X-linked hypophosphatemia (XLH), the prototypic vitamin D resistant disease, is caused by genetic defects that impair vitamin D action or phosphate metabolism. In other patients rickets is caused by genetic defects of the vitamin D receptor superfamily of receptors that inhibit vitamin D action. In the 1930's. More recently calcium deficiency has emerged as an important cause of nutritional rickets. In the first year of life the most rapidly growing bones are the skull, ribs, and wrists. Calciferol deficiency at this time leads to widened cranial sutures, frontal bossing, posterior flattening of the skull, bulging of costochondral junctions ("rachitic rosary"), and enlargement of the wrists. Nutritional deficiency of vitamin D remains a leading cause of rickets worldwide, despite the diminished prevalence of vitamin D deficiency that followed the fortification of milk with vitamin D. Hypocalcemic tetany and seizures can be the presenting features of rickets. The clinical features of calciferol deficiency are weakness, bone pain, bone deformity, and fracture. The most rapidly growing bones show the most striking abnormalities. In the first year of life the most rapidly growing bones are the skull, ribs, and wrists. Calciferol deficiency at this time leads to widened cranial sutures, frontal bossing, posterior flattening of the skull, bulging of costochondral junctions ("rachitic rosary"), and enlargement of the wrists. Nutritional deficiency of vitamin D remains a leading cause of rickets worldwide, despite the diminished prevalence of vitamin D deficiency that followed the fortification of milk with vitamin D in the 1930’s. More recently calcium deficiency has emerged as an important cause of nutritional rickets. In other patients rickets is caused by genetic defects that impair vitamin D action or phosphate metabolism.
gene expression relationships. Parallel polymorphisms in the ACHE and PON1 genes displayed apparent associations with both trait and state anxiety scores. Our findings indicate that a significant source of anxiety feelings involves inherited and acquired parameters of acetylcholine regulation that can be readily quantified, which can help explain part of the human variance for state and trait anxiety.

S2-17 Endocrinology of the Acutely Ill Child
The Hypothalamic-Pituitary-Adrenal Axis and Critical Illness
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Like the stress response, the inflammatory reaction of an individual is crucial for survival of the self and species. Also like the stress response, inflammation is tailored to the stimulus and time-limited. A fully fledged systemic inflammatory reaction consists of activation of immune and immune accessory cells and resultant stimulation of four major programs: (1) the acute phase reaction, (2) the sickness syndrome, (3) the pain program, mediated by the affective sensory and autonomic systems, and (4) the stress program, mediated by the stress system, i.e. the hypothalamic-pituitary-adrenal (HPA) axis and the locus coeruleus-norepinephrine/sympathetic system. The main effector substances of the systemic inflammatory response are inflammatory cytokines, such as TNFalpha, IL-1 and IL-6, chemokines, and other mediators of inflammation; the acute phase reactants, such as C-reactive protein (CRP), cell adhesion molecules, fibrinogen and plasminogen activator inhibitor 1, the effectors of the sensory affective system, such as substance P; and, of the stress system, namely hypothalamic CRH and vasopressin, cortisol, norepinephrine and epinephrine, and peripheral neuronal CRH. The sickness syndrome consists of anorexia/nausea, fatigue and/or depressed affect, somnolence, hyperalgesia, sleep disturbances, elevated temperature and an increased metabolic rate, all manifestations suppressed by glucocorticoids. Yet, peripheral neuronal CRH activated by stress or the inflammatory reaction, and substance P, activated by the inflammatory reaction potentiate inflammation. We recently found that during a systemic inflammatory reaction, as in ARDS or sepsis, there is inadequate activation of cortisol secretion and significant cytokine-induced glucocorticoid resistance, both suggesting beneficial actions of added glucocorticoids.

S2-18 Endocrinology of the Acutely Ill Child
Early Endocrine Predictors of Outcome
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Septic shock is the most severe clinical manifestation of sepsis. The systemic inflammatory response, such as in ARDS or sepsis, has a critical role in the outcome of the patient. Septic shock is a major player in the development and maintenance of the female phenotype in women, by regulating Müllerian duct formation and controlling steroidogenesis in the ovary. The WNT4 gene has been noted to be involved in sex differentiation in mice. We now describe a young woman who showed absence of structures derived from Müllerian ducts (monolateral renal agenesis and clinical signs of androgen excess. Her phenotype resembles the Mayer-Rokitansky-Kuster-Hauser syndrome (MRKH) and is also strikingly similar to that of the Wu4-knockout female mice. This constellation of findings prompted us to search for mutations in the WNT4 gene in this patient. The WNT4 gene of our patient carries a heterozygote single-base exchange corresponding to a Glu226Gly mutation in the WNT4 protein. The mutated WNT4 is unable to suppress the expression of the androgen-synthetic enzymes CYP17 and HSD3B2 in human ovarian cells. The mutant WNT4 is not lipid-modified, cannot be secreted and is therefore unable to activate its own signaling pathway. The mutated WNT4 has dominant negative properties, providing a clear genotype-phenotype correlation. This firstly-described loss-of-function mutation in WNT4 gene appears to cause developmental abnormalities in humans and identifies WNT4 as a major player in the development and maintenance of the female phenotype in women, by regulating Müllerian duct function and controlling steroidogenesis in the ovary.

S3-721 Recent Advances
WNT4 is Essential for Sexual Development in Women
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Differentiation of a testis or an ovary from the bipotential gonad is a complex developmental process involving various genes and hormones. Additional elements of the reproductive tract develop from an indeterminate stage via the differentiation of Wolffian (male reproductive tract anlage) and Müllerian (female reproductive tract anlage) ducts. Whereas factors involved in male sex differentiation are well studied, the pathways that regulate female sexual differentiation remain incompletely defined. To date, no genes have been demonstrated to play an equivalent role to that of SRY or SOX9 genes in testes development. Wnt4, one of a few factors with a demonstrated function in the ovarian-determination pathway has been noted to be involved in sex differentiation in mice. We now describe a woman who showed absence of structures derived from Müllerian ducts (monolateral renal agenesis and clinical signs of androgen excess. Her phenotype resembles the Mayer-Rokitansky-Kuster-Hauser syndrome (MRKH) and is also strikingly similar to that of the Wu4-knockout female mice. This constellation of findings prompted us to search for mutations in the WNT4 gene in this patient. The WNT4 gene of our patient carries a heterozygote single-base exchange corresponding to a Glu226Gly mutation in the WNT4 protein. The mutated WNT4 is unable to suppress the expression of the androgen-synthetic enzymes CYP17 and HSD3B2 in human ovarian cells. The mutant WNT4 is not lipid-modified, cannot be secreted and is therefore unable to activate its own signaling pathway. The mutated WNT4 has dominant negative properties, providing a clear genotype-phenotype correlation. This firstly-described loss-of-function mutation in WNT4 gene appears to cause developmental abnormalities in humans and identifies WNT4 as a major player in the development and maintenance of the female phenotype in women, by regulating Müllerian duct function and controlling steroidogenesis in the ovary.

S3-722 Recent Advances
Congenital Lipoid Adrenal Hyperplasia
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Congenital lipoid adrenal hyperplasia (CLAH), the most severe form of CAH, comprises combined mineralocorticoid, glucocorticoid and sex steroid deficiency, causing sex reversal in 46,XY infants and life-threatening salt-losing crises early in infancy of both sexes. CLAH results from mutations in the steroidogenic acute regulatory protein (STAR), commonly by the Q258X mutation found in most Japanese and Korean patients. Only one CLAH patient has been described presenting after 6 months of age, having a mutation (M225T) retaining partial function. We describe 8 patients in 6 Saudi families with CLAH. All were phenotypically female and hyperpigmented, presenting with hyponatremia, hyperkalemia, grossly elevated ACTH, and very low concentrations of serum cortisol, 17OH-progesterone and 17OH-pregnenolone; 5 were 46,XY and 3 were 46,XX. However, the ages of clinical presentation ranged from 1 month to 3 years, with four presenting at > 6 months. Genetic analysis of the STAR gene by PCR amplification of genomic DNA and sequencing showed that 7 patients in 5 families were homozygous for the novel missense mutation R182H, and the eighth patient was homozygous for the variant mutation M144R. Each mutant was re-created in a human STAR cDNA expression vector. Activity was assayed as pregnenolone produced by COS-1 cells co-transfected with a vector expressing a fusion protein of the cholesterol side-chain cleavage enzyme system and the following STAR constructs: empty vector (negative control), M144R, R182H, and wild-type STAR (positive control).

Surprisingly, the M144R and R182H mutants were wholly inactive, despite the novel mutation M144R. Each mutant was re-created in a human StAR cDNA expression vector. Activity was assayed as pregnenolone produced by COS-1 cells co-transfected with a vector expressing a fusion protein of the cholesterol side-chain cleavage enzyme system and the following StAR constructs: empty vector (negative control), M144R, R182H, and wild-type StAR (positive control).
The syndrome of inappropriate antidiuretic hormone secretion (SIADH) is a common cause of hyponatremia. Increased concentrations of vasopressin cause retention of free water, increased sodium excretion, and hyponatremia. We report two unrelated children, ages 3 and 6 months, who presented with hyponatremia (serum Na ~120 mmol/L). Their clinical appearance, chronic symptoms and laboratory findings were consistent with SIADH, yet they exhibited very low or unmeasurable ADH levels on repeated occasions. We hypothesized that these children had a gain of function defect in the ADH signaling pathway. ADH binds to the 7 transmembrane, G protein-coupled V2 vasopressin receptor (V2R) on the basolateral side of renal collecting duct cells to induce cAMP production and mobilization of aquaporin-2 channels to the apical cell membrane. The aquaporin channel allows free water to be reabsorbed, reducing serum sodium. Activating mutations in G protein-coupled receptors have been reported in other diseases, hence we considered that an analogous mutation in the V2 receptor might cause pseudo-SIADH in the absence of disease in these patients. DNA sequencing of each patient’s V2 receptor gene identified mutations (R137C or R137L) in each; R137H mutations have been previously shown to cause nephrogenic diabetes insipidus. We recreated each mutation by site-directed mutagenesis in a V2 receptor expression vector and co-transfected COS-7 cells with wild-type and mutant V2 receptor vectors and a cAMP responsive luciferase reporter plasmid. The R137L and R137C mutants induced 8 and 4 fold more luciferase activity than the wild-type V2 receptor, the empty vector or the inactivating R137H mutant. These novel gain of function mutations in the V2 receptor are the likely etiology of the patients’ SIADH-like clinical picture causing constitutive activation of the receptor and hyponatremia. These findings represent a previously unrecognized genetic disease, which we designate as pseudo-SIADH.

We have previously shown that intra-uterine food restriction during the last third of pregnancy induces a rise in maternal and fetal corticosterone levels, which in turn was responsible for the decreased beta-cell mass observed in the fetus. To determine whether glucocorticoids were also involved in normal pancreas development, glucocorticoid treatment of rat pancreatic buds in vitro was combined with the analysis of transgenic mice lacking the glucocorticoid receptor (GR) in specific pancreatic cell populations. In vitro treatment of embryonic pancreata with dexamethasone, a glucocorticoid agonist, induced a decrease of insulin mRNA levels of the 5’VNTR region of the human insulin gene are associated with variable expression of the protein and diabetes susceptibility. However, the mechanism linking this polymorphism and diabetes risk is unknown. The site of proinsulin expression (i.e. the in utero expression of h-proinsulin vs thyimic epithelial cell vs beta cell relevant to self tolerance is debated. In the mouse, insulin is encoded by two independent genes with proinsulin-2 (pro2) highly expressed in the thymus. In previous studies, we have demonstrated that 129 mice (non autoimmune prone) with a knock-out of the pro2 gene (pro2KO) are intolerant to pro2, while wild type (wt) mice are fully tolerant. In the present study, we investigated the site of expression of pro2 relevant to tolerance induction using thymus and bone marrow chimeras. CD4 T cells from wt animals transplanted with a pro2KO bone marrow were tolerant to pro2 (i.e. did not produce gamma-interferon in response to insulin and in vitro stimulators). In contrast, CD4 T cells from pro2KO animals transplanted with a wt bone marrow responded to pro2. This rules out a role for expression of pro2 in bone marrow-derived cells in induction of self tolerance. CD4 T cells from wt mice, thymectomized and grafted with a pro2KO thymus and a wt bone marrow were intolerant to pro2. This indicates that loss of pro2 expression in the thymic epithelial cells is sufficient to abolish tolerance to pro2. Our results demonstrate the functional role of thymic proinsulin expression in the regulation of beta cell autoreactivity. Future research in diabetes immunotherapy should aim at restoring central (thymic) T cell tolerance rather than pursue the so far unsuccessful attempts to manipulate peripheral tolerance.
Mechanisms in the Pulsatility of Growth Hormone Signaling
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Gene expression in mammalian liver is sexually dimorphic, and is regulated by sex-dependent patterns of pituitary GH secretion. DNA microarray studies and liver nuclear proteome analyses have established that GH is the major hormonal regulator of sex-dependent hepatic gene expression. Analysis of mice deficient in STAT5b, a GH pulse-activated intracellular signaling molecule and transcription factor, revealed that this factor is required for normal puberty growth and the profile of male liver gene expression. STAT5b deficiency leads to a loss of male-specific gene expression and is associated with increased expression of female-specific genes in male liver. Liver-enriched transcription factors, such as HNF4α, are also required for, and may act in concert with STAT5b to regulate sex-dependent liver gene expression. STAT5b is repeatedly activated in male liver by each successive plasma GH pulse, via JAK2-catalyzed tyrosine phosphorylation, followed by STAT dimerization and nuclear translocation. Down-regulation of the GH receptor-JAK2-STAT5b pathway in hepatocytes exposed to GH continuously underlies the much lower level of active liver STAT5b that is characteristic of adult females. Termination of GH receptor signaling to STAT5b is in part mediated by GH-inducible SOCS/CIS proteins, which bind to and inhibit the GH receptor-JAK2 complex by various mechanisms, rendering the hepatocyte temporarily unresponsive to GH. SOCS/CIS proteins synthesized in liver cells stimulated with GH continuously may contribute to the down-regulation of STAT5b signaling seen in adult female rat liver, by a mechanism that involves enhanced proteasome degradation of the GH receptor-JAK2 signaling complex. The recent discovery of human mutations in STAT5b linked to impaired growth highlights the significance of these studies for normal human physiology and development. [Supported by NIH grant DK33765].

Treatment with Long-acting GH
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Growth hormone (GH) action begins with binding of GH to the extracellular domain (ECD) of the cell surface GH receptor (GHR) in target tissues. The GH binding protein (GHBP) is a circulating form of the GHR that derives in humans from the extracellular domain (ECD) of the cell surface GH receptor (GHR) in target tissues. The GH binding protein (GHBP) is a circulating form of the GHR that derives in humans from the extracellular domain (ECD) of the cell surface GH receptor (GHR) in target tissues. GHBP binding to GHR complexes with the ECD of the GHR and this complex is internalized in the endosomal compartment and degraded into small peptides that have no biologic activity.

Surgical adenomectomy is a very infrequent option. Prolactin-lowering drugs, although available, are generally ineffective. Hyperprolactinemic amenorrhea is often associated with a pituitary adenoma. Both congenital and acquired anomalies in the structure of the uterus and vagina could produce amenorrhea: nevertheless, in the vast majority of patients, amenorrhea is related to an ovarian malfunction. Diagnostic work-up includes history, physical examination, laboratory data and imaging. Amenorrhea resulting from ovarian malfunction is associated with 4 distinct endocrine conditions. Hyperprolactinemia, amenorrhea is often associated with a pituitary adenoma. Surgical adenoectomy is a very infrequent option. Prolactin-lowering drugs, although available, are generally ineffective. Hyperprolactinemia

Amenorrhea: Diagnosis and Management
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Both congenital and acquired anomalies in the structure of the uterus and vagina could produce amenorrhea: nevertheless, in the vast majority of patients, amenorrhea is related to an ovarian malfunction. Diagnostic work-up includes history, physical examination, laboratory data and imaging. Amenorrhea resulting from ovarian malfunction is associated with 4 distinct endocrine conditions. Hyperprolactinemia, amenorrhea is often associated with a pituitary adenoma. Surgical adenoectomy is a very infrequent option. Prolactin-lowering drugs, although available, are generally ineffective. Hyperprolactinemia, amenorrhea is often associated with a pituitary adenoma. Amenorrhea resulting from ovarian malfunction is associated with 4 distinct endocrine conditions. Hyperprolactinemia, amenorrhea is often associated with a pituitary adenoma. Amenorrhea resulting from ovarian malfunction is associated with 4 distinct endocrine conditions. Hyperprolactinemia...
Suggested to prevent estrogen deficiency or for physiological reasons. If contraception is needed, oral contraception may be the choice for both cycle and fertility control. Hypergonadotropic amenorrhea is the result of the premature ovarian failure. There is no curative therapy for these patients, however, a long term hypogonadogenic condition should be treated with estrogen to cure symptoms and to prevent osteoporosis. Normalgonadotropic amenorrhea is caused by some disturbance in the ovarian function. Since these women have some ovarian activity, they are not hypoestrogenic and will bleed in response to progesterone withdrawal. Most of these patients are likely to have polycystic ovarian disease (PCO). Menstrual bleeding can be induced in these women by cyclical progesterone administration or the sequential use of estrogen plus progesterone. Oral contraception is indicated not only in patients who desire to be protected against pregnancy but also in women with acne and hirsutism.

**S5-24 Paediatric Gynaecology**

**Contraception During Adolescence**

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Sexual health for adolescents is based on three components: Recognizing sexual rights; sexuality education and counseling; and confidential high-quality services. Sexuality education needs to balance between prevention of pregnancy, prevention of STIs, and allowing for sexuality as a positive resource rather than a threat. Contraception needs to include prevention of both STIs and pregnancies. The first option is condoms backed-up by emergency contraception. A recent WHO study showed, that emergency contraception can be taken as a single dose of 1.5 mg levonorgestrel. Later there is a switch to oral contraceptives or other hormonal contraception in a longer relationship. Breast, pelvic and genital examination, and routine laboratory tests are not necessary before starting hormonal contraception. Condom use should not be stopped before it is reasonable certain that the partner is STI-negative. Other alternatives can be considered in special cases. Improved contraceptive methods do not automatically lead to reduced numbers of abortions. The prevention of unintended pregnancies requires a desire to use protection, a good contraceptive method, ability to obtain the contraceptive method, and ability to use it. High-quality sexual health services for adolescents call for specific clinics. These should have a youth-friendly atmosphere, where young people can feel comfortable. Unquestionable confidentiality is important. The providers must not moralize and judge the adolescents, but treat adolescents with respect indicating that young people are important. Services should be available at an affordable price, which preferably means free of charge. The threshold to come to the clinic should be low. When adolescent sexuality is not condemned but sexuality education and sexual health services are provided, it is possible to profoundly improve adolescent sexual health with comparatively small costs. Each year new groups of young people mature, requiring new efforts.

**S5-25 Sexual Dimorphism in Child Growth**

**Actions of Sex Steroids on the Growth Plate**

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Longitudinal growth is determined by a variety of hormones and growth factors. During puberty, estrogen contributes to the pubertal growth spurt through a stimulation of the somatotropic axis. A male patient with inactivated estrogen receptor (ER)-alpha gene and patients with aromatase mutations have confirmed the importance of estrogen in the regulation of growth plate fusion in females and males. This effect may be mediated via a direct effect on growth plate chondrocytes. Indeed, we now that both known estrogen receptors, ERalpha and EREbeta, are expressed in the growth plate, in both boys and girls, throughout pubertal development. Any functional role of ERalpha has yet not been defined in the human growth plate. However, recent data obtained in female mice with inactivated ERalpha and/or EREbeta, suggest that stimulation of ERalpha has the capacity to inhibit its skeletal growth and also to mediate growth plate fusion. An increased understanding about the effects of estrogen and interactions between estrogens and other endocrine factors within the epiphyseal growth plate is important for development of new treatment strategies in different disorders affecting longitudinal bone growth. Selective estrogen receptor modulators (SERMs) might be useful tools for modulation of pubertal growth. This possibility is supported by a recent report in rabbits showing that the SERM, raloxifene, acts as an estrogen receptor agonist on the growth plate without affecting the uterus. More studies are needed to further define the functional role of different ERs in the regulation of epiphyseal growth and growth plate fusion. This could open the door to more specific treatment modalities affecting longitudinal growth and growth plate fusion.

**S5-26 Sexual Dimorphism in Child Growth**

**Sexual Dimorphism in Intrauterine and Infantile Growth**

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Sexual dimorphism in the development of body composition during puberty has important implications for development of later adult disease risk. Overall, females gain 60% body weight between 11-18 years and males 78% between 12-19 years. This weight increase is partly attributed to gain in fat free mass of 30% in females and 50% in males. However, during later puberty, percentage fat mass tends to decrease in boys, while fat mass continues to rise by 1.14 kg/year in females. Fat mass is closely related to circulating leptin concentrations, and during puberty these levels tend to rise in girls, and fall in boys, reflecting their different changes in body composition. In girls, but not boys, leptin levels at the onset of puberty are predictive of subsequent gains in percentage fat mass. Sexual dimorphism in Adiponectin, IGF-1 and insulin levels is also observed during puberty, but the extent to which these reflect or determine change in body composition is unclear. Sex steroids are important determinants of “android” and “gyneco-cid” fat distribution; visceral adiposity being closely related to insulin resistance and cardiovascular risk factors. In males, central fat accumulation is associated with elevated blood pressure and subsequent cardiovascular risk. In females, central fat accumulation and insulin resistance are related to elevated androgen levels, particularly in girls with low birthweight followed by postnatal catch-up growth and proconvulsive: a sequence which leads to ovarian hyperandrogenism. In these subjects, body composition can be normalised by combination therapy with metformin and flutamide. Body composition in puberty “tracks” into adult life and common genetic variation is likely to be important. As yet, apart from recent links between aromatase and androgen receptor gene variants and androgen levels and body composition in girls, there are few data, however larger studies are currently in progress.
**S7-28** Capillary Blood Glucose Determination

**The Theoretical Basis of Glucose Monitoring**

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The aim of glucose monitoring is to give the possibility to the diabetic patient to master the control of his (her) glycemia. In type 1 diabetes, the main aim is to control the effect of the administered insulin. In type 2 diabetes, to estimate the quality of the achieved metabolic control. It is at the beginning of the 80s in the last century that glucose monitoring took its development, essentially due to the appearance of the first fingerprick devices. Within a few years, glucose monitoring widely replaced urinary monitoring, this one remaining nevertheless indispensable mainly in type 1 diabetes to detect ketosis episodes. During the 20 years which followed, glucose meters became smaller, the time of measurement dropped from two minutes to a few seconds, and the volume of the necessary drop of blood is now in the order of a few microliters only. More recently, a new concept appeared: continuous glucose monitoring; using a glucose sensor. Several configurations are possible, and some systems are already on the market. Thus, the CGMS developed by Minimed-Medtronic uses a glucose sensor having the shape of a needle implanted in the subcutaneous tissue; the GlucoDay system developed by Menarini uses a microdialysis fibre also implanted in the subcutaneous tissue. The GlucoWatch system developed by Cygnus uses the glucose extracted from the skin by iontophoresis. The ultimate goal is the control by the glucose sensor of the flow rate of a pump delivering insulin, leading to the development of an artificial beta cell. It is important to consider carefully the physiology of glucose concentration in blood an tissues to understand the results of glucose monitoring. We shall take two examples: 1) one suggests currently to measure glycemia in alternate sites, at the forearm for example. It is necessary to pay attention to the fact that there are differences between blood glucose measured at the forearm and at the fingertip, where blood is more arterial. In blood sampled at the forearm, changes in glycemia are slower, and one risks to underestimate episodes of hypoglycaemia. 2) Systems for continuous glucose monitoring often measure glucose not in blood but in interstitial fluid. There are also important differences between changes in glucose concentration in blood and in interstitial space, because insulin pulls glucose from this space to the surrounding cells. This explains for example that concentration in glucose in this liquid is often lower than glycemia. The existence of lags between changes in blood glucose and in interstitial glucose concentration must be taken into account if one envisages this type of system as a hypoglycaemic alarm. It will also be necessary to take into account this issue for the development of closed-loop insulin delivery systems: slow kinetics would lead to abnormal oscillations between hyper and hypoglycaemia.

**S7-29** Capillary Blood Glucose Determination

**Continuous Subcutaneous Glucose Monitoring in Children**

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Pediatric experience with continuous glucose sensors is increasing. As maintaining blood glucose levels within the target range can be an elusive goal in children with diabetes mellitus these monitors are used predominantly to identify factors that may contribute to glycemic instability. Several studies using such systems demonstrated facilitated and improved pediatric diabetes treatment, and patients received new insight and increased motivation. Also they have been shown to be of diagnostic value in assessing glucose fluctuations in MODY and Type 2 diabetes cases. A second reason for using these devices in children is to identify asymptomatic hypoglycaemia particularly at night. In this regard it is important whether under all circumstances changes in blood glucose are paralleled by glucose changes in the interstitial fluid. Recent data studying the two most widely applied continuous sensors, the GlucoWatch G2 Biographer and the continuous glucose monitoring system (CGMS), in pediatric patients indicated that they do not reliably detect hypoglycaemia. Thus, these devices perform better at higher glucose levels, suggesting they may be more useful in reducing HbA1c levels than in detecting hypoglycaemia. Finally, the sensors may also allow to characterize the day-to-day and within-day blood glucose variability. This is of particular importance as differences in blood glucose fluctuations may contribute to the development of diabetic late complications independent of HbA1c. Postprandial hyperglycaemia can exist despite excellent HbA1c target prandial glucose monitoring. The HbA1c is known to mask high and low fluctuations. Calculating the glucose area under the continuous glucose profiles has been shown to serve as indicator of glycemic variability. Thus, in addition to HbA1c and self-blood glucose monitoring, continuous subcutaneous glucose monitoring may turn out to be the third pillar in the assessment of metabolic control in pediatric diabetes patients.

**S7-30** Capillary Blood Glucose Determination

**Glucose Monitoring Systems in the Future**

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Nature’s biological master model for a lifelong truly continuous glucose monitoring (GM) system in vivo, e.g. within the pancreatic islet or the hypothalamus, and their inclusion into a feed back system for insulin secretion/production and a rather complex regulation of glucohomeostasis represents the challenging standard that has not been met by any marketed GM device so far. Despite the fact that for the foreseeable future of the next 5 – 10 years such a perfect GM system will probably not be available (except for a few 100 pancreas transplants/year), considerable efforts are invested globally into a large variety of minimally or non-invasive GM techniques for spot as well as continuous GM at different skin sites and depths, but also in the eye, the saliva or even the breath, to replace the current use of invasive strip based systems for capillary blood. The industrial technological research that specific fatty acids like isomers of conjugated linoleic acid (CLA) may derived from isolated islets of different species with the obvious need for some form of encapsulation to avoid local and/or systemic destructive reactions in vivo. Which of these GM system developments will finally satisfy the daily needs particularly of our pediatric patients remains speculative at present. Intravenous placement of an enzyme-based needle glucose sensor has been successfully studied in few adult patients with type 1 diabetes for several months, including 24 hours pilot closed loops with an insulin pump, but this approach is of questionable value for general use due to its very invasive nature and the related potential serious side effects. The combination of a more generally applicable GM system with an insulin pump remains the ultimate longterm goal of an artificial pancreas.

**S7-31** Capillary Blood Glucose Determination

**Endocrine Regulation of Adiposity**

**Regulation of Adipocyte Differentiation and Function**

**S. Mandrup**

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Adipocytes serve a main function as a site of storage of excess energy in the form of triglycerides. However, equally important is the secretion of factors (so-called adipokines) from adipocytes, many of which regulate whole body metabolism and homeostasis. Adipocyte differentiation and function are controlled by a large number of endocrine and paracrine factors. Some of the paracrine factors originate from the preadipocytes/adipocytes themselves, whereas others originate from non-adipocytes cell types like macrophages and endothelial cells. It has recently become increasingly clear that obesity can be seen as an inflammation of the adipose tissue in which macrophages accumulate in the tissue. The cytokines released by the macrophages have profound effects on adipocyte function. This talk will review the current knowledge on how endocrine and paracrine factors affect adipocyte differentiation and function. A large body of evidence suggests that specific fatty acids like isomers of conjugated linoleic acid (CLA) may have a significant effect on adipose tissue function. Our recent data on how isomers of CLA affect both differentiation and function of adipocytes will be presented. Using cultures of primary human in vitro differentiated adipocytes, we have shown that trans-10, cis-12 CLA, but not cis-9, trans-11, inhibits both differentiation of adipocytes as well insulin sensitivity and expression of adipocyte-specific markers in mature adipocytes.

**Symposia**

**Endocrine Regulation of Adiposity**

**Regulation of Adipocyte Differentiation and Function**

**S. Mandrup**

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Adipocytes serve a main function as a site of storage of excess energy in the form of triglycerides. However, equally important is the secretion of factors (so-called adipokines) from adipocytes, many of which regulate whole body metabolism and homeostasis. Adipocyte differentiation and function are controlled by a large number of endocrine and paracrine factors. Some of the paracrine factors originate from the preadipocytes/adipocytes themselves, whereas others originate from non-adipocytes cell types like macrophages and endothelial cells. It has recently become increasingly clear that obesity can be seen as an inflammation of the adipose tissue in which macrophages accumulate in the tissue. The cytokines released by the macrophages have profound effects on adipocyte function. This talk will review the current knowledge on how endocrine and paracrine factors affect adipocyte differentiation and function. A large body of evidence suggests that specific fatty acids like isomers of conjugated linoleic acid (CLA) may have a significant effect on adipose tissue function. Our recent data on how isomers of CLA affect both differentiation and function of adipocytes will be presented. Using cultures of primary human in vitro differentiated adipocytes, we have shown that trans-10, cis-12 CLA, but not cis-9, trans-11, inhibits both differentiation of adipocytes as well insulin sensitivity and expression of adipocyte-specific markers in mature adipocytes.
The identification and characterization of patients with morbid obesity due to mutations in single genes has shed light on the molecular mechanisms underlying the hypothalamic regulation of appetite, body weight and endocrine axes. Two severely obese cousins in a consanguinous family were found to have undetectable levels of serum leptin and were homozygous for a frameshift mutation in the ob gene. These children were severely hyperphagic, constantly demanding food and developed severe disabling obesity, impaired T cell mediated immunity and hypogonadotropic hypogonadism. Treatment with recombinant human leptin for up to six years led to sustained, beneficial effects on appetite, fat mass, hyperinsulinaemia and hyperlipidaemia. The major impact of leptin was on food intake with a marked reduction in caloric consumption during a test meal. Leptin administration permitted the full progression of appropriately timed puberty but does not appear to cause precocious activation of puberty in younger children. Mutations in the leptin receptor result in a similar phenotype. We have recruited over 1000 patients with severe, early onset obesity as part of the Genetics of Obesity Study (GOOS). Complete loss of pro-opiomelanocortin derived peptides results in isolated ACTH deficiency, red hair, pale skin and obesity. We have recently identified a second patient who is a compound heterozygote for mutations in pro-hormone convertase-1 which results in a complex endocrinopathy and enteropathy due to a failure of prohormone processing. Loss of function mutations in the melanocortin 4 receptor (MC4R) cause a dominantly inherited obesity syndrome that accounts for up to 6% of patients with severe, early-onset obesity. MC4R deficiency is characterised by hyperphagia, severe hyperinsulinaemia and increased linear growth. These studies provide evidence for the pivotal role of the leptin-melanocortin system in energy balance and neuroendocrine function in humans.

### S8-33 Endocrine Regulation of Adiposity

**International Consensus Development on Childhood Obesity**

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In light of the current worldwide epidemic in childhood obesity, a multidisciplinary group of professionals with a special interest in obesity met to develop a wide ranging consensus statement on all aspects of the problem. We hoped that by drawing upon current evidence that we could highlight issues relating to the care of obese children contribute towards prevention strategies and examine areas requiring further research.

The consensus process:

Sixty five professionals (paediatric endocrinologists, community paediatricians, adult endocrinologists, dietitians, psychologists and exercise physiologists) from eight countries convened for 3 days at the Dead Sea in Israel. Prior to the meeting seven groups were formed to address the areas of prevalence, treatment, prevention, psychology, diagnosis, risks and causes. Participants each undertook to address key questions, appraise the literature and draft a document which was circulated to their group. At the meeting the documents were discussed in groups and then by the entire meeting, recommendations were drawn up and agreement was reached.

The consensus conclusions:

- Debate was extensive, with emphasis on the environmental (as opposed to endocrine) causes of obesity, the need for lifestyle change and how this can be achieved. Controversial issues included
  - The use of BMI as a measure and the importance of the IOTF criteria
  - The value of waist circumference
  - The problem of obesity in infancy
  - Investigation for comorbidities
  - The need for prenatal prevention strategies
  - The definition of the metabolic syndrome in childhood

By the end of the meeting consensus was achieved and a document is in preparation for publication for the medical community. We believe that the development of the consensus statement will provide a valuable contribution towards the development of improved clinical care, help delineate approaches to prevention and signpost areas requiring further research.

### S8-34 Gene imprinting in Paediatric Endocrinology

**Basic Mechanisms of Imprinting**

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Although most genes are expressed from both alleles, some genes are genomically imprinted such that one allele, the maternal or paternal, is active while the other is silenced. This process is carried out at the molecular level by marking the maternal and paternal alleles in the gametes and maintaining this differential epigenetic pattern throughout development. While DNA methylation plays an important role in this regulatory mechanism, these gene regions are also characterized by asynchronous replication timing whereby one allele replicates early in S phase, while the other replicates later. This differential marking probably affects expression by modifying chromatin structure. Genomic imprinting is actually part of the more general phenomenon of random allelic exclusion, whereby some cells express the maternal allele while others express the paternal allele. This type of monoallelic expression is characteristic of the immune and olfactory receptor loci where it plays a key role in the generation of gene diversity.

### S8-35 Gene imprinting in Paediatric Endocrinology

**Role of Genomic Imprinting in Fetal Growth Restriction**

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Genomic imprinting is an epigenetic phenomenon resulting in monoallelic expression of genes in a parent of origin dependent manner. It is found almost exclusively in eutherian mammals, and is known to be an important regulatory pathway in growth, development and behaviour in the fetus. About 70 imprint genes have been found so far in the mouse, and for the majority the imprinting status is conserved in humans. Phenotypic effects observed from the disruption of imprinting in mouse and man are often growth-related and occur in utero. The paternal and maternal genomes have been shown to be non-complementary in terms of murine embryonic development, and it is these experiments that first illustrated the involvement of imprinting in normal fetal growth. Paternogenes containing two maternal genomes, give rise to embryos that do not develop well and die after implantation due to failure of the extra-embryonic components. Conversely, androgens have development of extra-embryonic tissues but failure of the embryo. Paternally expressed genes appear to be critical for placental development. Recently it has been reported that some genes in the mouse are only imprint in the placenta, and therefore may affect growth by influencing the fetal demand for, and the placental supply of, nutrients from the mother. Generally, imprint genes that are paternally expressed enhance growth and maternally expressed genes suppress it. This paternal versus maternal genome tug-of-war is the basis of the genetic conflict hypothesis. It is suggested that paternally derived genes influence nutrient acquisition by selecting more nutrients for the current fetus, while maternally derived genes balance the provision of nutrients to the current fetus protecting her potential for future successful pregnancies. These observations will be discussed with respect to human fetal growth restriction and the genetic aetiology of the growth restriction phenotype in Silver-Russell syndrome.

### S8-36 Gene imprinting in Paediatric Endocrinology

**Pseudohyoparathyroidism: a Spectrum of Imprinted Disorders Caused by Different Coding and Non-Coding Mutations in GNAS**

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Pseudohypoparathyroidism (PHP), i.e. hypocalcemia and hyperphosphataemia due to renal PTH resistance, was first described by Fuller Albright and his colleagues. These patients furthermore presented with clinical features now referred to as Albright’s hereditary osteodystrophy. Inactivating stimulatory G protein (Gs-alpha) mutations cause this form of PHP, PHP-Ia. Gs-alpha is encoded by exons 1-13 of GNAS, a complex gene which undergoes parent-specific methylation and gives rise to at least five different alternatively spliced mRNAs that are, with the
exception of Gs-alpha, transcribed from the non-methylated parental allele. Similar or identical GNAS mutations are also found in patients with pseudohy-
poparathyroidism (PPHP) and progressive osseous heteroplasia (POH). In con-
trast, most patients affected by PHP-Ib have no mutations in one of the 13 exons 
of GNAS that encode Gs-alpha. However, a familial form of PHP-Ib with an auto-
sonal dominant mode of inheritance and paternal imprinting (AD-PHP-Ib) was 
mapped to a 2.5-Mb locus on 20q13.3 comprising a portion of GNAS. Moreover, 
most familial PHP-Ib cases show a loss of methylation at the exon A/B differen-
tially methylated region (DMR) of GNAS. Recently, a 3-kb deletion located 
approximately 220-kb upstream of exon A/B was identified in more than 20 AD-
PHP-Ib kindreds, but not in healthy controls. The deletion was shown to be inher-
ited maternally and to be associated with loss of methylation at exon A/B alone. 
The identified deletion likely disrupts a cis-acting element necessary for establish-
ment and/or maintenance of the methylation imprint at GNAS exon A/B of the 
maternal allele. The loss of exon A/B methylation results in suppression of Gs-
alpha transcription in the renal cortex leading to PTH resistance. Since the 3-kb 
deletion was not identified in some AD-PHP-Ib families and many sporadic cases, 
additional genetic defects may be involved in the development of PHP.