Similar to last year Endocrine Meeting, in the No. 5 issue of Endocrinologia Pediatrica On Line, in this issue we will make an effort to review and comment some of the most interesting aspects of the 88th Meeting of the Endocrine Society, Boston 2006, the most relevant professional international event in this field. The venue was the Boston Convention and Exhibition Center, Boston, Massachusetts, USA, Saturday 24-27, 2006. The Meeting included 16 Plenary Lectures, 74 Symposia (3 speakers in each one) including basic, basic/clinical or translational, and clinical subjects, 84 Meet the Professor Sessions, 54 Oral Presentations (6 subjects in each one) adding up to 324 Oral Free Communications, 90 Poster Sessions (2702 Poster presentations), divided in 41 Basic Endocrinology, 12 Basic/Clinic or Translational, and 37 Clinical Sessions. There also were 8 Controversial Subject Debates, 9 Update Sessions, 3 New Technology Conferences, and 5 Workshops. Furthermore, over 200 companies exhibited to display a wide range of products of interest to researchers and clinicians. Moreover, several Ancillary Symposia and Satellite Meetings were held around the central Meeting. The number of subjects registered was approximately 8800.

The home site of the Society is [www.endo-society.org](http://www.endo-society.org)

As it happened last year, to review all aspects of this huge Meeting, including many simultaneous sessions, is an impossible task. The Editorial Committee can answer specific questions and the visitors of this site are encouraged to consult the Abstracts, which are are available at the official site of the Endocrine Society ([www.abstracts2view.com/endo](http://www.abstracts2view.com/endo)). Free registration is available (and required) to read the abstracts/talks.

In the Opening Ceremony, the President of the Society, Dr. Andrea Dunaif, made comments on two important aspects of her Presidency, one, her efforts in public policy advocacy for public and media awareness of the importance of endocrinology on health and scientific advance, relating endocrinology to the obesity epidemic and widespread type 2 diabetes; two, the publication of clinical guidelines, thus promulgating excellence in clinical endocrine practice. During the Meeting, two of these guidelines were presented: “Evaluation and Treatment of Adult Growth hormone deficiency” and “The Use of Testosterone Therapy in Adult Men with Androgen Deficiency Syndromes”; already available. They were developed in the last year by special task forces on each topic and were recently published in the May/June 2006 edition of The Journal of Clinical Endocrinology and Metabolism.

ENDO 2006 covered four broad themes: a) the endocrine brain, b) endocrine controversies, c) metabolic syndrome & growth factor signaling, and c) stem cell & transplantation.
ESTROGEN & CVD.

Michael E Mendelsohn, Molec Cardiol Res Inst, Tufts-New England Med Ctr, Tufts Univ Sch of Med, Boston, MA

The heart and blood vessels express functional estrogen receptors (ERs), and significant progress has been made over the past decade in understanding the importance of ERα and ERβ in cardiovascular physiology and disease. In this presentation, data regarding the specific role of ERs in cardiovascular physiology and pathophysiology will be reviewed and current controversies will be highlighted. Potential molecular and cellular explanations for the controversy that has arisen from the Women’s Health Initiative (WHI) trials of the cardiovascular effects of HRT on CVD will be discussed, with an emphasis on the relationship between the timing of HRT initiation and differences in the underlying vascular biology that exist between perimenopausal and older women. Several SSHR signaling concepts with implications for cardiovascular physiology that have emerged recently also will be discussed. These include the role of the ER co-regulatory protein SRC3 in vascular biology, rapid (‘non-genomic’) activation of vascular endothelial nitric oxide synthase by E2-ER, ligand-independent ER activation in vascular cells, gender differences in regulation of metabolic pathways important to cardiovascular diseases by other NHR like PPARγ and the LXRα, and genetic ER variants associated with altered cardiovascular risk in both sexes. The ways in which these newer pathways involving ERs can add substantial combinatorial complexity to the physiological effects of estrogen in target cardiovascular tissues will be highlighted.

DIABETES, OBESITY & THE BRAIN.

Michael W Schwartz, Univ of Washington/Harborview Med Ctr, Seattle, WA

The homeostatic regulation of body energy stored in the form of fat involves humoral mediators such as the hormones insulin and leptin that circulate at levels proportional to body fat content and, acting through neuronal receptors, elicit responses that promote stability of body fat content. An important physiological role for this neuroendocrine system is evidenced by severe obesity that results from mutations affecting its key molecular components. In recent years, our understanding of how information regarding body energy stores is communicated to the brain and subsequently transduced into behavioral and metabolic responses has benefited from the identification of neurons in the hypothalamic arcuate nucleus that integrate input from insulin and leptin as well as from nutrients such as free fatty acids and glucose. These neurons in turn regulate the response of hindbrain areas involved in the perception of satiety signals, allowing changes of body fat mass to be coupled to compensatory changes of intake during individual meals. A parallel circuit links the arcuate nucleus to the control of insulin sensitivity and endogenous glucose production. The ability of circulating insulin to inhibit hepatic glucose production depends in part on activation of this neuronal system, and selective restoration of leptin signaling to the arcuate nucleus of rats that otherwise lack leptin receptors exerts a pronounced, beneficial effect on systemic insulin sensitivity. Thus, hypothalamic integration of adiposity signals is essential to the regulation of both body fat mass and blood glucose levels.
Defects in this control system may therefore contribute to the link between obesity and type 2 diabetes.

THE NEUROENDOCRINOLOGY OF CRITICAL ILLNESS.
Greet Van den Berghe, Intensive Care Med, Catholic Univ of Leuven, Leuven, Belgium
Striking alterations within the hypothalamic-anterior pituitary axes characterize critical illness and the severity of these hormonal imbalances is associated with the risk of morbidity and mortality. Most attempts to treat some of these endocrine abnormalities, however, have shown to be ineffective or even harmful. The pathophysiological insight of a biphasic (neuro)endocrine response to critical illness has helped to clarify this controversy. The acute phase is characterized by an actively secreting pituitary, whereas the concentrations of most peripheral hormones are low, partly due to the development of target-organ resistance or to altered peripheral metabolism and binding of the target-organ hormones. In contrast, in prolonged critical illness, a uniform suppression of the (neuro)endocrine axes, predominantly of hypothalamic origin, contributes to the low serum levels of the respective target-organ hormones. The adaptations in the acute phase are considered to be beneficial for short-term survival. However, in the chronic phase of intensive-care dependent critical illness, the observed neuroendocrine alterations may contribute to the wasting syndrome of the critically ill and hereby hamper recovery and rehabilitation. With the exception of intensive insulin therapy, and perhaps hydrocortisone administration for a subgroup of patients with septic shock, no hormonal intervention so far has proven to beneficially affect survival. However, the combined administration of hypothalamic releasing factors holds promise as a safe therapy to reverse the neuroendocrine and metabolic abnormalities of prolonged critical illness by a balanced re-activation of the different anterior-pituitary axes.

PROLACTIN & ITS RECEPTOR: MORE THAN JUST THE LACTATION MEDIATOR.
Paul A Kelly, Inserm Unit 584, Fac Med Necker, Paris, France
The pituitary hormone prolactin (PRL) is best known for its effect on the induction and maintenance of lactation. However, we reported >300 separate actions which correlates with the near ubiquitous distribution of its receptor. In the late 1980s, cloning the cDNA encoding the PRL receptor just after that of the growth hormone (GH) receptor led to the identification of the GH/PRL receptor family, which has since expanded into the Class I cytokine receptor superfamily. The identification of the Jak/Stat signal transduction pathway in the 1990s elucidated one of the major means by which the actions of PRL (and all cytokines) are mediated. PRL-related (hormone and receptor) knockout models have essentially highlighted an irreplaceable role in lactation and reproduction, which suggests that most of the other reported actions in target tissues are more likely modulated by, rather than strictly dependent on PRL. Female homozygous PRL receptor knockout mice are completely infertile and show a lack of mammary development. Male and female homozygotes have markedly elevated serum prolactin levels, and in older animals, pituitary hyperplasia and adenoma are present. Maternal
behavior is severely affected in both heterozygous and homozygous animals. Bone formation is reduced in young animals and in adults (males and females). Finally, older males and females show a slight reduction in body weight, which seems to be due to reduced abdominal fat deposition in the knockout animals. The multiplicity of PRL actions in experimental animals is in direct opposition to the paucity of arguments concerning its involvement in human pathophysiology, other than effects on reproduction in hyperprolactinemic patients. There is a large body of literature showing that prolactin exerts growth-promoting activities in breast cancer, and possibly in prostate cancer and prostate hyperplasia. In addition, increasing evidence argues for the involvement of locally produced (autocrine) PRL, perhaps even more than pituitary-secreted (endocrine) PRL, in tumor growth. Because dopamine analogs are unable to inhibit PRL production in extrapituitary sites, alternative strategies need investigation. To that end, we recently developed delta 1-9-G129R-hPRL, a PRL receptor antagonist that is totally devoid of residual agonistic activity, and is thus the only pure antagonist available. We are currently testing its potency as an inhibitor of prostate and mammary tumor growth in various in vivo models.

THYROID HORMONE RECEPTORS IN BRAIN DEVELOPMENT & FUNCTION.

Juan Bernal, Inst de Invest Biomed, CSIC, Madrid, Spain

Thyroid hormones (TH) are very important for the mammalian brain both during development and in the adult. During maturation, TH influence late developmental processes such as myelination, neural cell migration, differentiation, and signalling. Genes regulated by TH have been described in the rodent brain during the postnatal period and in the adult. Although evidence for extranuclear sites of action is accumulating, most thyroid hormone actions in the brain are thought to be mediated by binding to nuclear receptors (TR) which are present in the rat from embryonic day 14, and in the human at least from the end of the first trimester of gestation. There are 4 TR isoforms, encoded by the TRα and TRβ genes, all of them expressed in brain, and TRα1 accounting for more than 70% of all TR protein. In the cerebellum, where neonatal hypothyroidism induces profound morphological alterations, TH promotes granular cell (GC) migration through TRα1, and Purkinje cell (PC) differentiation through TRα1 and TRβ. Paradoxically, TRα1 knock out mice do not display alterations in GC migration or PC differentiation. Furthermore, induction of neonatal hypothyroidism in the absence of TRα1 did not affect these cell populations (1). The developmental effects of hypothyroidism are therefore likely to be a consequence of unliganded receptor activity. In agreement with this, expression of a mutant TRα1, with low affinity for T3 and dominant negative properties affected cerebellar development and motor performance. These observations also indicate that the brain phenotype of hypothyroid animals may not reflect true physiological actions of the hormone, but interference of unliganded receptors with processes which do not implicate thyroid hormones during normal development. In addition to development, TRs are also involved in the function of the adult brain. TRα1 deletion, or expression of a dominant negative mutant receptor induced consistent behavioural changes in adult mice indicative of a state of extreme anxiety (2).
Morphological changes were found in the hippocampus of these mice affecting a subpopulation of inhibitory, GABAergic interneurons which, under normal conditions, express predominantly the TR\( \alpha \)1 isoform. These results demonstrate that TR\( \alpha \)1 has important roles in the adult brain, one of them being the maintenance of GABAergic interneurons and modulation of behaviour.

**STEROIDOGENESIS — GENERAL LESSONS FROM RARE DISEASES.**

*Walter L Miller, Dept of Pediat, Univ of California, San Francisco, San Francisco, CA*

Rare diseases are highly informative experiments of nature, revealing previously unappreciated functions; a recent, informative example is P450 Oxidoreductase (POR) deficiency. Three steroidogenic enzymes, 21-hydroxylase (P450c21), 17-hydroxylase/17,20 lyase (P450c17) and aromatase (P450aro) are members of the family of type II (microsomal) cytochrome P450 enzymes. There are 50 such human enzymes, including approximately 20 involved in biosynthetic pathways, 15 involved in drug metabolism and 15 orphan enzymes of unknown role. All 50 type II P450 enzymes require electron donation from POR. Knockout of the mouse POR gene causes embryonic lethality, thus a human POR deficiency disease seemed unlikely.

We identified POR mutations in 23 patients with hormonal evidence of partial combined P450c17 and P450c21 deficiencies (Nat Genet 36: 228, 2004; TEM 15:311, 2004; Am J Hum Genet 76: 729, 2005). Basal and ACTH-stimulated 17OHP is typically elevated, DHEA(S) and other C19 steroids are low and cortisol may be normal but unresponsive to ACTH. Most were infants with the Antley-Bixler skeletal dysplasia syndrome and genital ambiguity in both sexes: P450c17 deficiency caused incomplete masculinization in males and P450aro deficiency resulted in partial virilization in females. However, it has also been proposed that virilization during fetal life is secondary to an alteration in androgen metabolism. Thus the hormonal picture is complex, requiring detailed investigation. Some patients had no skeletal disorder, including males with androgen deficiency and females with a PCOS-like picture. We identified 21 POR missense mutations, re-created each by site-directed mutagenesis, expressed the mutants in bacteria, purified the mutant enzymes to homogeneity and assayed their activities in vitro. Conventional POR assays based on reduction of cytochrome c, a non-physiologic substrate, correlated poorly with clinical findings, but assays based on human P450c17 expressed in yeast, especially 17,20 lyase activity, correlated well. Others have found that liver-specific knockout of mouse POR yields reproductively normal mice with grossly disturbed drug metabolism. We are studying the impact of POR mutations or polymorphisms on drug metabolism by sequencing the POR gene in ethnically diverse human populations and examining the effects of POR variants on drug-metabolizing P450 enzymes. The combination of clinical endocrine investigation, human genetics, molecular biology, enzymology and pharmacogenomics has enhanced understanding of human disease in a way that no single approach could.

**THE DIVERSE WORLD OF ESTROGENS AND ESTROGEN RECEPTOR ACTIONS.**

*Benita S Katzenellenbogen, John A Katzenellenbogen, Depts of Molec and Integrative*
Physiol, and Cell and Devel Biol, Univ of Illinois and College of Med at Urbana-Champaign, Urbana, IL; Dept of Chem, Univ of Illinois at Urbana-Champaign, Urbana, IL

This presentation will focus on the intriguing estrogen receptor proteins, ER-alpha and ER-beta, and their important actions in reproductive as well as non-reproductive tissues. The estrogen receptors bind structurally diverse ligands, including endogenous estrogens, dietary phytoestrogens, and many synthetic compounds that function as selective estrogen receptor modulators and have therapeutic utility in normal tissues and hormone-dependent cancers, especially breast cancer. Acting via these two receptors, estrogens exert profound but distinctive effects on the regulation of gene networks and signaling pathways in target cells, as revealed by genome-wide microarray gene expression transcriptional profiling. The estrogen receptors also exert important effects post-transcriptionally. We have studied the biological activities of these two receptors using various genetic and structure-function approaches. Differences in the size, shape, and flexibility of the ligand binding pockets of ER-alpha and ER-beta have guided our synthesis of novel estrogen receptor subtype-selective ligands that have been used to probe their biological actions. We will present our current understanding of the genomic and membrane-initiated extranuclear actions of estrogens, the interactions of estrogen receptors with critical coregulator partner proteins, and how our knowledge of these mechanisms of action and ligand bioactivities will continue to impact medical approaches to improving the health of women and men.

THE FETAL ORIGINS OF ADULT DISEASE.

Susan E Ozanne, Dept of Clin Biochem, Univ of Cambridge, Cambridge, UK

A large number of epidemiological studies have shown that there is a relationship between poor early growth and the development of type 2 diabetes, insulin resistance and other features of the metabolic syndrome. The mechanistic basis of this relationship is not known and the relative importance of environmental and genetic factors remains the subject of much current debate. However, compelling evidence has emerged over the last fifteen years to suggest that early environmental factors such as nutrition play an important role. Studies of individuals who were in utero during a period of famine, the Dutch Hunger Winter, have shown a direct relationship between maternal nutrition and glucose tolerance in the offspring. Further support for the importance of the fetal environment has come from studies of monozygotic twins who were discordant for type 2 diabetes. These revealed that the diabetic twins had significantly lower birth weights than their non-diabetic co-twins. A number of animal models, including maternal protein restriction, maternal calorie restriction, maternal high fat feeding and maternal anaemia, have been developed to investigate the mechanisms by which the early environment determines future susceptibility to disease. The most extensively studied is the maternal low protein model where rats are fed a low (8 %) protein diet during pregnancy and lactation. The offspring have a low birth weight and develop many features of the metabolic syndrome including type 2 diabetes, and insulin resistance in adulthood. In vitro analysis has shown that the observed glucose intolerance and insulin resistance is associated with changes in
expression of key components of the insulin-signalling cascade (including protein kinase C zeta and the p110β catalytic subunit of PI 3-kinase) in muscle and adipocytes. We have demonstrated that the changes in profile of insulin signalling protein expression in muscle and fat from low protein offspring are strikingly similar to that observed in tissue biopsies from young men with a low birth weight. The molecular mechanisms by which a phenomenon that occurs in utero has a phenotypic consequence many years later are only just starting to emerge. However these proteins, which are susceptible to fetal programming, may be molecular markers of early growth restriction and thus risk of disease. If extended to a clinically accessible tissue in humans this would make targeted intervention strategies a realistic possibility.

SEX DETERMINATION
David Page, Massachusetts Institute of Technology, Cambridge, MA
In mammals, meiosis is initiated at different time points in males and females, but the mechanism underlying this difference is unknown. Female germ cells begin meiosis during embryogenesis. In males, embryonic germ cells undergo G0/G1 mitotic cell cycle arrest, and meiosis begins after birth. In mice, the Stimulated by Retinoic Acid Gene 8 (Stra8) has been found to be required for the transition into meiosis in both female and male germ cells. Stra8 is expressed in embryonic ovaries just before meiotic initiation, whereas its expression in testes is first detected after birth. They have examined the mechanism underlying the sex-specific timing of Stra8 expression and meiotic initiation in mice. Their work shows that signaling by retinoic acid (RA), an active derivative of vitamin A, is required for Stra8 expression and thereby meiotic initiation in embryonic ovaries. A developmental time course of Stra8 expression in germ cells of XX gonads has revealed an anterior-to-posterior wave of differentiation that lasts approximately 4 days, from embryonic days 12.5 to 16.5. They also discovered that RA is sufficient to induce Stra8 expression in embryonic testes and in vitamin A-deficient adult testes in vivo. Finally, their results show that cytochrome p450 (CYP)-mediated RA metabolism prevents premature Stra8 expression in embryonic testes. Treatment with an inhibitor specific to RA-metabolizing enzymes indicates that a cytochrome p450 from the 26 family (CYP26) is responsible for delaying Stra8 expression in embryonic testes. Sex-specific regulation of RA signaling thus plays an essential role in meiotic initiation in embryonic ovaries and precludes its occurrence in embryonic testes. Because RA signaling regulates Stra8 expression in both embryonic ovaries and adult testes, this portion of the meiotic initiation pathway may be identical in both sexes.

EVALUATION & TREATMENT OF ADULT GROWTH HORMONE DEFICIENCY (GHD): AN ENDOCRINE SOCIETY CLINICAL PRACTICE GUIDELINE.
Mark E Molitch, David Clemmons, Saul Malozowski, George R Merriam, Steve Shalet, Mary Lee Vance, Endocrinol, Metab & Molec Med, Northwestern Univ Feinberg Sch of Med, Chicago, IL; Endocrinol & Metab, Univ of North Carolina Sch of Med, Chapel Hill, NC; NIDDK, NIH, Bethesda, MD; Endocrinol, U of Washington - VA Puget Sound Hlth Care, Tacoma, WA; Endocrinol, Christie Hosp, Manchester, UK; Endocrinol, Univ of
Virginia Hlth Sci Ctr, Charlottesville, VA

These guidelines were developed by the writing group after a review of relevant, primary peer-reviewed publications and extensive peer review by members of the Endocrine Society. GHD can persist from childhood or be newly acquired. Confirmation through stimulation testing in adulthood is required unless there is a proven genetic/structural lesion persistent from childhood and IGF-1 levels are below the normal range when off treatment. Testing usually requires stimulation with insulin-induced hypoglycemia or GH releasing hormone (GHRH) + arginine, unless 3 or more other pituitary axes are deficient. A low IGF-I level usually indicates severe GHD unless there is concomitant catabolic illness but a normal IGF-I does not exclude GHD. GH therapy offers clinical benefits in several areas. Body composition improves with a decrease in fat mass and an increase in lean body mass. In most studies exercise capacity is improved. Bone mineral density improves but a reduction in fractures has not yet been shown. A number of cardiovascular parameters and risk factors improve but reductions in cardiovascular events and mortality have not yet been demonstrated. Most studies show an improvement in quality of life measures. Patients who have more severe clinical and biochemical abnormalities are the most likely to benefit. The risks of GH treatment are low. GH should not be administered to patients with active malignancy. To avoid adverse effects, GH dosing regimens should be individualized, starting with low doses and titrated according to clinical response, side effects, and IGF-I levels. Periodic monitoring is necessary for adverse effects and physiologic benefit. The final decision to treat adults with GHD requires thoughtful clinical judgment with a careful evaluation of the benefits and risks specific to the individual.

SYMPOSIA

A few Symposia and Oral Presentations have been selected.

MEDICATION-INDUCED BONE LOSS IN PEDIATRICS.
Laura K Bachrach, Dept of Pediat Endo, Stanford Med Ctr, Stanford, CA

Bone loss in adults has been associated with use of several medications including glucocorticoids, gonadotropin releasing hormone agonists (GnRHa), and depot medroxyprogesterone (DMPA). The dose and duration of drug causing bone loss and the modulating effects of host factors remain controversial. The response of the growing skeleton to these drugs may differ from adults and medications may influence bone size and geometry as well as mass. Furthermore, a reduction in the expected gains in bone size and mass may compromise lifetime bone health even if overt bone loss does not occur. Examining these more subtle skeletal effects is challenging given the limitations of bone densitometry in growing individuals. Glucocorticoids inhibit bone formation and enhance resorption through direct and indirect mechanisms. Studies of bone mineral using dual energy x-ray absorbiometry in children treated with long term systemic glucocorticoids have shown reductions in some, but not all, conditions. These data underscore the importance of malnutrition, inflammatory cytokines, and other skeletal risk factors related to the underlying disorder independent of steroids.
Systemic glucocorticoids have been shown to increase bone fragility in a large epidemiologic study from the United Kingdom; fractures were more common in children prescribed 4 or more courses of systemic glucocorticoids. Although GnRHa use in adults causes bone loss within months of initiating therapy, GnRH therapy for precocious puberty has not produced similar bone loss. Some studies have reported slower bone mineral accrual with GnRHa treatment but peak bone mass has been normal in women treated with GnRHa in childhood. DMPA, a highly effective hormonal contraceptive, has been linked to bone loss which appears to be greater in adolescents than in older women. Unhealthy lifestyles in DMPA users may contribute to this loss. Gains in bone mineral occur after DMPA is stopped but the potential for full recovery is still under investigation. Research is needed to better define the correlates of low bone mass, the risk of fracture, and potential interventions to reduce or reverse drug-induced skeletal changes.

PARTIAL ANDROGEN INSENSITIVITY SYNDROME.
Ieuan A Hughes, Univ of Cambridge Sch Clin Med, Cambridge, UK

Androgen insensitivity is defined as a clinical syndrome characterised by varying degrees of resistance to androgens in an XY male with testes which produce normal concentrations of age-appropriate testosterone. The partial form (PAIS) is manifest as a variable external phenotype which can range from ambiguous genitalia of the newborn to infertility in an otherwise normal male. Androgen insensitivity results from a defect in the androgen signalling pathway in which the androgen receptor (AR) is pivotal. Mutations causing androgen insensitivity are distributed throughout the AR gene with only minor hotspots discernible by reference to the AR mutation database (http://www.mcgill.ca/androgendb).

Only a minority of individuals with PAIS have an identified AR mutation. Functional assays of mutant receptors are not only useful to confirm causality, but also may provide information on structure:function dynamics of the AR. The phenotype in PAIS is not generally predicted by the genotype so that decisions taken in genetic counselling, certainly if prenatal diagnosis is considered, must be extremely guarded.

The majority of patients with the phenotype of PAIS in whom the AR is normal pose a diagnostic conundrum. A myriad of other disorders affecting fetal androgen production must be excluded yet, in a recent study of 111 patients truly defined as having PAIS, only 24 per cent had an AR mutation (1). Comparative analysis of the phenotype between the mutation positive and negative groups showed no distinguishing features other than a family history of genital anomalies. Furthermore, the sex of rearing could not be predicted despite applying a validated external masculinisation score to both groups. A number of possibilities may explain the incomplete virilisation in PAIS infants with a normal AR such as a strong association with reduced birth weight. It is important to investigate this category of PAIS cases further as they constitute a large proportion of infants born with abnormal genitalia, they are not fully characterised with respect to outcome at puberty and fertility in adulthood and any risk of recurrence cannot currently be predicted. It is anticipated that further progress in refining diagnosis will occur following the identification of new genes involved in the pathway of
androgen signalling.

**HOW WE DEFINE INSULIN-LIKE GROWTH FACTOR-1 DEFICIENCY?**

*Stephen M Rosenthal, Pediat, Univ of California-San Francisco, San Francisco, CA*

Insulin-like Growth Factor (IGF)-I insufficiency can result from defects at any point in the Growth Hormone (GH) Releasing Hormone (GHRH)/GH/IGF-I axis. The recent FDA approval of recombinant human (rh) IGF-I, alone or in combination with rhIGF Binding Protein-3 (rhIGFBP-3) for treatment of children with severe growth failure (height standard deviation score of less than or equal to -3.0) as a consequence of Primary IGF-I deficiency (IGFD) or in children with GH gene deletion that have developed neutralizing antibodies to GH underscores the importance of accurate diagnosis of these conditions. The term Primary IGFD has been used to denote a variety of disorders which excludes GHRH deficiency or resistance and direct GH deficiency, but which includes mutations of the GH receptor (GHR), post-GHR signaling defects, and IGF-I gene defects. The ability to correctly diagnose Primary IGFD requires, among other tools, accurate IGF-I assays, particularly at the lower part of the normal range. This talk will review current concepts in the classification of IGF-I deficiency, diagnostic considerations, and issues surrounding treatment of severely short children with rhIGF-I alone or as a binary protein complex with rhIGFBP-3.

**THYROID FUNCTION IN PREMATURE INFANTS.**

*Delbert A Fisher, Sci & Innovation, Quest Diag Nichols Inst, San Juan Capistrano, CA; Emeritus Pediat & Med, Harbor-UCLA Med Ctr, Torrance, CA*

In the United States the rate of premature birth (< 37 weeks gestation) has increased 25% since 1980, approximating 500,000 infants and 12.2% of total births in 2003. The cost of their care is near $14 billion yearly. 53% are low birth weight (LBW < 2500 g), 6% are very low birth weight (VLBW, 1000-1500) and 6% are extremely low birth weight (ELBW, < 1000 g). The large majority of the VLBW and ELBW infants range from 23 weeks (the periviable period) to 30 weeks gestation. They are born with immature organ and metabolic systems and have a high prevalence of neonatal morbidities, including respiratory distress, hypoxia, sepsis, intraventricular brain hemorrhage, gastrointestinal disorders, cardiac disorders, and endocrine-metabolic dysfunctions. Mortality approximates 20% and some 20% of survivors have subnormal cognitive function and 10% cerebral palsy. A significant correlation of morbidity and later cognitive dysfunction with low thyroxine (T4) concentrations in these infants has been reported, and low serum T4 concentrations have been associated with the neonatal morbidities. The association of low T4 levels and normal-low TSH values occurs in 90% of VLBW and ELBW infants during the first 1-2 weeks and has been referred to as Transient Hypothyroxinemia of Prematurity (THOP). Transient primary hypothyroidism with elevated TSH (usually due to iodine deficiency) occurs in less than 0.5% of neonates in iodine sufficient areas and permanent congenital primary hypothyroidism in 1:4000. Thyroid disorders to be considered in infants with THOP include permanent congenital secondary-tertiary hypothyroidism (1:25,000 infants), and primary hypothyroidism with a delayed rise in TSH
(1:40,000 infants), transient secondary-tertiary hypothyroidism (representing hypothalamic-pituitary immaturity) and the non-thyroidal illness (NTI or low T3) syndrome. The high correlation with the neonatal morbidities favors an NTI etiology for most infants with THOP, but whether THOP contributes to the neonatal mortality and the subsequent cognitive dysfunction is currently controversial. Several small clinical therapeutic trials have produced inconclusive results. One two-part study by van Wassenaer et al (NEJM 336:21,1997) and Briet et al (Pediatrics 107:712,2001) has suggested benefit of T4 treatment for infants 23-27 weeks gestation and possible risk of treatment for infants > 27 weeks and several thyroid hormone supplementation trials are in the planning or early operative stages.

MOLECULAR MECHANISMS OF GENOMIC IMPRINTING.
Anne Ferguson-Smith, Univ of Cambridge, Cambridge, UK

Genomic imprinting is an epigenetic marking process that causes genes to be expressed depending on their parental origin. To date approximately 100 imprinted genes have been identified that are expressed either from the maternally inherited chromosome homologue or the paternally inherited chromosome homologue. Imprinted genes have been shown to function in the regulation of pre-natal growth and development and in particular, play key roles in the development and function of the placenta. More recently imprinted genes have been implicated in post natal behaviour, adaptation to feeding and the regulation of metabolic processes. The functional non-equivalence of parental genomes that is caused by imprinting is the reason why androgenetic and parthenogenetic mammalian embryos fail.

Most of the imprinted genes studied to date are located in clusters. Much of what we know about imprinting control comes from the analysis of these domains and it has been shown that imprinted activity and repression is regulated by cis-acting imprinting control elements that influence the monoallelic expression of multiple imprinted genes in the cluster over long distances. These imprinting control elements are regulated by differential DNA methylation established in the developing male and female gametes. Subsequently, post-fertilisation, additional differential methylation can be acquired that may stabilise the imprinted state. There are two types of imprinting control element; those with a methylation mark on the paternally inherited chromosome and those methylated on the maternally inherited chromosome. The relationship between DNA methylation and modifications to core histones is of particular interest. Another recurrent theme at imprinted domains is the presence of non-coding RNAs which may play a role in determining regional epigenetic states. In this presentation, the mechanisms of imprinting will be reviewed and the implications for the epigenetic modulation of genome function will be considered.

THE ROLE OF IGF2 IMPRINTING IN FETAL GROWTH.
Ken K Ong, Med Res Council Epidemiol Unit, Dept of Paediat, Univ of Cambridge, Cambridge, UK

In both the mouse and human, the insulin-like growth factor-2 (IGF2) gene is imprinted with sole expression from the paternal allele. Experimental targeted disruption of the paternal Igf2 in the
mouse reduces embryonic and placental growth. Recent reports of specific deletions of either the placental or fetal Igf2 transcripts have highlighted various functions of Igf2 in the regulation of placental growth and nutrient transport.

Rare human mutations that lead to IGF2 overexpression are a major cause of the Beckwith-Wiederman syndrome, characterised by fetal overgrowth and Wilms tumour risk. More recently, hypomethylation defects in the imprinting control region-1 that lead to H19 overexpression and consequent IGF2 underexpression have been described to underlie the Russell-Silver syndrome of low birthweight and poor infant growth. In contrast to the mouse, where Igf2 is not expressed beyond weaning, in humans IGF2 normally becomes biallelically expressed in many tissues during postnatal life, and follow-up of individuals with these over- and underexpression syndromes may illuminate the continuing functions of IGF2 in children and adults.

The consequences of common genetic variations in IGF2 and its regulatory elements are now being increasingly explored. Common variants the insulin gene VNTR, H19 and IGF2 gene region have been variably associated with the Beckwith-Wiederman syndrome, IGF-II protein levels, size at birth and adult BMI. However, as yet no studies have been adequately powered to explore the parent-of-origin effects, which would be expected with these imprinted genes. A new generation of genetic epidemiology studies that include not only inherited variants but also gene methylation patterns should help us to more fully understand the role of IGF2 in human fetal growth and risks for various disease outcomes in later life.

ORAL PRESENTATIONS

DISSOCIATED TUBULAR-INTERSTITIAL TESTICULAR DYSFUNCTION IN A PATIENT WITH MCCUNE-ALBRIGHT SYNDROME: PATHOGENIC MECHANISM.
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McCune-Albright syndrome is characterized by polyostotic fibrous dysplasia, caf-au-lait skin lesions, and gonadotropin-independent gonadal activation. Somatic gain of function mutations in the Gs protein gene have been found in affected tissues. In the testis, Gs is normally involved in signal transduction of gonadotropins: LH in interstitial Leydig cells and FSH in seminiferous tubule Sertoli cells. We previously reported a 3 yr-old boy presenting with macro-orchidism, high serum levels of Sertoli cell markers anti-Mllerian hormone (AMH) and inhibin B, but low testosterone. The patients parents had given informed consent for the study. Testicular biopsy showed Sertoli cell hyperplasia with otherwise prepubertal features. A somatic R201H mutation was detected in the Gs protein gene in DNA extracted from testis. We aimed at understanding why only Sertoli cells showed hyperactivity, whereas Leydig cells remained quiescent. We
performed laser capture microdissection of testicular paraffin-embedded tissue in order to selectively isolate seminiferous tubules from interstitial tissue. DNA extracted from the different compartments was screened for R201H mutation by direct sequencing of PCR products obtained by nested PCR. Only DNA from seminiferous tubules displayed the mutation. Further, we compared the trans-activating capacity of a normal and a R201H Gs on the human AMH promoter activity in transient transfection assays. AMH promoter activity, evaluated by luciferase assay, was significantly higher (p<0.05) after transfection of R201H-Gs (3.7 0.9 relative luciferase units) than of wild-type Gs (2.1 0.7 relative luciferase units). We conclude that a somatic mutation R201H probably occurring in the Gs protein gene before testicular cell lineages differentiation in early embryonic life affected Sertoli but not Leydig cell precursors, which have different origin. The mosaic mutation, which mimicked the effect of FSH, resulted in isolated Sertoli cell activation evidenced by hyperplasia leading to prepubertal macro-orchidism and elevated AMH production, with no signs of Leydig cell activation. Molecular diagnostic procedures have allowed us to unravel the mechanism of disease underlying a gonadal disorder primarily affecting only one cell population. This represents an example of a novel diagnostic approach, focusing on the diagnosis of cell (e.g. Sertoli cell) disorder rather than on the classical diagnosis of organ (e.g. testis) disorder.

A NOVEL MISSENSE MUTATION OF KISS1 GENE IN A BOY WITH IDIOPATHIC GONADOTROPIN-DEPENDENT PRECOCIOUS PUBERTY.
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Kisspeptin and its receptor GPR54 have been implicated in the regulation of gonadotropin-releasing hormone (GnRH) secretion by the hypothalamic neurons. Recent evidence supports the role of this complex Kisspeptin/GPR54 as a trigger of puberty onset, stimulating the resurgence of the the pulsatile GnRH secretion. Kisspeptin is encoded by the KISS1 gene, which is highly expressed in placenta and brain, particularly in the hypothalamus and basal ganglia. Inactivating mutations in GPR54 and KISS1 genes were identified in a small number of normosmic isolated hypogonadotropic hypogonadism (IHH) cases. The aim of this study was to investigate KISS1 mutations or polymorphisms in patients with central idiopathic pubertal disorders. Forty five children (43 girls and 2 boys) with sporadic or familial (12%) idiopathic gonadotropin-dependent precocious puberty (GDPP) and 42 patients (32 males and 10 females) with IHH were studied. GPR54 mutations were ruled out in both groups as well as GnRH receptor mutations in the IHH group. Control population consisted of 100 normal individuals. Genomic DNA was extracted from peripheral leukocytes and the 3 exons of the KISS1 gene were amplified and automatically sequenced. No alteration was found in the IHH or control groups, except for six previously described polymorphisms, which were present in
similar proportions in both IHH and GDPP groups. A heterozygous missense mutation, P74S, was identified in a boy with sporadic idiopathic GDPP. He presented at the age of 1 yr with pubertal stage Tanner 3, elevated basal and stimulated LH (11.5 UI/L and 47.2 UI/L by RIA, respectively) and testosterone (600 ng/dL) levels and normal central nervous system MRI. Long term follow up (14 yr) showed satisfactory response to GnRH analogs and lack of other associated neurologic or systemic conditions. The probands mother and maternal grandmother, who had menarche at appropriate ages, also carried the P74S mutation. In contrast, this mutation was absent in 200 control alleles. Interestingly, the P74S mutation changes this highly conserved proline, an important amino acid for determining the secondary structure of proteins. Additionally, the mutated amino amino acid is located in a PEST sequence, which is associated with protein degradation. We suggest that the disruption of this highly conserved proline can increase the half life, and therefore the bioactivity of the KiSS1 peptide, resulting in GDPP.

GENETIC SCREENING IN 195 PATIENTS WITH COMBINED PITUITARY HORMONE DEFICIENCY: EXPERIENCE OF THE GENHYPOPIT NETWORK.

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Mutations in PIT1 (POU1F1), PROP1, LHX3, LHX4, and HESX1 transcription factor genes result in combined pituitary hormone deficiency (CPHD). The GENHYPOPIT network was launched as an international multicenter study to screen for genetic causes of CPHD. The aim of the study was to apply a genetic screening strategy based on endocrine and neuroradiological phenotype according to current knowledge, to establish the prevalence of gene defects in each category of patients and to provide a useful framework to determine the genetic aetiology and allow genetic counselling for individuals and families with CPHD. According to their phenotype, 195 CPHD patients belonging to 165 unrelated families were studied for POU1F1, PROP1, LHX3, LHX4, and HESX1. Patients selected had two pituitary hormone deficiencies or at least one deficiency with intracerebral malformations. The total prevalence of mutations was 13.3 %; and 52.4% in the subset of 20 patients with familial CPHD history. No mutation of HESX1 was observed in 16 patients harboring septo-optic dysplasia. A mutation of LHX4 gene, previously reported, was found in 1 familial case among 39 patients bearing pituitary stalk interruption syndrome (PSIS). In 109 patients without extrapituitary abnormalities, 20 had PROP1 mutations, including 8 patients with a family history of CPHD. Among 20 patients without PSIS, no LHX3 gene defect was found, even in patients with a neck rotation deficit. One POU1F1 gene defect was found in one patient among 12 presenting with the rare postpubertal association of thyrotroph and somatotroph deficits. Mutations in the PROP1 gene remain the first to be looked for, and POU1F1 mutations should be sought in the post pubertal population with GHD and TSHD. When extrapituitary
malformations are present, genetic analysis of PROP1, POU1F1, and possibly LHX3 do not appear required.