of patients with classical features of the
syndrome confirmed that the vast majority of affected individuals actually had abnormalities involving these regions. The tasks that are being addressed in this decade require animal models, genomics, in vitro expression systems, and a great deal of imaginative thinking to solve the puzzles of matching the individual gene functions to individual features of very complex phenotypes. The overall objective is to gain a complete explanation of the syndromes to foster novel strategies for alleviating the disabling aspects of the conditions.

**S1-8** Autosomal contiguous gene disorders caused by haploinsufficiency. Molecular mechanisms and animal models

**MOLECULAR GENETICS OF 22Q11 DELETION SYNDROMES-A REVIEW**

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Deletions of human chromosome 22q11 occur in approximately 1:4000 live births and are associated with a number of haploinsufficiency syndromes, including DiGeorge and velocardiofacial syndromes. Hypocalcemia is documented in around 60% of cases, although documented hypoparathyroidism is rare. The deleted region – or DiGeorge Chromosomal Region (DOCR) – is entirely cloned and sequenced. Despite this, human genetic techniques in isolation have so far failed to unequivocally demonstrate that haploinsufficiency of any specific gene is required to produce this syndrome. As many genes at 22q11 show conservation of syteny in the mouse, several laboratories have addressed the role of single genes and groups of genes using mouse models. Direct deletion, and Cre-lox mediated deletion and duplication have been used to create a shortest region of deletion overlap for a “murine Dgcr”. In 1999 Baldini’s group demonstrated that embryos hemizygous for the Dgcr have hypoaplasia of the 4th pharyngeal arch artery at E10.5, which gives variably penetrant great artery and heart defects, hypoplastic thymus and ectopic parathyroid glands. Further deletion mapping, transgenic rescue and gene targeting experiments have identified that the transcription factor Tbx1 as vital for pharyngeal arch artery morphogenesis/remodelling, and morphogenesis of the thymus and parathyroid glands. The variable penetrance and expressivity of the murine deletion syndrome reflects the situation seen in man, yet the models are, unsurprisingly, an imperfect phenocopy. Cellular studies and new animal models will undoubtedly be vital in understanding the developmental pathways affected by Tbx1 haploinsufficiency and the Tbx1 plays in the interactions of the pharyngeal endoderm and neural crest. References Jerome, L.A. and Papaiosannou, V.E. (2001). Nat.Genet. 27, 286-291. Lindsay, E.A., et al. (2001). Nature 401, 97-101. Merscher, S., et al. (2001) Cell 104, 619-629.

**S1-9** Autosomal contiguous gene disorders caused by haploinsufficiency. Molecular mechanisms and animal models

**THE PRADER-WILLI AND ANGELMAN SYNDROMES**

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The Angelman syndrome (AS) and Prader-Willi syndrome (PWS) are neurogenetic disorders, which are caused by the loss of function of imprinted genes in 15q11-q13. Imprinting is an epigenetic process by which specific chromosomal regions are differentially marked by the male and female germline. The nature of the parental marks is unknown, but DNA methylation and histone modifications play a major role in this process. Most of the imprinted genes in 15q11-q13 are expressed from the paternal copy only. Some of these genes encode proteins, whereas others encode small nuclear RNAs (snRNAs). Loss of expression of paternally expressed genes is associated with PWS, which is characterized by neonatal hypotonia, hypoglycaemia, hyperphagia and obesity, short stature, small hands and feet, craniofacial dysmorphism and mental retardation. The clinical findings point to the hypothalamus as the primarily affected organ. There are two genes (UBE3A and ATP10C) which are expressed from the maternal copy only. The expression of UBE3A occurs in brain cells only. UBE3A encodes an enzyme involved in intracellular protein degradation and is affected in AS, which is characterized by microcephalus, jerky movements, absence of speech, abnormal EEG pattern, severe mental retardation and frequent laughing. The most frequent lesions in PWS and AS are a deletion of the entire region or uniparental disomy. The deletions, which are of paternal origin in the case of maternal hypoparathyroidism, comprise approximately 4 million base pairs and include most of the imprinted domain plus several non-imprinted genes. Most often, they arise as a consequence of non-homologous cross-overs between duplicated DNA sequences which flank this region. Uniparental disomy is the presence of two homologous chromosomes from the same parent (maternal in PWS and paternal in AS) and most often results from a combination of meiotic and mitotic errors. In a small group of patients, the disease is due to aberrant imprinting and gene silencing. Incorrect imprints (a maternal imprint on the paternal chromosome in PWS and a paternal imprint on the maternal chromosome in AS) can occur de novo without any mutation in the DNA sequence or as the result of a microdeletion affecting the imprinting centre (IC), which controls the whole imprinted domain. The IC maps to the SNURF-SNRPN locus and appears to consist of two elements. One element is required for the maintenance of the paternal imprint during early embryogenesis. The second element is required for maternal imprinting in the female germline. There is tentative evidence that maternal expression of UBE3A is regulated through a paternally-expressed antisense transcript, which starts at the IC. The SNURF-SNRPN sense UBE3A antisense transcript serves as a host for the snoRNAs, which are encoded within introns of this transcript. Unlike other snoRNAs, these snoRNAs are probably not involved in the posttranscriptional modification of rRNA, because they lack any telic RNA complementarity. Between SNURF-SNRPN and UBE3A the snoRNAs appear to be the only conserved entities between human and mouse. From the mouse data this region appears to be critical for the neonatal PWS phenotype and therefore a lack of these snoRNAs may be causally involved in this disease.

**S1-10** Autosomal contiguous gene disorders caused by haploinsufficiency. Molecular mechanisms and animal models

**WILLIAMS-BEUREN SYNDROME**

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Williams-Beuren syndrome (WBS) is a neurodevelopmental disorder with multisystemic manifestations characterized by distinctive facial features, mental disability, supravalvular and other vascular stenoses, growth retardation and occasional infantile hypercalcemia. It was first reported in 1952 under the name of “Idiopathic Infantile Hypercalcemia”. Patients with WBS have cognitive deficits with a non-uniform profile, showing specific difficulties in visuospatial processing but adequate skills in face processing, language and sociability. The study of the pathophysiology of this disorder is likely to make important contributions towards the understanding of the genetic bases of cognition. Molecular basis WBS is a segmental aneusomy syndrome that results from a heterozygous deletion of contiguous genes at 7q11.23. The great majority of patients show a very similar deletion of ~1.5 Mb that includes a number of reported genes. However, with the exception of vascular stenoses caused by haploinsufficiency at the elastin gene, the other features of WBS have not been clearly attributed to specific genes. With the goals of defining the genes responsible for the phenotypic features, we and others are performing clinical-molecular correlations in typical and atypical patients. Based on the complete physical maps across the region from human and mouse, several novel genes located within the critical interval have been identified. Deletion mapping in the few atypical patients with smaller deletions have suggested that additive effects of haploinsufficiency for two or more genes may be necessary for the WBS phenotype. The genes responsible for abnormal cognition have been mapped to the telomeric edge of the deletion. Candidate genes in this region include CNL2, coding for a neuron-specific cytoplasmatic protein that localizes to the cytoskeleton via microtubules, and GTF2I, encoding a transcription initiator factor involved in both basal and activated transcription. The answer to the remaining open questions about WBS may rely on animal experimentation. We have established that the order of the intradeletion genes is fully conserved in mice. Therefore, mice carrying identical deletions to humans can be generated by using chromosomal engineering technology. Mutational mechanisms The fact that the great majority of WBS cases are sporadic indicates a high rate of de novo deletions, ~0.5×10-4 per gamete per generation. The deletions occur with similar frequency in the maternally or paternally inherited chromosome. Most deletions arise from crossover events between both chromosome 7 homologues during meiosis, although intrachromosomal rearrangements also occur. Large blocks of region-specific highly homologous segmental duplications have been found flanking the common deletion (called A, B and C). Deletion breakpoints in many WBS patients indicate that WBS could arise as a consequence of misalignment of duplicated sequences between two specific blocks of segmental duplications that are located in tandem and share >99% sequence identity over 105 Kb (blocks Bc and Bm, see figure). Thus, aberrant homologous recombination mediated by large segmental duplications is a precise mechanism that preferentially occurs in regions of extremely high sequence identity. In addition, at least two types of genomic rearrangements at 7q11.23 have been identified in progenitors transmitting the WBS chromosome: 1) –one third carry a chromosome with an inversion of the whole interval, 2) ~5% carry a chromosome with an abberant number of segmental duplications. These atypical alleles are found in the normal population as rare chromosomal polymorphisms. They have been generated as well through inter- or intra-chromosomal recombination events between the segmental duplications. Heterozygosity for such alleles would predispose to misalignment during chromosome pairing in meiotic prophase, and unequal crossing-over would lead to the WBS deletion. Therefore, it appears that several mechanisms account to explain the high mutation rate for de novo deletions causing WBS, being the segmental duplications involved in all the rearrangements. Sequence
analysis and comparative mapping of the genomic region in primates suggests that the human 7q11.23 chromosomal region evolved through serial, evolutionary tandem, complex rearrangements leading to segmental genomic duplications. In summary, WBS belongs to an increasing family of disorders that result from recurrent DNA rearrangements leading to abnormal gene dosage and involving unstable genomic regions generated during recent genomic evolution.

**S2-13 New insights into thyroid function**

### ONCOCGENIC MECHANISM OF THYROID CARCINOMAS

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The development of thyroid tumors offers a particular example of the correlation between loss of thyroid differentiation markers and activation of different oncogenes and/or loss of tumor suppressor genes function. One of the early events in thyroid tumor formation is the mutational activation of Ras oncoprotein. This process is found at similar frequencies in benign adenomas and differentiated carcinomas, suggesting that Ras mutations are an initiating event in thyroid neoplasia. How this initial event may influence thyroid cell differentiation is an important question arising from the above information and is the subject of many studies in thyroid carcinogenesis. Fully differentiated thyroid cells express a set of specific genes for thyroglobulin (Tg), thyroperoxidase (TPO), Na+/I- symporter (NIS), and the transcription factors TTF-1, TTF-2 and Pax-8. Thyrotophin (TSH) through its receptor (TSH-R) regulates the growth and differentiation of these cells. The expression of transforming Ras oncoproteins interacts with the establishment and maintenance of thyroid cellular differentiation, suppressing the expression of thyroid differentiation markers Tg, TPO and NIS. In parallel the transcription factors TTF-1, TTF-2 and Pax-8 are either not present or inactive. Several proteins have been identified as potential effectors of Ras signalling including Rap1, MAPK/ERK, BalGDS, and PI3K, although the mechanism of Ras mediated inhibition of thyroid cell differentiation remains essentially unclear. Another Ras protein family that includes RasA, Capn11 and Rap3, play a pivotal role in controlling mitotic and growth arrest of the thyroid. The implication of this effect of RasA is that RasA reverses the effect of RasT in TSH-mediated proliferation of thyroid cells has been demonstrated. To further analyze the RasA function in thyroid, we combined transient and stable transfection assays to express different mutant RasA forms in thyroid cells. Constitutively active RasA induces a fibroblast-like phenotype with organized actin fibers, whereas the expression of RasA negative dominant presented a rounded morphology and lost normal cytoskeletal architecture. The thyroid cells expressing constitutively active form of RasA results in TSH-independent proliferation, anchorage-independent growth and induces tumors when inoculated into nude mice. The tumors generated in these animals are highly undifferentiated and infiltrative. Tumors cells were poorly differentiated, showing small cytoplasm and large nuclei with abundant myosins. They grew in a solid, diffuse pattern with neither follicles nor papillary structures. Infiltration was observed mainly as satellite nodules. Bromodeoxyuridine immunostaining showed that proliferative cells were located mainly in peripheral regions of the tumor. Satellite tumor nodules were highly proliferative, mainly in the peripheral areas. Detailed necropsy of the mice revealed no other effects. The cells overexpressing active RasA lose the expression of thyroid differentiation markers. Specifically the expression of Tg was significantly reduced. This effect is mediated, at least in part, by a decrease in TGF-1 activity, since transient or stable expression of active RasA results in a reduction in the activity of the wild-type Tg promoter as well as an artificial promoter which activation depends only on TGF-1 activity. The similarity between RasA and other oncogenes and/or loss of tumor suppressor genes may influence thyroid cell differentiation. The expression of RasA in thyroid cells transformation increases the complexity of the signal transduction pathways involved in the control of thyroid cell proliferation and differentiation. Thyroid cells depend mainly on TSH for proliferation. This hormone stimulates thyroid cell proliferation through both PKA-dependent and independent pathways. After TSH stimulation, downstream effectors such as PI3K/Akt, Rac1 or RasA are involved in thyroid cell growth and function. Activation of the above proteins increases thyroid cell proliferation and modifies parameters of differentiation. The similarity between Ras-mediated thyroid oncogenesis, together with our observation that active RasA reverses the effect of active Ras on Tg promoter, suggest the existence of a cross-talk involving both...
proteins, as described for other cell systems. This to respect is interesting the fact that TSH increases RhoA expression together with an induction on G1-S transition, as parallel there is an increase in cyclin E-Cdk2 complexes formation and a down-regulation of p27kip1. New studies are currently being performed to explain the cell cycle proteins involved in RhoA- and Ras-mediated thyroid proliferation and subsequent transformation. Supported by Grant BMC2001-2087 (MCYT).

The peripheral hyperinsulinemia may contribute to the sexual dimorphism in growth patterns as it may lead to excess androgen production in girls. High androgen levels, insulin resistance and low IGF-I levels have been linked to risk of complications during puberty and genetic variability at INS VNTR, IGF-I and other loci may be important.

S2-14 New insights into thyroid function

MATERNAL FT4 LEVELS DURING PREGNANCY AND SUBSEQUENT INFANT DEVELOPMENT: IS THERE A CRITICAL SET POINT OF FT4?

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During the last decades it has been shown that low maternal FT4 concentrations during (early) gestation irrespective of elevated TSH levels (defined as hypothyroxinemia) might result in impaired infant development. There is growing evidence that, although iodine intake might be sufficient (although sometimes marginally) in most western countries in men and non-childbearing women, in a substantial number of pregnant women iodine intake might not meet the foetal needs, especially during early gestation. In a paper of 1999, 5 years aged children of mothers with TPO-Ab during late gestation showed a developmental delay of 10 IQ points compared to children of antibody negative women. In 1999, another study showed that 12 months aged children of women with maternal hypothyroxinemia (an FT4 below the 10th percentile) during early gestation had an IQ of 10 IQ points compared to women with normal FT4 levels. In 2001, a prospective follow-up study of 67 children of mothers with hypothyroxinemia during early gestation showed a developmental delay both at the age of one and two years compared to children of mothers with an FT4 between the 50 and 90th percentile. The question arises whether there exists a ‘preconception’ period in the mother - hence resulting in low FT4 levels during early gestation - which might predict impaired infant development. Because it is almost impossible to collect data of a large randomly allocated group of ‘preconception’ women we looked back at the data of the 1995 study. These children belonged to a group of 293 randomly selected women who were followed between 1989 and 1991 from 32 weeks’ gestation until 34 weeks’ gestation. From the 32 women with an FT4 below the 10th percentile at 32 weeks gestation and an FT4 below the ‘preconception’ level, n = 11. The children of these 11 women - at the age of 5 years - showed to have an IQ delay of 10 points compared to the children of the remainder group (t = 5.7, p < 0.001) with no differences in motor development (McCarthys Scales of Children’s Ability).

S3-15 Clinical and Molecular basis of neonatal diabetes

GROWTH, WEIGHT GAIN AND THE GENETIC SUSCEPTIBILITY TO TIDM AND ITS COMPLICATIONS

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The prevalence of type 1 diabetes mellitus (T1DM) is increasing, particularly in those diagnosed under 5 years. This may reflect changing patterns of early weight gain and feeding. Recent large epidemiological studies have noted increased infancy weight gain and more rapid growth in those children who subsequently develop diabetes. Height at diagnosis may be increased compared to controls and relatively taller stature may be observed in the high risk HLA siblings. However there is considerable genetic heterogeneity between different populations. Increasing weight gain during infancy and risk of T1DM has been linked to cows’ milk ingestion but recent data indicate that the two may be independent. Variation in IDDM2 the insulin gene related variable number of tandem repeats (VNTR) on chromosome 11 has also been linked to early weight gain. The class III INS genotype which is protective against T1DM is associated with increased fetal thyamic expression of insulin and thus putative improved immune tolerance. In contrast the class I allele which is associated with increased T1DM susceptibility is associated with increased postnatal insulin expression and weight gain. Weight gain continues to be a problem, particularly in girls with T1DM after diagnosis, whereas growth in stature tends to be slow and there is blunting of the pubertal growth spurt. These abnormalities of growth are related to impaired IGF-I generation due to reduced portal levels of insulin.

S3-16 Clinical and Molecular basis of neonatal diabetes

EPIDEMIOLOGY AND CLINICAL ASPECTS OF NEONATAL DIABETES

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Neonatal diabetes mellitus (NDM) is a rare (1/400 000 newborns) but potentially devastating condition. Most patients have intrauterine growth retardation and, at birth, develop hyperglycemia and failure to thrive, sometimes with dehydration. NDM occurs as two main variants, one transient (TNDM) and the other permanent (PNDM). TNDM demonstrates a developmental delay of insulin production that resolves after birth and accounts for 5% to 60% of cases of NDM. The majority of patients recover within the first year of life, but some continue to have impaired glucose tolerance, and the diabetes can recur in late childhood or adulthood. Two genetic anomalies have been described in 2 cases of PNDM, namely a deletion in the PDX gene (PFI1) and a mutation of the glucokinase gene which abolishes the enzymatic activity. We report the results of a nationwide study involving a network of pediatricians throughout France : 29 patients with TNDM (insulin therapy for fewer than 3 years) and 21 patients with PNDM were identified. Although patients with PNDM were less likely to have had intrauterine growth retardation and were older at diagnosis, no clinical features could distinguish PNDM from TNDM on an individual case basis. Permanent IDDM developed in five TNDM patients after 8 years of age, emphasizing the need for prolonged follow-up. Among the 19 TNDM patients surviving, two had paternal isodisomy of chromosome 6, seven from the maternal chromosome. These findings support the hypothesis that the foetal needs, especially during early gestation - which might predict impaired infant development. Because it is almost impossible to collect data of a large randomly allocated group of ‘preconception’ women we looked back at the data of the 1995 study. These children belonged to a group of 293 randomly selected women who were followed between 1989 and 1991 from 32 weeks’ gestation until 34 weeks’ gestation. From the 32 women with an FT4 below the 10th percentile at 32 weeks gestation and an FT4 below the ‘preconception’ level, n = 11. The children of these 11 women - at the age of 5 years - showed to have an IQ delay of 10 points compared to the children of the remainder group (t = 5.7, p < 0.001) with no differences in motor development (McCarthys Scales of Children’s Ability).

S3-17 Clinical and Molecular basis of neonatal diabetes

THE MOLECULAR BASIS OF PERMANENT AND TRANSIENT NEONATAL DIABETES

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Although rare (approx 1/400,000 live births), the basis of both permanent (PNDM) and transient (TNDM) forms of neonatal diabetes mellitus has proved most interesting in terms of the genetic mechanisms involved, the ways in which this understanding has come about and the information it gives us on pancreatic physiology. Currently there are four described types of PNDM for which a molecular basis has been identified. A single nucleotide deletion in the Insulin Promoter Factor-1 gene (IPF1) causes PNDM in the homozygous state due to pancreatic agenesis whilst causing MODY-4 in the heterozygous state. Frame shift or amino acid substitution mutations in EIF2AK3 (a regulator of protein synthesis in the islet cell) causing Wolcott-Rallison syndrome. Mutations in FOXP3 leading to immune dysregulation similar to that seen in the scurfy mouse causing X-linked immunodysregulation, polyendocrinopathy and enteropathy (IPEX). Most recently described is the homozygous state for glucokinase miss-sense mutations leading to PNDM in families with moderate glucose intolerance. Three well described genetic anomalies can cause TNDM. The first described was isodisomy of chromosome 6 (UPD) which accounts for around 50% of sporadic cases. The second was paternally inherited unbalanced duplications of 6q24 suggesting an
imprinting disorder. Most recently, a methylation defect has been identified in around 16% of sporadic cases lying within a TNDM imprinted CpG island. Two genes, ZAC and HYMA1, overlap the TNDM locus and imprinted region. Activity of ZAC's promoter is dependent on the methylation status of this CpG island; methylation being associated with absent/reduced activity. Further evidence that ZAC is indeed the gene for TNDM comes from work in a TNDM patient with a methylation defect where bi-allelic expression of ZAC in skin fibroblasts has recently been demonstrated. Activity of ZAC's promoter to the gene for TNDM is still awaited, as is an explanation of the effect of over-expression at this locus on pancreatic function.

**S4-18 Neuroendocrinology: Non-classical actions of hormones**

**NEUROACTIVE STEROIDS REGULATE THROUGH THE GABAA RECEPTOR THE HYPOTHALAMIC CONTROL OF REPRODUCTIVE FUNCTIONS**

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Hypothalamic neurons secreting the luteinizing hormone releasing hormone (LHRH), also known as the gonadotropin releasing hormone (GnRH), represent a key point in the regulation of reproductive functions. However, in spite of their crucial relevance, many aspects of the mechanisms controlling the gene expression and the release of LHRH are still obscure. Steroid hormones, and growth factors (two different signals which may reach the synthesis of bFGF. Altogether, the present observations indicate that their metabolites loose the property of interacting with the classical steroid receptors but acquire the capability to interact with receptors located on the hypothalamic neurons; this because of the relative paucity of steroid receptors in the hypothalamic-secreting neurons. Recent observations have indicated that several growth factors [e.g., transforming growth factors beta 1 and beta 2 (TGFbeta 1 and TGFbeta 2), transforming growth factor alpha (TGFalpha), basic fibroblast growth factor (bFGF)] may interact with the TGFalpha receptor, suggesting that estrogens modulate LHRH secretion. Moreover, LHRH neurons both directly and indirectly interact with growth factors secreted by THDA receptor present on neurons connected to LHRH neurons. A novel gene termed Nell-2 appears to play a role in glutamatergic neuron function as it is preferentially expressed in these neurons and its down-regulation leads to decreased LHRH release and delayed puberty. Astroglate regulates LHRH secretion by releasing growth factors and growth factors are also involved in the development of the brain and, therefore, the local formation of estradiol. Genetic disruption of erbB-1 or erbB-4 receptors in mice delays female sexual development due to impaired erbB ligand-induced LHRH release. The combined effect markedly exacerbates the delay in puberty, indicating that both erbB-1 and erbB-4 receptors are essential components of the glial-neuronal communication process required for normal female sexual development. Global analysis of the changes in gene expression that occur in the primate hypothalamus during the onset of puberty, revealed that broader changes in expression, involving genes encoding transcriptional regulators, as well as neuron-neuron and neuron-glia communication may also be required for neuroendocrine reproductive maturation.

**S4-19 Neuroendocrinology: Non-classical actions of hormones**

**TRANSGENIC AND GENOMIC APPROACHES TO THE STUDY OF FEMALE PUBERTY**

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The initiation of mammalian puberty requires an increased secretion of LHRH from the hypothalamus. This increase is determined by changes in the activity of neuronal and astrogial networks connected to LHRH neurons. The main inhibitory neurotransmitter controlling LHRH release is GABA, which delays the onset of puberty via activation of GABA-A receptors. However, this inhibition is not directly exerted on LHRH neurons, as these cells do not express the chloride channels required for GABA-A receptor-mediated inhibitory responses, and respond to GABA-A activation with excitation. Targeted expression of GAD-67 to LHRH neurons to mimic the activity of a subset of LHRH neurons to produce GABA during early development did not delay puberty, but altered LHRH neuronal migration and decreased postpubertal reproductive success. Thus, a direct action of GABA on LHRH neurons does not seem to be required for the timing of puberty, but instead is important for the normalcy of postpubertal reproductive function. Glutamatergic neurons represent the main excitatory neuronal system stimulating LHRH release at puberty. Glutamate stimulates LHRH neurons both directly and indirectly. LHRH neurons both directly and indirectly interact with growth factors secreted by TGFalpha and neuroeigens (NRG), two members of the epidermal growth factor family, acting via the tyrosine kinase receptors erbB-1 and erbB-4, respectively. Neuroeigens enhance erbB-dependent signaling via a glutamate-dependent activation of metabotropic and AMPA receptors located on astroglial cells. Genetic disruption of erbB-1 or astrocytic erbB-4 signaling in mice delays female sexual development due to impaired erbB ligand-induced LHRH release. The combined effect markedly exacerbates the delay in puberty, indicating that both erbB-1 and erbB-4 receptors are essential components of the glial-neuronal communication process required for normal female sexual development. Global analysis of the changes in gene expression that occur in the primate hypothalamus during the onset of puberty, revealed that broader changes in expression, involving genes encoding transcriptional regulators, as well as neuron-neuron and neuron-glia communication may also be required for neuroendocrine reproductive maturation. Epineuronal and astroglial networks connected to LHRH neurons to mimic the ability of a subset of LHRH neurons to produce GABA during early development did not delay puberty, but altered LHRH neuronal migration and decreased postpubertal reproductive success. 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**S4-20 Neuroendocrinology: Non-classical actions of hormones**

**NEUROPROTECTION BY SEX STEROIDS AND IGF-I**

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There is now evidence that sex steroids, in addition to their participation in neuroendocrine regulation and sexual behavior, have neuroprotective properties. Systemic administration of the neurotoxin kainic acid (KA) to induce degeneration of hippocampal hilar neurons was used in these studies. Estradiol and its receptor testosterone are neuroprotective in this model in both male and female rats. In addition, KA induced the expression of the enzyme aromatase in the brain and, therefore, the local formation of estradiol. Genetic or pharmacological inhibition of brain aromatase resulted in increased neurodegeneration after the administration of KA and blocked the neuroprotective effect of estradiol. These findings indicate that local formation of estradiol in the brain is neuroprotective. The mechanism of action of estradiol as a neuroprotectant is currently being explored. Here we present evidence that insulin like growth factor-I (IGF-I), a trophic factor for neurons an glia, interacts with estrogen in the promotion of neuronal survival. Both systemic administration of
17 beta-estradiol, as well as intracerebroventricular infusion of IGF-I, prevented hilar neuronal loss induced by KA in adult ovariectomized rats. To determine the role of IGF-I receptors in the neuroprotective effect of estrogen, we studied a specific IGF-I receptor antagonist, the peptide JB1. This antagonist was infused for 7 days in the lateral cerebral ventricle to neutralize the local action of IGF-I in the hippocampus. The neuroprotective action of 17 beta-estradiol was abolished under these conditions. This finding indicates that IGF-I receptor activation is necessary for the beneficial effect of estradiol on hippocampal neurons. Furthermore, the neuroprotective effect of IGF-I was blocked by infusion in the lateral cerebral ventricle of the estrogen receptor antagonist ICI 182,780, indicating that activation of estrogen receptors is also necessary for the effect of IGF-I on hippocampal neurons. These findings suggest that co-activation of both ERs and IGF-I receptor is necessary for neuroprotection and hiliar hippocampal neurons from KA toxicity. To explore the possible mechanisms involved in the interaction of estradiol and IGF-I in neuroprotection, we examined whether there is interaction of ER and IGF-I signaling in the CNS. Analysis of the distribution of ERs, alpha and beta, and IGF-I in the rat brain by confocal microscopy, revealed that acetylcysteine co-express ER beta and IGF-I. This finding indicates that interactions of the signaling pathways of ERs and IGF-I are possible at the cellular level in the CNS, both in neurons as well as in glial cells. To determine whether there is cross-regulation of ERs and IGF-I in the CNS, we infused antagonists of these receptors, or IGF-I, in the lateral cerebral ventricle of adult ovariectomized rats. Infusion for 7 days of an ER antagonist (ICI 182,780, 10-7M) down-regulated IGF-I receptor expression in the hippocampus. This suggests that ERs regulate the expression of IGF-I in the CNS. We also assessed whether IGF-I regulates the expression of ERs. Infusion for 7 days of IGF-I (10-7M) in the lateral cerebral ventricle up-regulated ER alpha in the hippocampus. In contrast, infusion for 7 days of the IGF-I receptor antagonist JB1 (20 micrograms/ml) resulted in the down-regulation of ER alpha levels. These findings suggest that there is cross-regulation of ERs and IGF-I in the rat brain. We also assessed whether IGF-I signals through ERs in the brain. Administration of estradiol activated brain ERK1, ERK2 and Akt (protein kinase B, PKB), mediators of the effects of IGF-I. Immunoprecipitation experiments suggest that the activation of IGF-I signaling by estradiol may be in part mediated by the interaction of ER alpha and IGF-I and the consequent phosphorylation of the IGF-I receptor. This interaction is induced by estradiol and probably occurs at the membrane level. Furthermore, a synergistic action of estradiol and IGF-I in the phosphorylation of Akt was observed. Phosphorylation and activation of Akt results in the phosphorylation and inactivation of the pro-apoptotic molecule Bad and the prevention of apoptosis. Therefore, the synergistic activation of Akt by estradiol and IGF-I in the brain may be involved in the neuroprotection. It is known that activation of Akt also regulates the expression of the anti-apoptotic molecule Bcl-2. In addition, both IGF-I and estrogen induce Bcl-2 expression in the CNS. We assessed, therefore, whether estrogen and IGF-I may interact to regulate Bcl-2 in the brain. We tested whether IGF-I is necessary for the expression of Bcl-2 in the hypothalamus in response to estradiol. Animals receiving estradiol alone had increased expression of Bcl-2 in the hypothalamus compared to animals receiving vehicle. However, infusion in the lateral cerebral ventricle of the IGF-I receptor antagonist JB1 blocked the effect of estradiol on Bcl-2. This suggests that IGF-I receptor activation is necessary for the induction of Bcl-2 by estradiol in the adult CNS. Infusion of Bcl-2 may represent molecular targets for regulation of the interaction of estrogen and IGF-I in the promotion of neuronal survival. In summary, these findings indicate that estradiol and IGF-I signaling interact to protect neurons from excitotoxic cell death. This study has been supported by DFG/SI (PN98-0110) and Commission of the European Communities, specific RTD programme “Quality of Life and Management of Living Resources”, QLK/C-2000-00179.
demonstrated on growth and body composition (1,2). Patients: The effects of GH (Genotropin®, Pharmacia Corporation) treatment in 14 Swedish children with PWS, who started treatment prepubertally, have been followed during seven years. Initially, they all participated in a randomised controlled trial with two different doses; 0.033 mg/kg/day for 15 subjects and 12 subjects served as controls for the first year then treated with GH at a dose of 0.066 mg/kg/day. This trial was conducted to assess the effects of GH treatment on growth, body composition and behaviour. After 2 years of GH treatment, children were separated into two groups: one group continued GH treatment for 6 months and then restarted with GH at a dose of 0.033 mg/kg/day. Results: During the first year of GH treatment there was a dramatic increase in height SDS and change in body composition in both groups with decreased fat mass and increased lean mass. The attained height percentile has persisted until 6 years after the GH treatment. Seven years after the start of GH treatment, the height is within average for age in the normal population. Four subjects have reached final height, all within ± 2 SD of target height. BMI SDS improved dramatically during the first GH treatment year, but worsened during the 6 months without treatment. After restart of GH treatment, BMI has been stabilized. During the first year of GH treatment, ratio lean/fat improved remarkably, but deteriorated during the 6 months period of discontinued treatment. Following the restart of GH treatment, ratio lean/fat re-improved and has persisted during treatment. In all patients, fasting insulin, glucose and HbA1c remained within normal ranges during these years of GH treatment. The parents reported an improvement of physical and psychological activity, as well as a, stabilization of temper during GH treatment. Conclusion: Children with Prader-Willi syndrome have a sustained beneficial effect on height and body composition after seven years of GH replacement therapy when a low-calcic diet is maintained. References 1) Lindgren AC, Hagens E, Müller J, Blicke S, Rosenborg M, Brismar T, Ritzén EM 1998 Growth hormone treatment of children with Prader-Willi syndrome affects linear growth and body composition favourably. Acta Paediatr 87:28–31 2) Carrel AL, Myers SE, Whitman BY, Allen DB 1999 Growth hormone improves body composition, fat utilization, physical strength and agility, and growth in Prader-Willi syndrome: a controlled study. J Pediatr 134:215–221

**S5-25** Non-classical aspects of GH therapy

**THE EFFECT OF IGF-I DEFICIENCY AND REPLACEMENT THERAPY ON THE HEMATOPOIETIC SYSTEM IN PATIENTS WITH PRIMARY GH INSensitivity (LARON SYNDROME)**

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Introduction: Previous studies performed in vitro and in rodents have revealed that Growth Hormone (GH) and Insulin-like Growth Factor-I (IGF-I) have a concert with bone marrow cytokines, stimulatory effects on both the red and white cell lineages. Primary IGF-I deficiency - Laron Syndrome (LS) - a unique model in man caused by defects in GH signal transmission, enables to study the specific role of IGF-I on hematopoiesis. Objective: To assess retrospectively, the effect of IGF-I deficiency and therapy (Rx) on the red and white cell lines of the hematopoietic system including platelets, as reflected by repeated peripheral blood counts. Patients/Material and Methods: We studied 10 children with LS (6M, 4F mean age 6.9±4.28) treated with rhIGF-I (180 µg/kg/d) for 4-6 years and 5 adult patients (1M, 4F mean age 32.8±4.16) treated with rhIGF-I (120-150µg/kg/d) for 9 months. Data on the blood counts were collected from the patient’s charts before and during treatment. Blood samples were drawn at 0 time, weekly during the first month, monthly during the first year and once every three months thereafter. Statistical analysis of the change over time was performed using repeated-measures ANOVA. Results: Untreated children and adolescents with LS had red cell indices in the lower normal range. The monocyte count was elevated in all age groups. The main findings registered before treatment and those registered after 5 years of IGF-I treatment are shown in the table. Nine months treatment of the adult patients showed a similar trend. Conclusions: The present investigation, the first of its kind, shows that IGF-I has a stimulating effect on hematopoiesis. In contrast, the lower dose of IGF-I Rx had a reducing effect on monocytes and platelets, an effect not described so far. The mechanism by which IGF-I mediates these effects needs further elucidation.

| Parameter | n | % | IGF-I | p-value | Parameter | n | % | IGF-I | p-value |
|-----------|---|---|-------|--------|-----------|---|---|-------|--------|
| RBC       | 465±0.06×10⁶ | 465±0.06×10⁶ | 465±0.06×10⁶ | 0.001    | MCV      | 72.2±7.5 | 72.2±7.5 | 0.001    |
| Hb        | 11.9±0.6 | 11.9±0.6 | 11.9±0.6 | 0.001    | Platelets | 425±0.06×10⁹ | 425±0.06×10⁹ | 0.001    |
| Mc         | 34.9±0.1 | 34.9±0.1 | 34.9±0.1 | 0.001    | MCV      | 72.2±7.5 | 72.2±7.5 | 0.001    |

**OR1-26** Oral Presentation 1: Growth: Clinical

**THE EFFECT OF IGF-I DEFICIENCY AND REPLACEMENT THERAPY ON THE HEMATOPOIETIC SYSTEM IN PATIENTS WITH PRIMARY GH INSensitivity (LARON SYNDROME)**

Z. Laron 1), P. Liloa 2), B. Sivan 2)
1Endocrinology & Diabetes Research Unit, Schneider Children's Medical Center of Israel, Petah-Tikva, Israel

Introduction: Previous studies performed in vitro and in rodents have revealed that Growth Hormone (GH) and Insulin-like Growth Factor-I (IGF-I) have a significant effect on the bone marrow, including monocytes and platelets, an effect not described so far. The mechanism by which IGF-I mediates these effects needs further elucidation.

| Parameter | n | % | IGF-I | p-value | Parameter | n | % | IGF-I | p-value |
|-----------|---|---|-------|--------|-----------|---|---|-------|--------|
| RBC       | 465±0.06×10⁶ | 465±0.06×10⁶ | 465±0.06×10⁶ | 0.001    | MCV      | 72.2±7.5 | 72.2±7.5 | 0.001    |
| Hb        | 11.9±0.6 | 11.9±0.6 | 11.9±0.6 | 0.001    | Platelets | 425±0.06×10⁹ | 425±0.06×10⁹ | 0.001    |
| Mc         | 34.9±0.1 | 34.9±0.1 | 34.9±0.1 | 0.001    | MCV      | 72.2±7.5 | 72.2±7.5 | 0.001    |
Introduction: Recombinant GH treatment improves growth in prepubertal children with short stature and former IUGR. However, the effect in patients close to pubertal onset has not been evaluated. Objective: The aim of this randomized controlled trial was to evaluate the effects of GH on adult height of short children with former IUGR, treated close to the onset of puberty. Patients/Material and Methods: Patients were included with the following criteria: birth length ≤ 5SD, height ≤ 2.5 SD for age, age >10.5 yr (F) or >12.5 yr (M), bone age ≥ 9 yr (F) or 10 yr (M), pubertal stage (Tanner) 1 (78%) or 2. They were randomized (2:1) to receive GH (Maxomax, Sanofi-Synthelabo, France), 1.4 IU/kg/week or no treatment. Treatment was discontinued after 2.7 ± 0.6 yr. Adult height was measured when growth velocity was <2 cm/yr or bone age ≥ 21.5 yr (F) or 16 yr (M) and was available for 89% of the treated and 70% of the control per protocol patient population. Results: The results (see table, means ± SD; *p ≤ 0.005; **p ≤ 0.002) demonstrate a 0.6 SD (3 to 4 cm) increment in adult height in the treatment group. Multivariate analysis confirmed the independent effect of treatment with a gain of 0.25 SD/yr of treatment. Bone age delay at baseline was also an independent predictor. Tolerance to the treatment was excellent. Conclusions: In conclusion, GH increases adult height in children with intra-uterine growth retardation, even when given at a late stage of the growth process.

| Inclusion | Year 1 | Year 2 | Year 3 |
|-----------|--------|--------|--------|
| LM(kg)    | 19.8   | 21.0   | 23.7   |
| LM(SDS/W) | 0.45   | 0.38   | 0.56   |
| FM(kg)    | 7.5    | 6.8    | 8.0    |
| FM(SDS/W) | 29     | 23.4   | 24.2   |
| BMI(kg/m²)| 0.477  | 0.488  | 0.526  |
| BMC(kg)   | 599    | 631    | 746    |
| BMC(SDS/W)| 0.03  | -1.71  | -1.25  | -1.34 |

Conclusions: These data demonstrate that GH treatment induces a dramatic improvement in body composition, particularly by increasing lean mass and by preventing bone loss in JCA patients receiving long-term GC.

Conclusion: In conclusion, GH increases adult height in children with former IUGR, treated close to the onset of puberty. The results (see table, means ± SD; *p ≤ 0.005; **p ≤ 0.002) demonstrate a 0.6 SD (3 to 4 cm) increment in adult height in the treatment group. Multivariate analysis confirmed the independent effect of treatment with a gain of 0.25 SD/yr of treatment. Bone age delay at baseline was also an independent predictor. Tolerance to the treatment was excellent. Conclusions: In conclusion, GH increases adult height in children with intra-uterine growth retardation, even when given at a late stage of the growth process.

CONCLUSIONS:

- GH treatment improves adult height in children with former IUGR close to pubertal onset.
- The effects are independent of bone age delay at baseline.
- Tolerance to treatment was excellent.
- These results confirm the independent effect of GH treatment in improving adult height in this population.

First year height velocity

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First year height velocity
Introduction: Optimisation of pubertal growth in GHD remains a major challenge. Objective: Patients/Material and Methods: Data from patients with idiopathic GHD and spontaneous puberty (N=317; 187 male) from KIGS (Pharmacia International Growth Database) served as the basis for modelling: maxGH <10µg/L; pub. start = B2 or testes >3 ml; >5 yrs on GH; >2 yrs prepub on GH; age at spont. = pub. 9-16 yrs; near final HT (HTvel last yr <2 cm). Statistical analysis and prediction modelling were the same as reported previously (1, 2).

Results: Median [10th%/90th%] values were: S= start (GH); P=puberty onset, T=final: BW(SD)= -0.6 [-2.4/0.9]; MPH(SD)= -0.6 [-2.1/1.1]; age(S) yrs= 8.6 [3.9/11.9]; P=11.7; HT(S)SDS=-2.7 [-4.1/1.7]; age P yrs= 12.7 [10.6/15.0]; HT P SDS = -1.2 [-2.3/0.1]; mean dose GH P= 59 IU/kg wk [0.36/0.82]; age F yrs= 14.6 [16.8/14.8]; HT F SDS = -1.1 [-2.6/0.5] (33 GHD patients from Tubingen used for validation had very similar anthropometrical characteristics.) 70% of the variability was explained with an error term of the model: TPG(cm)= 57.0 + [-3.0 x H(birth)-1.3 x (HT P SDS minus MPH SDS)] + [6.4 x mean GH dose (IU/kg/wk) + (11.3 in males only). The ranking predictors in their order of importance was: HT distance to target HT at puberty onset+age-dose GH. Studentised Residuals (S=observed HTvel – predicted HTvel) and error in the validation group was 0.38 ± 1.86 thus different from zero.

Conclusions: The four parameter growth prediction model for TPG from a large cohort offers a high degree of explained variability, has a small prediction error, utilizes readily available and standardized variables, including treatment modalities; and was validated with an independent cohort. The height achieved at puberty onset is the most important predictor, followed by age at GH start.

(1) Ranke et al. (1999) J Clin Endocrinol Metab 84:1174-1183 (2) Ranke et al. (2000) J Clin Endocrinol Metab 85:4212-4218

OR-3-32 Oral Presentation 2: Adrenal Disorders

NOVEL ACTH RECEPTOR ASSAY FOR FUNCTIONAL TESTING OF MUTATIONS IN PATIENTS WITH FAMILIAL GLUCOCORTICOID DEFICIENCY

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Introduction: The ACTH receptor (MC2R) is a key element of the hypothalamic-pituitary-adrenal axis. MC2R is expressed only in the adrenal cortex, but is one of five G-protein coupled melanocortin receptors whose activation can result in ACTH stimulation of hypothalamic CRH production and ACTH stimulation. Inactivating mutations of the MC2R lead to the familial glucocorticoid deficiency syndrome (F GD). Patients with FGD show a decrease in cortisol production by the adrenal cortex, but do not show any additional symptoms. Our objective was to establish an improved rapid accurate assay for MC2R activity and to test this assay by expressing novel mutations in two patients with FGD.

Patients/Material and Methods: We based our assay on OS-3 cells, which are derived from mouse adrenocortical Y1 cells, and are unresponsive to ACTH but have an intact cAMP signal transduction pathway (a generous gift of Prof Schimmer, Toronto). These cells were co-transfected with a vector expressing human MC2R cDNA and a cAMP-responsive luciferase reporter plasmid. The luciferase activity that contained sixteen CRE units (CRE-luc). MC2R receptor activity was studied at both basal and after stimulation with various concentrations (10-17 to 10-5 M) of synthetic (1-24)ACTH. Leukocyte DNA was prepared from two patients with FGD. Sequencing of exon 2, which contains the protein-coding sequence, showed three gene mutations. By site-directed mutagenesis these mutations were built in the MC2R expression vector and their activity was tested. Results: OS-3 cells transfected with the CRE-luc reporter alone were minimally responsive to ACTH, but co-transfection with the human MC2R cDNA expression vector increased the luciferase activity ten-fold. We report for the first time that parents of two patients with FGD were heterozygous for the same mutation in the CRE-luc and either wild-type or any of the three mutant MC2R's yielded no activity without exogenously added synthetic (1-24)ACTH. We found that the addition of (1-24)ACTH showed an EC50 of 5x10-18 M for the wild type receptor, which is similar to values reported with stable transfection assays using Y1 cells. Genetic studies of our patients revealed a homozygous mutation R137W in one patient and a compound heterozygote S74I and Y254C mutation in the other. In our assay the R137W mutant had an EC50 of 5x10-18 M and there was no measurable response with either the S74I or Y254C mutants. Conclusions: Our novel assay based on OS-3 cells provides excellent sensitivity in a transient transfection system; thus assessing a more rapid and efficient measurement of ACTH receptor activity than was previously possible with stable transfection systems.

OR-3-31 Oral Presentation 2: Adrenal Disorders

PHYSICAL AND BEHAVIOURAL MASCULINISATION ARE RELATED TO GENOTYPE IN GIRLS WITH CONGENITAL ADRENAL HYPERPLASIA (CAH)

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Introduction: Females with congenital adrenal hyperplasia (CAH) are more masculine and less feminine than their peers and their behaviour is thought to result from exposure of the fetal brain to high concentrations of androgens. Using 2 highly sensitive measures: the Child Game Participation Questionnaire [CGPQ] and the Child Behaviour and Attitude Questionnaire [CBAQ] (Meyer-Bahlburg et al, 1994) we have reported that Manchester CAH girls were significantly more masculine and less feminine than diabetic (D) girls (p<0.000 for both) and that the D girls were not significantly different from USA control group.

Objective: Several studies have demonstrated a relationship between genotype and biochemical phenotype in CAH, and the aim of this study was to assess whether such a relationship exists between genotype and physical and behavioural masculinisation.

Patients/Material and Methods: In Manchester, mutation detection based on allele specific PCR is used for genetic diagnosis of CAH. Mutations screened include cyp21 deletion and large gene conversion, and the following pseudogene-derived mutations: 655 A/C>G, 2108 C>T, 999 T>A. The patients were either virilized at birth (girls) or had siblings with congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency does not usually occur within the first days of life. We hypothesized that the 11b-hydroxysteroid dehydrogenase type 2 (11b-HSD) responsible for conversion of cortisol (C) to cortisone (E) are involved in the temporary maintenance of sufficient mineralocorticoid activity by increasing the concentration of the mineralocorticoid active steroid cortisol. Patients/Material and Methods: The patients were either virilized at birth (girls) or had siblings with congenital adrenal hyperplasia (CAH). Infants in G1-3 were more virilised at birth than those in G4: G1-3, 3(17%) were P2, 3(17%) were P3, 11 (60%) were P4 and 1 (6%) was P5, whereas in G4, 2 (40%) were P2, 2 (40%) were P3, 1 (20%) was P4 and none was P5. Serum testosterone, K, Na and 170HP were all recorded at birth in 14 infants: only K was correlated with M (r=0.6, p=0.03), with 23% of the variance in K being accounted for by G (p=0.02). Conclusions: Genotype is the main determinant of both physical and behavioural masculinisation in girls with CAH.
endorphin were normal. Repeated administration of hCRH, or a combined stimulation by hCRH and vasopressin, evoked responses of ACTH to 17-38

Conclusion: While declining plasma aldosterone levels precede the onset of salt wasting in CAH patients, plasma cortisol concentrations are maintained in

4.

OR2-37 Oral Presentation 3: Thyroid Disorders

DILUTE, BLOCK AND REPLACE THE THYROID DURING RADIO-IOIDE TREATMENT

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Introduction: When using 123/131-I-meta-iodobenzylguanidine ( MIBG) in children with neuroblastoma, the thyroid is protected from radiation by potassium-iodide. Despite this prophylaxis, up to 64 % of patients will develop thyroid dysfunction. Objective: We introduce a new thyroid prophylaxis by ways of dilution, blockade and replacement of thyroid hormone when using radioactive MIBG in children. Patients/Material and Methods: First from 1999 - 2001, 34 children receiving 123/I-MIBG for known or suspected neuroblastoma, the thyroid was protected using dilution (potassium-iodide), blockade ( thiouazol) and replacement (thyroxine) prophylaxis. Informed consent was obtained. Baseline thyroid parameters were determined. Prophylaxis started one day before 123/I-MIBG, until four weeks after the last 131/I-MIBG. Follow-up measurements were performed one month after stopping prophylaxis and every three months thereafter. Results were compared to a historical control group who had received potassium-iodide only for thyroid protection. Results: In all patients thyroid parameters received 131I-MIBG for treatment. One patient died due to an aberrant thyroid function at baseline. Preliminary data of 22 patients was evaluated. Two patients showed transient elevation of plasma thyrotopin (> 4.5 mU/L). One patient developed hypothyroidism after only one dose of 123I-MIBG. Three patients developed a thyrotopin elevation after 6, 6 and 15 months. In all other patients thyroid function remained normal ( 81.8 % versus 60 % after two and a half years, p= 0.008). Scintigraphic visualization of the thyroid was diminished significantly in comparison to the control group; 5 versus 21.5 % (p<0.001). Conclusions: In inclusion, protection of the thyroid during 123/I-1-MIBG treatment by this new trias of thyroxine, thiamazol and potassium-iodide is more accurate than with potassium-iodide alone.

OR2-35 Oral Presentation 2: Adrenal Disorders

ACTH DEFICIENCY CAUSED BY TPIT-TBX19 GENE MUTATION: EXTENSIVE CLINICAL EXPERIENCE

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Introduction: In the mouse, the Tpit gene encodes a novel T-box factor present in a spectrum of congenital adrenal hyperplasia based on a large series of unselected Japanese patients with this entity. Patients/Material and Methods: Ninety three Japanese patients with congenital adrenal hyperplasia including 7 patients recruited from the Japanese literature between 1961 and 1996. Results: 1) Sex ratio: There were 53 males, 33 females, and 7 unknowns. 2) Consanguinity: Four patients were born by consanguineous marriage, and the rest of 89 patients were born by nonconsanguineous marriage. 3) Prognosis: There were 73 survived patients and 20 deceased patients, though all the patients born after 1981 survived. 4) Autopsy findings: Ten patients were autopsied, with all disclosing swollen adrenals laden with fat (adrenal weight = 5)Male gonadal function: Fifty XY patients were completely feminized, and 2 XY patients were ambiguous in external genitalia. All survivors were raised as female.6) Female gonadal function: All 33 female patients were normal female in external genitalia. At least 15 patients reached adolescence and young adulthood. All of the 15 manifested pubertal signs and experienced menarche at the mean age of 12.3 years. One patient had probable ovarioly menstrual bleeding as manifested by elevated serum progesterone. 7)STAR mutations: At least 28 patients were studied, with Q258X mutation accounting for some 70 % of affected alleles. Conclusions: The data indicate that congenital adrenal hyperplasia is phenotypically a rather uniform disorder. Female gonadal function is much less compromised as compared with that of male, and the follicle may even luteinize and produce progesterone in exceptional patients.

OR2-34 Oral Presentation 2: Adrenal Disorders

CLINICAL AND MOLECULAR SPECTRUM OF 93 JAPANESE SUBJECTS WITH CONGENITAL LIPOID ADRENAL HYPERPLASIA

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Introduction: Congenital adrenal hyperplasia is caused by mutations in the genes for the steroidogenic acute regulatory protein(StAR), affecting both XX females and XY males in the adrenal function. However, there is an apparent sex dimorphism in the gonadal function of affected subjects. Objective: The purpose of this study is to better define clinical and molecular spectrum of congenital lipid adrenal hyperplasia based on a large series of unselected Japanese patients with this entity. Patients/Material and Methods: Ninety three Japanese patients with congenital lipid adrenal hyperplasia including 7 patients recruited from the Japanese literature between 1961 and 1996. Results: 1) Sex ratio: There were 53 males, 33 females, and 7 unknowns. 2) Consanguinity: Four patients were born by consanguineous marriage, and the rest of 89 patients were born by nonconsanguineous marriage. 3) Prognosis: There were 73 survived patients and 20 deceased patients, though all the patients born after 1981 survived. 4) Autopsy findings: Ten patients were autopsied, with all disclosing swollen adrenals laden with fat (adrenal weight = 5)Male gonadal function: Fifty XY patients were completely feminized, and 2 XY patients were ambiguous in external genitalia. All survivors were raised as female.6) Female gonadal function: All 33 female patients were normal female in external genitalia. At least 15 patients reached adolescence and young adulthood. All of the 15 manifested pubertal signs and experienced menarche at the mean age of 12.3 years. One patient had probable ovarioly menstrual bleeding as manifested by elevated serum progesterone. 7)STAR mutations: At least 28 patients were studied, with Q258X mutation accounting for some 70 % of affected alleles. Conclusions: The data indicate that congenital adrenal hyperplasia is phenotypically a rather uniform disorder. Female gonadal function is much less compromised as compared with that of male, and the follicle may even luteinize and produce progesterone in exceptional patients.

OR3-38 Oral Presentation 3: Thyroid Disorders

NKX2-1/TTF-1 MUTATIONS BEYOND THE THYROID: THE EXTENDED PHENOTYPE, EVIDENCE FOR DOMINANT TRANSMISSION AND NEW CASES

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Introduction: The NKX2-1/TTF-1 gene is expressed not only in the developing thyroid but also in other embryonic regions, the basal ganglia as well as in the lung. Recently we described the first five patients with NKX2-1 haploinsufficiency leading to variable hypothyroidism, choreoathetosis and pulmonary problems in accordance with the particular expression profile of this gene during embryogenesis (JCI 2002, 109,475-480). Results: Here we describe a new patient with a heterozygous C649A mutation in the DNA-binding-homeodomain leading to hypothyrotropinemia (TSH max. 25µU/ml), muscular hypotonia, developmental delay and frequent pulmonary infections. Again in this patient the leading symptom was the neurological defect which underlines the predominant role of NKX2-1 in the brain. The same conclusion was drawn when the mental outcome of the two oldest of the recently reported patients was investigated. Despite adequate treatment borderline mental retardation (IQ 80) and even oligophrenia was found. While the IQ of 80 could be explained by NKX2-1 haploinsufficiency alone, oligophrenia was probably the result of a contiguous gene defect in this patient with a gross chromosomal deletion. The same mechanism might be the reason for the observation of oligodendria in this deletion patient since the PAX9 gene -
which was shown to be haploinsufficient in oligodendroglioma (Stockton et al. Nat.Gen2000)- was deleted togetehr with the NXK2-1 gene. Variability of phenotype among the patients with NXK2-1 haploinsufficiency was further underlined by the new demonstration of inheritance of one NXK2-1 mutation in one family. The same mutation leading to severe congenital hypothyroidism and muscular hypotonia in the child only resulted in mild movement disorder without congenital hypothyroidism in the mother. Conclusions: In conclusion, haploinsufficiency of NXK2-1 seems to result mainly in a neurological disorder of muscular hypotonia and choreothetosis associated -in part- with mental retardation. Hypothyroidism is not a consistent finding in NXK2-1 haploinsufficiency but these new data underline that NXK2-1 represents an important candidate gene for patients with congenital hypothyroidism and adverse neurological outcome despite adequate treatment.

### OR3-39 Oral Presentation 3: Thyroid Disorders

**PENDRED’S SYNDROME: CLINICAL AND MOLECULAR STUDIES IN SIX ITALIAN FAMILIES**

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**Introduction:** The disease-gene for Pendred’s syndrome (PS) has been recently characterized and named PDS. It codes for pendrin, which is highly expressed at the apical surface of the thyroid cell and functions as a transporter of chloride and iodide. Pendrin is also expressed at the inner ear level where it appears to be involved in the maintenance of the endolymph homeostasis in the membranous labyrinth and in the kidney where it mediates chloride/formate exchange and bicarbonate secretion. Mutations in the PDS gene and the consequent impaired function of pendrin leads to the classical phenotype of PS, i.e. dyshormonogenic goiter and congenital sensorineural hearing loss. Up to date renal function has not been investigated in humans. **Objective:** The objective of the study was to perform a detailed clinical, radiological and molecular analysis of Pendred’s syndrome.

**Patients/Material and Methods:** Six Italian families presenting with a clinical diagnosis of PS have been submitted to a full investigation of thyroid function and of inner ear malformations. In order to assess possible differences have been found between individuals with different mutations. Clinically, all patients had goiter with positive perchlorate test, euthyroidism or differences have been found between individuals with different mutations. No significant alterations of the renal function were found. The objective of the study was to perform a detailed clinical, radiological and molecular analysis of Pendred’s syndrome. Conclusions: Pendred’s syndrome is a complex disease where to separate the renal function from the inner ear malformations is crucial. No significant alterations of the renal function were found. Conclusions: Pendred’s syndrome is a complex disease where to separate the renal function from the inner ear malformations is crucial.

### OR3-40 Oral Presentation 3: Thyroid Disorders

**PRE-OPERATIVE MOLECULAR STUDIES IN THE DIAGNOSIS OF THYROID NODULAR DISEASE IN CHILDREN**

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**Introduction:** Up to date methods of research based on polymerase chain reaction (PCR) and reverse-transcription (RT) resulted in the ability to search and detect some markers even in poorly cellular biological material. This is why the RT-PCR has been employed in the proximal regulation of neoplastic lesions in thyroid. Fine-needle aspiration biopsy (FNAB), the most important diagnostic method in thyroid nodular disease (TND), does not always correlates with the postoperative histopathological diagnosis (false negative FNAB result). **Objective:** The aim of the study was to improve the clinical cytological evaluation of the material obtained with biopsy US-guided with a RT-PCR based technique to detect carcinoma even in minimally invasive technique. Patients/Material and Methods: Leftover cells in the needle after US-guided biopsy were submitted to a full investigation of thyroid function and of inner ear malformations. In order to assess possible differences have been found between individuals with different mutations. No significant alterations of the renal function were found. The objective of the study was to perform a detailed clinical, radiological and molecular analysis of Pendred’s syndrome. Conclusions: Pendred’s syndrome is a complex disease where to separate the renal function from the inner ear malformations is crucial. No significant alterations of the renal function were found. Conclusions: Pendred’s syndrome is a complex disease where to separate the renal function from the inner ear malformations is crucial.

**Conclusions:** Our results are the first evidence that the Gal-3 expression, previously documented in thyroid carcinoma follicular origin is also present in HT (false positive result). This study shows some limitations in the usefulness of Gal-3 expression especially in such cases of HT which coexist with nodule(s) of benign behaviour. Immunocyto- and immunohistochemical studies or RT-PCR in situ would be of great value to visualize target cells for Gal-3 in these forms of TND as well as a cellular distribution, nuclear vs cytoplasmic, of Gal-3 which was shown to be haploinsufficient in oligodendroglioma (Stockton et al. Nat.Gen2000)- was deleted togetehr with the NXK2-1 gene. Variability of phenotype among the patients with NXK2-1 haploinsufficiency was further underlined by the new demonstration of inheritance of one NXK2-1 mutation in one family. The same mutation leading to severe congenital hypothyroidism and muscular hypotonia in the child only resulted in mild movement disorder without congenital hypothyroidism in the mother. Conclusions: In conclusion, haploinsufficiency of NXK2-1 seems to result mainly in a neurological disorder of muscular hypotonia and choreothetosis associated -in part- with mental retardation. Hypothyroidism is not a consistent finding in NXK2-1 haploinsufficiency but these new data underline that NXK2-1 represents an important candidate gene for patients with congenital hypothyroidism and adverse neurological outcome despite adequate treatment.

### OR4-41 Oral Presentation 4: Reproduction

**IS THERE A ROLE FOR PREPUBERTAL ESTROGEN IN CHILD GROWTH?**

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**Introduction:** Serum estrogen levels are higher in prepubertal girls as compared to boys. Low level of estrogen have a growth promoting effect as evidenced by acceleration of growth following low dose estrogen treatment in girls with central precocious puberty during pituitary GNRH suppressive therapy. 2/3 of the growth deficit in patients with Turner syndrome is inflicted during prepuberty and it may partly be due to prepubertal estrogen deficiency. **Objective:** The role of estrogen in prepubertal growth was modeled in Turner syndrome patients with and without spontaneous puberty. The working hypotheses were that i) patients with prepubertal spontaneous puberty have normal ovaries estrogen secretion during prepuberty, whereas those without spontaneous puberty have no or subnormal secretion; ii) girls with normal prepubertal estrogen will grow better than those without or lower estrogen secretion; iii) estrogen deficiency contributes to the prepubertal growth failure in Turner syndrome. **Patients/Material and Methods:** The prepubertal growth of 24 Turner syndrome patients who later entered puberty spontaneously (+pub) was compared to that of 54 patients who had no spontaneous puberty (-pub) and required hormone replacement therapy. Two-third of the +pub patients were matched by karyotype for each -pub patient. Growth data of each child was fitted to the ICP childhood model at age 1-10 years and the individual estimated parameters of patients from each group were pooled by a weighted average, shown to have normal distribution, comparing the parameters by student t-test. **Results:** Analysis of the growth parameters and the curves as a whole revealed no significant differences between the +pub (67.17±9.90) + 9.63[±6.99]*y = 0.42[±0.65]*y^2, mean ± SD) and the +pub Turner syndrome patients (64.92[±10.33] + 7.21[±4.97]*y^2 - 0.16[±0.37]*y^2). Likewise, bone maturation was similar for the 2 groups. **Conclusions:** Comparable growth in girls with and without spontaneous puberty in childhood rejects a role for estrogen in prepubertal growth or a role for estrogen deficiency in the prepubertal growth retardation of girls with Turner syndrome.

### OR4-42 Oral Presentation 4: Reproduction

**AGE-DEPENDENT SYNERGISTIC STIMULATORY EFFECT OF IGF-I/IGF-II AND PROINFLAMMATORY CYTOKINES ON LEYDIG CELL STEROIDGENESIS IN VITRO**

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**Introduction:** It is well established that severe inflammation reduces plasma testosterone. At least partly, this effect is mediated by proinflammatory cytokines interacting at all levels of the hypothalamic-pituitary-gonadal axis. The role of other mechanisms have demonstrated that hyperinsulinemia (Ht) - (L-1) and luteinizing hormone (LH) - (L-2) FSCG-driven
steroidogenesis by adult Leydig cells in vitro. In contrast and surprisingly, we recently been found to have additively normalizing effects on the endocrine-
Combined anti-androgen and insulin-sensitizing therapy has steroidogenesis by adult Leydig cells in vitro. In contrast and surprisingly, we combined treatment with a low-dose androgen-receptor blocker (flutamide 125 µg/ml, inclusion, each girl had hirsutism, hyperinsulinism (oGTT, peak insulin unknown. The hormonal interactions involved in the cytokine-driven steroidogenic effects are unknown. The proinflammatory effects of inflammatory mediators comprised IGF-I/IGF-II on Leydig cell steroidogenesis in vitro. Patients/Material and Methods: Immature and adult Leydig cells were prepared from 40- and 80-day-old rats respectively by enzymatic digestion and Percoll density gradient centrifugation. Functionally the isolated immature Leydig cells correspond to pubertal Leydig cells in humans. The cells were >90% pure as judged by steroidogenic enzyme staining. Cytokines and IGF-I and IGF-II were recombiant proteins of rat origin. Lipo polysaccharide (LPS) from E. coli was used as a source of endotoxin. Results: LPS dose-dependently stimulated basal testosterone production by immature rat Leydig cells after 24 h. of culture. The effect was mimicked by IL-1α and to a lesser extent by tumour necrosis factor-α. The efficacy of IL-1α to stimulate steroidogenesis was equal to that of hCG. Adult Leydig cells did not respond to the cytokines. In combination with an optimal concentration of IL-1α, IGF-I and IGF-II alone had no effect. Conclusions: The age-dependent direct steroidogenic effect of proinflammatroy cytokines and IGF-I/IGF-II on Leydig cells may be part of a systemic anabolic rescue response to a severe inflammatory insult. It may also contribute to a paracrine defence system in the testis protecting the developing germ cells from harmful effects of inflammation.

OR4-43 Oral Presentation 4: Reproduction

COMBINED LOW-DOSE ANDROGEN-RECEPTOR-BLOCKING AND INSULIN-SENSITIZING TREATMENT TO NORMALIZE BODY COMPOSITION, ENDOCRINE-METABOLIC STATUS AND OVULATORY FUNCTION IN NON-OBESE ADOLESCENTS WITH HYPERINSULINEMIC HYPERANDROGENISM
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Introduction: Combined anti-androgen and insulin-sensitizing therapy has recently been found to have additively normalizing effects on the endocrine-metabolic status of non-obese young women with hyperinsulinemic hyperandrogenism, dyslipidemia, and anovulation. Excess central adiposity, an additional cardiovascular risk factor, has been reported in non-obese women with hyperinsulinemic hyperandrogenism. Objective: We assessed the effects of a combined low-dose androgen-receptor blocker (flutamide 125 mg/d) and an insulin sensitizer (metformin 1275 mg/d) on body composition and on the endocrine-metabolic status of non-obese adolescents with hyperinsulinemic hyperandrogenism. Patients/Material and Methods: The study population included 30 girls; mean age 15 yr; BMI 21.7 Kg/m2. At inclusion, each girl had hirsutism, hyperinsulinism (oGTT, peak insulin >150µU/mL), dyslipidemia and ovarian hyperandrogenism (diagnosis by GnRH-agonist test); monthly ovulation rate was monitored by weekly serum progesterone measurement. The study population was randomized for an onset of treatment that was either delayed by 3 mo (n=14, controls) or not delayed (n=16, treated); in untreated girls, all study indices remained stable during the control phase of 3 mo. Clinical, hormonal and body composition variables were assessed in all girls before and 6 mo after start of treatment. Results: Combined flutamide-metformin treatment was accompanied by marked decreases in hirsutism score, serum insulin and androgens, by an increase in serum SHBG and by a decrease in luteinizing hormone (LH) and testosterone levels. Testosterone levels were restored in 29/30 girls, and monthly ovulation rates rose from 14% to 67% within 6 mo. The correction of endocrine-metabolic abnormalities was accompanied by a striking decrease in waist-to-hip ratio and in truncal and abdominal fat mass, and by an increase in lean body mass (all p<0.001), without changing total body weight. Conclusions: The beneficial effects of combined flutamide-metformin therapy in hyperinsulinemic hyperandrogenism without obesity are herewith extended at three levels: 1)body composition, 2)low-dose flutamide (125 mg/d) and 3)young age (mean 15 yr). These results suggest that central adiposity in non-obese adolescents with hyperinsulinemic hyperandrogenism is, at least in part, contrary to their endocrine-metabolic condition.

OR4-44 Oral Presentation 4: Reproduction

BERTOLI CELL SECRETIONS ARE NORMAL IN INFANTS WITH KLINEFELTER SYNDROME AND DECLARE ONLY AFTER PUBERTAL MATURATION
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Introduction: Klinefelter syndrome is a major cause of male infertility because of complete failure of spermatogenesis or partial arrest of sperm cell maturation. Thanks to the availability of specific assays, low serum inhibin B (InhB) levels have been recently documented in adult patients, which is consistent with the well known hypersecretion of FSH. However immunoreactive alpha inhibin is normally secreted in adults with Klinefelter syndrome. Little is known about Sertoli cell markers in very young children with XXY caryotype. Objective: Since Klinefelter syndrome can now be recognized by prenatal cytotyping in the course of prenatal screening for Down’s syndrome, we had the opportunity to investigate Sertoli cell secretions in neonates and infants presently shown as having a XXY caryotype, with the aim to follow-up the evolution of InhB and anti-mullerian hormone (AMH) levels during infancy and beyond.

Patients/Material and Methods: A total of 9 infants were investigated by repeated sampling (2 to 4 specimens) from birth to the age of 12-18 months. Testosterone, gonadotropins,InhB and AMH were measured by means of highly sensitive immunoassays. Reference values established in our laboratory for InhB were (pg/ml):80-230 in the first month of life, 150-240 at 1-3 months, 170-300 at 4-8 months, 40-220 at 9-18 months. In addition, Sertoli cell markers were measured in 3 adolescent boys with Klinefelter syndrome aged 17-19 years. Results: 1- In all infants, testosterone levels exhibited the physiological rise at 3-6 months: 2- All InhB levels (pg/ml, mean +/- sem)were in the normal range whatever the age of sampling: 150 +/- 21 before 1 month, 257 +/- 25 at 1-3 months, 224 +/- 19 at 4-8 months, and 125 +/- 20 at 9-18 months. InhB levels were positively correlated with both FSH and testosterone levels. 3- AMH levels (pmol/l, mean +/- sem)were also in the normal range for age: 370 +/- 41 before 1 month, 658 +/- 78 at 1-3 months, 553 +/- 32 at 4-8 months, and 633 +/- 167 at 9-18 months. 4- By contrast, in the 3 postpubertal boys with XXY caryotype, InhB and AMH levels were undetectable or very low. Conclusions: These data demonstrate for the first time that Sertoli cell production of InhB and AMH is normally secreted in infants and that a secondary failure occurs at the time of puberty. The relationship between the secondary failure of Sertoli cell secretions and the adult defect in spermatogenesis emphasizes the role of germ cells in the regulation of InhB and AMH production. Indeed, it was recently reported that normal adult germ cells are able to express the inhibin B subunit gene. In addition, normal germ cell may be needed for full competence of Sertoli cells.

OR4-45 Oral Presentation 4: Reproduction

LONGITUDINAL SERUM INHIBIN B LEVELS IN PREPUBERTAL AND ADOLESCENT BOYS WITH KLINEFELTER SYNDROME (KS)
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Introduction: Boys with KS are usually born with normal testes but during early childhood germ cells start degenerating leading to almost total absence of germ cells already before puberty (1). During puberty the degeneration of Klinefelter testes progresses leading to the adult pattern, which includes Sertoli cell only tubules, complete hyalinisation of most seminiferous tubules, and Leydig cell hyperplasia. Inhibin B is a testicular peptide hormone that prepubertally is produced by the Sertoli cells in normal boys. Inhibin B secretion is stimulated by FSH and has a negative feed-back regulation on FSH secretion. During normal puberty the secretion of inhibin B after puberty is a joint product of Sertoli cells and certain germ cells (2). Objective: Our aim was to study longitudinal changes in serum inhibin B in patients with KS before, during and after puberty as a marker of testicular changes in these patients. Patients/Material and Methods: 7 boys with KS (karyotype 47,XXY) were followed with longitudinal serum inhibin B measurements. The mean (range) age of the boys at the first and last measurements was 9.3 yrs (4.3-12.6) and 14.3 yrs (12.3-17.6), respectively. In addition, longitudinal serum inhibin B levels were obtained from 9 young men with KS aged 16.7-29.5 yrs. Results: Before puberty all boys had normal inhibin B levels and exhibited initially the normal increase in inhibin B levels by the onset of rapid pubertal growth. However, when inhibin B levels in adult men with normal spermatogenesis remain at the higher levels reached during puberty, inhibin B levels in boys with KS decreased shortly after the onset of puberty. Samples from boys beyond Tanner stage 4 showed levels below the normal range and close to the detection limit. In adult patients with KS inhibin B levels was generally below detection limit. Conclusions: The normal inhibin B levels in prepubertal boys with KS suggest that their Sertoli cell function may be relatively normal during childhood and early puberty. The initial rise in inhibin
B levels in early puberty are presumably reflecting a FSH stimulated increase in inhibin B production by immature Sertoli cells; mimicking the normal increase in inhibin B production by a newly developed functional testicular tissue in late puberty and adulthood was associated with a dramatic decrease in inhibin B to unmeasurable levels. This decrease in inhibin B presumably reflects a progressing degeneration of tubular structures in KS and is also consistent with the assumption that after the puberal differentiation of the Sertoli cells, inhibin B production becomes germ cell dependent. 1) Müller et al. Int J Androl. 18:57-62, 1995. 2) Anderson et al., J Clin Endocrinol Metab. 83:4451-8, 1998

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**Oral Presentation 5: Growth: Basic**

**DOWNREGULATION OF GS-PROTEIN TRANSMEMBRANE SIGNALLING IN CHILDREN WITH A VARIANT OF SHORT STATURE RESPONSIVE TO EXOGENOUS GROWTH HORMONE**

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**Introduction:** Objective: We hypothesized that downregulation of Gα-protein mediated transmembrane signalling is one of the mechanisms underpinning so-called idiopathic short stature. Patients/Material and Methods: In view of the complexity of the GNAS1 locus, encoding Gs-protein-coupled receptors; Gs-protein function was judged, as previously described. Normal signalling was observed in 25/58 children; loss of Gα-protein function was evidenced in 24 children (18 boys, 6 girls) and, unexpectedly, gain of function was noted in 9 children. Clinical and endocrine indices are listed below (mean ± SEM; +p<0.05 vs Normal Test; *p<0.01; **p<0.001). Subsequently, in a cohort of short children with loss of Gα-protein signalling, we explored the growth response to growth hormone (GH 50 mcg/kg/d) within a randomized, controlled study (n = 10; age 5.1 ± 0.8 y; height -2.9 ± 0.2 SDS; growth velocity 6.3 ± 0.7 cm/yr; IGF-I 2.6 ± 0.3 SDS). Results after 1 yr are as follows: untreated (n = 4) vs GH-treated (n = 6); change in height SDS 0.4±0.1 vs 1.1±0.1++; growth velocity (cm/yr) 6.1±0.8 vs 10.5±0.5++; IGF-I SDS -1.5±0.4 vs 0.6±0.4++. No adverse events were attributed to GH. Molecular studies of the GNAS1 locus were initiated. So far, a functional polymorphism consisting of a 36 bp insertion and 2 bp substitutions, of body weight compared to control rats, whereas IGF-I ameliorated it (6%). In skeletal muscle, proteolysis induced by glucocorticoids is mainly accomplished by the ubiquitin (Ub) pathway whereas IGF-I opposes this process of two key events in the chondrocyte life cycle: chondrogenesis and terminal differentiation (TD). Patients/Material and Methods: The ATDC5 chondrocyte cell line was fully characterised by gene profiling using RT-PCR in order to determine the onset of chondrogenesis and TD. Dex was added in quadruplicate to the cells at concentrations 10⁻⁷, 10⁻⁶ and 10⁻⁵M during the chondrogenic (days 6-10) and the TD (days 10-15) periods. Alkaline phosphatase (ALP) activity (a marker of differentiation), cell number, proliferation rate and proteoglycan concentrations were determined at day 10 and day 15. In an attempt to reverse the effects of Dex on proliferation, GH and IGF-I (both 500 ng/ml) were added together with the GC. Results: RT-PCR analysis indicated that the cells expressed the GC and IGF-I receptor already from day 6 and the GH receptor from day 9. During chondrogenesis, Dex over the three concentrations caused a significant reduction in cell proliferation (28%), cell number (27%) and proteoglycan synthesis (47%) and increased ALP activity (106%); (p<0.01 in all), as compared to the control values. No significant effects were noted during TD. The addition of IGF-I completely reversed the anti-proliferative effects of Dex by significantly stimulating proliferation above control values (126%) (Table 1). Dex, GH and IGF-I together had the highest proliferation rate with a significant elevation (p<0.05) above IGF-I treatment alone. Conclusions: All significant effects of Dex occurred during the chondrogenic period. IGF-I stimulated cell proliferation and completely reversed and superceded the adverse Dex effects. Studies with this cell line will allow a more focussed approach towards studying the mechanisms underlying GC induced growth retardation as well as investigating the potential benefit of growth promoting therapy.

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**Oral Presentation 5: Growth: Basic**

**THE POTENTIAL OF GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTOR-I TO AMELIORATE THE EFFECTS OF DEXAMETHASONE DURING CHONDROGENESIS**

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**Introduction:** Glucocorticoids (GC) are used extensively in paediatric practice for their potent anti-inflammatory and immunosuppressive effects. However a principal side effect is growth retardation that is partly due to the inhibition of the endochondrial ossification process within the growth plate. Growth Hormone (GH) and Insulin-like growth factor-1 (IGF-I) are important regulators of bone growth and may ameliorate some of the GC effects. Objective: To assess the effects of Dexamethasone (Dex), GH and IGF-I on chondrocyte proliferation in the ATDC5 chondrocyte cell line. This line mimics the in vivo differentiation process of two key events in the chondrocyte life cycle: chondrogenesis and terminal differentiation (TD). Patients/Material and Methods: The ATDC5 cell line was fully characterised by gene profiling using RT-PCR in order to determine the onset of chondrogenesis and TD. Dex was added in quadruplicate to the cells at concentrations 10⁻⁷, 10⁻⁶ and 10⁻⁵ M during the chondrogenic (days 6-10) and the TD (days 10-15) periods. Alkaline phosphatase (ALP) activity (a marker of differentiation), cell number, proliferation rate and proteoglycan concentrations were determined at day 10 and day 15. In an attempt to reverse the effects of Dex on proliferation, GH and IGF-I (both 500 ng/ml) were added together with the GC. Results: RT-PCR analysis indicated that the cells expressed the GC and IGF-I receptor already from day 6 and the GH receptor from day 9. During chondrogenesis, Dex over the three concentrations caused a significant reduction in cell proliferation (28%), cell number (27%) and proteoglycan synthesis (47%) and increased ALP activity (106%); (p<0.01 in all), as compared to the control values. No significant effects were noted during TD. The addition of IGF-I completely reversed the anti-proliferative effects of Dex by significantly stimulating proliferation above control values (126%) (Table 1). Dex, GH and IGF-I together had the highest proliferation rate with a significant elevation (p<0.05) above IGF-I treatment alone. Conclusions: All significant effects of Dex occurred during the chondrogenic period. IGF-I stimulated cell proliferation and completely reversed and superceded the adverse Dex effects. Studies with this cell line will allow a more focussed approach towards studying the mechanisms underlying GC induced growth retardation as well as investigating the potential benefit of growth promoting therapy.

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**Oral Presentation 5: Growth: Basic**

**IGF-I ADMINISTRATION RESTORES HEART WEIGHT IN DEXAMETHASONE TREATED RATS ROLE OF THE UBIXIUTIN PATHWAY**

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**Introduction:** In skeletal muscle, proteolysis induced by glucocorticoids is mainly accomplished by the ubiquitin (Ub) pathway whereas IGF-I opposes this effect. Heart muscle is a striated muscle similar to skeletal muscle, but it is considered as more resistant to proteolysis in catabolic situations. Objective: To determine the role of the Ub pathway in heart during catabolic situations, we examined the expression of different components of this pathway in heart from dexamethasone (Dex) treated rats. Patients/Material and Methods: Two groups of 5 rats each were made catabolic by treatment with Dex (5mg/kg/day) for 3 days. One of these groups also received IGF-I 3.5mg/kg/day). A third group of 5 rats were not treated. Results: Dex caused a 12% loss of body weight compared to control rats, whereas IGF-I ameliorated it (6%). In Dex treated rats, heart weight was reduced by 9% compared to controls (0.710 ± 0.01 vs 0.783 ± 0.02 g, p<0.01). In Dex/IGF-I treated rats, heart weight was similar to controls (0.82 ± 0.04 vs 0.783 ± 0.02 g, p>0.05) and heavier than in Dex (p<0.001). Ub, Ub conjugating enzyme 14kDa (E2-14kDa), and proteasomal subunit C-3 mRNA were studied by Northern hybridisation. Ub mRNA was decreased by 32% in Dex and by 25% in Dex/IGF-I compared to control rats (p<0.01 respectively). E2-14kDa mRNA was increased by 155% in...
Dex (p<0.01) and by 58% in Dex/IGF-I treated rats when compared to controls. C-3 mRNA did not change in Dex but was decreased by 50% in Dex/IGF-I treated rats (p<0.05). Ubiquitinated proteins were studied by Western immunoblot from heart lysate. In Dex treated rats, some ubiquitinated proteins (above 30 kDa) were increased up to 4-fold compared to control rats. Treatment with Dex/IGF-I restored the ubiquitinated proteins to control levels. Protein levels of actin were decreased in Dex by 83% (11.2±4 vs 64.6±4 p<0.01) and almost totally restored them (29.7±8 vs 64.8±4 p<0.05). Immunoprecipitation of actin revealed that Dex caused a significant increase in ubiquitinated forms of actin compared to controls and IGF-I decreased them. Conclusions: We conclude that the lower heart weight in Dex group is associated with increased degradation of certain proteins through the ubiquitin pathway and that IGF-I opposes these effects. In heart, in contrast to skeletal muscle, Dex does not induce a dramatic increase of mRNAs encoding different components of the Ub pathway. This most likely indicates a more selective or limited activation of the Ub pathway in heart muscle.

**OR5-49** Oral Presentation 5: Growth: Basic

**INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 (IGFBP-3) INDUCES CDK INHIBITOR P21 TO REGULATE MUSESCHYNAL CHONDROGENIC STEM CELL PROLIFERATION**

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**Introduction:** Growth is the most sensitive and accurate marker of a child's health. Skeletal growth results from the condensation of mesenchymal chondrocytes that proliferate and differentiate and differentiate into articular and diaphyseal chondrocytes. The ordered proliferation and maturation from MSCC to chondrocytes, termed chondrogenesis, is regulated by the combined actions of different growth factors. Insulin-like growth factors (IGFs) seem to have a central role in chondrogenesis. Insulin-like growth factor binding protein-3 (IGFBP-3), well characterized as IGF carrier, has been reported to have intrinsic bioactivity that is independent of IGF binding. We have previously reported that IGFBP-3 has an IGF-independent antiproliferative effect on MSCC. Objective: Aim of the present study is to determine if the growth inhibitory effect of IGFBP-3 is through the CDK inhibitor p21. **Patients/Material and Methods:** We used RCJC.1C5.18 stem cells that in culture from MSCC stage understand spontaneously to terminal chondrocyte differentiation. We employed an IGFBP-3 mutant with abolished binding for IGFs termed GGG-IGFBP-3. MSCC were transfected with IGFBP-3 and GGG-IGFBP-3. Results: A remarkable morphological change of the cells transfected with IGFBP-3 and IGFBP-3 mutants was noted, cells detached and changed phenotype. IGFBP-3 and GGG-IGFBP-3 had a dramatic antiproliferative effect. Twenty-four hours after transfection, cells transfected with IGFBP-3 and IGFBP-3-3GGG mutant reached the lowest cell number compared with untransfected cells, respectively 30±5% of control for IGFBP-3 (p<0.01) and 10±3% of control for GGG-IGFBP-3 (p<0.01). To determine whether IGFBP-3 induced increased expression of p21 in RCJC.1C5.18 cells, we analyzed protein expression by Western blotting. The level of p21 in IGFBP-3 and GGG cells increased 24 hours after transfection by (more than 80% of control) compared with untransfected or transfected with empty vector. Conclusion: These results show a novel IGFBP-3 and GGG-IGFBP-3 independently of IGF-3. IGFBP-3 regulates the number of MSCC during the chondrogenesis process, inducing p21 protein expression.

**OR5-50** Oral Presentation 5: Growth: Basic

**LEPTIN AS A GROWTH FACTOR: REVERSES THE GROWTH LIMITING EFFECTS OF RESTRICTED CALORIC INTAKE**

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**Introduction:** Leptin, secreted by adipose tissue acting to curb appetite and increase energy expenditure as body fat stores increase. Various studies have shown that leptin also exerts its effects on sites other than the central nervous system such as hematopoietic precursor cells, hepatocytes and granulosa cells. Our group has recently shown that chondrocytes in the growth centers express leptin receptors. We have shown that leptin induces both proliferation and differentiation of chondrocytes thus acting as a direct skeletal growth factor under in-vitro conditions. We have demonstrated that leptin stimulates IGF-I and IGF-I-Receptor gene expression and abundance in the growth centers, and has a direct growth dependent effect on the endochondral ossification (IBMR In press).

**Objective:** To study the effect of leptin on an in-vivo model of endochondral ossification in mice. **Patients/Material and Methods:** Three groups of 21-day-old mice participated (n=6 each): a leptin group, which received recombinant leptin in a dose/weight dependent manner and was fed ad-libitum for 12 days and two control groups which were injected with vehicle only: one was fed ad-libitum and the other was pair-fed. Results: We found that leptin acts to limit caloric intake in the leptin group by 17% when compared to controls, and consequently resulted in a weight gain 9.7% short of the control group. The pair-fed group attained a similar weight gain, as did the leptin group. In spite of the marked differences in weight gain between the leptin and control mice fed ad-libitum, the tibial growth was the same in both groups. In contrast, the tibial growth of the pair-fed group, which reached the same body weight as the leptin group, was attenuated. The tibial length of the leptin treated mice was not significantly different from the tibial length in the control (ad-libitum) group. Tibial length in the leptin treated leptin fed group was 12.5% shorter than the pair-fed (caloric-restricted) group. **Conclusions:** Leptin reversed the effect of caloric restriction on growth thus preventing the growth deceleration observed in the caloric-restricted (pair-fed) group. This effect enabled the continued growth of skeletal bone despite a significant reduction in caloric intake. Based on our in-vitro and in-vivo experiments we assume that this effect is by mechanism by which leptin acts to regulate the interplay between caloric intake and growth.

**OR5-51** Oral Presentation 6: Fetal and Neonatal Endocrinology

**IN-VIVO STUDIES ON PLACENTAL VEGF EXPRESSION IN HUMAN PREGNANCIES COMPLICATED BY TWIN-TROTTERIA C. TAEKOANN**; R. TROLLMANN**; K. AMANN**; W. WENZEL**; W. RASCHER**; J. DOETSCH**

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**Introduction:** Vascular endothelial growth factor (VEGF) and its receptors have been found to be involved in paracrine adaptive mechanisms of utero-placental and fetal tissues to prolonged hypoxia/ischaemia. Objective: The purpose of our study was to investigate placental expression of VEGF and VEGF receptors in a prospective study. Methods: Placental tissue was collected from pregnancies complicated by pre-eclampsia and 15 gestational age-matched controls. Messenger RNA levels for VEGF and its receptors Flt-1 and KDR were measured by TaqMan RT-PCR. VEGF protein expression was determined by immunohistology. Results: In birth asphyxia associated with severe hypoxic-ischemic encephalopathy of the neonates (HIE grade 2-3, n=5), placental mRNA ratio VEGF/ß-actin (4.09±0.76) and KDR/ß-actin (0.56±0.08) were significantly above the values of mild asphyxia (HIE grade 0-1, n=15; 0.88±0.14, p<0.001, resp. 0.03±0.01, p<0.01) and controls (p<0.01 resp. p<0.05). Increased levels of VEGF/ß-actin (3.62±0.35), KDR/ß-actin (0.78±0.11) and Flt-1/ß-actin (0.1.37±0.38), were only present in pre-eclamptic and gestational age-matched controls compared to controls (1.83±0.17, p<0.01; 0.31±0.03, p<0.05; 0.04±0.01, p<0.01; resp.). VEGF expression could be confirmed by immunohistology. Conclusions: Our results imply that placental VEGF system is involved in local fetoplacental adaptive response to chronic as well as to acute perinatal placental hypoxia/ischaemia. In acute birth asphyxia, the degree of placental tissue hypoxia/hypogena seems to determine the up-regulation of VEGF.

**OR5-52** Oral Presentation 6: Fetal and Neonatal Endocrinology

**BPDZ 154 IS A NOVEL AND POTENT ACTIVATOR OF ATP-SENSITIVE POTASSIUM CHANNELS IN PANCREATIC -CELLS; IMPLICATIONS FOR HYPERINSULINISM**

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**Introduction:** Diazoxide is an agonist of ATP-sensitive potassium (KATP) channels in β-cells and since it inhibits insulin release is used in the treatment of hyperinsulinism caused by insulinomas or Hyperinsulinism in Infancy (HI). However, the responsiveness of patients to diazoxide is variable and complicated by severe hypoglycaemia including fluid retentions, hypoglycaemia, thrombocytopenia, hypotension, facial changes and generalized hypertrophic.
**OR6-54 Oral Presentation 6: Fetal and Neonatal Endocrinology**

**GLUCOCORTICOIDS MODULATE THE EXOCRINE/ENDOCRINE BALANCE DURING PANCREATIC DEVELOPMENT BY DECREASING PDX-1 AND INCREASING PTF1-P48**

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**Introduction:** Intra-uterine undernutrition impairs pancreatic beta cell development in rats. We have previously shown that this impairment is, at least partly, due to fetal glucocorticoid overexposure. **Objective:** The aim of this work was to understand the cellular and molecular mechanisms by which glucocorticoids (GC) alter pancreatic differentiation We hypothesized that the effects of GC might be mediated by the factors controlling pancreatic development. Pancreatic precursor cells express the transcription factor Pdx-1. During exocrine cell differentiation, the pancreatic precursor cells stop to express Pdx-1 but express the transcription factor PTF1-p48. The expression of Pdx-1 and PTF1-p48 was studied by semi-quantitative duplex radioactive RT-PCR. Insulin-Pdx1 positive cells numbers or amylase-positive cell area were quantified by immunohistochemistry. **Results:** A significant 35% decrease in Pdx-1 mRNA levels was observed in E15 pancreatic buds treated in vitro with DEX, whereas the mRNA levels of PTF1-p48 were increased by 500%. In line with these results insulin-Pdx1-positive cell numbers were decreased by 35% whereas amylase-positive cell area was increased by 200% under the same experimental conditions. These results suggest that GC favour the differentiation into exocrine cells and inhibit endocrine cell differentiation. To investigate whether GC could also inhibit Pdx-1 expression in differentiated beta cells, various models of exposure to GC were used: fetal or adult islets treated in vitro with 100mM Dexamethasone (DEX). The expression of Pdx-1 and PTF1-p48 was studied by semi-quantitative duplex radioactive RT-PCR. Insulin-Pdx1 positive cells numbers or amylase-positive cell area were quantified by immunohistochemistry. **Conclusions:** In conclusion, these results highlight the importance of glucocorticoids in controlling the exocrine/endocrine balance during pancreas development. Overexpression to glucocorticoids during fetal life impairs beta-cell development while favouring exocrine cell development by selectively decreasing Pdx-1 and increasing PTF1-p48 mRNA levels. The effects of glucocorticoids on Pdx-1 in mature islets may suggest an additional negative effect on beta-cell function.

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**OR6-55 Oral Presentation 6: Fetal and Neonatal Endocrinology**

**PROGRAMMING OF ADULT METABOLISM BY DEXAMETHASONE IN UTERO PERSISTS INTO THE NEXT GENERATION IN RATS; A NON-GENETIC MECHANISM FOR THE INHERITANCE OF LOW BIRTHWEIGHT**

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**Introduction:** Epidemiological studies demonstrate a link between low birthweight and later hypertension, glucose intolerance and cardiovascular disease. These effects may extend into subsequent generations, with evidence for a multi-generational effect on birthweight. Two major hypotheses propose that early life events retarding fetal growth can ‘programme’ subsequent cardiovascular development or alternatively, that genes determining low birthweight also determine cardiovascular risk. **Objective:** To investigate whether glucocorticoids during fetal life impair beta-cell development, the administration of dexamethasone to pregnant rats reduces Pdx-1 expression and increases PTF1-p48 expression. The effects of glucocorticoids on Pdx-1 in mature islets may suggest an additional negative effect on beta-cell function.

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**OR6-53 Oral Presentation 6: Fetal and Neonatal Endocrinology**

**TRANSACTIVATION PROPERTIES OF NATURAL ANDROGEN RECEPTOR GENE MUTATIONS IN TRANSFECTED HUMAN GENITAL FIBROBLASTS**

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**Introduction:** Androgen receptor (AR) mutations resulting in androgen insensitivity syndrome (AIS) have usually been characterized by investigation of androgen-induced activation of reporter genes in transiently transfected cell lines. However, several groups reported striking discrepancies between a severe AIS phenotype and considerable function of mutant AR-constructs in vitro (e.g., Val866Met). We hypothesized that this discrepancy may in part be due to the artificial character of the used immortalized cell lines (e.g., COS1, CHO). Human genital fibroblasts are ontogenetically derived from the urogenital mesenchyme and may provide a relevant co-factor environment with respect to androgen actions in sexual differentiation. **Objective:** Development of a genital fibroblast-based model for investigation of the influence of the androgen receptor and its natural mutations on reporter gene activation **Patients/Material and Methods:** Normal 46, XY genital skin fibroblasts expressing a normal AR were transiently transfected with androgen responsive (ARE)2TATA-Lux or MMTV-Luc reporter genes in either the presence or the absence of a co-transfected wild-type AR (pSVAR). In addition, AR-negative genital fibroblasts of gonadal origin were derived from a CAIS-patient with no AR-expression because of a deletion in exon 1 leading to a frameshift and a premature stop codon. Either pSVAR0 or AR-plasmids containing the mutations Leu172stop, Arg774Cys (CAIS), Leu172Phe (PAIS), or Val866Met (CAIS) were co-transfected in addition to the reporter genes. We investigated whether this discrepancy may in part be due to the artificial character of the used immortalized cell lines (e.g., COS1, CHO). Human genital fibroblasts are ontogenetically derived from the urogenital mesenchyme and may provide a relevant co-factor environment with respect to androgen actions in sexual differentiation. **Objective:** Development of a genital fibroblast-based model for investigation of the influence of the androgen receptor and its natural mutations on reporter gene activation. **Results:** The transactivation efficiency of the reporter constructs was measured by luciferase reporter activity. The transfected wild-type AR constructs were transcribed and translated in the presence of the androgen receptor and its natural mutations on reporter gene activation. **Conclusions:** Our data suggest that genital fibroblasts of gonadal origin were more effective than diazoxide in those patient tissues where KATP channel activity was more effective than diazoxide in vitro. With improved selectivity for functional defects in KATP channels, these types of compounds may be used to treat KATP channel dysfunction.
maternal weight gain or litter number; gestation was 1 day longer in FIDEX mothers (p<0.01). F2DEX offspring had reduced birthweight (5.65±0.06g, n=108; 6.0±0.06g, n=101, p<0.001) and elevated liver PEPCK (2.55±0.3; 1.48±0.07 nmol/min/mg protein, p<0.001). Conclusions: Thus, low birthweight and elevated PEPCK activity are transmitted to a second generation after in utero dexamethasone treatment. Human and animal studies suggest that fetal glucocorticoid overexposure may be a key mechanism linking maternal environment, fetal growth and later cardiovascular risk. These results link this environmental mechanism with intergenerational inheritance, suggesting that an adverse in utero experience in one generation may masquerade as an inherited/genetic predisposition to cardiovascular disease in subsequent generations.

OR7-56 Oral Presentation 7: Small for Gestational Age (SGA) GAMMA-GLUTAMYLTRANSFERASE (GGT) LEVELS IN FORMERLY SMALL FOR GESTATIONAL AGE (SGA) INFANTS

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Introduction: Serum GGT levels are raised in NIDDM patients, and a strong relation with central obesity has been described. Objective: To examine the association between serum GGT and intrauterine growth and glucose tolerance in adolescents. Patients/Material and Methods: The relationship between GGT, body composition, lipid parameters, glucose metabolism, and uric acid (UA), was studied in 90 adolescents [30 former SGA children with IGT, 30 former SGA children with normal glucose tolerance (NGT), and 30 controls; age range 14.2-21.6 years, all Tanner stage V]. Results: Mean serum GGT was higher in SGA+IGT children compared with the controls (0.25±0.12 and 0.24±0.22 vs. 0.16±0.06 nmol/L*sec, each P<0.05). Adolescents with IGT were characterized by the highest body mass index and by higher percent body fat as well as by higher serum concentrations of UA and lipoprotein(a), and showed the strongest correlation between GGT and birth weight (BW; r= -55), waist-to-hip ratio (WHR; r= 60), UA (r= 70), and low-density lipoprotein cholesterol (LDL-C; r= -55), each P<0.001. For the whole group multiple step forward regression analysis demonstrated that age (partial r= -29, P<0.01), BW (partial r= -31, P<0.01), WHR (partial r= -24, P<0.05), sum of skinfolds (partial r= 26, P<0.05), triglycerides (partial r= -29, P<0.05), LDL-C (partial r= -22, P<0.05), and UA (partial r= -29, P<0.01), were independently associated with GGT (R2= 69, P<0.001).

Conclusions: Serum GGT levels are higher in former SGA subjects independent of glucose tolerance and do correlate positively with estimates of body fatness, and inversely with birth weight, suggesting that the deterioration of metabolic variables found in patients with NIDDM is already present in former SGA infants with IGT.

OR7-57 Oral Presentation 7: Small for Gestational Age (SGA) COMBINED EFFECT OF GENETIC AND ENVIRONMENTAL FACTORS ON THE INSULIN RESISTANCE (IR) ASSOCIATED WITH INTRA-UTERINE GROWTH RETARDATION (IUGR)

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Introduction: Insulin sensitivity measured in subjects born with IUGR shows scattered values, reflecting a variable susceptibility to IR. Genetic polymorphisms of key molecules of the adipose tissue are known to influence the expression of the IR syndrome in Humans. Objective: We investigated whether the PPAR-gamma2/Pro12Ala and the beta3 adrenergic receptor (ADRB3)Trp64Arg gene polymorphisms might affect the IR associated with IUGR. Patients/Material and Methods: Gene polymorphisms were genotyped in 1777 normal born subjects (BW<3rd perc) and 231 age-matched controls (25thperc. BW<75th perc) who underwent an OGTT at the age of 20 years. Results: As previously demonstrated, the IUGR group showed significantly higher serum insulin concentrations than controls both at fasting and after stimulation, whereas no significant difference in serum glucose concentrations was observed. The frequencies of the PPAR-gamma2 Ala allele (0.22) and the ADRB3 allele (0.11) were not different between the two groups. In neither group did the polymorphisms affect current body size or glucose tolerance. Effects of the two polymorphisms on IR indices after adjustment for BMI and gender are summarized on the following table. The ADRB3 polymorphism was associated with IR indices in the two groups but in opposite ways: the Arg variant was associated with a significantly higher insulin excursion under OGTT in the IUGR group while an inverse association was observed in controls. Insulin excursion was also increased in the IUGR-born subjects who carried the PPAR-gamma2/Ala variant whereas no significant effect was observed in controls.

These effects on IR indices were significantly potentiated by BMI in the IUGR group. Conclusions: Our data exemplify the strong interaction between intrauterine environmental and genetic factors in the development of the IR associated with IUGR. They also point to the key role of adipose tissue in this association, including during the post-natal period.

| ADRB3  | Trp/Trp | Arg/Carrier | Control (n=231) |
|--------|---------|-------------|----------------|
| Fastiging insulin (µU/mL) | 5.63±8.4 | 8.2±0.04 | 5.0±0.22 | 4.6±0.22 | ns |
| AUC insulin (10±3; µU/mL/ml-1) | 4.30±0.4 | 7.5±4.7 | 0.05 | 3.7±2.0 | 0.05 |
| PPARGamma2 | Pro/Pro | Ala carrier | Pro/Pro | Ala carrier | p |
| Fastiging insulin (µU/mL) | 5.74±4.7 | 6.5±4.6 | 0.01 | 5.0±2.3 | 4.9±1.9 | ns |
| AUC insulin (10±3; µU/mL/ml-1) | 5.40±5.5 | 6.5±3.0 | 0.03 | 4.5±2.2 | 4.4±2.6 | ns |

OR7-58 Oral Presentation 7: Small for Gestational Age (SGA) PREDICTION OF RESPONSE TO GROWTH HORMONE (GH) IN SHORT CHILDREN BORN SMALL FOR GESTATIONAL AGE (SGA) (N = 682): ANALYSIS OF DATA FROM KIGS

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Introduction: GH treatment is emerging as a suitable mode of treatment for short stature in children born small for gestational age (SGA). Since prediction models are useful tools in children with GHD and Turner Syndrome (TS) on GH, prediction models were developed for these diagnoses. Objective: Patients/Material and Methods: The following data of SGA children (N=448) from KIGS (Pharmacia International Growth Database) and from Pharmacia treatment trials (234) were analyzed: birth weight (BW) = -1.28 SD for GA; GA >30 wks; prepubertal; onset of GH <10 (f)/<12 (m) yrs. Statistical analysis and prediction modelling as reported previously (1, 2). Results: Median values [100% ranges] in N = 682 (m = 67%) of 1st year patients were: BW (SDS) = -2.5 [-4.0/-1.6]; MPH (SDS) = -2.7 [-4.0/-1.9]; GH dose = 0.04 mg/d [0.02/0.07]; HTvel before GH therapy (cm/yr) = 4.8 [3.2/6.7]; HTvel during 1st year of treatment = 8.4 [6.2/11.2]. (N = 68 used for validation had identical data.) 52% of the variability was explained with an error SD of 1.3 cm/yr with the model: HTvel (cm/yr) = 9.4 + [0.31 x age start (yrs)] + [0.30 x weight start (SDS)] + [56.5 x GH dose (mg/kg/d)] + [mid-parent height (SDS)]. The ranking predictors in order of their importance were: GH dose > age > weight > MPH. Standardised residual (observed HTvel - predicted HTvel): error SD in the validation group was -0.18 +/- 1.0, thus not different from zero. Second year prediction models (data not shown) were also developed. Conclusions: The 4-parameter prediction model is similar to models for GHD and TS with respect to the high numbers investigated, high degree of explained variability, small prediction error, being validated with an independent cohort and utilizing readily available and standardized variables including treatment modalities. GH dose is the most important predictor, followed by age at GH start. Sensitivity to GH ranking is GHD > SGA > TS. (1) Rankel et al. (1999) J Clin Endocrinol Metab 84: 1174-1183 (2) Ranke et al. (2000) J Clin Endocrinol Metab 85: 4212-4218
OR7-59 Oral Presentation 7: Small for Gestational Age (SGA)
HIGH DOSE GH TREATMENT IN CHILDREN WITH SHORT STATURE DUE TO INTRA-UTERINE GROWTH RETARDATION (IUGR) BEFORE THE AGE OF FIVE: A FRENCH COLLABORATIVE STUDY
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Introduction: GH treatment improves height velocity and height SDS in children with short stature due to intra-uterine growth retardation (IUGR). Among several questions that remain to be answered, the age at which the treatment should be started for the best outcome is important. Objective: This is a 2-study, in which short-height (≤2SDS) children with IUGR (birth length (BL)(2SD for gestational age (GA)) aged from 2 to 4 years are treated with high doses of rhGH(0.6mg/kg/ week, Granidipen® 5mg liquid formulation administered daily (sc) with Grandipen® BD) during 3 years and then stratified in a treated or untreated group (randomization at entry). The preliminary results of the first part of the study are reported here.

Patients/Material and Methods: 47 patients (23 boys, 24 girls) have been already included in this study and have achieved the first year of GH treatment. 17 of them have achieved the second year of GH treatment. Results: Their mean GA was 37.6±3.5 weeks, their mean BL 43.3±8.0 cm, -3.1±1.5SDS/GA. Their mean CA at inclusion was 3.8±0.9 years. Auxological characteristics (target height (TH), height velocity (HV), height (H), bone age (BA)) and plasma IGF1 levels (ng/ml) at baseline and during GH treatment are reported below. (*p<0.001 vs baseline values levels (ng/ml) at baseline and during GH treatment are reported below. (*p<0.001 vs baseline values levels (ng/ml) at baseline and during GH treatment are reported below. (*p<0.001 vs the preceding year) We observed a dramatic increase in HV during GH treatment, leading to a mean gain of HSDS of 2.7±1.07 after 2 years of treatment. In parallel, mean IGF1 levels significantly increased, slightly above the normal range. No excessive bone maturation was observed. Conclusions: This is one of the most important gain in HSDS observed among the several studies published up to now, probably due to the young age of the patients.

OR7-60 Oral Presentation 7: Small for Gestational Age (SGA)
PUBERTY IN CHILDREN BORN SMALL FOR GESTATIONAL AGE (SGA) DURING GROWTH HORMONE (GH) TREATMENT
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Introduction: In several studies, children born SGA are treated with growth hormone (GH). Important determinations of final height are the height and age at start of puberty and the magnitude of pubertal growthspurt. There are no data about pubertal in GH treated children born SGA. Objective: To investigate the pubertal in GH treated children born SGA. Patients/Material and Methods: 79 prepubertal short children (25F/50M) with birth height and birth weight < -2SDS, with a height < -2SDS. Patients were randomly and blindly assigned to either one of two GH dosage groups (1 vs 2 mg/m2 body surface/day – 0.035 vs 0.07 mg/kg/day). Height, weight and pubertal stage were measured every three months. Pubertal spurt (start puberty till final height (FH)) and duration of puberty were determined in 45 children who had attained FH. The results were compared to normal Dutch children1. Results: Age and height at onset of puberty and duration of puberty were not significant different between the two groups. In both groups height SDS was significantly different from zero. Boys receiving 2 mg had a significantly higher pubertal spurt compared to boys receiving 1 mg. Conclusions: GH treatment, regardless of dose had no significant influence on pubertal onset and duration of puberty in children born SGA compared to the normal population. GH dose had a significant influence on the pubertal spurt in males born SGA.1 Frederiks et al.,(2000) pediatric research 47:316

OR8-61 Oral Presentation 8: Neuroendocrinology and Puberty
PREPUBERTAL ACCELERATION OF PULSATILE GONADOTROPIN RELEASING HORMONE (GNRH) SECRETION IN VITRO BY GHRELIN
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Introduction: Body weight and energy balance are tightly regulated through hypothalamic neuropeptides with peripheral feedback from leptin and the recently discovered Ghrelin. Using explants of the prepubertal male rat hypothalamus, we have previously shown that leptin could increase pulsatile GnRH secretion through some anorectic signals (CART, α-MSH and CRH). Objective: Since Ghrelin has orexigenic effects mediated by NPY-containing neurons, which project to orexin and melanocortin hormone (MCH)-containing neurons, we hypothesized that Ghrelin could affect GnRH secretion with the involvement of such neuropeptides.

Patients/Material and Methods: Retrochiasmatic hypothalamic explants from 15-day-old male rats, the mean ± SD GnRH interpulse interval (60 ± 4 min) whereas the NPY Y5-receptor antagonist alone had no effect. Incubation using 10-7 M of Orexin A or 10-7 M of NPY Y5-receptor selective antagonist (58 ± 4 min). We have shown previously that NPY could accelerate pulsatile GnRH secretion in prepubertal male rat via the Y5-receptor subtype. Using hypothalamic explants, the decrease in GnRH interpulse interval caused by Ghrelin was totally prevented by 10-6 M of a NPY Y5-receptor selective antagonist (58 ± 3 min) whereas the NPY Y5-receptor antagonist alone had no effect. Incubation using 10-7 M of Orexin A or 10-7 M of MCH had no effect on the GnRH interpulse interval (60 ± 60 ± 3 ± 0 min, respectively). Conclusions: We conclude that the new peripheral hormone, Ghrelin, is involved in the stimulatory regulation of GnRH secretion in the prepubertal hypothalamus, together with leptin. The signaling pathway mediating Ghrelin effects could involve NPY whereas Orexin A and MCH do not seem to be involved. Supported by the Belgian Study Group for Pediatric Endocrinology, the Belgian FRSM and the Faculty of Medicine at the University of Liège.

Data at onset of puberty and pubertal spurt are expressed as mean (SD), duration of puberty is expressed as median (interquartile range).

| Age (yrs) | Height (cm) | Height SDS | BMI (SDS) | Bone age (GUS yrs) | Duration GH therapy (yrs) | Pubertal spurt (cm) |
|----------|-------------|------------|-----------|-------------------|--------------------------|---------------------|
| 12±0.6   | 144.7±7.8   | -1.0±0.6   | -0.5±1.3  | 12±2.7            | 4.5±2.2                  | 4.0±2.5             |

* Significant lower than zero p<0.05 * 1 mg vs 2 mg p<0.023

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HORMONE AND ENDOCRINE REGULATION OF PULSATILE GONADOTROPIN RELEASING HORMONE (GnRH) SECRETION IN VITRO BY GHRELIN
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**OR8-62** Oral Presentation 8: Neuroendocrinology and Puberty

**NOVEL INACTIVATING GNRH RECEPTOR MUTATION ALA171THR IMPEDES CONFORMATIONAL MOBILITY OF TRANSMEMBRANE DOMAINS 3 AND 4**

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**Introduction:** Sequential ligand binding and conformational mobility are critical in the activation of rhodopsin-like G-protein coupled receptors (GPCR), including the gonadotropin releasing hormone receptor (GnRH-R). **Objective:** Here we identify and characterize the novel inactivating GnRH-R mutation Ala171Thr located in transmembrane helix 4 (TMH4), clinically causing familial hypogonadotropic hypogonadism. **Results:** Patients/Material and Methods: The human embryonic kidney 293T cells transiently expressing the Ala171Thr mutant GnRH-R showed a lack of hormone-induced phospholipase C activity in vitro. In cells transfected with Ala171Thr GnRH-R complementary DNA, specific receptor binding of [125I]labeled GnRH agonist was undetectable. Mutations in the Ala171Thr codon 612.0x792.0

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**MUTATIONS IN THE GONADOTROPIN-RELEASING HORMONE RECEPTOR GENE IN A GERMAN GIRL WITH HYPOGONADOTROPIC HYPOGONADISM**

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**Introduction:** Congenital hypogonadotropic hypogonadism (HH) is a genetically heterogeneous condition characterized by a functional deficit in hypothalamic GnRH production or action. **Objective:** To elucidate the pathogenesis of hypogonadotropic hypogonadism and the molecular basis underlying the severity of the disease. **Patients/Material and Methods:** The 16.6 year-old girl was referred to our department for investigation of primary amenorrhea. She had spontaneous thelarche and pubarche at about 13 years. On physical examination she presented with breast development at Tanner stage 3 and pubic hair at Tanner stage 3 and normal olfaction. Hormonal evaluation revealed decreased gonadotropins (FSH 0.1 U/l, LH 0.0 U/l) and a prepubertal growth pattern. Intra-operative examination revealed decreased GnRH-binding and prevented GnRH induced stimulation of inositol phosphate accumulation in vitro.

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**DOMINANT AND RECESSIVE INHERITED MUTATIONS IN THE MC4R GENE IN PATIENTS WITH SEVERE EARLY-ONSET OBESITY**

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**Introduction:** Mutations in the MC4 receptor (MC4R) gene are the most frequent monogenetic causes of severe obesity occurring in 3 - 5% of studied obese populations. In contrast to mutations in other related genes the mode of inheritance has been described as autosomal dominant for most MC4R mutations but the molecular mechanisms of this phenomenon is still unclear. **Patients/Material and Methods:** We examined 62 patients with severe early-onset obesity. All endocrine functions in these patients were normal despite elevated leptin levels. **Results:** We were able to detect 2 heterozygous (Y35X, D90N) and one recessive homozygous mutation (C271R) in three unrelated families. Functional characterization wild-type mutant receptors were transiently transfected into COS-7 cells and cAMP accumulation as well as binding properties were investigated. The Y35X mutation resulted in a truncated receptor with a complete loss-of-function and lacks any cell surface expression. For the D90N mutant a slight increase in cAMP accumulation after agonist challenge could be detected. Interestingly, this mutation is highly expressed on the cell surface. The C271R mutant responded to agonist stimulation but maximal cAMP response is reduced compared to the wild-type receptor and EC50 values are shifted towards higher agonist concentrations. The complete or nearly complete loss-of-function of the two heterozygous mutations (Y35X and D90N) would suggest that an autosomal dominant mode of inheritance would be caused by a gene dosage effect. In contrast to these mutations, the partial loss-of-function of the homozygous C271R mutation is in accordance with an autosomal recessive mode of inheritance. **Conclusions:** Further investigation of mutant MC4-R will provide more insight into the receptor function and thereby body weight regulation.

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**MODULATION OF IGF-I EXPRESSION IN HYPOTHALAMIC NEUROPEPTIDES (GH) AND GHRP-6**

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**Introduction:** Growth hormone-releasing peptides (GHRPs) are synthetic molecules that stimulate growth hormone (GH) secretion from the anterior pituitary, acting directly at the level of the pituitary, as well as on the hypothalamus. The mechanism of action of these peptides begins with the recognition of a specific membrane receptor whose ligand, ghrelin, has recently been discovered. However, the transduction pathways of GHRPs are largely unknown. Both GH and GHRPs can stimulate IGF-I production in specific areas of the brain, including the hypothalamus, although the physiological meaning of this remains to be clarified. **Objective:** The purpose of this study was to analyze the mechanism of action of GHRP6 and GH on hypothalamic neurons and to establish a new model to study signal transduction in the hypothalamus. **Patients/Material and Methods:** Two fetal hypothalamic neuronal lines were used: RACA6 and RCF 8. Cell cultures were treated with GHRP-6 or GH. At different time-points, total RNA was isolated and IGF-I measured by RT-PCR analysis. **Results:** GHRP-6 increased IGF-I mRNA levels in a dose-dependent manner in Raca6 cells, with the maximum effect occurring at a dose of 25 µg/ml and after 4 hrs of treatment. GH also increased IGF-I levels in these cells. Neither GHRP-6 nor GH had an effect on IGF-I levels in RCF8 cells, which could be because RC8 cells do not have receptors for these ligands. **Conclusions:** GHRP-6 and GH modulate IGF-I expression in Raca6 cells, but not in RCF8 cells. The cell line Rca 6 appears to be a good model to study GHRP and GH signal transduction in hypothalamic neurons. RCF8 cells are a good negative control for these studies and may be useful for the study of signal transduction by other factors.

**OR8-66** Oral Presentation 9: Glucose Metabolism

**CONTINUOUS SUBCUTANEOUS INSULIN INFUSION THERAPY IN CHILDREN AND ADOLESCENTS WITH TYPE I DIABETES MELLITUS: IDENTIFICATION OF FACTORS CONTRIBUTING TO OUTCOME**

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**Introduction:** Currently there is increased demand to use continuous subcutaneous insulin infusion (CSII) therapy in the management of type I diabetic patients. The potential advantages that make CSII popular include better metabolic control, flexible lifestyle, fewer injections and less hypoglycemia. On the other hand it is still not very well known to what extent these expectations are fulfilled in children and adolescents. **Objective:** The aim of the study is to compare the efficacy and safety of two insulin treatment regimens, CSII and multiple daily injections (MDI) of insulin in a large group of children and adolescents with type I diabetes and to identify the factors that contribute to outcome. **Patients/Material and Methods:** Eighty-eight patients who had requested & completed at least 6 months of CSII therapy and one hundred and seventy-six age, sex, diabetes duration, HbA1c and BMI matched patients on MDI therapy were included. Glycemic control and adverse event rates were compared for the prior MDI period with CSII therapy period in the study group.
and also between study group and control group. **Results:** CSII therapy decreased HbA1c levels significantly at 3 and 6 months of therapy (7.6±0.9 vs 7.4±0.9 vs 7.4±0.5, respectively) with improving BMI and frequency of hypoglycemia and frequency of DKA. Also compared to the control group on MDI therapy, the CSII group had significantly lower HbA1c levels during therapy. To identify the factors that are contributing to outcome the study group was divided into 3 subgroups; improved, unchanged and worsened based on a 0.5% change from baseline HbA1c levels. The HbA1c levels were similar at baseline in all 3 subgroups, however the worsted group had significantly higher HbA1c levels 3 months prior to CSII than the other subgroups and this improved prior to the start of CSII. To assess the effect of age, glycemic control was compared in patients younger & older than 15 years. The older group had significantly higher HbA1c levels than the younger group prior to CSII. A pre-existing HbA1c level of 8.25% and higher predict patients who worsen by 0.5% or more at 3 months with 70% sensitivity and 69.9% specificity. No relation was found between outcome and other parameters such as duration of diabetes, gender or BMI. **Conclusions:** Almost half of patients improved metabolic control as reflected by 0.5% decrease in HbA1c levels by switching from MDI to CSII therapy, but the remained did not. Previous glycemic control can predict the outcome of SCII therapy. Identifying those most likely to benefit from this therapy will aid in appropriate allocation of his expensive source.

**INTRODUCTION**

**Objective:** To examine the influence of inheritance on insulin requirement in children and adolescents with type 1 diabetes. **Patients/Methods:** As part of a national prospective quality study of childhood diabetes in Norway 445 type 1 diabetes patients were included from eight hospitals in the eastern part of the country. The prevalence of type 2 diabetes in their first and second degree relatives was recorded together with clinical data. Mean age was 13.0 years (range 2-20 years), mean diabetes duration 5.5 years (range 1-17 years), mean HbA1c 8.63% (reference range 4.1-6.4%). **Results:** The total daily insulin requirement was significantly higher in the patients with first and second degree relative with type 2 diabetes (n=94) than in the patients without such relatives (n=351). 1.04 ±0.35 U/kg bodyweight vs 0.94 ±0.38 U/kg (p<0.05). By multiple regression analysis the following factors were independent predictors of insulin requirement: duration of the disease (p<0.01), body weight (p<0.01), blood glucose control estimated by HbA1c (p<0.05), and heridity of type 2 diabetes (p<0.05). Herity of type 2 diabetes explained 14% of the variation in daily insulin requirement per kg bodyweight. In the regression analysis an increase of one year in the duration of the disease resulted in an increase of 0.024 U/kg in the insulin requirement, an increase of 10 kg body weight an increase of 0.034 U/kg, an increase of 1% HbA1c an increase of 0.038 U/kg and heridity of type 2 diabetes an increase of 0.08 U/kg. **Conclusions:** Herity of type 2 diabetes is an important predictor for insulin requirement in children and adolescents with type 1 diabetes mellitus. This may indicate inheritance of insulin resistance in these patients.
Expression of the Insulin Receptor-Related Receptor, the Insulin Receptor and the Neurotropin Receptors TrkA and P75 is Differentially Regulated by Glucose and by B-Cell Toxic Cytokines (IFNg and IL-1b) in INS-1 B-Cells

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Introduction: The insulin receptor-related receptor (IRR), a member of the insulin receptor tyrosine kinase family, has structural and functional homology with the insulin receptor (InsR). In contrast to InsR, expression of the IRR is largely reduced to NGF-sensitive neurons, and outside neuronal tissues to renal tissue or pancreatic b-cells. In neurons, the IRR is strictly co-expressed with the high affinity NGF-receptor (TrkA). Until now, the ligand, gene regulation and biological function of the IRR are largely unknown.

Objective: To assess mRNA expression of IRR in relation to InsR, TrkA and as controls to receptors of the insulin- or the NGF-receptor families. To stimulate differential expression we selected extreme glucose levels, that induce b-cell apoptosis, and the cytokines IFNg and IL-1b that strongly induce apoptosis via their own specific signaling pathways.

Patients/Material and Methods: Apoptosis in INS-1 b-cells was quantified by FACS analysis of annexin-V / PI positive cells and of UTP-FITC-positive cells (TUNEL-method). For expression analysis, INS-1 cells were stimulated for 24 hrs with several glucose levels, IFNg (10 ng/ml), IL-1-b (10 ng/ml), or TNFa (10 ng/ml). RNA of three independent stimulations was isolated, cDNA templates for RT-PCR were adjusted to equal b-actin expression levels for further semi-quantitative expression analysis.

Results: Apoptosis of INS-1 cells was strongly induced by low (25 mg/dl) and high (1000 mg/dl) glucose levels, compared to glucose levels of 250 mg/dl. When added to 250 mg/dl glucose, IFNg and IL-1b both strongly induced apoptosis. INS-1 b-cells expressed all receptors within the insulin-receptor family (IRR, InsR, IGF-IR, IGF-II/M6PR) and the NGF-receptor family (TrkA, TrkB, TrkC, p75). Low glucose (25 mg/dl), highly stimulated mRNA expression of IRR (3.7 ± 0.6), TrkA (2.5 ± 0.2) and InsR (5.5 ± 0.7) compared to 250 mg/dl glucose. In contrast to this, cytokines differentially influenced expression patterns of these receptors. IL-1b suppressed IRR (0.31 ± 0.13) but stimulated InsR (4.6 ± 1.0) expression, while IFNg suppressed expression levels of TrkA (0.21 ± 0.15) but not of IRR or InsR.

Conclusions: Expression of IRR at the mRNA-level is co-regulated with the InsR or the TrkA by withdrawal of glucose (25 mg/dl) but is differentially regulated by IFNg or IL-1b, respectively. INS-1 cells could serve as a model to study physiological expression and regulation of the IRR and related receptors of both the insulin and NGF receptor family.