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Abstracts

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Reproduktion und Endokrinologie

OP 1-1

The microRNA miR-145 contributes to the pathogenesis of endometriosis by targeting pluripotency factors and cytoskeletal elements

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microRNAs are small noncoding RNAs which regulate gene expression at the posttranscriptional level. Endometriosis is a disease characterized by ectopic growth of endometrial tissue in distant locations such as the ovary or peritoneum, leading to reduced fertility. The objective of the present study was to identify and confirm target genes and proteins of miR-145, previously shown to be misexpressed in endometriotic tissue, and to study the functional consequences of miR-145 dysregulation *in vitro*. Ectopic expression of miR-145 induced a significant 34% reduction in proliferation of the endometriotic cell line 12Z, and a significant 80% inhibition of matrigel chamber invasiveness ($p < 0.05$). qPCR revealed a significant downregulation of mRNA expression for the cell adhesion molecule JAM-A, and the cytoskeletal elements fascin and PODXL by about 70%. Expression of the pluripotency factors SOX2, KLF4, and OCT4 was downregulated by about 50% upon ectopic miR-145 expression ($p < 0.01$). Flow cytometric investigations revealed a decrease in the fraction of side population cells upon ectopic miR-145 expression. Downregulation of JAM-A and fascin was confirmed at the protein level by Western blotting. In addition, direct miR-145-dependent downregulation of JAM-A via its 3´UTR was confirmed using luciferase assays. We conclude that miR-145 modulates endometriotic cell proliferation and invasiveness by targeting the expression of cell adhesion molecules, cytoskeletal elements and pluripotency factors. Ectopic expression of miR-145 may emerge as a novel future therapeutic concept in endometriosis.

OP 1-2

Posttranscriptional inhibition of the receptor tyrosine kinase coreceptor Syndecan-1 results in decreased invasiveness and proliferation of human endometriotic cells

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Syndecan-1 is cell-surface heparan sulfate proteoglycan that acts as a co-receptor for growth factors and chemokines. *In silico* analysis revealed that Syndecan-1 is a potential target for posttranscriptional regulation by miR-10b, one of the microRNAs showing decreased expression in endometriosis. Characterized by ectopic growth of endometrial tissue, this diseases is associated with reduced fertility and pain symptoms. To study the function of Syndecan-1 and its potential regulation by miR-10b, the human immortalized epithelial endometriotic cell line 12Z was transiently transfected with the pre-microRNA miR-10b or a Syndecan-1 siRNA. The direct regulation of the proteoglycan by miR-10b was demonstrated by a 3´UTR luciferase assay. Changes in the Syndecan-1 expression were further confirmed by qPCR, FACS and Western blotting. The ectopic expression of miR-10b led to a significant lower (20%) invasiveness in matrigel assays and well as a lower (14%) proliferation rate as determined by MTT assay. Ectopic expression of a Syndecan-1 construct lacking its endogenous 3´UTR abolished the invasion -inhibiting effect of miR-10b, confirming Syndecan-1 as a major relevant target for this phenotype. In contrast, the siRNA knockdown of Syndecan-1 led to a significant lower (65 %) invasiveness in these cells. We conclude that Syndecan-1 is a new target molecule of miR-10b potentially involved in the pathogenesis of endometriosis. Syndecan-1 expression modulates endometriotic cell invasiveness, thereby facilitating the establishment of endometriotic lesions at ectopic sites. Our data suggest that a pharmacological modulation of either miR-10b or Syndecan-1 could be a promising therapeutical concept for this disease.

OP 1-3

Oncofertility - Experience with Cryopreservation and transplantation of ovarian tissue

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Introduction: Cryopreservation of ovarian tissue with subsequent retransplantation following a period of recurrence -free survival is a promising technique for fertility preservation in patients facing gonadotoxic treatment. Seventeen live-births have been reported worldwide from this procedure. It can be expected that in the near future more and more cancer patients who have been cured of their disease are likely to request reimplantation of their cryopreserved ovarian tissue. However, there are still many unanswered questions regarding the best location to transplant ovarian tissue and extending the longevity of tranplanted ovarian tissue. This recitation summarizes the Erlangen expertise on cryopreservation and transplantation of ovarian tissue following 7 retransplantations**.**
Method: Ovarian tissue from 7 cancer patients (n=3 hodgkin, n=2 anal cancer, n=2 breast cancer) were cryopreserved with a slow freezing protocol. After cancer remission, the cryopreserved ovarian tissues were retransplantated orthotopically (either into the pelvic wall near or into the remaining ovary).
Results: All patients regained ovarian function between 8 and 24 weeks after transplantation as shown by follicle development and estrogen production. All these women have had regular menstrual cycles and in 4 patients, oocytes from the transplantated ovarian tissue could be retrieved and fertilized. Recurrence of the primary disease was not observed in any of these cases. To date, one patient delivered a healthy child 16 months after transplantation.
Conclusion: Cryoperservation and autotransplantation of ovarian tissue is an encouraging method of reestablishing fertility in women with iatrogen POF. We believe that ovarian cortex cryopreservation should be offered before gonadotoxic chemo- or radiotherapy in all cases where there is a high risk of POF. The network "FertiProtekt" (www.fertiprotkt.de) makes recommendations for fertility preservation in patients with different tumor diseases.

OP 1-4

Mitochondrial redox profile after CryoTop vitrification of in vitro matured murine metaphase II oocytes or germinal vesicle oocytes from preantral follicles

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Introduction: There is so far no evidence for reduced quality and late adverse effects of vitrification of mouse preantral follicles or human metaphase II stage oocytes. Redox regulation and control of metabolic homeostasis are critical for stress resistance and normal embryonic development. *In vitro* maturation (IVM) may influence oocyte quality. Improved culture conditions possibly help to improve stress resistance and developmental potential.
Methods: mRNA coding for a fusion protein with a mitochondrial translocation peptide, glutaredoxin-1 and redox sensitive roGFP2 sensor was microinjected into mouse germinal vesicle (GV) oocytes, followed by IVM and CryoTop vitrification at MII stage, or into GV oocytes of preantral follicles, followed by vitrification and warming 24h later. Inner mitochondrial redox potential (E^m_{GSH}) was quantified in live oocytes. To improve oocyte quality, IVM medium was supplemented with L-carnitin or glutathione ethyl ester (GEE).
Results: The fusion protein was expressed efficiently in mitochondria of GV oocytes. Vitrification did not significantly affect E^m_{GSH} in GV oocytes of preantral follicles compared to controls (-360,4mV vs. -359,9mV; n=33 and 60). Vitrification at MII stage following IVM significantly altered E^m_{GSH} ($p< 0.001$; -324,4mV control; -335,7mV vitrified). It appears that L-carnitin or GEE supplementation during IVM alters E^m_{GSH} but further studies are required to test whether this improves tolerance to vitrification.
Conclusions: Vitrification of healthy, GV arrested oocytes in preantral follicles does not appear to alter redox regulation significantly. However, vitrification affects metaphase II oocytes derived from IVM without cumulus. Therefore, stress tolerance to vitrification appears reduced after sub-optimal IVM. L-carnitin or GEE modifies cellular redox homeostasis and thereby may improve oocyte quality and stress tolerance. Observations appear relevant for clinical applications of IVM and vitrification.

OP 1-5

Natural cycle IVF: evaluation of 591 cycles

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Introduction: Over the last decade, natural cycle (NC) IVF/ICSI has proved to be easy, safe and an alternative to conventional IVF/ICSI cycles. Among others, patients with poor ovarian response or with ovarian hyperstimulation syndrome (OHSS) might benefit. The aim of our retrospective cohort study was to analyse the fertilization rate and pregnancy rate in NC patients.
Material and methods: From May 2007 to December 2011 n=152 infertile couples underwent n=591 cycles of NC-IVF/ ICSI. At a follicle diameter of ≥17 mm, ovulation was triggered with HCG. Oocyte retrieval was performed 35 hours later without anaesthesia. Depending on male fertility parameter oocytes were fertilized by IVF/ICSI. A maximum of 2 embryos was transferred two or three days later.
Results: The mean age was 36.3 ± 4.6 years (mean±SD), mean BMI was 23.5 ± 4.0 kg/m² and mean FSH levels on day 3 were 9.0 ± 6.7 IE/l. Patients had tried to conceive for 5.0 ± 3.2 years.
Oocyte retrieval was performed in n=458 NC (77.5%), in 302 cases retrieval was successful (IVF n=112, ICSI n=190). Altogether, 194 Embryos (IVF n=79, ICSI n=105) were transferred in 184 embryo transfers (fertilization rate: 64.2%). Finally, 18 (11.8%) patients conceived, one patient twice (pregnancy rate: 10.3%). Pregnancies resulted in one biochemical pregnancy (0.5%), 7 (3.8%) miscarriages, 4 (2.2%) live births and one pregnancy of unknown outcome (0.5%). 6 pregnancies are still ongoing (3.3%).
Conclusion: Since only 3 cycles of conventional IVF/ICSI are partially paid by health insurance funds in Germany, a cost-saving alternative is highly appreciated by the patients. So far, NC did not meet the expectations and pregnancy rate is quite low due to unfavourable preconditions (e.g. patients age, ovarian reserve).

OP 1-6

Loss of Lin28 and PGP9.5 expression in the postnatal testis of 41,XX^{Y*} mice

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Background: The male genetic disorder Klinefelter's syndrome (KS) is characterized by a supernumerary X-chromosome. Patients present a heterogenous phenotype frequently associated with hypergonadotropic hypogonadism, cognitive and metabolic deficits. In the majority of patients complete germ cell loss is found after puberty indicating problems in the maintenance of the spermatogonial stem cell (SSC) population that might be affected by altered expression of stem cell relevant genes. Therefore, utilizing our mouse model, we examined the SSC marker Lin28 and PGP9.5 expression in order to characterize the SSC population in 41,XX^{Y*} mice.
Material and methods: Immunohistochemical staining (LIN28A, PGP9.5) in testes of different developmental stages (1, 3, 5, 10, 14, 21 dpp and 30 wks pp; n=3 each) of male mice with a 41,XX^{Y*} karyotype and 40,XY* controls.
Results: Lin28 and PGP9.5 were expressed in control testes over all developmental stages. Postnatally, almost all gonocytes were stained positively and afterwards a decreasing but still substantial subpopulation of SSCs was positively stained up to the adult state. In contrast, in 41,XX^{Y*} mice at 1 dpp Lin28 already fewer positive cells were found, and from 3 dpp onwards Lin28 expression was no longer observed, although positive staining for PGP9.5 was still present up to 5 dpp, before it was not longer detectable. At 14 dpp the germ cells were depleted in 41,XX^{Y*} mice.
Conclusion: In contrast to previous studies we found evidence that germ cell loss in male mice with a supernumerary X chromosome is already visible postnatally. Our results on the mouse model point to a very early disturbance of the germ cell fate, a finding that might have implications for fertility preservation treatment of Klinefelter patients.

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OP 1-7

Crosstalk between Arylhydrocarbon receptor (AhR and Peroxisome Proliferator-Activated Receptor (PPAR) signaling pathway in human granulosa cells

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There is much evidence that environmental contaminants binding to the AhR and/or to PPARs might contribute to adverse effects on reproduction.
DEHP (di-(2-ethylhexyl) phthalate, a common plasticizer) is known as ovarian toxicant in rodents. It alters gene expression predominantly by activating PPARs. These are transcription faktors that are critical for several metabolic pathways but also for normal ovarian function by mediating steroidogenesis.
TCDD (tetracholordibenzodioxin) as a strong ligand of the AhR, a widespread nuclear transcription factor with an important role in many tissues and in ovarian physiology too, leads to altered gene expression of gonadotropin and estrogen receptors as well as to a decreased estradiol synthesis in the human granulosa cell line KGN. The KGN cell line, expressing PPARs as well as AhR, offers the opportunity to study regulatory mechanisms independent from overriding endocrine control.
KGN cells were stimulated with DEHP and/or TCDD with or without simultaneous exposure to specific antagonists of AhR, PPARalpha or PPARgamma. The expression of target genes and functional granulosa cell markers were analysed by quantitative real-time PCR. ELISAs have been performed to measure estradiol synthesis.
Expression analysis shows a PPAR-dependent increase of AhR and CYP1B1 transcription under DEHP exposure. A TCDD DEHP mix shows no additive effects in expression of these genes. Expression of the PPARα and PPARγ target genes 17beta-HSD and PEPCK were unaffected by TCDD exposure. ERalpha, FSH-R and LH-R expression was upregulated under DEHP exposure. Upregulation did not occur when specific PPARα and PPARγ antagonists were simultaneously applied.
DEHP exposure of the human granulosa cell line KGN induced an activation of AhR and AhR target genes and expression of gonadotropin and estrogen receptors via the PPAR signaling pathway. An activation of PPAR signaling downstream of the AhR pathway was not detectable.

OP 1-8

Thyroid autoimmunity does not impair assisted reproductive technology outcome in fertile women

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Introduction: Thyroid autoimmunity (TAI) has been associated with adverse pregnancy outcomes in subfertile women with spontaneous and ART induced pregnancies. The underlying pathophysiology is still elusive. Its association with other syndromes, thyroid dysfunction and higher age has been discussed. TAI itself has been associated with female subfertility. However, a higher prevalence of TAI is described in various disorders like endometriosis, which themselves can hamper fertility and pregnancy outcome. To further investigate the contribution of TAI to complications in ART-induced pregnancies, we examined the impact of TAI on maternal and neonatal outcome in euthyroid fertile women, who underwent intracytoplasmatic sperm injection (ICSI) only because of male infertility reasons.

Materials and methods: In 835 fertile, euthyroid patients (age: 31.4±4.3 ys., BMI: 23.7±4.2 kg/m^2), TAI-status was correlated retrospectively with maternal (pregnancy (PR), birth (BR) and miscarriage rate (MR) and neonatal outcome (gestational age, birth size and weight) of ICSI procedure. TSH, age, BMI and multiple pregnancy were used as covariates. TAI-positive and -negative groups did not differ in age and TSH level.

Results: TAI status did not influence maternal and neonatal outcome in women undergoing ICSI. Maternal age was correlated with lower PR (OR: 0.942 (95%CI: 0.91-0.97); p=0.0003) and BR (OR: 0.934 (95%CI: 0.09-0.97); p< 0.0001).

Conclusion: Our study in a preselected cohort suggests that age but not TAI *per se* has a negative impact on ICSI success in fertile, euthyroid women undergoing ICSI. Association between TAI and miscarriage or preterm delivery could be due to its association to higher age and TSH or fertility limiting diseases.

Hypophyse und Schilddrüse

OP 2-1

Cardiac risk in patients with treatment naïve (ACT), first-line medically controlled (MED) and first-line surgically cured (SUR) acromegaly in comparison to matched data from the general population

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Coronary risk factors in patients with acromegaly after first-line transsphenoidal surgery (TSS) or first-line somatostatine analogue (SSA) treatment have rarely been examined. Aim was an evaluation of risk factors and left ventricular hypertrophy (LVH) in 3 different patient groups with ACT, MED and SUR acromegaly and a calculation of the Framingham Weibull Risk Score (FS).
Design: Retrospective comparative matched case-control study.
Patients and methods: 40 acromegalic patients (aged 45-74 years, 23 men) were matched with respect to age and gender to 200 controls from the general population. 13 patients had ACT, 12 MED and 15 SUR acromegaly. Risk factors were assessed after 12 months of treatment. Only patients normalized for IGF-I in MED and SUR group were included. FS and odds ratios (OR) from multiple conditional logistic regression (matched for age and gender, adjusted for BMI) were calculated.
Results: Compared to controls ACT patients had higher HbA1c levels (6.9±1.4 vs 5.5±0.7% (p< 0.0001)) and an increased prevalence of LVH (30.8 vs 3.2% (p=0.007)). MED and SUR groups were similar for gender, age, disease duration and IGF-I levels at diagnosis. Compared to controls, MED patients had an increased diastolic blood pressure (89±9 vs 79±11mmHg (p=0.001), prevalence of LVH (41.7 vs 1.7% (p< 0.0001), prevalence of diabetes mellitus (33.3 vs 10.0% (p=0.03)), higher HbA1c levels (6.8±1.3 vs 5.5±0.7% (p=0.0005)) and a higher FS (21.2±9.7 vs 12.4±7.7% (p=0.002), OR 1.11 [1.02-1.21] (p=0.01)) while in the SUR group only higher prevalences of LVH (40.0 vs 4.1% (p< 0.0001)) and HbA1c levels (6.4±1.2vs5.5±0.8% (p=0.006)) were found compared to controls.
Conclusion: When comparing treatment naive, medically treated and surgically cured patients with acromegaly to age- and gender-matched subjects from the general population, we have found an increased cardiovascular risk in patients at 12 months after first-line SSA treatment but not in patients after first-line surgery.

OP 2-2

Metabolic changes in adult patients with craniopharyngiom

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Introduction: Patients with craniopharyngeoma often have multiple pituitary insufficiencies and serious metabolic changes leading to obesity. We hypothesized, that adult patients with craniopharyngioma have more metabolic complications compared to adult patients with non-functioning pituitary adenoma (NFPA).
Methods: 33 patients (m=16, f=17, median age: 48 years [26-77]) with craniopharyngioma and 33 patients (m=24, f=9, median age: 66 years [44-80]) with NFPA were included in the study. Most of the patients had multiple hormonal pituitary insufficiencies. We performed an oral glucose tolerance test (OGTT) lasting 3h with assessment of glucose and insulin and measured HDL and LDL as well as total cholesterol at baseline. Furthermore, we evaluated clinical parameters as height, weight, blood pressure, waist and hip circumference. Total body fat mass (=FM) was determined by dual-x-ray-absorptiometry. For analysis of insulin resistance (IR) homeostatic model assessment (HOMA) was used.
Results: Patients with craniopharyngioma were significantly smaller than patients with NFPA (168cm [136-186] vs. 175cm [158-191], p=0.016) but they did not significantly differ in weight (91kg [54-122] vs 82kg [54-127]. They had significantly higher fasting glucose levels (82.5 mg/dl [68-120] vs 73.5 mg/dl [54-112], p=0.004) and higher FM (38.48% [20.90-54.90] vs 32.48% [17.90-53.70], p=0.017). Furthermore, significantly more patients with craniopharyngioma had an IR (HOMA-IR>2.5, n=25 [76%] vs. n=16 [49%], p= 0.041). There were no differences in blood pressure, lipid levels, fasting insulin levels as well as glucose and insulin levels during OGTT.
Conclusion: Patients with craniopharyngioma have more metabolic complications than patients with NFPA. As both groups had multiple pituitary insufficiencies, hypothalamic damage caused by the craniopharyngeoma might be an additional factor. The diminished height might be due to an onset in childhood or puberty.

OP 2-3

Positive association of serum prolactin concentrations with all-cause and cardiovascular mortality: Results from a 10-year follow-up of the Study of Health Pomerania (SHIP)

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Background: Increased serum prolactin (PRL) concentrations have been associated with adverse cardiovascular risk profiles, but the relation between PRL and mortality risk is unknown.
Methods: We evaluated 3,929 individuals (1,946 men and 1,983 women) aged 20 to 81 years (mean 50.3 years) from the population-based Study of Health in Pomerania. Associations of continuous (per standard deviation [SD] increase) and categorized (sex-specific tertiles) serum PRL concentrations with all-cause and cause-specific mortality were analyzed separately for men and women by age- and multivariable-adjusted Cox regression models.
Results: During a median follow-up period of 10.1 years (38,231 person-years), 419 deaths (10.7%), 132 cardiovascular deaths (3.4%), and 152 cancer deaths (3.9%) were observed. After multivariable adjustment, we observed a positive association of PRL with all-cause mortality in men and women (hazard ratio [HR] per SD increase: 1.17; 95% confidence interval [CI], 1.07 to 1.29 and 1.22; 95% CI, 1.03 to 1.46, respectively). Similarly, individuals with PRL concentrations in the highest tertile (as compared to lowest PRL tertile) experienced the highest mortality risk (men: HR, 1.75; 95% CI, 1.32 to 2.32 and women: HR, 1.66; 95% CI, 1.08 to 2.56), with a significant trend across PRL tertiles (p for trend < 0.05). Cause-specific mortality analyses yielded similar associations for cardiovascular, but not for cancer death.
Conclusions: This is the first study to report an independent positive association of PRL concentrations with all-cause and cardiovascular mortality. Further studies are required to confirm our findings and to elucidate the potential role of PRL as a useful biomarker of cardiovascular risk and mortality assessment.

OP 2-4

A new thyrotropin receptor mutation in a patient with specificities of congenital hypothyroidism reveals extended insights into mechanisms of signal transformation

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In our study we investigated details of a family case of congenital hypothyroidism and thyroid dysgenesis caused by a newly identified homozygous thyrotropin receptor (TSHR) mutation. The mutation is located at position 579 in exon 10, leading to an exchange of alanine to valine (p.Ala579Val). Additionally, we explored functional properties of this pathogenic TSHR variant in transmembrane helix (TMH) five by in *vitro* studies to gain deeper insights into its functional mechanism.
The receptor variant Ala579Val was tested in cell-systems for signaling capabilities and cell surface expression compared to wild type. Tight interactions of Ala579 with residues of the second extracellular loop (ECL2) were suggested by a structural TSHR model and we evaluated this hypothesis by testing different side chain variations at position 579.
Patient's TSHR mutaion revealed a decreased cell surface expression down to 30% and a completely impaired signaling capacity compared to wild type. Substitutions with other more bulky and branched side chains compared to the small alanine also resulted in a complete loss of cAMP and IP signalling, whereas serine receptor variant resulted in a complete functional receptor.
The newly identified TSHR substitution Ala579Val leads to impaired functional parameters. Our results indicating that Ala579 is interacting tightly with the ECL2, which is of high priority for signaling regulation at TSHR. The Patient's mutation Ala579Val modifies by repulsion these interactions, which likely leads to a relative spatial shift of ECL2 and other receptor components to each other. Such mutated receptor is incompetent for signal transformation from the extra- to the intracellular site.
Finally, we here describe a case of congenital hypothyroidsm, caused by a new thyrotropin receptor mutation.

OP 2-5

Molecular fine needle aspiration (FNA) diagnostics is possible in routine air dried FNA smears

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Although FNA is currently the most sensitive method to select suspicious nodules for surgery it has some inherent limitations, e.g. indeterminate samples. As rearrangements (*PAX8/PPARG, RET/PTC)* and point mutations (*BRAF, NRAS*) were detected in follicular carcinomas (FTC) and papillary carcinomas (PTC), its detection in FNA smears could improve the diagnosis.
RNA and DNA was extracted from 104 routine air dried FNA smears and corresponding FFPE samples. *PAX8/PPARG* and *RET/PTC* rearrangements were detected by FRET-PCR while *BRAF* and *NRAS* point mutations were detected by FRET-PCR and HRM-PCR.
The comparison of FRET- and HRM-PCR for the detection of point mutations in a subset of 50 FNA and FFPE samples revealed a significant improvement by HRM-PCR: while the number of questionable results and PCRs with no result could be reduced, the number of mutations detected could be increased.
PAX8/PPARG rearrangements were detected in 5 FNA smears and in 3 FFPE samples with overlapping results in 2 FNA and corresponding FFPE samples. *RET/PTC* rearrangements were detected in 4 FNA smears and in 3 FFPE samples with overlapping results in 2 FNA and corresponding FFPE samples.
BRAF mutations were detected in 10 FNA smears and in 19 FFPE samples with overlapping results in 7 FNA and corresponding FFPE samples. *NRAS* mutations were detected in 12 FNA smears and in 22 FFPE samples with overlapping results in 6 FNA and corresponding FFPE samples.
In summary, these results show for the first time the feasibility to extract RNA from routine air dried FNA smears to detect *PAX8/PPARG* and *RET/PTC* rearrangements with RT-PCR. Moreover, HRM is more reliable and more sensitive than FRET-PCR in the detection of point mutations in FNA smears. These promising methodological findings are a first step to the introduction of molecular analysis of routine air dried FNA smears in every day practice. However, prior to this larger series of FNA and FFPE samples have to be analyzed.

OP 2-6

Standardized documentation for external quality management and patient-centered research: German/Austrian register for connatal hypothyreoidism of the APE / AQUAPE

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Optimal management of neonates identified by screening as potentially affected by hypothyroidism is an important topic for pediatric endocrinologists. Therefore, the working group on quality management in pediatric endocrinology (AQUAPE) is focusing on this group of patients. A software for standardized, longitudinal documentation of patients has been developed and is available free of charge for all participating institutions (http://www.peda-qs.de / Hypothyreose). Twice yearly, anonymized data are exported by the centers and transmitted for central analysis. Inconsistent data are reported back to the centers for correction. Data are then integrated into the register database and benchmarking reports are compiled for each institution, comparing individual treatment results among all participating institutions. Parameters reported include number of patients per institution, age at first presentation, initial TSH, age at initiation of therapy, % of confirmed diagnoses, complete diagnostics according to current guidelines, as well as subsequent growth, BMI and intelligence. By October 2011, 55 centers have contributed a total of 9097 visits from 997 patients with congenital hypothyroidism. Mean birth-weight was 3281 g, mean height 50.4 cm and mean duration of gestation 39.1 weeks. 35 % of patients were male, 65 % female. Screening TSH averaged 169 µU/ml [Q1-Q3: 66-246], confirmation TSH 206 µU/ml [85-234]. Screening TSH correlated closely with confirmation TSH ($r=0.56$, $p< 0.0001$) and inversely with total T4 ($r=-0.46$, $p < 0.0003$). On average, diagnosis was made on day 8.7 and therapy started on day 9.8. In 81 % of patients, a starting dose of 50µg L-thyroxin/day was used. At age 10 years (n=120), mean height-SD score was -0.13 SD [-0.73-0.14], and mean BMI-SD-Score 0.27 [-0.38-1.19]. This multicenter register of patients with congenital hypothyroidism reflects structure, process and outcome of current quality of care by pediatric endocrine services.

OP 2-7

Allosteric modulation of the TSH receptor: Switching from agonism to antagonism and reverse

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The thyrotropin receptor (TSHR), a G-protein-coupled receptor, has, in addition to its extracellular orthosteric hormone binding site, an allosteric binding region in the transmembrane domain. Previously we have demonstrated, that several signalling-sensitive amino acids surround this allosteric binding site for small molecule ligands (SMLs). Modelling driven mutagenesis led to distinct silencing mutations and constitutively activating mutations (CAM) in this area, changing TSHR conformation to an inactive or active state. All these identified signalling-sensitive amino acids can be mapped onto a structural model of TSHR. They indicate locations where a SML may interact and switch the receptor to an inactive or active conformation respectively.
Here we studied the effects of SMLs on these signalling-sensitive amino acid residues at TSHR. The partial antagonistic effect of SML c52 was reversed to an agonistic effect, if tested at the silencing TSHR mutant M572A. Such a reversing effect of c52 was also observed at the CAM Y667A at position 7.42 of transmembrane helix 7. c52 is a derivative of the partial agonist org41841, and differs only by an enlarged substituent. In our molecular model c52 is wedged between these two residues and the enlargement points into a cluster of silencing mutations. Both of these two mentioned mutations lead to a relocation of c52 similar to position of the agonist, which allows contact to amino acid site chain M637, a signalling-sensitive point for TSHR activation.
Our modifications are switching agonism to antagonism and reverse by changing either small molecule ligand or by mutation of residues covering the binding pocket. Detailed knowledge about discriminative pharmacophores on both counterparts, ligand and receptor, provides a basis for our long term goal, the rational design of further high affinity SML antagonists that have the potential to interfere with pathogenic activation of the TSHR, for instance in Grave`s orbitopathy.

OP 2-8

Activating mutations of the calcium-sensing receptor: Evidence for functional difference between mutations causing autosomal dominant hypocalcemia and Bartter syndrome type 5

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Introduction: Activating mutations of the calcium sensing receptor (CaSR) cause autosomal dominant hypocalcemia (ADH) characterized by low serum calcium, inappropriately low PTH and relative hypercalciuria. Four activating CaSR mutations cause additional renal wasting of sodium, potassium and other salts, a condition called Bartter syndrome type 5. It is not clear on a molecular level why these 4 activating mutations have an additional renal phenotype.

Methods: Five activating CaSR mutations and all 4 known mutations causing Bartter syndrome type 5 were expressed in human embryonic kidney 293T cells and receptor signalling was studied by measurement of intracellular free calcium ($[Ca^{2+}]_i$) in response to different concentrations of extracellular calcium ($[Ca^{2+}]_o$). To investigate the effect of calcilytics, cells were stimulated with 3 mM $[Ca^{2+}]_o$ either in the presence or absence of NPS-2143 (300 nM or 1 µM).

Results: All 9 mutations demonstrated left shifted dose response curves (EC_{50}: wt: 4.0 mM, ADH: 1.6 - 2.8 mM, Bartter: 1.3 - 2.3 mM) as expected. The 5 mutations causing ADH displayed higher maximum induced increases in $[Ca^{2+}]_i$ than the 4 mutations causing Bartter Syndrome Type 5. Repetitive stimulation by 3 mM $[Ca^{2+}]_o$ decreased the intracellular calcium response of wildtype CaSR and of 4 of 5 ADH mutants but enhanced the cytosolic calcium response in all 4 Bartter mutants. When co-expressed with wildtype CaSR all 5 ADH mutations were sensitive to the calcilytic NPS-2143, while only 2 of 4 Bartter mutations could be attenuated by NPS-2143.

Conclusion: CaSR mutations causing Bartter syndrome type 5 appear functionally different from mutations causing ADH according to their differential activation of the cytosolic calcium pathway. These changes in signalling behaviour may contribute to the distinct clinical phenotypes.

Nebenniere

OP 3-1

Investigating the role of SLC26A2 in primary aldosteronism

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A correlation between high aldosterone to renin ratio (ARR) and a locus at 5q32 was observed following a genome wide association study with subjects from KORA F4 survey. Hypothesizing that this locus may contain genes of relevance for the etiology of primary aldosteronism (PA), we investigated SLC26A2, a sulfate transporter, after observing significantly higher expression in mouse adrenal glands, even though we did not find any reports on effects of this gene in adrenal function in the literature. Effects of aldosterone regulators on this gene were evaluated using human adrenocortical cell line NCI H295r, demonstrating up-regulation in the presence of K^+ ions or forskolin. However, *in vivo* tests with aldosterone effectors angiotensin II and KCl had suppressive effects on gene expression in adrenal glands of treated mice. To assess possible effects of SLC26A2 as a transporter on kidney function, we have investigated its expression in a human collecting duct cell line, and detected an increase in response to treatment with aldosterone. A shRNA-mediated four-fold knockdown of SLC26A2 in H295r cells yielded a highly significant increase in aldosterone output; this effect of knockdown was sustained after incubation with aldosterone stimulators. We have also investigated adrenal glands from SLC26A2 knock-out mice; with results suggesting a tendency towards higher expression for adrenal enzymes with more specific role in aldosterone synthesis and expression in the zona glomerulosa (CYP11B2, HSD3B6), and a tendency towards lower expression for enzymes with a more unspecified expression pattern (STAR, CYP11A1) or a more fasciculata specific distribution (HSD3B1). Further investigation into the correlation of adrenal SLC26A2 expression and aldosterone secretion is ongoing.

OP 3-2

Analysis of *TP53* polymorphisms in 142 patients with adrenocortical cancer

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Introduction: Adrenocortical carcinoma (ACC) can be a manifestation of the Li-Fraumeni tumour syndrome, which is in 70% due to germline mutations in *TP53*. Some *TP53* polymorphisms have also been shown to impact on p53 function and seem to influence cancer risk and clinical outcome in several tumour entities. Data on *TP53* polymorphisms in adult patients with ACC are so far only available for R72P polymorphism in exon 4. We therefore investigated 11 *TP53* sequence alterations in a large cohort of adult Caucasian patients with sporadic ACC.
Methods: Peripheral blood for DNA extraction was collected from 142 ACC patients. Polymorphism analysis was performed by amplification and sequencing of exons 2-11 and adjacent intron sections of *TP53*. Correlation with clinical data was investigated and in 5 cases distribution of polymorphic genotypes was compared to data available from literature.
Results: We investigated 7 exonic and 4 intronic polymorphic sites located throughout *TP53*. Genotype frequencies of analysed *TP53* polymorphisms among ACC patients and control groups significantly differed in 3 of 5 polymorphisms: PIN2 (74+38C>G), PIN3 (96+41_96+56ins16) and PIN6 (672+62G>A). For R72P and PIN9 (993+12T>C) no significant genotype differences were found. Survival analysis of ACC patients with regard to the different genotype variants of the polymorphism showed no significant differences for each of the analysed polymorphisms.
Conclusion: To our knowledge this is the first *TP53* polymorphism analysis of a large group of Caucasian ACC patients concentrating on more than 10 polymorphic sites. Single *TP53* polymorphisms do not seem to influence clinical outcome. However different distribution of polymorphic genotypes in ACC patients compared to control groups suggests a possible influence of *TP53* polymorphisms on the development of ACC.

OP 3-3

Chromaffin progenitor cells from bovine adrenal medulla: Characterization and dopaminergic differentiation

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Chromaffin cells and sympathetic neurons derive from a common sympathoadrenal progenitor cell. Unlike sympathetic neurons, chromaffin cells are able to proliferate throughout life. Similar to neuronal progenitors that form neurospheres, isolated bovine chromaffin cells formed spheroid clusters called chromospheres (CS). Such clusters are heterogeneous structures that support enrichment of progenitors. Our data establish enrichment of progenitor cells within CS that revealed increase of neural progenitor markers such as nestin, Mash1, Sox1 and Sox10.
In this study, we investigated two major hall marks of stemness: self-renewal and differentiation. Clonal growth of cells from primary spheres resulted in secondary spheres directly providing evidence of progenitor's self-renewal. Moreover, sphere initiation capacity that depends on seeding density slightly decreased after 4 weeks of culturing. Under differentiation conditions, bovine chromaffin precursor cells were capable to derive neurons positive for tyrosine hydroxylase (TH), dopamine β hydroxylase. Shift towards neuronal differentiation was accompanied with simultaneous downregulation of neural progenitor markers such as Hes1, Hes5, nestin and Notch2 and upregulation of neural markers such as β III tubulin, MAP2 and Pax6. Moreover, frequency of generated dopaminergic neurons positive for TH was significantly elevated after treatment with retinoic acid (RA) and ascorbic acid (AA). Unlike neurons derived by standard procedure, stimulation of neurons derived after RA and AA treatment revealed increased dopamine production. In addition, typical neuronal excitability and existence of voltage-dependant channels was found after patch-clamping confirming functional gain of generated neurons.
In summary, our data establish the presence of progenitor/stem cells with pronounced ability to differentiate into dopaminergic neurons promising source in the treatment of neurodegenerative diseases such as Parkinson's disease.

OP 3-4

Purification of recombinant human aldosterone synthase from *E. coli* enables the analysis of clinically relevant mutants on the molecular level

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Aldosterone, the most important human mineralocorticoid, is involved in the regulation of the blood pressure and has been reported to play key a role in the formation of arterial hypertension, heart failure and myocardial fibrosis. Aldosterone synthase (CYP11B2) catalyzes the biosynthesis of aldosterone by 11β-, 18-hydroxylation and 18-oxidation of 11-deoxycorticosterone in the adrenal cortex. Since more than 20 years, all attempts to purify recombinant human CYP11B2 in significant amounts for detailed analysis failed, due to its hydrophobic nature as a membrane protein. Here, we present the successful expression of human CYP11B2 in E. coli, its purification and enzymatic characterization. Co-expression with molecular chaperones GroEL/GroES yielded approx. 90 nmol/L culture. The activity of the enzyme was tested by in vitro conversion assays of the substrate 11-deoxycorticosterone. Determination of steroids was done using HPLC and gas chromatography-mass spectrometry (GC-MS). Dissociation constants of substrate and intermediates were analyzed which revealed an immense increase from 1.34 ± 0.13 µM for DOC to 115 ± 6 µM for B. Since the enzyme is, furthermore, of clinical importance concerning the severe autosomal recessive disorder of corticosterone methyl oxidase (CMO) deficiency leading to salt-wasting and failure to thrive in early infancy, we expressed and analyzed mutants of CYP11B2 related to CMO. The analysis of mutants on the molecular level revealed for the first time that reduced activity is attributed to a weaker substrate binding caused by residue alteration.

OP 3-5

Development and validation of a LC-MS/MS method for routine determination of aldosterone and its application in pseudohypoaldosteronism

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Reliable assays for the detection of aldosterone plays a major role for the clinical evaluation of pseudohypoaldosteronism, hypoaldosteronism and hyperaldosteronism. The correct and sensitive measurement is hampered by various preanalytical and method specific difficulties with immunoassays.
We established an assay for measuring aldosterone in small sample volumes. Combined with our previous Multi-Steroidassay we determine simultaneously aldosterone and progesterone, deoxycorticosterone, corticosterone, 17-hydroxyprogesterone, 11-deoxycortisol, 21-deoxycortisol, cortisol, cortisone, androstenedione, testosterone and dihydrotestosterone. 0.1 mL plasma was extracted by solid phase extraction (SPE) and analyzed using an UPLC-MS/MS in MRM mode. The calibration curve was linear and reproducible in the range of 50-2000 pmol/L aldosterone. The limit of detection was 10 pmol/L and the limit of quantification was 30 pmol/L. We compared our assay with RIA and ELISA assays for aldosterone (DRG-Diagnostics); the coefficients of determination were 0.87 for RIA *vs* LCMSMS and 0.81 for ELISA *vs* LCMSMS. The aldosterone concentration was about 1.3 times higher analyzed by nonextractive RIA and 20% lower analyzed by ELISA compared to LC-MS/MS. With this validated assay preliminary reference ranges for children aged 0-18 years were measured. As an example for its clinical application plasma from patients with pseudohypoaldosteronism was measured revealing 15-fold to 45-fold multiples of the median of the aldosterone reference value.
The detection limit and the sensitivity of this assay allow measuring aldosterone simultaneously with 11 other steroid hormones in one injection and overcome antibody related problems of the established immunoassays.

OP 3-6

Therapeutic shRNA mediated ß-catenin knockdown in a tumor model of adrenocortical carcinoma

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Activation of the Wnt/ß-catenin pathway is frequently observed in adrenocortical carcinoma (ACC) and has recently been shown to be associated with a more aggressive tumor phenotype. We have developed a novel therapeutic approach by generation of stable NCIh295R transfectands with a ß-catenin shRNA expression plasmid (shß7) inducible by doxycyclin (dox). ß-catenin knockdown efficacy was investigated in vitro by quantitative Real-Time PCR on NCIh295R[shß7] and mock transfected cells NCIh295R[TR]. While no significant changes were detectable for NCIh295R[TR], a significant reduction in ß-catenin expression was detectable after treatment with dox for NCIh295R[shß7] cells (-dox: 100±8%, +dox: 18±1%; p=0.003). Accordingly, we detected a significant reduction in cell viability (-dox: 100±20%, +dox: 54±15%; p=0.04) and increase in apoptosis (-dox: 100±9%, +dox:525±37%; p=0.03) for NCIh295R[shß7] upon dox treatment while no changes were detectable for NCIh295R[TR]. Moreover, we evaluated the therapeutic efficacy of a ß-catenin knock-down in subcutaneous NCIh295R[TR] and NCIh295R [shß7] xenografts in athymic nude mice. Short term treatments revealed after 9 days of dox administration a significant decrease in intra-tumoral ß-catenin expression for NCIh295R[shß7] (-dox: 100±40%, +dox:11±5%;p=0.007) while NCIh295R[TR] remained unaffected. In long-term therapeutic experiments mice were treated 3 days after tumor induction continuously with dox. 31 days after tumor induction mice were euthanized and tumors excised. While no significant differences regarding tumor weight [mg] were detectable for NCIh295R[TR] tumors (-dox: 334.6±149.6%, +dox: 259.2±111.2%) and untreated NCIh295R[shß7] tumors (116.8±48.5%), no tumors were detectable for the dox-treated NCIh295R[shß7] group even after dissection (0±0%,p< 0.001). In summary, these experiments provide evidence that inhibition of Wnt/ß-catenin pathway in ACC has therapeutic potential that should be evaluated in more detail in the future.

OP 3-7

Prevalence of benign and malignant secondary neoplasms in patients with primary aldosteronism

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Context: Primary aldosteronism (PA) is the most common cause of secondary hypertension. In vitro, aldosterone excess can cause oxidative stress leading to DNA damage. Single case reports describe a coincidence of PA with renal cell carcinoma and other tumors. However, no data on the prevalence of benign and malignant neoplasms in patients with PA exists.
Methods: In the multicentre MEPHISTO study the prevalence of benign and malignant tumors was investigated in 338 patients with confirmed PA both retro- and prospectively. The SHIP cohort of patients with primary hypertension served as a matched control group.
Results: Of the 338 patients 50 (15%) had been diagnosed with at least one tumor at any time of their life. In total, 62 neoplasms were identified which were in 44% of benign and in 47% of malignant dignity (9% unknown). 41% (n=11) of all benign secondary neoplasms were derived from endocrine tissue (thyroid, parathyroid and pituitary). The remaining benign neoplasms were located in skin (19%), lung, brain, prostate (4% respectively), female reproductive organs (7%) or were characterized as lipoma, hemangioma and tumor of the sebaceous glands (together 22%). By contrast, only 10% (n=3) of the malignant tumors were of endocrine origin (thyroid carcinomas). Most of the malignant tumors were skin tumors (21%). Both renal cell carcinoma and prostate cancer were diagnosed in 4 patients (each 14%). Less frequently diagnosed malignant tumors were colonic cancer (10%), breast cancer (7%) and malignant tumors in lung, gastrointestinal tract, larynx, reproductive organs or brain (each 3%).
Conclusion: In this cohort of PA patients a high prevalence of benign endocrine neoplasms was found. Interestingly, a relatively high prevalence of renal cell carcinoma (14% of malignant neoplasms) was observed. Renal cell carcinoma generally accounts for only 3.3-4.4% of all malignant tumors in Germany. Probable pathophysiological backgrounds are subject of ongoing studies.

OP 3-8

PET-tracers for differential diagnosis in primary hyperaldosteronism - *in vitro* studies

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Objective: The major diagnostic problem in primary aldosteronism is the differentiation between bilateral hyperplasia and aldosterone producing adenoma which is essential for further treatment. Adrenal vein sampling is regarded as the current gold standard; however it is an invasive, highly examiner-dependent method. Molecular imaging targeting the aldosterone synthase (CYP11B2) which is expressed specifically in aldosterone producing adrenal tissue may be a useful alternative. CYP11B2 is highly homologous to 11ß-hydroxylase (CYP11B1) (93%). We, therefore, aimed to develop a PET tracer which binds to CYP11B2 with both high affinity and high selectivity.
Methods: We have synthesized more than 90 new compounds so far mostly containing a fluorine atom which enables radiolabelling with ^{18}F for PET imaging. Compounds were tested for inhibition of aldosterone/cortisol (corticosterone) in NCI-H295 cells, murine Y1 cells expressing human CYP11B1/CYP11B2 and in V79 chinese hamster fibroblasts expressing rat CYP11B1/CYP11B2.
Results: After structural optimization, 7 fluorinated CYP11B2 inhibitors could be identified (IC50 values for inhibition of aldosterone synthesis up to 5.5 nM, selectivity factors for inhibition of aldosterone vs cortisol synthesis in murine Y1 cells mostly >100).
Conclusion: We developed several fluorinated inhibitors of CYP11B2 exhibiting high affinity and selectivity to the target enzyme. These compounds may be suitable for specific molecular imaging in primary hyperaldosteronism. Establishment of radiosynthesis and *in vivo* evaluation is subject of ongoing studies.

Adipositas und Metabolismus

OP 4-1

A high fat diet alters energy intake, body weight and hypothalamic circadian clock gene expression in GIPR $^{-/-}$ mice

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Introduction: An intact circadian clock system is essential for the regulation of energy homeostasis and food intake. CLOCK-mutant mice and Per2 knock out mice on a high fat diet (HFD) are hyperphagic and develop obesity. Glucose-dependant insulinotropic polypeptide receptor knock out (GIPR$^{-/-}$) mice are resistant to diet induced obesity when exposed to a HFD most likely due to increased energy expenditure. This study was conducted to investigate whether GIPR$^{-/-}$ mice on a HFD exhibit altered expression of hypothalamic clock genes that might be related to food intake and adiposity compared to wild type (WT) mice on HFD.
Methods: Male WT and GIPR$^{-/-}$ offspring on a C57Bl/6J background were kept on a normal rodent chow (CD, 10% fat) until the age of 25 weeks and then switched to HFD (60% fat) for 20 weeks. Body weight (BW) and food intake (FI) were determined weekly. At the age of 45 weeks, body composition using NMR-spectroscopy and hypothalamic gene expression of circadian clock genes using quantitative RT-PCR were assessed.
Results: No significant differences in BW and FI were observed between WT and GIPR$^{-/-}$ mice during the first 25 weeks on CD. After exposure to HFD, GIPR$^{-/-}$ mice gained significantly lower body weight (43.98±0.93 g vs. 50.61±0.88 g in WT) and body fat (41.06±0.58 % vs. 43.65±0.41 % in WT) at the age of 45 weeks (both $P< 0.01$). However, their cumulative energy intake on the HFD was significantly increased compared to WT mice (44.17±1.04 vs. 40.23±0.57 kcal/gBW, respectively, $P< 0.01$). For hypothalamic circadian clock gene expression, GIPR$^{-/-}$ mice showed a significant down regulation for CLOCK (0.70-fold), Per2 (0.79-fold), Bmal1 (0.71-fold) and RevErbα (0.66-fold) compared to WT mice (all $P< 0.05$).
Conclusion: Despite decreased BW and adiposity GIPR$^{-/-}$ are hyperphagic compared to WT mice when exposed to a HFD. The increased energy intake could be explained by reduced expression of circadian clock genes in the hypothalamus.

OP 4-2

Exogenous peptide YY3-36 and Exendin-4 further decrease food intake, whereas octreotide increases food intake in rats after Roux-en-Y gastric bypass

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Background: Patients show an elevated postprandial satiety gut hormone release after Roux-en-Y Gastric bypass (gastric bypass). The altered gut hormone response appears to have a prominent role in the reduction of appetite and body weight (BW) after gastric bypass. Patients with insufficient BW loss after gastric bypass have an attenuated postprandial gut hormone response in comparison with patients who lost an adequate amount of BW. The effects of additional gut hormone administration after gastric bypass are unknown.
Methods: The effects of peripheral administration of peptide YY3-36 (PYY3-36; 300 nmol kg(-1)), glucagon-like peptide-1 (GLP-1) analogue Exendin-4 (20 nmol kg(-1)) and somatostatin analogue octreotide (10 µg kg(-1)) on feeding and BW were evaluated in rats after gastric bypass.
Results: Gastric bypass rats weighed ($P < 0.01$) and ate less on postoperative day 5 ($P < 0.001$) and thereafter, whereas postprandial plasma PYY and GLP-1 levels were higher compared with sham-operated controls ($P < 0.001$). Administration of both PYY3-36 and Exendin-4 led to a further decrease in food intake in bypass rats compared with saline treatment ($P=0.02$ and $P < 0.0001$, respectively). Similar reduction in food intake was observed in sham rats ($P=0.02$ and $P < 0.001$, respectively). Exendin-4 treatment resulted in a significant BW loss in bypass ($P=0.03$) and sham rats ($P=0.04$). Subsequent treatment with octreotide led to an increase in food intake in bypass ($P=0.007$), but not in sham rats ($P=0.87$).
Conclusion: Peripheral administration of PYY3-36 and Exendin-4 reduces short-term food intake, whereas octreotide increases short-term food intake in rats after gastric bypass. The endogenous gut hormone response after gastric bypass can thus potentially be further enhanced by additional exogenous therapy with pharmacological doses of gut hormones in patients with insufficient weight loss or weight regain after surgery.

OP 4-3

Transgenic mice with beta cell-specific p8 overexpression display preserved glucose tolerance during high fat diet- or insulitis-induced beta cell damage

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Objective: p8 is induced by pancreatitis and protects acinar tissue from inflammatory damage. Within the endocrine pancreas, own previous work has demonstrated *in vitro* that p8 is a glucose-induced mediator of beta cell proliferation and a cellular stress-induced inhibitor of beta cell apoptosis. To evaluate the beta cell protective role of p8 *in vivo*, we generated transgenic mice with beta cell-specific overexpression of p8 under the control of the rat insulin promoter I (tg). Previously, we have introduced the basic characterisation of phenotype. Here we compare effects of high fat diet (HFD)- vs. insulitis-induced beta cell damage on glucose tolerance and beta cell mass in tg mice and syngenic wild type controls (wt).
Results: tg mice and wt mice were fed with normal chow or HFD with 60% fat for 10 weeks. Compared to normal chow controls HFD-fed animals significantly gain weight and display enhanced unfasted insulin levels (insulin resistance). Significant loss of beta cell mass and reduced glucose tolerance (ipGTT) demonstrate the detrimental effect of chronic nutrient-induced lipotoxicity. tg-HFD mice demonstrate a significantly enhanced first phase insulin secretion (ipGTT) which is associated with a significantly less beta cell loss as compared to wt-HFD animals. The more pronounced inflammatory stress by multiple low dose streptozotocin-induced insulitis significantly reduced glucose tolerance (first and second phase insulin secretion) in wt mice while tg animals demonstrate glucose clearance similar to untreated controls. This indicates that ectopic p8 production has a substantial anti-inflammatory capacity.
Conclusion: p8 inhibits apoptosis and protects beta cell mass from damage by chronic HFD-induced lipotoxicity and insulitis. More pronounced protection of beta cells during insulitis raises the question whether p8 can modulate inflammatory response. Therefore, we currently analyse beta cell mass and lymphocyte infiltration.

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OP 4-4

Identification of a Melanocortin-4 receptor interaction region

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During the last two decades the knowledge about mechanisms of body weight regulation has increased with the identification of the leptin-melanocortin-pathway. This pathway integrates peripheral signals in hypothalamic nuclei to maintain energy homeostasis. A key player in hypothalamic weight regulation is the melanocortin 4 receptor (MC4R), a G-Protein coupled receptor (GPCR). This receptor is expressed in several brain regions like the nucleus paraventricularis (PVN) where it modulates food intake but also in penis tissue. So far, it was not possible to develop a specifically highly potent agonist for the MC4R in the PVN that influence food intake in humans.
We could show that the MC4R is able to homo- and heterodimerize. One possible option to target the MC4R, to modulate energy intake, is the activation of a PVN specific MC4R heterodimer pair. Here, the first step is the elucidation of MC4R domains responsible for interaction.
We generated MC4R/cannabinoid-1-receptor (its phylogenetic closest relative who did not interact with the MC4R) chimeras to locate the MC4R dimerization domains. We substituted mainly the transmembrane helix 3 (TMH3) and the transmembrane helix 4 (TMH4) and the intracellular loop 2 (icL2) of MC4R by CB1R. We determined cell surface expression, ligand binding as well as signalling and dimerization properties.
We identified one receptor region, the interface of TMH3/icL2, being responsible at least in part for interaction of MC4R. However, identification of single amino acids responsible for dimerization was not possible due to the fact that most likely more than one position is involved in receptor-receptor interaction.
In this study we identified the first MC4R receptor region that could serve as a contact point for homodimerization. This is a crucial step to gain new insights into MC4R physiology and the new information could be useful for the design of new MC4R ligands.

OP 4-5

GIP-dependent regulation of endothelial factors and cytokine release in Human Umbilical Vein Endothelial Cells (HUVEC), human macrophages and their coculture

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Introduction: GIP is actively involved in the pathogenesis of obesity. The latest investigations showed that GIP may cause proinflammatory effects in the adipose tissue. We tested the hypothesis that GIP can lead to the inflammation-associated response in endothelial cells.
Methods: HUVEC were incubated with different concentrations of glucose and [*D-Ala2*]GIP (10 pM to 100 nM) as well as with DPPIV-inhibitor and human GIP. The proinflammatory effects were investigated in coculture with human monocyte-derived macrophages differentiated with GM-CSF. The coculture was incubated with 100 nM GIP and different glucose concentrations for 4 hours. The mRNA expression of proinflammatory cytokines (CCL2, IL6, TNFα), cell adhesion molecules (SELE, ICAM1, VCAM1, EDN1) and endothelial nitric oxide synthase (NOS3) was measured by RT-PCR.
Results: GIP increased concentration-dependently the gene expression of TNFα, CCL2, ICAM1, VCAM1 ($p < 0.05$ for all) in HUVEC. Glucose enhanced significantly the effect of GIP on IL6, SELE, CCL2, ICAM1, VCAM1 gene expression. In experiments with the DPPIV-inhibitor the NOS3 and IL6 expression was increased compared to control ($p < 0.05$). GIP stimulation in high glucose medium resulted in the increased expression of IL6, TNFα, CCL2, ICAM1, EDN1, NOS3 in human macrophages. However, this effect was lower in the coculture. The gene expression of ICAM, EDN1 and NOS3 in coculture decreased significantly under the influence of glucose and GIP.
Discussion: GIP increases the gene expression of proinflammatory cytokines and adhesion molecules in HUVEC and macrophages. In cocuture this effect was weaker. Thus, the proinflammatory effect of GIP is tissue specific and dependent on the tissue interactions. In addition, the endothelial effect of GIP seems to be dependent on the balance between pro-and anti-inflammatory factors.

OP 4-6

A role of complement C5a anaphylatoxin receptor (C5aR) in adipose tissue inflammation

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Obesity is associated with the development of insulin resistance and type 2 diabetes mellitus. Adipose tissue (AT) inflammation is a critical player for the development of insulin resistance and is characterized by the accumulation of macrophages in the obese AT and their polarization into the M1 pro-inflammatory macrophage subtype. Complement is a versatile system involved in activation of innate immunity, inflammation and tissue remodeling. The central reactions in the complement cascade are the cleavage of C3 and C5 that leave behind the active anaphylatoxins C3a and C5a that act through their cellular G-protein-coupled receptors, C3aR and C5aR, and the opsonins C3b and C5b. Complement components have been implicated as regulators in AT inflammation, however, the exact influence of the C5a-C5aR axis on the accumulation and activation of pro-inflammatory immune cells within the AT in the course of obesity is poorly characterized. Here we found, that expression of C5aR was increased in AT of obese mice compared to lean mice. Moreover, we performed the high fat diet (HFD)-induced obesity model in mice sufficient or deficient in the C5aR. Interestingly, obese mice lacking C5aR displayed improved glucose tolerance. This was associated with reduced AT inflammation due to C5aR deficiency. In particular, while the number of total leukocytes and infiltrated CD4+ or CD8+ T-cells did not differ between C5aR-sufficient and -deficient mice, we observed that the numbers of proinflammatory M1 macrophages (defined as F4/80+CD11b+CD11c+ and / or F4/80+CD11c+CD206- cells) were significantly reduced in C5aR-/- mice, as compared to WT controls. In addition, we could detect increased expression of the M2-derived anti-inflammatory cytokine IL-10. These results suggest that the C5a-C5aR axis contributes to macrophage polarization into M1 cells in the obese AT and thereby to insulin resistance development.

OP 4-7

Accumulation of myocellular diacylglycerol species relate to insulin resistance in human skeletal muscle

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Skeletal muscle determines insulin resistance in humans with or at risk of type 2 diabetes (T2DM), but the underlying cellular mechanisms are still unclear. Possible mechanisms are alterations in adipocytokines linked to inflammation, mitochondrial function and intramuscular lipid metabolites [ceramides, diacylglycerols (DAGs)] which have been tested in animal studies, but not yet proven in humans.

We examined 16 healthy humans (CON, n=16, 30±5 years, BMI: 24±2 kg/m^2) and measured circulating cytokines (n=6: TNFα, IL-6, sICAM, adiponectin, RBP-4), intramuscular DAG and ceramide contents, protein kinase C isoforms (PKC ß, Δ, θ) expression and activities as well as mitochondrial function before and after 2.5h and 4h infusion of lipid (Intralipid 20%) or glycerol infusion. Mitochondrial function was examined in permeabilized muscle fibers with high-resolution respirometry. Obese glucose tolerant (OB, n=5, 26±2 years, 45±3 kg/m^2) and T2DM (n=4, 61±1 years, 35±3 kg/m^2) were examined without intervention. A hyperinsulinemic-euglycemic clamp combined with [6,6-$^2H_2$]glucose was performed.

Compared to glycerol infusion, glucose disposal was reduced by 57% during lipid infusion ($p< 10.10^{-7}$), by 78% in OB ($p< 0.005$) and by 88% in T2DM ($p< 0.001$). Plasma cytokines and mitochondrial oxidative capacity remained unchanged during lipid infusion. Associated with lipid-induced insulin resistance, muscular DAG, but not ceramide content increased ~2fold ($P< 0.005$) at 2.5h and remained elevated (~1.4fold, $P< 0.05$) at 4 h of lipid infusion. At 4 h, the activity of PKCθ was raised by 64% ($P< 0.005$). Cytosolic DAG were increased ~3-fold in OB and T2DM ($P< 0.0001$) compared to CON at baseline.

These results support the concept that DAG-induced activation of PKCθ plays a key role in causing lipid-induced insulin resistance in human skeletal muscle. Neither circulating adipocytokines nor muscular ceramides contribute to acute lipid-induced muscle insulin resistance in humans.

OP 5-1

Differential TNFα -synthesis and signaling in endocrine tumors after treatment with the Tumor-Vascular-Disrupting Agent ASA404 (vadimezan)

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Recently, we investigated effects of the Tumor-VDA ASA404 in tumor models for neuroendocrine tumors of the gastroenteropancreatic system and adrenocortical carcinoma 24 hours after treatment of BON and NCIh295 tumor bearing mice with ASA404 (A), paclitaxel (P) or the combination (A+P). We detected for BON tumors extensive necrotic areas as well as a significant decrease in cell proliferation, increase of apoptotic cells and reduction of microvessels after A or A+P treatment while no comparable effects were detectable in NCIh295 tumors. As TNFα -signaling is assumed to mediate parts of A induced effects we thenceforth utilized these models with their different responsiveness for characterization regarding TNFα stimulation and signaling. We detected in both groups a significant increase of TNFα serum levels compared with controls ($p< 0.05$), but no significant differences between both tumor entities upon A treatment (BON: 2818 ±999%; NCIh295: 1165±422%, $p=0.18$). However, intra-tumoral TNFα content was significantly increased for BON tumors after A treatment while no differences were detectable for NCIh295 (basal: 100%; BON: 1178±263%, $p=0.007$; NCIh295: 220±86%, $p=0.23$). In vitro we detected a TNFα dependent 4-fold higher induction of apoptosis (basal: 100%; BON: 823±35% vs. NCIh295: 244±12%; $p=0.007$) and increase in IKK beta activity for BON but not for NCIh295 cells (basal: 100%; BON: 140±4%. $p< 0.001$; NCIh295: 108±5%; $p=0.32$). Basal TNF receptor 1 expression was not significantly different, but we detected high levels of Toll-like-receptor (TLR)-4 in the BON tumor model in vitro and in vivo while receptor expression appeared to be abrogated for NCIh295. TLRs are widely expressed in cytokine producing cells and TNF alpha is an important downstream mediator of TLR-4 signaling. Thus,ASA404 treatment holds promise in the treatment of GEP-NETs. Furthermore, the utilized tumor models might help to delineate resistance mechanisms involved in VDA induced anti-tumor activity.

OP 5-2

Intracranial transplantation of human craniopharyngioma tumor cells into NMRI $^{nu/nu}$ - mice

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Objectives: Adamantinomatous craniopharyngiomas (adaCP) are histological benign epithelial tumors of the sellar region, causing severe endocrinological deficits by invading the pituitary gland and the hypothalamus. Current treatment options are limited rendering the molecular pathobiology of adaCP to be elucidated. Herein, we generated a first *in vivo* brain tumor model of adaCP in order to be able to study underlying pathomechanisms of tumor development and progression and to test new pharmacological treatment strategies.
Methods: Human primary adaCP cells ($50x10^3$ - $150x10^3$) were obtained from three different surgical specimens and stereotactically implanted into the brain of six week old NMRI $^{nu/nu}$ - mice (8 females, 12 males; n = 20). Sham treated control mice (n = 6) received phosphate buffered saline injections. All animals obtained analgetic treatment for one week. Eleven weeks after tumor cell inoculation, all brain specimens were microscopically reviewed using haematoxylin/eosin as well as immunohistochemical stainings.
Results: Histology confirmed tumor growth in 6 out of 20 treated mice (30 %). Tumors had an average diameter of 250 µm, were predominantly situated at subdural location and showed histological hallmarks of human adaCP including calcifications, wet keratin and whirl-like cell clusters. Immunohistochemistry displayed proliferating tumor cells (Ki67) with distinct cytokeratin expression (KL1) and cells with nuclear β-catenin accumulation. Only small scars with hemosiderin deposits but no tumors were observed in any of the control animals.
Conclusion: To the best of our knowledge, here we present a first animal model of human craniopharyngiomas in brains of nude mice applying heterotopic human tumor cell implantation. Transplanted cells generated solid tumors with histomorphological and immunohistochemical hallmarks of human adaCP. This *in vivo* mouse model is promising to study growth characteristics and assess novel drug targets of human adaCP.

OP 5-3

Tailored antitumor therapy by in vivo evaluation of individualized anti-proliferative strategies in a tumor transplant model of anaplastic thyroid cancer

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Despite various attempts in modifying usual treatment modalities, anaplastic thyroid cancer (ATC) is still associated with unfavorable prognosis. Results of preclinical investigations are often of limited assignability to clinical tumor biology. Individualized multimodal treatment regimens, involving novel, growth inhibiting drugs might be a future option.

Tumor tissue, freshly prepared from a patient operated for ATC, was xenotransplanted to nude mice immediately after removal. While the patient obtained a hyperfractionated external beam radiation, mice cariing xenotransplanted tumors grown to a size of 5mm were randomized into intervention and control groups (10 mice each) and treated by multikinase inhibitors (MKI - Sorafenib[S]: VEGF-, PDGF- und RET, Vandetanib[V]: VEGF- und EGF and MLN8054[M]: Aurorakinase A-C). Antiproliferative, antiangiogenic and proapoptotic effects were evaluated.

Diagnosis of ATC was confirmed by pathologic examination. Treatment of xenotransplanted tumors by MKI reduced the tumor volume between 56% [M] und 35% [V]. Reduction of tumor proliferation was between 35% [V] and 14% [S]. Reduction of tumor vascularity between 77% [V] und 28% [S] was accompanied by decreased pEGF-R/pVEGF-R2 receptor activity [V and S]. An up to 28% increase in tumor apoptosis was detected by caspase-3 analysis. An adjuvant therapy of the patient by oral Sorafenib application resulted in a tumor-free follow-up one year after surgery.

Successful in vivo evaluation of novel, growth inhibiting drugs on xenotransplantated fresh tumor tissue allows prospective analysis for possible clinical application and justifies further investigation. Based on the individual results a tailored clinical drug application seems possible.

OP 5-4

CGB and OCT4 promoters are differentially methylated in different human tumor cell lines - a model for testicular tumor differentiation

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Objectives: Chorionic gonadotropin (CG) is expressed at high levels in most cancer tissues, such as testicular seminomas. Until now the mechanisms, causing and driving the CG expression are not quite clear. It has been shown before that in seminomas an interaction of OCT4 and CG exists in which OCT4 represses CG expression and that an overall hypomethylation of the OCT4 gene in tumors can be observed.The study tries to clarify to role of methylation for CG beta-subunit (CGB) and OCT4 expression using seminoma (T-Cam-2), choriocarcinoma- and embryonic carcinoma (CC, BeWo and EC, 2102EP) cell lines as a model.
Methods: T-Cam-2, BeWo and 2102EP cell lines, DNA-isolation, Pyrosequencing, qPCR, IHC, Deazitabine treatment
Results: In EC cells, low OCT4 promoter methylation is associated with high OCT4 expression in combination with high CGB promoter methylation and low mRNA expression. In CC, the effect is the just the opposite as shown by qPCR, histology and methylation. T-Cam-2 cells display a CGB promoter methylation pattern intermediate between choriocarcinoma and embryonic carcinoma cells while OCT4 promoter methylation is low. Complete DNA demethylation by deazitabine treatment of T-Cam-2 cells lead to a high CGB mRNA expression, whereas OCT4 mRNA expression remains stable. Using the EC cells as a control, neither increased CGB nor OCT4 expression could be observed.
Conclusion: By studying different cell lines, we developed a model linking the repressive effect of OCT4 on CGB expression to differential DNA-methylation levels of CG and OCT4 promoters. In further studies, we will evaluate if this model can be transferred to the *in vivo* situation by determining the methylation status of CGB and OCT4 promoters in patients with different germ cell tumors. This might improve the ability to detect and differentiate early testicular cancer types by determining CGB/OCT4 status using methylation specific tests.

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OP 5-5

Myostatin-targeting siRNA increases skeletal muscle mass, reduces visceral body fat content and improves markers of lipid and glucose metabolism in normal mice

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Introduction: Inhibition of myostatin, a negative regulator of muscle growth, has been demonstrated to increase muscle growth and reduce body fat. One potential approach to inhibit myostatin activity is the knock down of its gene expression by small interfering RNAs (siRNAs).
The aim of this study was to investigate whether myostatin-targeting siRNA treatment and siRNA in combination with physical activity affects muscle growth, body fat content and markers of glucose and lipid metabolism in normal mice.
Methods: The mice were treated with myostatin-targeting siRNA via osmotic mini-pumps and performed a treadmill based exercise protocol in terms of strength training during a period of 4 weeks. Effects on the growth of the gastrocnemius muscle, expression of genes involved in myostatin signaling, body fat, serum levels of leptin, myostatin related proteins and several molecular markers of glucose and lipid metabolism (PPARα, γ, δ and GLUT4) in liver and muscle were investigated.
Results: The siRNA treatment led to an increased muscle growth resulting from the increased muscle fiber size and activation of satellite cell markers. In addition, the myostatin-targeting siRNA affected lipid and glucose metabolism. The treatment resulted in decreased visceral fat content along with decreased leptin levels and improved markers for insulin sensitivity as shown by increased PPARα and GLUT4 expression in gastrocnemius muscle and increased PPARγ expression in liver. The performed strength training was able to enhance the siRNA activity on skeletal muscle adaptation and myostatin related proteins in serum.
Conclusion: Taken together, these results suggest that the myostatin-targeting siRNA increases skeletal muscle growth and improves lipid and glucose metabolism in normal mice. Therefore it might be a useful strategy not only for the treatment of muscle wasting diseases but also for the treatment of metabolic disorders such as obesity or type 2 diabetes.

OP 5-6

Inhibition of the PI3-Kinase/mTOR pathway and influence on the viability of human PTEN-deficient lipoma cells

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Introduction: The development of lipomatosis is one symptom of the PTEN-Hamartoma-Tumor Syndrome (PHTS), which can lead to severe complications due to airway or digestive system obstruction. Rapamycin treatment, as so far only available therapeutical option besides surgery, has proved to be only partially successful.
Aims: We aimed to test *in vitro* whether pharmacological inhibition of PI3-Kinase or mTOR leads to reduced viability or the induction of apoptosis in human PTEN-deficient lipoma cells.
Methods: Cells from a lipoma of a patient with PHTS were established and maintained in long term culture. Cell differentiation was determined by Nile-Red lipid staining and cell counting. Viability was assessed using the WST-1 assay and apoptosis was measured flow-cytometrically after annexin V/propidium-iodide staining.
Results: Lipoma cells had a lifespan of 91 population doublings (PD) with a doubling time of 25 h. They preserved a capacity for adipocyte differentiation of 55.1±4.2% for 29 PD as measured by the rate of lipid accumulating cells. PTEN mRNA and protein levels were decreased compared to controls and a constitutive phosphorylation of the kinase AKT was ascertained. Rapamycin as inhibitor of mTORC1 decreased viability by 43.4±1.9% and adipocyte differentiation by72.7±5.4% (p=0.0001) at a concentration of 100 nmol/L. However, no induction of apoptosis could be detected. Rapamycin analoga had a lesser effect on cell viability at the same concentration. mTORC1/2 inhibitors pp242 and WYE-354 (both 1 µmol/L) decreased cell viability by 47.1±3.6% and 56.5±3.0%, whereas the PI3-Kinase inhibitor LY294002 (100 µmol/L) had the greatest effect with 77.9±4.6% reduction. A slight induction of apoptosis was seen after incubation with LY294002 (100 µmol/L).
Conclusion: Inhibitors of mTORC1/2 and PI3-Kinase reduced cell viability to a greater extent than rapamycin. They may be of therapeutical value for patients with PHTS.

OP 5-7

N-methylserotonin as a potential active constituent of *Cimicifuga racemosa* for treatment of hot flashes

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Introduction: Hot flashes are a disorder of thermoregulation due to the lack of estrogens and are a common climacteric complaint. Hormone replacement therapy ameliorates hot flashes which however is associated with side effects like increased breast cancer risk. *Cimicifuga racemosa* (CR) is used to relieve hot flashes, but the active constituent(s) are yet unknown. Based on *in vitro* assays, recently it has been suggested that N-methylserotonin (NMS) may be the active compound of CR which ameliorates hot flashes [1]. To investigate whether NMS can affect body temperature *in vivo*, adult ovariectomized (ovx) rats were used as an established animal model for the endocrine situation of a menopausal women.
Methods: 3 month old female rats were ovx and implanted with temperature sensitive transponders which measure subcutaneous temperature continuously. Body temperature was read in 5 min intervals for 120 min.
Results: Mean temperature increased significantly within 24 hrs after ovx by 4 ± 0.5 °C. Thereafter mean temperature remained constant. In individual ovx animals skin temperature fluctuated largely with peaks (i.e. hot flashes) occurring every 20-40 minutes. To assess the effect of NMS on hot flashes temperature was recorded 45 minutes before and 1.5 hrs after i.p. injection. Already 10 minutes after injection of 5 mg NMS /kg body weight temperature decreased significantly. Maximal reduction of temperature was observed 30 minutes after injection (3.2°C, $p < 0.01$ vs pretreatment level). The temperature-lowering effect of NMS lasted up to 1.5 hours. The effect of NMS was dose-dependent with a minimum dose of 1 mg/kg body weight to achieve a significant reduction of skin temperature
Conclusions: We demonstrate for the first time with an animal model for menopausal hot flashes that NMS dose dependently reduces skin temperature. Thus we suggest that NMS is an active constituent of CR to relieve menopausal hot flashes
1 Powell et al. J Agric Food Chem. 2008; 56: 11718

OP 5-8

Cdc42 controls osteoblast differentiation

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Cdc42 (cell division cycle 42) is a Rho GTPase involved in cell morphology, cycle progression and adhesion. We therefore examined the effect of conditional deletion of cdc42 in the osteoblasts.
There were no changes in bone mineral density in conditional knockout mice (cKO) using the collagen-1(I) promoter (p=NS vs. CT). Thus cdc42 does not seem to play a role in differentiating osteoblasts. We then used the osterix promoter to examine the role of cdc42 in early osteoblasts. Both 3 week old homozygote and heterozygote cKO mice had a significantly decreased BMD as measured by pQCT. The decrease was more pronounced in homozygote cKO mice suggesting a dose response for the absence of cdc42 in the osteoblasts (CT: 282±17; cdc42 fl/+ cKO: 264±11, p< 0.05 vs. CT; cdc42 fl/fl cKO: 228±16g/cm³, p< 0.05 vs. CT). Histomorphometry analysis of the bones showed a decrease in osteoblasts in cKO (ObN/BS: CT: 64±13 vs. cKO: 44±14 ObN/mm BS, p< 0.05). In vitro studies of calvarial osteoblasts showed a significant decrease in nodule formation in line with decreased osteoblast activity (p< 0.05). Osteocalcin expression (determined by qPCR) was also diminished (CT: 21±6 vs. cKO: 0,7±0,5 relative mRNA-expression, p< 0,05). This suggests that osteoblast differentiation is diminished in the absence of cdc42. Because examination of the bone marrow showed a significant increase in adipocytes (CT 12% vs. cKO: 55%, p< 0.05) we asked whether cdc42 is involved in fate determination of progenitor cells, pushing them towards osteoblastic differentiation. PPARγ, a key molecule for adipogenic differentiation showed a 6-fold increase in mRNA expression in homozygote cKO cells (p< 0.05).
In summary, deletion of cdc42 in early osteoblasts leads to an increase in adipocyte differentiation and a decrease in bone mineral density. Thus, cdc42 seems to be a key modulator of progenitor differentiation towards osteoblasts, and hence a potential target for anabolic bone therapy.

Hypophyse/Neuroendokrinologie I

P1 1-1

Prevalence of AIP gene mutations in young patients with acromegaly

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Introduction: Familial and sporadic growth hormone secreting pituitary adenomas are associated with mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene. Acromegaly patients harbouring an AIP mutation (AIPmut) tend to have larger and more aggressive tumors and are diagnosed at a younger age. We investigated the prevalence of AIPmut in acromegaly patients that were diagnosed at ≤ 30 yrs of age.
Methods: The German Acromegaly Register database was screened for patients that were diagnosed at ≤ 30 yrs of age. Eleven centers participated and 62 patients (33 men) consented to AIPmut analysis. DNA was analyzed by direct sequencing and multiplex ligation-dependent probe amplification (MLPA) was used to search for extensive deletions. Given are means ± SD.
Results: In the 63 patients the mean age at diagnosis was 23.2 ± 4.4 yrs, 3 patients had a history of familial acromegaly, in 1 patient familial acromegaly was discussed, and 58 cases were sporadic, 5 patients had a microadenoma, 50 a macroadenoma and in 7 patients the adenoma size at diagnosis was unknown. AIP gene sequence variations were detected in 5 patients (3 men, age at diagnosis 24.2 ± 4.3 yrs, 2 with a positive familiy history, 5 with an invasive macroadenoma). The AIPmut detected were c.47G>A, c.490C>T, c.844C>T and c.911G>C and a gross deletion (del ex 1-2) by MLPA in 1 patient. The patient with the AIPmut c.47G>A had also a menin gene mutation (c.881 del G) and clinically MEN-1 syndrome. The patients with AIPmut c.490C>T and c.911G>C had a history of familial acromegaly. The other 2 patients had apparently a sporadic somatropinoma (2/62 or 3.2 %).
Conclusion: The prevalence of AIPmut in this unselected cohort of young patients with acromegaly was lower than previously reported. Since the number of cases with an AIPmut was low, no specific risk profile could be delineated except for a positive family history. In the latter patients genetic testing for AIPmut appears warranted.

P1 1-2

Expression, signalling and function of somatostatin receptors and clinical efficacy of somatostatin receptor agonists in acromegaly

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Introduction: Somatostatin receptor agonists (SSA) are currently the main medical treatment for acromegaly and are clinically effective in 40 - 70% of patients. Expression of somatostatin receptors (SstR) is a prerequisite for the action of SSA, but it is controversial whether the SstR subtype expression pattern alone determines the efficacy of SSA. We studied predictive factors for the therapeutic response to SSA.
Methods: We prospectively studied all acromegalic patients undergoing pituitary surgery over a period of 18 months at our institution. 73 patients were eligible, 45 patients gave their informed consent and for 35 patients primary adenoma cell cultures and a complete clinical follow up could be obtained. We examined the expression of SstR subtypes and gsp mutations and the responsiveness of cAMP-, calcium- and ZAC1-signalling to SSA in primary adenoma cells. The clinical response to SSA was judged by IGF-1 levels.
Results: SstR 2 was the most abundant subtype and was expressed at levels comparable to 19 normal pituitaries in about half of the patients. In vitro octreotide treatment (100nM) suppressed the calcium and cAMP pathways and enhanced ZAC1 expression in 33, 27 and 18 of 35 samples respectively. No sample was completely unresponsive in all 3 pathways. 14 of 35 patients received SSA. IGF−1 levels could be normalized in 7 patients, 5 showed a partial response and 2 patients showed no response. SstR subtype expression pattern did not correlate with clinical outcome, but there was a significant correlation between the stimulation of ZAC1 expression by octreotide and the IGF-1 response to SSA.
Conclusion: The vast majority of adenomas are responsive to octreotide on the cellular level in vitro even if a full clinical response cannot be obtained in vivo. This indicates the presence of important determinants for the clinical sensitivity to SSA downstream of SstR such as ZAC1 expression, which appears to be a promising predictor of therapeutic outcome.

P1 1-3

Epidemiology of pituitary tumors - Multicenter study (to assess the patient data) of a geographical region

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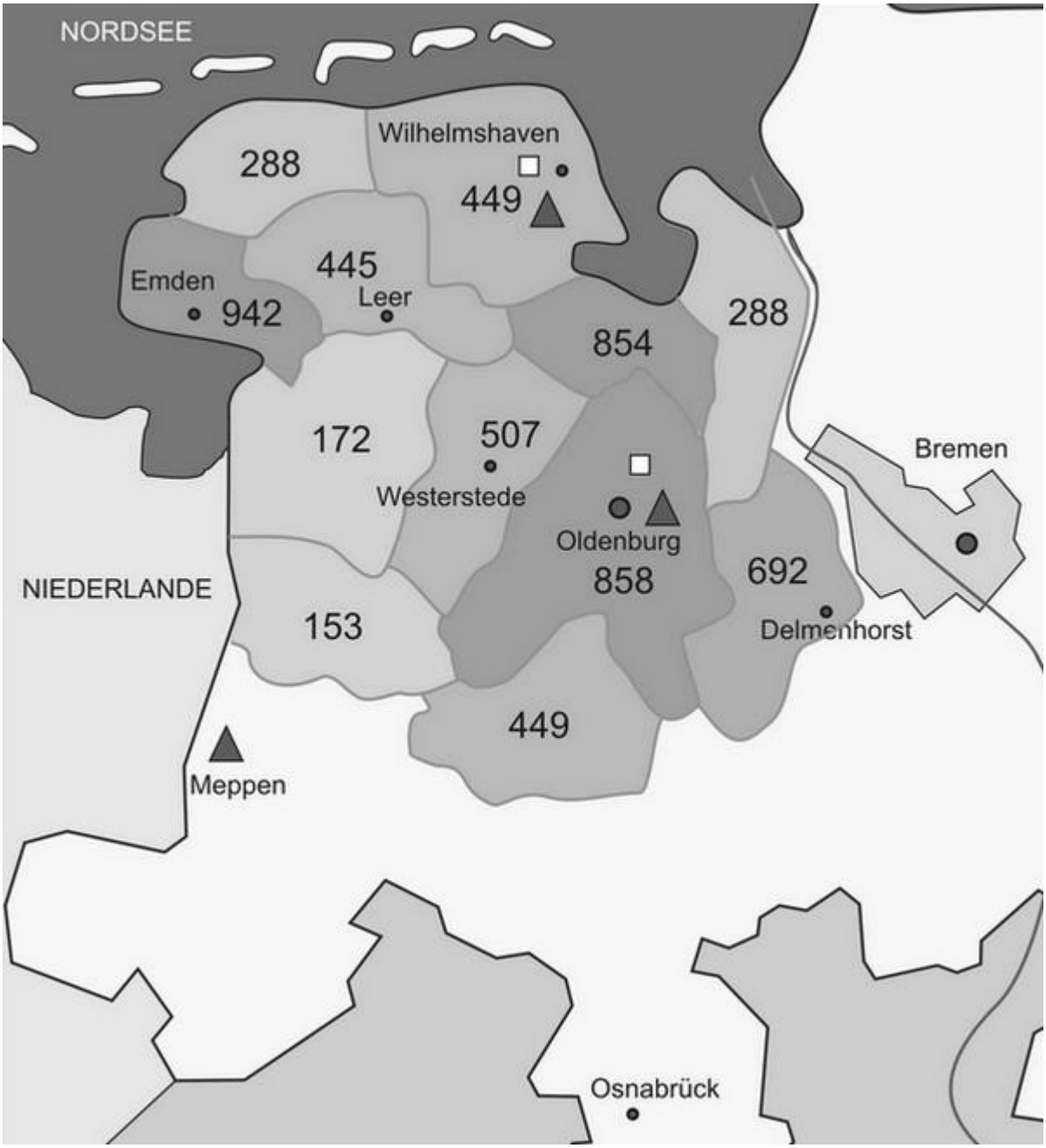
Data covering prevalence and incidence of pituitary diseases is sparse. Beckers et al. found a threefold-elevated occurrence of pituitary adenomas with 940/1 Mio in a small Belgian population (N=71000), if contrasted to current literature. The Weser-Ems region in the Northwest of Germany, separated in 12 districts has a population of 1.65 Mio and is locally treated by three neurosurgical departments and two specialized endocrine clinics. The current study comprises all adult pituitary patients, who were treated in the years 2009 to 2011 at one of these institutions. Captured were only macroadenomas, if non-secretory. Displayed is the prevalence of pituitary diseases per 1 Mio, allocated to the different administrative districts, with populations between 50 000 and 350 000 inhabitants (fig.).



[prevalence of pituitary tumors per Mio]

The analysis gives evidence about frequency and access to specialized endocrine care in dependence on regional infrastructure. Raised data suggests clinical relevant pituitary disease in 950/1 Mio. The pathology is distributed over 750/1 Mio pituitary adenomas and 200/ 1Mio other diseases affecting the pituitary region, like

craniopharyringiomas, sellar meningiomas and hypophysitis. Acquired data also reflects differences in applied care. Patients with neurological deficits and complex endocrine deficiencies could be filtered effectively and were treated comprehensively by the regional specialists (e.g. craniopharyngioma). This is in sharp contrast to rural patients with pituitary adenomas, who frequently were undetected/not diagnosed for considerable periods of time and left untreated.

P1 1-4

Long-term-outcome in Acromegaly: Analysis of 1344 patients from the German Acromegly register

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Introduction: Acromegaly is a rare disease with increased morbidity and mortality. Control of growth-hormone excess and normalization of insulin-like growth factor I (IGF-I) reduces morbidity and reverses increased mortality. Currently available therapeutic options have been demonstrated in study populations to allow for disease control in the vast majority of acromegaly patients. Epidemiological data, however, about therapeutic outcome under “real life” conditions are scarce.
Methods: 1344 patients (age 55.3 ± 14.1 yrs, 57% women) were included from 42 centers participating in the German Acromegaly Register and that had their last follow-up visit within the last 3 yrs. Patients data were collected 10.8 ± 9.2 (median 8.62) yrs after the initial diagnosis (age at diagnosis 44.8 ± 13.7 yrs). Controlled disease was defined by an IGF-I within the center-specific reference range. Given are means ± SD.
Results: 1200 patients (89%) had at least one surgical intervention, 298 patients (22%) underwent radiotherapy, and 573 patients (43%) received medical treatment (somatostatin analogs (n=407), dopamine agonists (n=140), pegvisomant (n=122), combinations (n=90)). In 917 patients (68%) acromegaly was controlled according to a normal IGF-1 (158 ± 58 ng/ml). In patients with an initial diagnosis (ID) dated back > 2 yrs (n=1012) IGF-1 was normal in 78 % of cases. After surgery 33% of the patients were controlled without any further therapy. 35% of the patients with adjunctive radiotherapy had a normal IGF-1 13.75 ± 9.9 yrs post irradiation. Patients treated medically were controlled in 63% of cases (77% ID > 2 yrs). 47% of the patients with an elevated IGF-1 (432 ± 228 ng/ml) received no medical therapy.
Conclusion: Most patients with acromegaly were controlled under real-life conditions in Germany. However, long-term outcome could be improved by exploiting medical treatment options especially in those patients who were not controlled by surgery and/or radiotherapy.

P1 1-5

Use of intraoperative ultrasound for resection of ACTH-secreting pituitary adenomas

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In Cushing´s disease (CD) MRI may fail to detect a pituitary tumor. It was reported that intraoperative transsphenoidal ultrasound identified microadenomas in CD as hyperechoic structures more than 70 %. We report on the intraoperative use of direct contact high-frequency ultrasound (US).
Out of 33 patients (24 female, 9 male, age 20-71 ys, mean follow up 12 ms) with typical endocrinological findings for CD (4 with persistent hypercortisolism, 3 recurrences), in 21 cases microadenomas were suspected or identified by MRI, 3 macroadenomas were visible, in 9 cases MRI (27 %) was negative. For surgery in microadenomas an end fire US-probe (B-mode frequency range 7.5-13 Mhz, field of view 5 mm, penetration 20 mm) was introduced after wide opening of sellar floor. The pituitary gland was scanned. In macroadenomas a side fire probe was introduced into the resection cavitiy to evaluate the parasellar and suprasellar compartments for resection control.
Results In 21 out of 29 cases (72 %) with proven microadenomas US identified the tumors as hyperechoic masses, 5 were negative, 2 false positive, and 2 were questionable. In the 7 cases with negative preoperative or false negative MRI, US identified the adenomas correctly. In all cases posterior pituitary lobe could be distinguished from anterior lobe as hypoechoic. In 2 out of 3 macroadenomas, resection was completed after identification of suprasellar tumor remnants by US. In 31 out of 33 patients postoperative decline of serum cortisol to subnormal levels revealed remission of CD (remission rate 94 %). In one case after early reoperation remission was accomplished. Two recurrences occurred after 8 and 14 months, respectively (6 %).
Intraoperative US-scanning of the pituitary gland may enable the surgeon to identify intrapituitary pathologies even in MRI-negative cases. In macroadenomas perpendicular ultrasound improves resection control by real time investigation of the parasellar and suprasellar compartments.

P1 1-6

Copeptin as a marker for anterior and posterior pituitary function during insulin induced hypoglycemia

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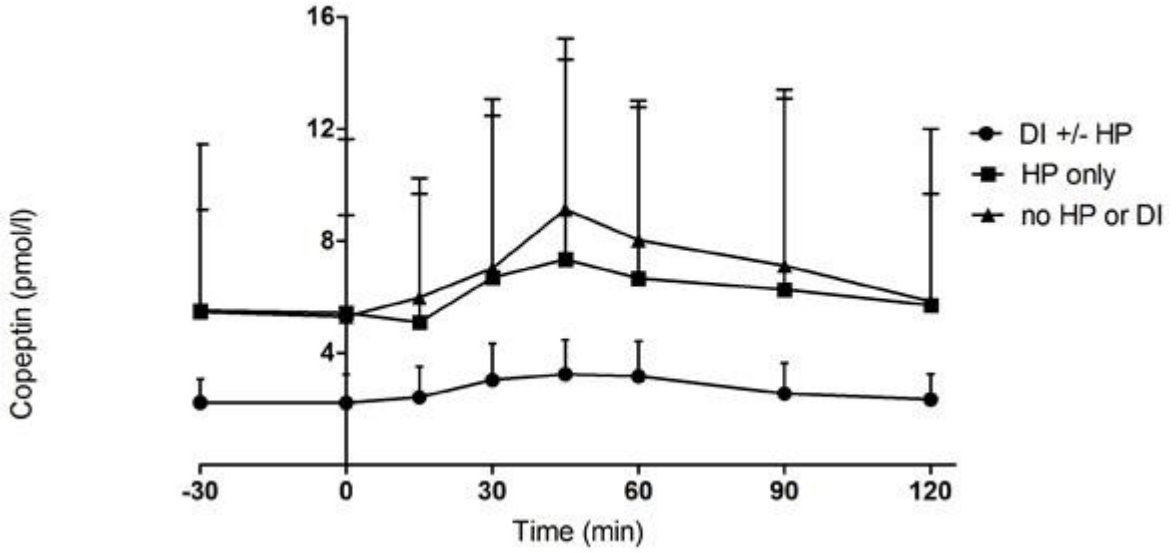
Background: Copeptin (COP), the C-terminal peptide of pro-vasopressin is stoichiometrically released with arginine vasopressin (AVP) in the posterior pituitary. A role of COP in the diagnosis of central diabetes insipidus (DI) has recently been established. AVP is thought to stimulate cortisol secretion via ACTH under stress conditions. However, the extent of such interaction is largely unknown.
Methods: 118 patients (mean age 47.7±13.6 yrs) with suspected hypopituitarism (HP) were prospectively studied by insulin tolerance testing. Blood samples for COP and other endocrine parameters were serially collected. Serum COP was measured by a sandwich immunoassay.
Results: 64 patients had HP defined as low GH or cortisol response, 12 had DI ± HP, and 42 patients had neither HP nor DI. Symptomatic hypoglycaemia was safely induced in all individuals. Patients with DI showed lower baseline (2.2±1.0pmol/l, means ±SD) and stimulated (3.6±1.5pmol/l) COP levels than individuals with no HP or DI (5.3±3.6 pmol/l and 10.3±7.0pmol/l, resp.) or patients with HP and no DI (5.2±5.6pmol/l and 8.9±9.0 pmol/l, resp.). Stimulated COP was higher in males (9.6±7.8pmol/l) than in females (7.9±8.3pmol/l). COP levels in patients insufficient of the HPA axis were lower (8.0±8.7pmol/l) than in those with GH deficiency only (11.2±9.5 pmol/l). There was a moderate correlation between stress-induced maximum plasma ACTH and COP.



[Copeptin levels depending on Diagnosis]

Conclusions: Our results show that both basal and stress-induced COP levels are low in patients with central DI. Blunted copeptin responses in patients with HP in correlation with low ACTH responses suggest a link of the HPA and AVP systems during hypoglycaemia-induced activation.

P1 1-7

Bacterial lipopolysaccharides (LPS) stimulate angiogenic factor production in normal and tumoral pituitary cells under basal and hypoxia-mimicking conditions

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The role of transient or chronic bacterial infectious or inflammatory processes in the initiation and progression of tumors is still a matter of debate in general oncology. We have previously reported that folliculostellate (FS) pituitary cells as well as subsets of pituitary tumor cells express functional toll-like receptors 4 (TLR4), the target of the bacterial cell wall component LPS. The latter could induce the production of IL-6 in FS cells and TLR4-expressing pituitary tumors. As ligands of toll-like receptors have also been reported to influence angiogenic factor production, the effect of LPS on the secretion of vascular endothelial growth factor-A (VEGF-A) was studied in the TLR4-positive FS cell line TtT/GF, in TLR4-negative lactosomatotroph GH3 cells (negative control), in primary cell cultures of rat pituitaries and a small series of somatotroph and nonfunctioning human pituitary adenoma cell cultures. LPS (0.1 - 1000 ng/ml) dose- and time-dependently (24 to 96 hrs) stimulated the basal VEGF-A secretion only in TtT/GF cells but not in GH3 cells. Interestingly, LPS also significantly enhanced the secretion of VEGF-A under hypoxia-mimicking conditions (treatment with cobalt chloride), which per se strongly stimulate VEGF-A. This suggests that LPS stimulates VEGF-A through a hypoxia-inducible factor-1 (HIF-1) independent mechanism. In normal rat pituitary cell cultures and in some, but not in all human pituitary adenoma cell cultures studied, LPS also significantly stimulated the release of VEGF-A under both basal and hypoxia-mimicking conditions. The effects of LPS on other angiogenic factors (e.g. IL-8) and its mechanism of action are currently under investigation.

P1 1-8

Treatment of two patients with pituitary adenoma and meningioma with temozolomide

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Objective: Due to the lack of treatment alternatives beyond surgery or radiotherapy in patients with recurrent and aggressive pituitary tumors, patients have been exposed to a chemotherapy with temozolomide. We herein present two cases in which the patients harboured both, pituitary adenomas and surgically uncurable meningiomas.
Case I: In 1990, a 42-year-old patient was diagnosed with a non-functioning pituitary adenoma. He underwent three operations until 1994 when he received conventional radiotherapy (50.4 Gy). Between 2006 and 2010 he was again operated four times. A more recent MR scan did not only show a recurrent pituitary adenoma but also multiple meningiomas of which only a few were resectable. After another operation, he completed 12 cycles of chemotherapy with temozolomide (according to the “Stupp-Scheme”). Repeat follow-up MR scans revealed not only a significant adenoma shrinkage, but also size reduction of the meningiomas.
Case II: In 1990, a 27-seven-year old patient was diagnosed with a prolactinoma. The tumor was resistant to dopamine-agonistic therapy. After three operations until 1993 she underwent conventional radiotherapy (50 Gy). In 2008 she presented with a rapidly enlarging large skull base tumor but a normoprolactinaemia. She had another resection and histology report showed a petroclival meningioma and a prolactinoma. Postoperatively, she received temozolomide for 6 months. Unfortunately the tumor progressed during therapy now causing hydrocephalus and brain stem compression.
Conclusion: We herein report two patients who suffered from recurrent, aggressively growing pituitary tumors. In addition, they also developed difficult to treat meningiomas. Whereas in the male patient all tumors responded well, in the female patient chemotherapy could not prevent further tumor progression. These results are in perfect agreement with the current literature in which the response rate of temozolomide in pituitary tumours is estimated between 50-60%.

P1 1-9

Dual intrasellar pathologies

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Objective: Dual intrasellar pathologies are rare findings. We herein present three patients who all underwent transsphenoidal surgery in our department for secreting pituitary diseases and in whom two distinct intrasellar pathologies were confirmed postoperatively.
Case I: A 47-year-old female suffered from Cushing´s disease. Her MR scan depicted a left sided pituitary adenoma. She underwent transsphenoidal resection and the adenoma was resected. Histology revealed a prolactinoma and hypercortisolism persisted. On the post-op MR scan another, this time right sided abnormality was visible and IPSS confirmed the pituitary origin of the ACTH-hypersecretion. She underwent a second operation during which an ACTH-secreting adenoma was found and resected.
Case II: A 39-year-old female presented with hyperprolactinaemia. The MRI depicted a left sided adenoma and dopamine-agonist therapy was initiated. In the age of 44, IGF-1 levels increased and oral glucose testing confirmed acromegaly. A newly performed MRI scan showed a second pituitary adenoma on the right side. She underwent transsphenoidal resection of both separate adenomas, and histology confirmed a prolactin- and a GH-secreting adenoma.
Case III: In a 46-year-old female with Cushing disease the MRI suggested a cystic intrasellar adenoma. She underwent transsphenoidal surgery. Initially, a purely cystic lesion was found in the center of the pituitary gland. After sectioning the gland a right sided microadenoma of 3 mm was identified and resected, which was not visible on preoperative imaging. Histology proved a Rathke´s cleft cyst and an ACTH-secreting pituitary adenoma.
Conclusion: All our patients had at least one intrasellar lesion detected on preoperative imaging. Microadenomas, especially in Cushing´s disease might me missed unless the entire pituitary gland is completely exposed exposed and sectioned during surgery. All three patients are still in biochemical remission.

P1 2-1

Combined use of methylisobutylnitrile-(MIBI)-scintigraphy and aspiration cytology to assess the risk of malignancy of thyroid nodules and stratify patients for surgical or non-surgical therapy - a retrospective cohort study

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Introduction: Differentiation between benign and malignant thyroid nodules is essential. Aim of the study was to evaluate an approach for cold thyroid nodules.
Materials: retrospective cohort study, 391 consecutive patients (1.1.2004 - 31.12.2006) with at least one cold thyroid nodule; assessment by a standardized procedure including ultrasonography, ^{99m}Tc-pertechnetate-scintigraphy, laboratory tests, fine needle aspiration cytology (FNAC), ^{99m}Tc-MIBI-scintigraphy. Surgical therapy was recommended in patients with positive MIBI-scans and / or positive FNAC or according to clinical complaints.
Results: 57.3% (224/391) had one cold nodule, 17.9% (70/391) several cold, 24.8% (97/391) cold and hot nodules. MIBI-scan was classified "positive" (16.1%, 63/391), "weakly positive" (19.2%, 75/391) and "negative" (64.7%, 253/391). FNAC was classified benign (87.9%, 247/281), nondiagnostic (6.8%, 19/281) and suspicious / malignant (5.3%, 15/281). 127 patients received surgery revealing malignancy in 13.3% (17/127) which were predominantly papillary (64.7%, 11/17) and follicular carcinoma (23.5%, 4/17). MIBI-scintigraphy was "positive" (64.7%, 11/17) or "weakly positive" (23.5%, 4/17) in most patients with malignant findings. FNAC was unavailable in 23.5% (4/17) with malignancy, positive in 38.5% (5/13) and negative in 61.5% (8/13). Among patients receiving surgery, sensitivity, specificity, negative and positive predictive value for MIBI-scintigraphy were 88.2%, 35.5%, 95.1% and 17.4%, for FNAC 38.5%, 90.6%, 90.6% and 38.5% respectively and for the combination (MIBI-scan + FNAC) 92.3%, 30.6%, 96.3% and 16.9%. Benign MIBI-positive nodules were predominantly follicular adenomas (68%, 33/48).
Conclusion: The evaluation of cold thyroid nodules by MIBI-scintigraphy is helpful for therapeutic decisions: MIBI-negative findings support nonsurgical therapy, while MIBI-positive nodules have an increased risk of malignancy supporting surgery, however the positive predictive value was low.

P1 2-2

Primary hyperaldosteronism and the prevalence of hyperparathyroidism in Germany

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Introduction: The role of parathyroid hormone and its influence on aldosterone as well as the effect of aldosterone excess on the calcium phosphate homeostasis are so far not clearly understood. Our aim was to identify the prevalence of hyperparathyroidism in a large cohort of patients with primary aldosteronism (PA) and to focus on the changes in calcium, phosphate, parathyroid hormone (PTH) and 25-hydroxyvitamin D (25OH vitamin D).
Methods: A.) The German Conn Registry was screened for patients with the diagnosis of 'hyperparathyroidism'; B.) Intact PTH (iPTH) levels were measured in 27 newly diagnosed patients with PA; C.) Blood and 24h urine samples from 11 patients with an aldosterone producing adenoma (APA) from the German Conn Registry were analysed for calcium, phosphate, iPTH and 25OH vitamin D at time of diagnosis and one year after adrenalectomy.
Results: A.) 16 out of 394 evaluated patients with PA were diagnosed with hyperparathyroidism (4.1%). Three of them had a parathyroid adenoma, the remaining 13 were diagnosed as having “secondary” hyperparathyroidism. B.) Of 27 newly diagnosed patients 13 (48.15%) had an elevated iPTH at diagnosis. C.) Following adrenalectomy, patients with APA had a significant decrease in iPTH levels (from 55.5 to 44.5 ng/l, P = 0.05), a decrease of urinary calcium excretion (from 2.2 to 1.0 mmol/l, P = 0.003), an increase in serum calcium (from 2.39 to 2.46 mmol/l, P = 0.037), and serum phosphate concentrations (from 2.97 to 3.44 mg/dl, P = 0.015). 25OH vitamin D levels were low initially and increased post-operatively (from 21.4 to 24.8 ng/ml, not significant).
Conclusion: PA patients have a high prevalence of hyperparathyroidism, mainly resembling “secondary” hyperparathyroidism. Aldosterone excess alters calcium homeostasis and leads to a significantly higher calcium excretion with significantly lower serum calcium and elevated iPTH levels which normalize with treatment.

P1 2-3

Current practice to diagnose and treat thyroid nodules in Germany

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The diagnosis and management of thyroid nodules is described in recently revised guidelines. However, the current practice to deal with this clinical problem is unknown.
We therefore retrospectively investigated the insurance claim and prescription data of 1.129.556 AOK insured patients who were first diagnosed with nodular goiter or single thyroid nodule in april - june 2006 in Germany. We analyzed the diagnostic examinations during the 9 months before the diagnosis nodular goiter or single thyroid nodule was first made and all further measures and treatments (including surgery or radioiodine) during the succeeding two years.
The diagnoses of single/ multinodular goiter were made by general practitioners (50,3%/54,8%), internists (37,8%/ 36,2%) nuclear medicine physicians (4,9%/ 4,6%) and others with ultrasound (75,8%/ 64,2%), szintigraphy (35,1%/ 31,9%), TSH determination (87,6%/86,5%), Calcitonin determination (5,6%/ 10,4%), Anti-TPO determination (13,5%/ 22,2%), TRAK determination (10,2%/ 14,5%) and FNAB (8,1%/ 5,3%). During the 2 years after diagnosis 2,5% / 0,2% of single thyroid nodule and 2,5% / 0,2% of multinodular patients treated by general practitioners, 2,6% / 0,2% and 2,9%/ 0,2% treated by internists, 6,1%/0,9% and 7,7%/ 0,2% treated by nuclear medicine, 14,7% / 0% and 23,2%/ 0% treated by surgeons underwent thyroid surgery or radioiodine respectively. Only 13,6%/ 8,4% of all single/ multinodular patients who underwent surgery had a FNAB. Medical treatment consisted of L-thyroxine therapy for 18%/27% and L-thyroxine-iodine combination for 26%/34% of all non-operated single/ multinodular patients. Iodine therapy could not be evaluated
since its use does not require a prescription.
The different specialists use different diagnostic and therapeutic options with different frequencies. The reasons for the variations and discrepancies need further elucidation. However, the need for guideline dissemination and training appears obvious.

P1 2-4

Impact of Iodide supplementation on the murine TAZ10 autoimmune thyroiditis model

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Introduction: Recently, various studies revealed an association between excessive iodide (ID) intake and autoimmune thyroiditis. TAZ10 mice spontaneously develop an autoimmune thyroiditis due to autoreactive TPO-specific T cells. In this murine model, development of autoimmune thyroiditis is determined by a significant gain in weight, elevation of serum TSH-levels and cellular infiltration of the thyroid. In our work we intended to investigate the impact of physiological and excessive ID supplementation on the course of disease in mice which are immunologically prone to autoimmune thyroiditis.
Methods: Mice were supplemented with 15 ng versus 30 ng ID per ml drinking water, which equates to a daily supplementation of 200 µg versus 400 µg ID per 70 kg. The autoimmune thyroiditis defining parameters were analyzed after 10 and >23 weeks and compared to untreated control mice. Furthermore, immunological effects were monitored by FACS.
Results: After 23 weeks we could significantly demonstrate increased thyroid infiltrates with CD8+ cells by FACS and immunofluorescence staining in those mice supplemented with 15 ng/ml ID (0.9 ± 1,5% CD8+ cells) and 30 ng/ml ID (0,8 ± 1,4% CD8+ cells) when compared to the control mice (0.13 ± 0.2% CD8+ cells). However, no significant differences were displayed when comparing the gain in weight (15 ng/ml ID: 31.3 ± 6.5g, 30 ng/ml ID: 31.1 ± 4.8g, control: 29.4 ± 4.1g) and serum TSH levels (15 ng/ml ID: 46.6 ± 17.7 µIU/ml, 30 ng/ml ID: 46.6 ± 16.8 µIU/ml, control: 44.6 ± 20.1 µIU/ml) in the supplemented versus control groups. Immunological monitoring revealed a significantly higher number of FOX+/CD25+ cells in mice supplemented with 15 ng/ml ID besides an enhanced production of Interleukin-1 in all treated mice.
Conclusions: Physiological and excessive iodide supplementation has no impact on the course of disease in mice which are immunologically prone to autoimmune thyroiditis.

P1 2-5

Determination of 3-Iodothyronamine and thyroid hormones in mouse thyroids using LC-MS/MS

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Since the re-discovery of the endogenous signaling molecule 3-Iodothyronamine (3-T1AM) in 2004, the main question about endogenous serum and tissues levels of this deiodinated and decarboxylated thyroid hormone (TH) metabolite challenged the scientific community. 3-T1AM is a putative modulator of TH responses owing to its metabolic effects which largely antagonize T3 actions. Up to now, 3-T1AM was demonstrated in tissues and blood of several species like humans, mice and rats. The identification and quantification of 3-T1AM was either performed using liquid chromatography- tandem mass spectrometry (LC-MS/MS) or using a highly sensitive monoclonal antibody-based chemiluminescent immunoassay developed in our lab.
T4-substituted thyroid cancer patients after thyroidectomy/radioiodine treatment still present with normal to elevated plasma 3-T1AM levels suggesting that extrathyroidal tissues contribute significantly to its synthesis. Nevertheless, the role of the thyroid in 3-T1AM biosynthesis and physiological function is still elusive. In rodents, approximately 50% of TH produced by the thyroid gland is in the form of the inactive prohormone thyroxine (T4). Since 3-T1AM is suggested to be a major end-product of T4 metabolism, we analyzed the TAM and TH profile in mouse thyroids. Here, we present a liquid-liquid extraction method for the isolation of TH and TAM from tissue and a sensitive LC-MS/MS method that allows the simultaneous detection of all analytes in one analytical run. All detected TAM and TH metabolites were quantified against authentic synthetic molecular standards. Using this technique, we determined 3-T1AM in mouse thyroids to be in the pmol/g concentration range. T4 and T3 as well as 3,5-T2 were detectable in the same concentration range. In the future, we will evaluate thyroids of mice with different genetic TH axis modifications to investigate the physiological role of 3-T1AM and a possible 3-T1AM-based regulation of TH biosynthesis.

P1 2-6

Cubilin exon polymorphism and its effects on vitamin D levels in differentiated thyroid carcinoma

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Introduction: Cubilin is a multiligand endocytic receptor located in various epithelial cells including the human thyroid gland. It mediates the vitamin D uptake into the cells by binding vitamin D metabolites complexed with the vitamin D binding protein (DBP). A relation between lower levels of circulating $1,25(OH)_2D_3$ and differentiated thyroid cancer (DTC) was reported by our group previously. The aim of the present study was to investigate the role of Cubilin (rs1801222) exon polymorphism in patients with DTC and healthy controls (HC). Also its influence on the vitamin D status in DTC was evaluated.
Methods: Patients (n = 230; 153 females and 77 males) with DTC (follicular, n = 44 or papillary, n = 186) and HC (n = 294; 130 females and 164 males) were genotyped for the Cubilin polymorphism (rs1801222) using real time PCR. Furthermore, the $25(OH)D_3$, and $1,25(OH)_2D_3$ plasma levels in patients were measured by radioimmunoassay.
Results: No difference was observed between DTC patients and the HC in the genotype frequencies. However when patients were devided into papillary (PTC) and follicular thyroid cancer patients and grouped according to four $25(OH)D_3$ categories (severely deficient, deficient, insufficient, sufficient), in the deficient PTC group the allele G was more prevalent (75% vs. 54.9%), while the allele A was found to be less prevalent (25% vs. 45.1%; p = 0.004) compared to the HC. Furthermore, the genotype analysis revealed that the genotype GG was more frequent (58.3% vs. 29.3%), whereas the genotypes AG (33.3% vs. 51.2%) as well as AA (8.3% vs. 19.5%; p = 0.01) were less frequent than in the HC. Additionally, deficient PTC patients with genotype GG showed significantly lower $1,25(OH)_2D_3$ plasma levels compared with the HC (p = 0.0004).
Conclusion: A combination of the allele G of the Cubilin polymorphism (rs1801222) and a deficient vitamin D status with lower $1,25(OH)_2D_3$ activation appear to be associated with an increased risk for PTC.

P1 2-7

Cinacalcet effects on the perioperative course of patients operated on for secondary hyperparathyroidism

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Introduction: Since its registration in 2004, the calcimimetic agent cinacalcet has been established as an alternative treatment for secondary hyperparathyroidism. Working by allosteric activation of the calcium-sensing receptor, cinacalcet can lower parathyroid hormone and calcium in those patients. The influence of calcimimetics on the perioperative course has been unclear so far.
Materials and methods: We retrospectively analyzed the data of patients with primary operation for secondary hyperparathyroidism between 2004 and 2011, comparing the perioperative course of patients with and without preoperative cinacalcet treatment.
Results: 56 patients had cinacalcet therapy, and 54 patients had no calcimimetic medication prior to surgery. Gender, age, haemodialysis, and medical treatment were similar in both groups. Also parathyroid hormone- levels were similar pre- and postoperatively (preoperative: 1,249 ±676 pg/ml vs. 1,196 ±601 pg/ml; postoperative: 86 ±220 pg/ml vs. 62 ±91 pg/ml). Patients with cinacalcet preoperatively had significant lower calcium- levels pre- (2.49 ±0.25 mmol/l vs. 2.61 ±0.24 mmol/l) and postoperative (1.75 ±0.37 mmol/l vs. 1.86 ±0.35 mmol/l), and a higher rate of oral calcium- substitution postoperatively (*93%* vs. *74%*). The risk for postoperative persistent disease was slightly higher in these patients compared to those without preoperative cinacalcet therapy (*5%* vs. *0%*, not significant).
Conclusion: Cinacalcet did not alter the perioperative course in secondary hyperparathyroidism patients to our experience.

P1 2-9

Diagnostic determinants and follow-up of subacute thyroiditis in a retroperspective case series of 115 patients

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Introduction: Subacute thyroiditis (SAT) is a self-limiting inflammatory disease associated with painful thyroid enlargement and profound malaise. SAT can be confused with the common sore throat, leading to delayed confirmation of the diagnosis.

Methods: A retrospective chart review identified 115 patients with SAT who had been referred to an outpatient clinic between 2008 and 2010.

Results: SAT was more prevalent in females (5:1) and most often occurred in the 5th decade. A history of a previous viral infection was found in 31%, but SAT prevalence was not affected by season. ESR was elevated in 93% and CRP in 67% of cases. 44% of the patients presented with a goiter, while 45% suffered from hyperthyroidism. Ultrasound identified hypoechoic areas in all but 7% of cases. Twenty-two percent of patients had anti-thyroid antibodies. A thyroid scan was performed in half of the patients and was not diagnostic in 52%. FNA was performed in 45% of cases and not diagnostic in half of the cases. While one third of the patients were diagnosed within one week of symptom onset, confirmation of SAT in the other two thirds was delayed for up to six months. 85 patients (74%) required medication, either with NSAID (19), prednisolone (33) or both (33). Mean duration of prednisolone treatment was 30 days with a starting dose of 36±18 mg/day. SAT recurred in 7%, but only 2 patients required surgery. Permanent hypothyroidism was found in 57 patients (50%).

Conclusion: Female gender, a typical history and presentation, hypoechoic areas in thyroid ultrasound, increased ESR and CRP were relevant, while thyroid scan and FNA appeared not to be as helpful as expected to confirm the diagnosis of SAT. In contrast to previous data, half of the patients developed permanent hypothyroidism.

P1 3-1

How Brown Adipose Tissue (BAT) controls triglyceride metabolism: From lipoprotein lipase (LPL) and CD 36

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Introduction: Recently, we described a powerful role of brown adipose tissue (BAT) in the clearance of triglycerides-rich lipoproteins (TRL) and plasma glucose. In this study we focus on the molecular basis of the lipid uptake into BAT and the pathophysiological relevance of lipoprotein lipase and the membrane receptor CD 36 for hyperlipidemia and obesity.
Methods: For BAT activation and inactivation, mice were kept for 24 h at 4 °C or 22 °C, respectively. Different mouse genotypes (CD36 deficient, adipocyte specific LPL deficient using cre-lox technology and fabp4-cre) were used with respective litter mate controls. TRL metabolism was investigated using nanocrystals and BODIPY-triglyceride labels. During turnover studies, LPL was manipulated using heparin or tetrahydrolipstatin.
Results: It was found, that both LPL activity and CD 36 are necessary for the BAT lipid uptake. This was especially true during cold exposure. However, ucp1 was induced by cold exposure independent of cellular lipid uptake. Compensatory, BAT strongly increased de novo lipogenesis releasing palmitoelate into plasma.
Conclusion: LPL and CD 36 act as a gatekeeper in a highly organ-specific manner facilitating entry of lipids into BAT cells for subsequent combustion. During cold exposure, this pathway reduces plasma lipids and facilitates heat production. However, when adipocyte LPL or CD 36 are absent, increased de novo lipogenesis takes place in BAT.

P1 3-2

TRIB3 related modulation of AKT activity in HepG2 cells

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Introduction: TRIB3 belongs to a family of kinase-like proteins with potent signaling regulatory functions. In vitro studies have shown that TRIB3 can inhibit AKT activation in hepatocytes and PPARγ activation in adipocytes, thus impairing insulin action in both tissues. Furthermore TRIB3 is increased in experimental conditions of insulin resistance as well as in patients with diabetes mellitus type 2.
Methods: We examined the correlation between TRIB3 expression and insulin sensitivity in HepG2 cells. Therefore the cells were treated with either TRIB3 siRNA for gene silencing or TRIB3 plasmid for gene overexpression. Moreover HepG2 cells were exposed to palmitic acid to investigate the effect of a TRIB3 Knockdown in insulin resistant cells. After insulin stimulation insulin sensitivity was assessed by western blotting determination of phospho(Ser473)-protein kinase B (Akt) levels. TRIB3 expression was measured by reverse transcription-polymerase chain reaction and western blotting.
Results: TRIB3 plasmid treatment specifically induced TRIB3 expression 200fold in HepG2 cells. In line with increased insulin resistance, we found that overexpression of TRIB3 led to a decreased phosphorylation of AKT in HepG2 cells. In contrast, no difference in AKT phosphorylation was seen after siRNA induced knockdown of TRIB3. Exposure of HepG2 cells to palmitic acid led to insulin resistance and TRIB3 induction of 235%. In this context TRIB3 siRNA treatment specifically reduced TRIB3 expression by 70%. However, we found that knockdown of TRIB3 in HepG2 cells treated with palmitic acid did not lead to an increased phosphorylation of AKT.
Conclusion: Together these data support the observation, that TRIB3 overexpression results in insulin resistance. However, a TRIB3 knockdown does not improve insulin sensitivity in untreated cells as well as in palmitic-acid induced insulin resistance in HepG2 cells.

P1 3-3

Glucagon induziert das Saeugetier *Indy*-Gen-Homolog in primaeren Rattenhepatocytenv

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Die verringerte Expression des *Indy* Gens (*Indy* = ***I*** am ***n***ot ***d***ead ***y***et) führt in *D. melanogaster* zu einer Zunahme der Lebensspanne durch Mechanismen, die denen der kalorischen Restriktion sehr aehnlich sind. Das Saeugetier-*Indy*-Homolg (SLC13A5, *mIndy*) kodiert einen Trikarboxylat-Transporter und wird vorwiegend in der Leber exprimiert. Reduktion des *Indy*-Homologs in Saeugetieren verhindert die Adipositas und die Insulin Resistenz, die durch eine Hochfettdiaet und waehrend des Alterungsprozesses entstehen. Es ist bislang unbekannt, durch welche Mechanismen *Indy* reguliert wird.
Hepatozyten wurden aus männlichen Wistar-Ratten isoliert und für Promotorstudien mit *Indy*-Promotor-/Luciferase-Reportergenplasmiden transfiziert. Die Hepatozyten wurden für 24h kultiviert, mit Glucagon stimuliert, die *Indy* mRNA-Expression mittels q-RT-PCR bestimmt und die Aktivierung des *Indy*-Promotors durch Luciferaseaktivitätsmessungen analysiert.
Glucagon induzierte zeit- und dosisabhaengig die Ratten-*Indy (rIndy)* mRNA mit einem Maximum bei 10 nM nach 2h(6-fach,$P < 0.05$). Ein 2.2 kb Fragment des *rIndy*-Promotors wurde mit Glucagon (10nM) inkubiert. Glucagon steigerte die Aktivität des Fragments des *rIndy* Promotors bis -1917 in Rattenhepatocyten 2.5-fach($P < 0.05$). Die Glucagon-abhängige Aktivierung war in einem verkürzten r*Indy*-Promotor-Fragment bis -324 stärker (10-fach,$P < 0.01$), eine Verkürzung bis -290 hob die Glucagon-abhängige Aktivierung auf. Studien mit Forskolin und dem PKA-Aktivator 6-Bnz-cAMP fuehrten ebenfalls zu einer sign. Induktion der *rIndy* mRNA und der Promotoraktivitaet. ‚Site directed mutagenesis' des CRE-Elements (CREB-Bindungsstelle) im Indy-Promotor verhinderte komplett die Glucagon-stimulierbare *rIndy* mRNA Induktion und Promotor Aktivitaet.
Glucagon reguliert die *rIndy*-Expression zeit- und dosisabhaengig in primaeren Hepatocyten. Der Effekt wird vermutlich ueber den cAMP/PKC/CREB Signalweg vermittelt und koennte wesentliche Bedeutung fuer den Stoffwechsel in Saegetieren haben.

P1 3-4

Exendin-4 upregulates the expression of Wnt4, a novel regulator of pancreatic beta-cell proliferation

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Objectives: Wnt-signaling is a novel regulator of beta-cell physiology. GLP-1 activates the canonical Wnt-signaling pathway in beta-cells and stimulates beta-cell proliferation through Wnt-signaling. It is not known how the expression of Wnt-signaling molecules in beta-cells is regulated. Therefore, we investigated the effect of antidiabetic drugs and glucose on the expression of Wnt-signaling molecules in beta-cells. Furthermore, we examined effects of Wnt4 on beta-cells.
Methods: Isolation of primary murine beta-cells. Stimulation of beta-cells with glucose insulin, tolbutamide, rosiglitazone, metformin, exendin-4 and Wnt4. Quantification of the expression of Wnt molecules, the insulin gene and the TNFalpha gene by PCR and western blotting. Transient transfection of beta-cells. Knockdown of Wnt4 by three different siRNA sequences in INS-1 beta-cells. Proliferation assays of INS-1 beta-cells ([^{3}H]-thymidine uptake). Measurement of insulin secretion.
Results: The expression of Wnt4 in beta-cells on the mRNA level and on the protein level is significantly increased by Exendin-4 (twofold). Treatment with insulin, tolbutamide, metformin, rosiglitazone and glucose had no effect on the Wnt4 expression. The expression of Frizzled-4, LRP5, Wnt10b and TCF7L2 was not regulated by any of the stimuli. Wnt4 antagonised the activation of canonical Wnt-signaling in beta-cells and depletion of endogenous Wnt4 inhibited INS-1 beta-cell proliferation. Wnt4 and exendin-4 increased the expression of TNFalpha-mRNA in primary beta-cells. Wnt4 had no effect on the glucose-stimulated insulin gene expression or on insulin secretion.
Conclusions: These data demonstrate that stimulation with the GLP-1 receptor agonist exendin-4 increases the expression of Wnt4 in beta-cells. Wnt4 modulates canonical Wnt-signaling and acts as a regulator of beta-cell proliferation and inflammatory cytokine release. This might be a novel mechanism through which GLP-1 can regulate beta-cell proliferation.

P1 3-5

Acute oral fat load increases incretin hormone secretion in rats

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Oral carbohydrate intake stimulates secretion of incretin hormones (GIP, GLP-1). Some studies suggest that also intake of protein and fat affect incretin secretion, but data are scarce. We previously observed higher incretin levels in rats fed a low-carb, high-fat diet compared to controls. To better understand the mechanism of fat induced incretin secretion we now studied GIP secretion during an acute fat tolerance test (FTT).
Methods: Using oral gavages, Wistar rats received a bolus (6ml/kg) of fish-oil, sunflower oil or tap water (control). Rats were on regular chow before FTT. FTT was performed in rats fasted for 16h ("F", n=15) and in non-fasted rats ("NF", n=12). Chilled EDTA tubes preloaded with protease- and DPP-4 inhibitors were used for plasma collection at 0, 15, 30, 60, 120 and 180min after the bolus. Glucose (glucose-oxidase method), insulin (Alpco), active GLP-1 (EDI) and total GIP (Millipore) were measured. Statistical comparison was done by ANOVA.
Results: GIP levels steadily increased after fish- and sunflower oil, but remained unchanged in controls. After 180min, GIP concentrations were massively increased in all rats gavaged with oil when compared to controls (mean±SEM; [pg/ml]; F-H_20: 45.4±13.2, F-sun: 995±70.2, F-fish: 701±111; NF-H_20: 58.6±10.7, NF-sun: 595±170, NF-fish: 385±80; $p< 0.01$), accompanied by increased GLP-1 levels. However, insulin levels did not change significantly in any group. Baseline glucose was higher in NF compared to fasted rats. In fasted rats, glucose remained unchanged throughout the FTT, but in NF rats, sun- and fish-oil load significantly lowered glucose after 30min when compared to controls.
Conclusion: FTT significantly increases GIP secretion in rats independent of the fat source and fasting status. Higher GIP levels did not acutely stimulate insulin secretion, but were associated with lower glucose in non-fasted rats. Therefore, fat induced increases in GIP might play a role in metabolism independent from insulin.

P1 3-6

Identification of a novel Hepatocyte Nuclear Factor 1 alpha (HNF-1α) mutation in a patient with Maturity-Onset Diabetes of the Young (MODY) and hepatic adenomas

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Context: Maturity-onset diabetes of the young (MODY) type 3 is caused by hepatocyte nuclear factor 1 alpha (HNF -1α) mutations. Bi-allelic inactivation of HNF1-α is associated with hepatic adenomas.
Objective: We describe a 20-year old, lean, female patient with newly diagnosed diabetes mellitus. An oral glucose tolerance test (OGTT) revealed normal fasting glucose level (4.5mmol/l) but a pathological increase after 1 and 2 hours (12mmol/l and 16.9mmol/l, respectively). Glutamic acid decarboxylase and IA2/ICA512 autoantigen antibodies were not detectable. Multiple hepatic nodules were identified by ultrasonography and magnetic resonance imaging. MODY 3 was suspected.
Methods: Needle biopsy of a hepatic lesion was performed. OGTT and hepatic ultrasonography studies were conducted on the patient's parents. HNF-1α genes of the patient and her parents were sequenced; identified alterations were analyzed in vitro.
Results: Histological results of needle biopsy were consistent with hepatic adenoma. Analysis of the patient's HNF-1α gene revealed a previously reported Ala98Val polymorphism and a novel Gln495Stop mutation, both heterozygous. The patient's father was found positive for the heterozygous Gln495Stop mutation and an OGTT revealed impaired glucose tolerance. Imaging of the liver did not discover hepatic lesions. The novel Gln495Stop mutation, but not the Ala98Val polymorphism, leads to a dominant-negative HNF-1α protein variant which blocked HNF-1α wild-type mediated gene expression *in vitro*.
Conclusion: The novel Gln495Stop mutation causes a repression of HNF-1α target genes and is most likely the cause of our patient's diabetes and hepatic adenomas. It may also cause her father's impaired glucose tolerance.

P1 3-7

Declining UVB irradiation and the Vitamin D status of German type 1 diabetes patients

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Background: Type 1 diabetes (T1D) is an autoimmune disease caused by the destruction of insulin-producing pancreatic beta cells. A possible risk factor for T1D are low cholecalciferol ($25(OH)D_3$) concentrations. The $25(OH)D_3$ concentration depends on alimentary intake but mostly on human skin production that is influenced by exposure to UVB radiation (290-315nm). Our aim was to investigate whether UVB radiation has changed in the past years and if this influences patients' $25(OH)D_3$ concentrations.
Methods: We analysed the $25(OH)D_3$ concentration of 424 in- and outclinic patients (age 41±18.24 years, male n = 216; female n = 208) with T1D from 2004 to 2008 at the University Hospital Frankfurt am Main. Additionally, daily UVB levels of this period were obtained by the German Federal Environmental Agency (Langen) for the Frankfurt region. Wilcoxon-Mann-Whitney test, Pearson product-moment correlation coefficient and Spearman´s rho were used for statistical analyses.
Results: $25(OH)D_3$ levels of patients with T1D were in summer (Apr-Oct) significantly higher than in winter (Nov-Mar) season (18.3 ng/ml versus 15.0 ng/ml; p = 0.005). Furthermore, the $25(OH)D_3$ concentration of T1D patients obtained in summer 2004 to 2008, declined significantly (22.5 ng/ml versus 19.4 ng/ml; regression coefficient = b = -2.25; $p = 3 \times 10^{-4}$) whereas it remained stable in winter (11.5 ng/ml versus 18.0 ng/ml; b = 0.65; p = 0.26) during the same period. During this observation period, UVB radiation declined significantly in summer (b = -0.43; $p = 3 \times 10^{-5}$) but no change was found in winter ($b = 6 \times 10^{-4}$; p = 0.99). Finally, we detected a significant correlation between the $25(OH)D_3$ concentration and UVB radiation (rho = 0.44, p = 0.01).
Conclusion: Our results suggest that decreasing UVB radiation in summer possibly due to changes in stratospheric ozone layers and caused by alteration of solar activity may lead to declining vitamin D levels in German patients with T1D.

P1 3-8

Vitamin B-12 deficiency associated with metformin treatment may result in diabetic neuropathy in type 2 diabetic patients in daily practice

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Introduction: In a recent study, vitamin B-12 deficiency was observed in 10 % of metformin-treated type 2 diabetic patients, but neurological status of the patients was not documented. Therefore, we decided to examine to which extent vitamin B-12 deficiency can be detected in metformin-treated diabetic patients in daily practice and which impact vitamin B-12 deficiency - if present - could have on the development of diabetic neuropathy in these patients.
Methods: 88 consecutive type 2 diabetic patients with metformin treatment for ≥ 1 year (37 females, 51 males, age, 66 ± 2 years [SEM], duration of diabetes, 10 ± 2 years, metformin, 1450 ± 120 mg/day for 6 ± 2 years) were included in this cross-sectional study. We measured vitamin B-12 levels, folate, 25-OH-vitamin D and PTH by enzyme immunoassay, additionally we performed a routine screening of all patients for signs or symptoms of diabetic neuropathy.
Results: 10 % of the patients had vitamin B-12 deficiency, a total of 29 % of the patients had vitamin B-12 levels < 200 pmol/l. Patients with decreased vitamin B-12 levels had received higher doses of metformin for longer duration than patients with vitamin B-12 levels > 200 pmol/l ($p < 0.05$). Moreover, they exhibited an increased prevalence of diabetic neuropathy, had decreased folate levels and decreased vitamin D levels ($p < 0.05$). In patients with decreased vitamin B-12, diabetic neuropathy developed after shorter duration of diabetes and despite better HbA1c levels than in patients with normal vitamin B-12 ($p < 0.05$).
Conclusions: In this cross-sectional study in daily practice, there is evidence for neurological consequences of metformin-associated vitamin B-12 deficiency, which therefore must be considered as eventually harmful for metformin-treated patients. Thus, patients should be regularly screened and vitamin B-12 replacement started as early as necessary, together with the replacement therapy of mostly associated vitamin D-deficiency.

P1 3-9

Autoantibodies against serotoninergic 5-HT_4 receptor in patients with heart failure

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Introduction: Serotoninergic 5-HT_4 receptors have been detected in several tissues including the heart. An autoimmune mechanism may underline the pathogenesis of heart failure. The aim of this work was to look for autoantibodies to the 5-HT_4 receptor in patients with heart failure.
Methods: We looked for the presence of autoantibodies against 5-HT_4 receptor as well as angiotensin II type (AT1), β_1-adrenoceptor and muscarinic M2 receptors In 176 patients sera with heart failure (female: n = 96, male: n= 80) and in 108 controls (female: n = 69; male: n = 39).
Results: The prevalence of 5-HT_4 receptor autoantibodies was 18.8 % (n=33) in the group of patients with heart failure and 4.6 % (n=5) in the control group ($p < 0.002$). The prevalence of autoantibodies against AT1 was 1.7 (n=3), β_1-adrenoreceptor 0.6 (n=1) and muscarinic-receptor M2 4.2 (n=5). Female patients with diabetes and heart failure had a positive trend ($p = 0.07$) to the presence of 5-HT_4 receptor autoantibodies. In the group of female heart failure patients we found a significant correlation with the presence of coronary heart disease ($p = 0.05$).
Conclusion: The clinical relevance of 5-HT_4 receptor autoantibodies has to be further studied. The prevalence of 5-HT_4 receptor autoantibodies was highly significantly in patients with chronic heart failure. It was also a significant correlation between these autoantibodies and the female subgroup with coronary heart disease. It is conceivable that the increased prevalence of autoantibodies against the 5-HT_4 receptor in patients with heart failure is more than just an epiphenomenon.

P1 3-10

Functional analysis of genetic variants and vitamin D status in immune cells by RT-PCR

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Introduction: Vitamin D insufficiency has been linked to Type 1 Diabetes and studies related about significant correlations with polymorphisms of vitamin D (*CYP2R1*, *CYP27B1*) and cholesterol biosynthesis (*DHCR7*) genes. To investigate possible associations, gene expression in immune cells, vitamin D status and genetic variants were analyzed in healthy controls (HC).
Methods: From 23 HC monocytes (Mo), T-helper cells (Th) and natural killer cells (NK) were isolated and after cell lysis extracted RNA was reverse transcribed into cDNA. Furthermore gene expression of DHCR7, VDR, CYP2R1, CYP27B1 and the endogenous control GAPDH were measured by Taqman assay. Finally, *DHCR7* (rs-12785878*), CYP2R1* (rs-10741657), *CYP27B1* (rs-10877012), *VDR Fok* (rs-1073580) polymorphisms were genotyped and 25-hydroxyvitamin D_3 [25(OH)D_3] plasma levels were measured using radioimmunoassay. Statistical analyses were performed using the $2^{-\text{delta-delta-Ct}}$ values by Kruskall-Wallis-test.
Results: All studied immune cells showed a significantly different gene expression of DHCR7 (NK=100, Mo=10, Th=$116x10^3$; p=0.001), CYP2R1 (NK=238, Mo=20, Th=$162x10^3$; p=0.001) and CYP27B1 (NK=15, Mo=6, Th=$72x10^4$; p=0.001). By dividing the HC into 25(OH)D_3 deficiency (< 20ng/ml) and 25(OH)D_3 sufficiency (>20ng/ml), significant down regulation of VDR expression in NK and Th cells from HC with vitamin D deficiency compared to those with vitamin D sufficiency was observed (p=0.019 and p=0.037 respectively). No associations between the gene expression levels and the investigated polymorphisms in all immune cells were detected. However, the CYP27B1 expression was significant higher in NK cells from HC with vitamin D deficiency and the "CC" CYP27B1 genotype than those with the "AC" genotype (p=0.03).
Conclusion: Vitamin D deficiency or its deficiency in combination with the CYP27B1 polymorphism seems to interact with cellular immune effects like VDR or CYP27B1 expression illustrating the need for an individualized therapy.

P1 3-11

High dose vitamin D in type 1 diabetes or Addison´s disease 1 (ViDDA 1 study)

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Background: Vitamin D levels (VD) are significantly lower in patients with type1 Diabetes (T1D) and Addison´s disease (AD). VD insufficiency is implied as risk factor for the development of autoimmune disorders. Few data exists as to how immune system is affected by the VD status.
Aim: We are investigating the safety and efficacy of a three-month 25(OH)D³ supplementation of 4.000 IE/d on the VD status, gene expression in immune cells, inflammatory markers as well as on the disease activity compared to three-months placebo.
Materials: Randomized, double-blind, placebo-controlled trial with two parallel cross-over groups. During the first three months patients are randomized to treatment with 6 drops (4000 IE/d) Vigantol or placebo-oil (medium chain triglycerides) followed by three months vice versa. Planned study end is December 2012, whereby we aim at the recruitment of 180 patients.
Methods: We are studying the VD effect on cellular immune status and inflammation markers and correlate to imprinting by various genes (VDR, CYP2R1, CYP27B1, DBP, CYP24, DHCR7) and HLA-haplotypes. We quantify T -lymphocyte subpopulations and analyze mRNA-expression of genes relevant for VD metabolism and immune system (VD cascade, IL-10,IL-12). Renal function and calcium levels (serum and urin fractional excretion) are included in our safety parameters.
Results: Of the 20 recruited patients, n=13 are diagnosed with T1D (90%) and n=7 with AD (10%), 70% (n=14) are female and 30% (n=6) male. Average age is 38±19,22 yrs. According to the time of disease manifestation,we classify patients into two groups,n=11(55%)diagnosed in the last 5years and n=8 (45%) before. So far no events of hypercalcemia or renal dysfunction have been documented. Our results will show whether high dose VD can normalise surrogate markers of altered immunity and chronic inflammation.

P1 4-1

Compound A, a selective glucocorticoid receptor modulator does not impair bone in a mouse model of glucocorticoid-induced bone loss

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Glucocorticoids (GCs) regulate various physiological processes, including bone remodeling. Compound A (CpdA) is a novel GC receptor modulator with the potential of an improved benefit/risk profile. Here, we tested the effects of CpdA on bone in a mouse model of GC-induced bone loss.
Bone loss was induced in FVB/N mice by implanting slow-release pellets containing either placebo, prednisolone (PRED) (3.5 mg), or CpdA (3.5 mg). After a total of 4 weeks, mice were killed to examine the effects on the skeleton using quantitative computed tomography, bone histomorphometry, serum markers of bone turnover, and gene expression analysis of bone. To assess the underlying mechanisms *in vitro* studies were performed with human bone marrow stromal cells (BMSCs) and murine osteocyte-like cells (MLO-Y4 cells).
PRED reduced the total and trabecular bone density in the femur by 9% and 24% and in the vertebral body by 11% and 20%, whereas CpdA did not influence these parameters. Histomorphometry confirmed these results and further showed that the mineral apposition rate was decreased by PRED whereas the number of osteoclasts was increased. The decreased mineralization rate was paralleled by a decline in serum P1NP, a reduced tibial expression of osteoblast markers, and increased mRNA levels of dickkopf-1 (DKK-1). In addition, serum CTX-I and the tibial RANKL/OPG ratio were increased by PRED. None of these effects were observed with CpdA. In line with the *in vivo* data, CpdA also had no effect on the RANKL/OPG ratio in MLO-Y4 cells. Finally, CpdA also failed to transactivate DKK-1 expression in bone tissue, BMSCs and osteocytes.
This study underlines the bone-sparing potential of CpdA in an experimental model of GC-induced bone loss and shows that by preventing the induction of RANKL and DKK-1, GC-induced bone loss can be ameliorated.

P1 4-2

Adipocyte-secreted factors increase osteoblast proliferation and the OPG/RANKL ratio to influence osteoclast formation

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Objective: Studies have shown a positive relation between the body mass index and the bone mineral density. It is not clear, whether adipocytes secrete signaling molecules which directly act on osteoblasts. Therefore, we have investigated the effect of fat cell-secreted factors on the proliferation and differentiation of preosteoblasts and the underlying molecular mechanisms.
Methods: We have used following cells: preosteoblastic MC3T3-E1, mononuclear RAW264.7, primary human preosteoblasts, primary human adipocytes. Cell culture medium was conditioned by the isolated adipocytes. Preosteoblast proliferation was measured by ^{3}H-Thymmidine incorporation. Differentiation to osteoblasts was determined by the expression of differentiation markers on the mRNA and protein level, to osteoclasts by TRAP staining. Furthermore, we determined the bFGF level in fat cell-conditioned medium and in serum samples of adipose patients.
Results: Stimulation with fat cell-conditioned medium increased the proliferation of MC3T3-E1 and primary human preosteoblastic cells (2.8-fold and 1.5-fold; $p < 0.0001$), which could be inhibited with inhibitors of tyrosine kinases, FGF receptor I and PI3K. By analogy, we could show adipocytes to secrete bFGF and recombinant bFGF to imitate this effect. The OPG/RANKL ratio in primary human preosteoblasts was 9-fold higher after stimulation with fat cell-conditioned medium. In addition, osteoblasts, which were stimulated with fat cell-conditioned medium, inhibited the formation of osteoclasts.
Summary: Human adipocytes secrete factors which directly act on preosteoblasts and modulate their interaction with osteoclasts. Herefrom, more osteoblast precursors and a reduced bone resorption results. These *in vitro* findings reflect the higher bone density among obese people and attribute it to direct effects of adipocytes on preosteoblasts.

P1 4-3

An O-glycosylation site in fibronectin inhibits osteoblast function and bone formation both *in vitro* and *in vivo*

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Fibronectin is an extracellular matrix protein required for normal osteoblast function.
Oncofetal fibronecton (oFN) is an isoform, which includes an O-glycosylation site in the variable region at AA 33. It is increased in patients with chronic cholestatic liver disease, inhibits nodule formation by osteoblasts *in vitro* and bone formation *in vivo* (Kawelke et al. JBMR 2008). The aim of our work is to characterize the site responsible for these effects and define the receptor involved for possible modification in the treatment of osteoporosis.
There are 7 N- and 3 O-glycosylation sites in fibronectin, two of which are localized in the variable region, and one that is common between plasma FN (pFN) and oFN. Deglycosylations of both pFN and oFN were performed and success was confirmed based on western blotting and glycoprotein staining. Treatment of osteoblasts *in vitro* revealed normalization of nodule formation by the O-deglycosylation of oFN ($p < 0.05$), but no effect of O-deglycosylation of pFN (p=NS), suggesting that indeed the O-glycosylation in the variable region of oFN is responsible for the effects we saw. Transfection of osteoblasts with a construct containing a point mutation at AA 33 of the variable region resulted in enhanced nodule formation ($p < 0.05$ vs. CT) and an increase in osteocalcin mRNA expression ($p < 0.05$ vs. CT). Inhibition of this site with a specific antibody (FDC6) resulted in a normalization of nodule formation ($p < 0.05$ vs. CT). Furthermore, injection of oFN in mice with conditional β1 integrin deletion in osteoblasts (colα1(I)-cre β1-/-) did not show a difference in the response to *in vivo* administration of oFN. Taken together, these findings suggest that the receptor involved does not contain β1 integrin.
Thus we have identified a site in fibronectin that is able to inhibit osteoblast and bone formation. Identifying the site of interaction of this fibronectin fragment with osteoblasts may open new avenues for osteoblast modulation.

P1 4-4

Impact of pregnancy, puerperium and lactation on bone metabolism

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Background: To provide the growing fetus/infant calcium alterations in maternal bone metabolism take place during pregnancy and postpartum. The aim of this study was to show possible alterations in bone mineral density and biochemical markers of bone turnover during pregnancy, puerperium and lactation.
Methods: In this prospective longitudinal clinical study bone mineral status was analyzed by quantitative ultrasonometry (QUS) at the distal metaphyses of phalanges II-V in healthy women thrice during pregnancy (10.9±1.4; 24.9±1.3; 34.4±1.4 weeks of gestation; n=49), once postpartum (pp 7.1±1.3 wks., n=43) and once during lactation (pp 29.1±5.7 wks., n=35). Anamnestic and biometric data were collected. Biochemical markers of bone remodeling (osteocalcin OC, ß-Crosslaps, TRAP-5b) and osteoprotective factors (osteoprotegerin OPG) were determined.
Results: Decrease in AD-SoS ($p < 0.004$), T-score ($p < 0.003$) were observed throughout pregnancy. In puerperium an increase in T-score ($p < 0.045$) was detected. During lactation there was a non-significant decrease in all QUS parameters. In breastfeeding < 2 mo. AD-SoS and T-score in- ($p < 0.049$) and maternal OC concentrations decreased ($p < 0.046$); lactation >6 mo. resulted in a non-significant reduction in QUS parameters and a significant rise in OC concentrations ($p < 0.028$) compared with non-lactating women. All biochemical markers of bone turnover were elevated during pregnancy ($p < 0.001$). Markers of bone resorption (TRAP-5b, ß-Crosslaps) continuously rose and peaked in puerperium. Markers of bone formation (OC) rose ($p < 0.001$) after having decreased in the 2. trimester and peaked during lactation. There was a negative correlation between pp QUS parameters and OC- and TRAP-5b concentrations ($p < 0.002$).
Conclusion: Pregnancy is associated with bone loss and a high bone turnover. In addition, women with a prolonged duration of lactation show higher bone loss and a higher bone formation rate in comparison to non-lactating women.

P1 4-5

Parity-dependency of estrogen response in bone and uterus of Lewis rats

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The commonly used preclinical animal model of postmenopausal osteoporosis is the mature rat. Castration induced cessation of ovarian estrogen production consequently results in bone volume reduction. Amongst studies using this approach, there is no consensus whether nulliparous or pluriparous rats are more appropriate. This is important as persistent changes in skeletal mass, chemistry and mechanical properties occur during pregnancy and lactation. Here the aim of the study was to compare responsiveness to ovariectomy and to E2 substitution between nulli- and pluriparous rats. Therefore, female Lewis rats aged 12 months were subjected to ovariectomy or to sham surgery. Eight weeks after surgery, half of the Ovex animals received E2 pellets *s.c.* (4.5 µg/kg bw/d). 4 weeks later, animals were sacrificed, uteri were collected and tibiae were stored for micro-computed tomography analysis.
No differences between the uterus wet weights of the nulli- and pluriparous rats were observed with regard to Ovex and sham surgery. In contrast, a significantly higher uterotrophic effect was obvious in the nulliparous group. Furthermore, analysis of uterine gene expression revealed significantly different responses to endogenous estrogen depletion and to a subsequent E2 substitution, e.g. the increase of the *Esr*1 mRNA level in the Ovex group compared to the intact animals was higher amongst the nulliparous rats. Related to this, the E2 induced changes of responsive genes were observed to be higher in the nulliparous group. Analysis of trabecular bone parameters of the intact animals revealed no significant differences between nulli- and pluriparous animals. The E2-induced increase of a number of bone parameters was again found to be more pronounced in the nulliparous animals.In conclusion, in terms of the estrogen responsiveness of the uterus and the bone, we observed significant differences on regulation of uterine gene expression and on bone physiology dependent on parity status.

P1 4-6

Fibronectin isoform EDA increases osteoblast activity

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Fibronectin is produced by osteoblasts and laid down with collagen to form osteoid and then bone. There are several fibronectin isoforms, whose function remains poorly understood. Studies in conditional knockout mice using the collagen α1(I) promoter attached to cre in homozygote fibronectin conditional knockout mice had shown that fibronectin is required for osteoblast differentiation (Bentmann et al. 2010). The failure of circulating fibronectin to overcome the decrease in differentiation implied a role for an isoform produced by the osteoblast. Indeed, osteoblasts produce 9-fold more fibronectin containing the extra domain A (EDA) than is found in the circulation. We therefore hypothesized, that EDA is required by osteoblasts to induce their differentiation via a feedback loop. To examine this question we generated transient transfection constructs of the EDA isoform and a control construct containing the plasma isoform of fibronectin and hence is without EDA. Transfecting these two constructs into freshly isolated newborn calvarial osteoblasts followed by culture in differentiation media showed that parameters of osteoblast differentaion in vitro were all increased: the number of nodules stained by von kossa: CT:0.0177±0.0019 vs. EDA:0.0243±0.0015nodules/mm^2, $p< 0.05$, the area of the von kossa stained nodules: CT:1.93±0.34 vs. EDA:3.87±0.51% , $p< 0.05$, and finally osteocalcin as measured by quantitative real time PCR: CT:1.38±0.43 vs. EDA:6.03±1.82RE, $p< 0.05$. Because EDA is known to bind to α4β1 integrin. We used an inhibitory antibody to determine whether this affects nodule formation. In preliminary experiments no effect could be detected (p=NS, n=4/group), suggesting that α4β1 integrin did not mediate the signal originating from EDA fibronectin.
Thus, EDA fibronectin increases osteoblast differentiation and supports in vitro mineralization to a larger degree than plasma fibronectin. Identification of the receptor involved may open new therapeutic avenues.

P1 4-7

Prepubertal pig offspring show sex-specific changes in bone mass density following *in utero* estradiol-17β exposure

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Estradiol-17β (E2) is important for both trabecular and cortical bone mass development in females, but only for the latter in males. Studies in mice have shown that early estrogen exposure mainly affects female bones leading to an increase in bone mass density (BMD) in adulthood. In the present study, adult sows were exposed to E2 through food supplementation during gestation. In addition to the control group receiving only the carrier, the treatment groups received 0.05, 10 and 1000 µg E2/kg body weight daily, respectively. Male and female prepubertal offspring (n=11/group) were slaughtered at 56 and 63 days of age, respectively, and plasma E2 and testosterone was determined by ELISA. Furthermore, BMD was assessed at the proximal tibia, the distal and the proximal femur using quantitative computer tomography. No treatment effect was found in male offspring regarding BMD, bone area, and bone length. In contrast, female offspring of the 1000 µgE2/kg body weight daily group showed a significant reduction in the total BMD at the metaphysis of the distal femur compared to controls (mean ± SEM: 320 ± 9 and 290 ± 6 mg/cm³; $P< 0.05$), which mainly stemmed from a lower trabecular BMD (mean ± SEM: 292 ± 5 and 273 ± 5 mg/cm³; $P< 0.05$). Furthermore, total BMD was also reduced at the proximal tibia ($P< 0.05$). In spite of these differences, E2 and testosterone concentrations were unaffected not only in male but also in female offspring at the time of slaughter. In summary, females were sensitive to in utero estrogen exposure regarding bone development. Contrasting to the findings in mice, we found a decrease in BMD, which may be caused by an imprinting effect on bone cells set during pregnancy. Thus, to characterize bone cell activity and further endocrine regulators of bone development the determination of plasma osteocalcin, type I collagen C-telopeptide breakdown product (CrossLaps) and insulin-like growth factor 1 (IGF1) concentrations are currently under way.

P1 4-8

Comparison of different methods for determination of 25-hydroxyvitamin D (25-OHD): Influence on the prevalence of vitamin D deficiency

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Introduction: Vitamin D deficiency is an important cause of bone diseases. The availability of automated methods provides the possibility to analyze large sample sizes. However, method standardization is still problematic. In 2011 Roche Diagnostics (Mannheim, Germany) changed the method for 25OHD with the Elecsys analyzer. We compared the two Roche methods and an ELISA reference method.
Methods: 25OHD was determined in serum samples of 80 ambulant consecutive patients (54 female, 26 male; mean age 65.8 ± 13.0 years). Some of these patients were supplemented with vitamin D. 25OHD was determined with three assays: Elecsys Vitamin D3 (Roche old), Elecsys Vitamin D total (Roche new; both from Roche Diagnostics; Mannheim Germany) and 25OHD direct ELISA Kit (Immundiagnostik, Bensheim, Germany). All methods are competitive, both Roche methods are automated. The pretreated serum samples were incubated with a polyclonal antibody (Roche old), vitamin D-binding protein (Roche new) or a monoclonal antibody (25OHD direct ELISA Kit).
Results: Mean 25-OH-D values for patients:
Roche (old): 40±27 nM;Roche (new): 80±33 nM;ELISA 97±36 nM
Correlation coefficient Roche(old) versus Roche (new): 0.69 Correlation coefficient Roche(old) versus ELISA : 0,41
Correlation coefficient Roche(new) versus ELISA: 0,73
Percentage of Vitamin D-deficiency (25-OH-D < 50 nM): Roche (old): 73%;
Roche (new): 16%; ELISA 6%
The Bland-Altmann-Plot shows concordance between Roche(new) und ELISA(Immundiagn.).
Conclusion: The old Elecsys Vitamin D3 assay yields significantly lower levels of 25OHD than the Elecsys Vitamin D total assay and the percentage of vitamin D deficient patients is significantly higher with the old method. The 25OHD levels determined with the Elecsys Vitamin D total method (new) correlate very well with with the 25OHD direct ELISA, therefore, the new Roche method shows high plausibility. The method of 25OHD assay has important influence on clinical interpretation.

P1 4-9

Long term higher urinary calcium excretion within the normal physiologic range predicts impaired diaphyseal bone status in healthy children: the importance of potential renal acid load

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Introduction: Reduced bone mineral density (BMD) and bone mass have been observed in children with idiopathic hypercalciuria. Whether urinary calcium excretion at the higher end of the normal physiologic range can influence bone health in healthy children independent of dietary intake is unknown.
Methods: Urinary calcium was quantified in 603 24-h urine samples from 154 healthy children and adolescents who had ≥3 urine collections and parallel 3-day weighed dietary records during the 4 years preceding proximal forearm bone analyses by peripheral quantitative computed tomography (pQCT). Urinary potential renal acid load (uPRAL) was determined according to urine ionogram by subtracting measured quantitatively important mineral cations from nonbicarbonate anions.
Results: Urinary calcium excretion showed to be negatively associated with volumetric (v)BMD ($p < 0.05$), cortical bone mineral content (BMC) ($p = 0.05$, trend) and cortical cross-sectional area (CSA) ($p = 0.09$, trend), but not with total CSA or Strength-Strain Index ($p > 0.1$) in the total population sample. Stratified analyses based on the median split of uPRAL showed that the negative associations of calcium excretion with vBMD, cortical BMC, and cortical CSA were especially pronounced in those children with higher uPRALs ($p < 0.01$), but not in those with low uPRALs ($p > 0.3$).
Conclusion: Long-term higher calciuria within the physiological range predicted reduced diaphyseal bone mass and bone density particularly in healthy children and adolescents with long-term unfavorable higher dietary acid load, e.g., with lower fruit and vegetable intake.

P1 4-10

Serum tryptase levels should be included in the evaluation of secondary osteoporosis

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Introduction: Mastocytosis is a rare and complex disease characterized by increased mast cell infiltration in various tissues combined with a diversity of different symptoms. Osteoporosis can be a secondary complication of mastocytosis, in some patients the reason for devastating fractures.
Methods: We retrospectively studied 93 patients with suspected mastocytosis by analyzing patient records for basic patient data, specific laboratory parameters for mastocytosis and osteoporosis, dual-x-ray-absorptiometry (DXA) and skin or/and bone biopsy analysis.
Results: Our population included 26 patients with histologically confirmed indolent systemic mastocytosis, 37 patients with a cutaneous form and two patients with a systemic form other than indolent systemic mastocytosis. In 57 patients bone density (BMD) was measured by DXA. 70,2 % (n=40/57) of these patients had osteopenia and/or osteoporosis (T-score ≤ -1). Mean age of these patients was 52 years ±14.7. Bone alkaline phosphatase was higher in patients with osteoporosis (MW±SD 20.9 ±9.7 µg/l) compared to those with osteopenia (MW±SD 6.83±1.04 µg/l, $p>0.05$). Thirteen fractures in 11 patients were documented, predominantly in patients with a systemic form of mastocytosis (8/2). Patients with fractures had a significant lower T-score in lumbar spine BMD (n=10, MW±SD -2.36±1.62) than patients with no reported fracture (n=47, MW±SD -1.27±1.38, $p < 0.05$). There was a significant negative correlation between serum tryptase and DXA results of the lumbar spine ($R=-0.37$, $p< 0.05$). Five patients were initially diagnosed during their osteoporosis evaluation by serum tryptase levels.
Conclusion: In patients with mastocytosis an evaluation for osteoporosis would be reasonable. In addition, serum tryptase levels should be included in the evaluation of secondary osteoporosis. The mechanisms that cause osteoporosis in patients with mastocytosis is poorly understood and require further investigations.

P1 5-1

Young age in Klinefelter-patients increases chances for successful testicular sperm extraction: Predictive factors for a positive spermatozoal yield

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Introduction: Klinefelter`s Syndrome (KS) with an incidence of 1:600 males is one of the most frequent numeric chromosome aberrations, leading to an irreversible damage of fertility in 97% of the patients. To have the option for a later intracytoplasmic sperm injection (ICSI), microsurgical testicular sperm extraction (m-TESE), and in case of a positive result, the cryopreservation of spermatozoa can be performed.
Methods: Between 2008 and 2011 we analysed the m-TESE-results of 47 non-mosaic KS-patients (karyotype 47,XXY). Correlations between and prediction of a positive spermatozoal yield in m-TESE-samples and age, maldescended testes, hormone parameters as well as hormonal treatment before surgery were tested.
Results: In 40 % of the patients (19/47, group 1) we found a mean of 16 ± 23 spermatozoa (mean ± standard deviation, range 1-84 spermatozoa). In 60 % (28/47, group 2) no spermatozoa were found. In binomial regression analysis younger age (22 ± 8 vs. 27 ± 8 years), testosterone levels ≥ 7.5 nmol/l and hCG-treatment before surgery were positive predictors for a successful m-TESE ($p = 0.05$, $p = 0.009$, $p = 0.07$, respectively). No spermatozoa were found, if testosterone levels were < 7.5 nmol/l. Chances to find spermatozoa in m-TESE are 1.9fold higher if testosterone levels are ≥ 7.5 nmol/l ($p = 0.03$, 95% confidence interval 1.4 - 2.6, chi-square-test).
Conclusions: KS-patients should be counselled concerning fertility issues and offered m-TESE as early as possible to find spermatozoa for cryopreservation with the option for a later ICSI therapy. Testosterone levels ≥ 7.5 nmol/l have a positive influence on m-TESE-results. Exogenous testosterone treatment, which has been stopped at least 3 months prior to surgery, does not seem to have negative effects.

P1 5-2

Slow-freezing vs. vitrification in ovarian tissue preservation

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Introduction: Chemo- and radiotherapy often result in infertility and premature ovarian failure. Slow-freezing is currently the standard for cryopreservation of ovarian tissue prior to chemotherapy. Vitrification could be an effective alternative; however the data on the efficacy of the procedure are still conflicting. We therefore performed a systematic comparison of cryodamage after vitrification versus slow-freezing of human ovarian tissue.
Material and methods: Ovarian tissue biopsies were collected from 21 patients. The tissue was cut to pieces, which were randomly frozen using either slow-freezing or vitrification. After thawing, the follicle morphology was assessed. In order to test the viability of the ovarian tissue *in vitro* and the effect of sphingosine-1-phosphate or activin A we established an ovarian tissue culture system and analyzed the estradiol biosynthesis, as well as follicular apoptosis and proliferation by immunohistochemistry. To test the viability and the developmental competence of tissues *in vivo* we used a xenografting human-mouse model.
Results: 366 primordial and primary follicles (192 slow-freezing; 174 vitrification) were analyzed afther thawing. We found no statistically significant difference (P=0.696) of the morphological qualities after slow-freezing or vitrification. In media without supplements the vitrified tissues released more estradiol into the media than tissues after slow-freezing. However, no statistically significant difference was reached (P=0.6). During the culturing of ovarian tissues in the presence of S1P (P=0.273) or activin A (P=0.486) there were also no significant different releases of estradiol. The xenografting model showed higher survival rates and better morphological qualities in the slow-freezed tissue.
Conclusion: Vitrification shows *in vitro* a trend towards better morphological integrity as compared to slow freezing. In an *in vivo* model the slow-freezing procedure leads to better results. However, larger sample numbers need to be analyzed for more confident conclusions to be drawn.

P1 5-3

Cumulative live-birth rates (CLBR) considering the total number of embryos replaced in 26504 consecutive ovum donation cycles (OD)

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Background: CLBR instead of the raw live-birth rate per cycle is a good IVF success estimation, but the number of embryos transferred (ET) is not considered with this approach and survival curves drawn from CLBR are poorly defined. Our aim was to describe the cumulative outcome of OD per total number of ET needed to achieve a live-birth.
Design: Retrospective cohort analysis.
Methods: CLBR and 95% confidence intervals (CI) was estimated by Kaplan-Meier method according to the sum of embryos transferred (SoET) in each set of treatments (SOT), defining SOT as all consecutive OD cycles performed in a couple until they abandoned, or reached a live-birth, including fresh and frozen embryo replacements, excluding preimplantational genetic screening.
Results: A total number of 9659 patients from 26054 OD cycles were analyzed, 9657 SOT and 59422 ET. Patient's mean age was 39.9(CI39.8-40.0) years old. CLBR reaches 64.8%(CI95%63.6-65.9) when SoET=5, meaning an increase of 6.5% per embryo. However, this amount is reduced to 2.0% per embryo, given that the CLBR is 85.2% (CI95%84.0-86.3), when the SOeT=10 embryos. CLBR when SoET=15 embryos is 92.4%(CI95%91.3-93.5), increasing only 1.4% per embryo added compared with CLBR when SOeT=10 embryos. When 25 embryos have been replaced, CLBR is 96.8%(CI95%95.6-97.8), with only an increase rhythm on their live-birth chances of 0.44% per embryo added. OD presents the same curves of CLBR when categorized by age, confirming the most important relevance of oocyte quality rather than the endometrial receptiveness.
Conclusions: CLBR depending on SoET provide with realistic, and precise information regarding the likelihood of OD success, and can be used to inform patients and counsel in the decision-taking process.

P1 5-4

Analysis and determination of origin from microparticles in follicular fluid

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Introduction: G-CSF, IL-15, AMH and other biomarkers are known as predictors for quality and maturity of the oocyte. Their concentration can be referred to the oocyte's level of maturation as well as to fertilisation-, pregnancy- and baby-take-home-rates.
Microparticles (MP) are vesicles between 0,1-1µm in size that are released by cells in case of activation, cell death and sheer stress. Their concentration differs specifically between males and females and varies during the menstrual cycle. Beside procoagulant effects MP also show immune stimulation and serve as means of transport. The existence of MP in human follicular fluid (FF) had never been examined by other groups. Therefore we established a protocol for their detection and subtyping.
Methods: Study subjects were treated with controlled ovarian hyperstimulation during an IVF/ICSI-procedure. On the day of puncture undiluted FF was collected, centrifuged and then stored at -80°C until final examination.
For further analysis the samples were processed as described by Nieuwland et al. Finally they were incubated with Annexin-V-FITC and specific PE-labelled antibodies (anti-LIF, -CD45, -EGF-rec., -CD14). MP were measured via flow cytometry and their concentration was calculated according to Berckmans et al.
Results: In all samples (n=15) MP could be found and were mainly (~80%) marked by Annexin V. Most of the patients also showed CD45 positivity for a small amount of FF-MP.
Labelling with anti-EGF-rec. or anti-CD14 was only successful in particular cases; LIF-expression could never be detected.
Conclusions: FF contains MP which can be identified by Annexin V and measured via FACS-analysis. Subtyping of a part of these MP - mainly by anti-CD45 - is possible. The origin of the remaining MP fraction needs to be further examined with additional antibodies. Whether FF-MP can be used as predictive markers for oocyte maturity or to what extend they are related with fertilisation- and pregnancy-rates is part of our ongoing research.

P1 5-5

Analysis of morphological changes in serially observed bovine embryos

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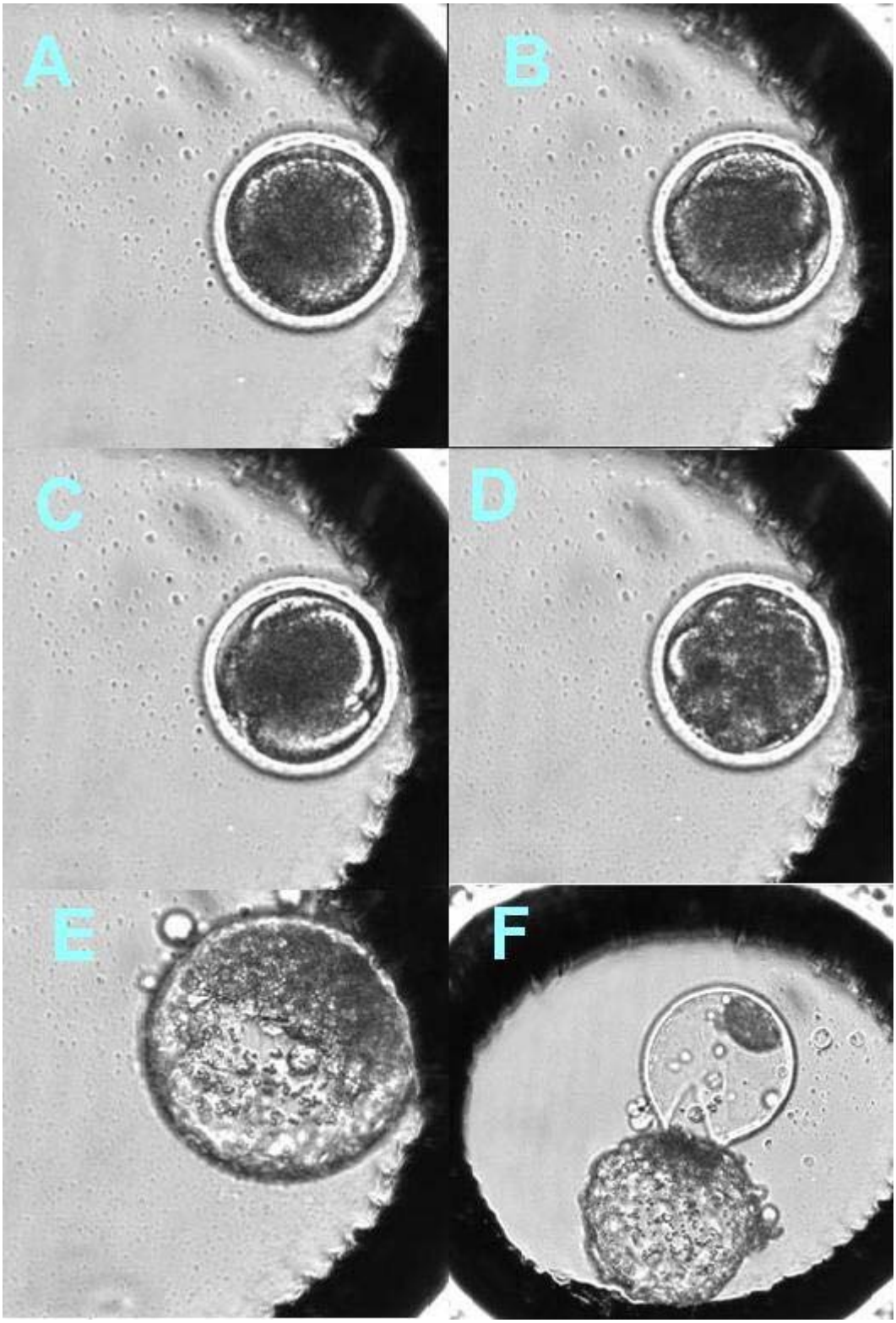
Time-lapse monitoring has recently become an important method for determining the developmental potential of oocytes and early embryos. In this study, a monitoring system for cattle embryos was established in order to analyze the most critical stages in early embryo development for proteome and 3D multichannel CLSM analysis. Oocytes were recovered from abattoir ovaries. After 23 h maturation, the oocytes were in vitro fertilized and subsequently cultured for 7 d. To guarantee an identification of the individual embryo, a special Well-of-Well culture dish was used. While being cultured, the embryos were photographed every 5 min using the Primo Vision system, to set time lapse videos with regard to time of the first and second cleavage as well as normal and abnormal cleavages. This way, it is possible to obtain developmental time profiles for individual embryos that can be correlated with different endpoint analyses. In order to collect 2- and 4-cell embryos with a high developmental capacity for proteome analysis, it is necessary to determine parameters for early embryonic development. In the present study the relationship between the duration of the first and second cell cycle and the embryo developmental rate was high. In addition a wide time window for the first two cell cycles and an early cleavage in embryos that reached later embryonic stages was observed.



[Time profile of an individual embryo]

A: fertilized oocyte 18 hpi; **B**: 2-cell-stage 27 hpi;
C: 4-cell-stage 28 hpi; **D**: Morula 91 hpi; **E**: Expanded Blastocyst 157 hpi; **F**: Hatched Blastocyst 169 hpi; (hpi = hours post insemination)

P1 5-6

Age-specific reference ranges for serum testosterone and androstenedione concentrations in women measured by liquid chromatography-tandem mass spectrometry

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Objective: Radioimmunoassay-based sex hormone measurements offer only limited precision and specificity in the low concentration range of women. Therefore, we aimed to establish age-specific reference ranges for serum sex hormone concentrations in women using mass spectrometry and quantile regression.
Methods & results: Data from 985 women aged 20-80 years, recruited for the prospective Study of Health in Pomerania (SHIP), were included in the analyses. Quantile regressions models were performed to calculate the age-specific 2.5th and 97.5th percentiles for sex hormone concentrations in women. Serum total testosterone (TT) and androstenedione (AD) concentrations were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Measured concentrations of sex hormone-binding globulin (SHBG) and TT were used to calculate free testosterone (free T).TT, AD, and free T concentrations showed a distinct age-related decline across 10-year age groups (one-way ANOVA $p < 0.001$). Sex hormone reference ranges for TT, AD, and free T were determined across each single year of age and for 10-year age groups. Reference ranges over the whole age range of 20-80 years were 0.35 - 1.97 nmol/L for TT, 0.89 - 4.56 nmol/L for AD, and 0.0025 - 0.0253 nmol/L for free T. Separate reference ranges were provided for pre- and postmenopausal women, as well as after inclusion of women using oral contraceptives or hormone therapy (N = 1,357).
Conclusion: This is the first study to establish age-specific reference ranges for LC-MS/MS-measured TT, AD, and calculated free T concentrations based on quantile regression analyses, accurately accounting for the observed low concentration range and the strong age-dependency of these sex hormones in women.

P1 5-7

Profiling intact steroid sulfates in biological samples by liquid chromatography-tandem mass spectrometry (LC-MS-MS)

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For the initialization of a biological response via specific receptors, steroid hormones have to be in an unbound, free form. Circulating sulfated steroids - so far considered as biologically inactive metabolites intended for elimination - are not able to pass the cell membrane. However, the discovery of membrane bound uptake carriers for sulfated steroids, such as SOAT (sodium dependent organic anion transporter) and its colocalization with intracellular steroid sulfatase and sulfotransferases, points to a biological role of steroid sulfates. To further characterize their biological functions, a highly sensitive and specific analytical method was required. LC-MS-MS is the technique of choice permitting physicochemical analysis of the intact steroid conjugate with highest reliability thus avoiding interferences e.g. cross reactivity associated with immunoassays. Our workflow consisted of various sample purification procedures such as protein precipitation and solid phase extraction with the use of stable isotope labelled analogues as internal standards. After short liquid chromatographic separation the steroids were identified and quantified via specific mass transitions by electrospray triple quadrupole mass spectrometry in the negative ionization mode. We are currently able to simultaneously measure estrone sulfate, estradiol sulfate, 16-OH-dehydroepiandrosterone sulfate, androstenediol sulfate, dehydroepiandrosterone sulfate and pregnenolone sulfate in one profile. The method can be applied to various biological media such as plasma, cell lysates, methanolic extracts and was assessed for linearity, sensitivity, stability, recovery, matrix effects, as well as precision.

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P1 5-8

Role of steroid sulfates in the regulation of steroid hormone biosynthesis

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Since the discovery of the co-localization of estrogen receptors, steroid sulfatase and estrogen sulfotransferase in the same tissue, new hypotheses on the role of sulfated steroids and a system that controls the availability of free steroids arised. Moreover, the abundance of conjugated steroids is often much higher compared to their free, unbound forms. However, detailed investigations about the interplay between steroid biosynthesis and steroid conjugation are missing so far. In this work, the influence of sulfated steroids on reactions catalyzed by the cytochromes P450 (CYP) CYP11A1 and CYP17 are investigated in a reconstituted *in vitro* system. CYP11A1 initiates the steroid biosynthesis by catalyzing a side chain cleavage of cholesterol yielding pregnenolone, while CYP17 is essential for sex hormone biosynthesis, converting pregnenolone to dehydroepiandrosterone and progesterone to androstenedione. The recombinant expression and subsequent purification of the bovine enzymes and their respective reaction partners (adrenodoxin and adrenodoxin reductase for CYP11A1 as well as cytochrome P450 reductase and cytochrome b5 for CYP17) was successfully performed. Conversion tests with CYP11A1 and CYP17 proved the *in vitro* systems to be functional and useful to examine the influence of sulfated steroids. The addition of DHEA shows no effect on the CYP11A1 dependent cholesterol side-chain cleavage. The relative activity is 0.25±0.055 for the control and 0.26±0.014 for the sample incubated with a 5-fold excess of DHEA. However, the CYP11A1 dependent activity in the presence of DHEA-sulfate is increased by 50% to a relative activity of 0.39±0.074. These results indicate interesting relationships between steroid hormone biosynthesis and steroid sulfonation. Further tests on the impact of sulfated steroids on CYP11A1 and CYP17 catalyzed reactions are in progress.

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P1 5-9

Elucidation of molecular determinants for dysfunction of LH/CGR-Exon10 mutant causing Leydig cell hypoplasia type II

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The activation of the human LH/CG Receptor by lutropin (LH) and choriogonadotropin (CG) is essential for normal sexual development and fertility. A reported case of Leydig cell hypoplasia type II was caused by a complete deletion of the Exon10 in the LH/CGR gene which results in a lack of 27 amino acids within the hinge region. The deficient receptor LH/CGR-Exon10 impairs LH but not CG action.To understand this malfunction at the structural level we investigated the molecular determinants of the activation process to elucidate the difference in receptor activation of both hormones. Since it is reported that the LH/CGR exist as a dimer and behave as a single signalling unit, when activated with CG, we performed a cAMP-bioassay combining binding and signalling defective mutants including LH/CGR-Exon10. Our results indicate a different activation mechanism for LH and CG not only for LH/CGR-Exon10 but also for mutants with a intact hinge region. While CG is able, LH fails to bypass the lack of Exon10 by trans-activating the neighbouring protomer. Similar results were achieved with a complete hinge region but different signalling disturbing positions, thus LH indicates a cis-activation mechanism in which each protomer bind and signal on its own. Substantiating this, we studied the oligomerisation of LH/CGR-Exon10. Fluorescence correlation spectroscopy revealed no disturbance in oligomerisation of the LH/CGR-Exon10, indicating that the native interaction between LH and LH/CGR-Exon10 in a cis-activated process is rather disturbed due to the lack of the Exon10 part.
In summary, lack of Exon10 does not affect the trans-activation mechanism or oligomerisation. The dysfunction LH/CGR-Exon10 is rather caused by disturbed nativ cis-interaction between LH and receptor. We discovered on LH/CGR different molecular activation mechanisms, trans-activation by CG and cis-activation by LH stimulation.

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P1 5-10

Cardiovascular risk factors in Klinefelter patients and healthy controls: A prospective clinical trial

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Background & aim: Klinefelter syndrome (47,XXY; KS) is a common chromosome disorder, affecting 1:600 men. Patients have been described to exhibit relevant metabolic patterns related to a pro-inflammatory status, resulting in a high prevalence of insulin resistance and cardiovascular impairment. Testosterone deficiency is a common feature in these men.
Material and methods: A prospective clinical trial involving Klinefelter patients (n=130), assessing a wide area of cardiovascular, inflammatory and metabolic factors as well as sex steroids and questionnaires in comparison to age -matched healthy male and female controls (2xn=50). A significant range of genetic and epigenetic investigations completes the approach.Here, we present preliminary and novel clinical data comparing Klinefelter patients to healthy male controls in regard to cardiovascular and metabolic parameters.
Results: Klinefelter patients had a higher waist circumference and BMI in comparison to controls. Decreased insulin sensitivity, higher levels of triglycerides and lipoprotein (a) as well as lower concentrations of HDL-cholesterol were found in patients. Levels of hs-CRP were elevated in Klinefelter patients. Consequently, prevalence of the Metabolic Syndrome (Harmonized Criteria) was markedly higher in Klinefelter men than in controls (52/130 vs 5/50). Corroboratingly, carotid artery intimia-media thickness was increased and flow mediated dilatation of the brachial artery was decreased. These differences were statistically significant. Metabolic disadvantages of patients were enhanced by low testosterone concentrations and already present in the sub-cohort younger than 40 years.
Conclusion: Men with the Klinefelter Syndrome exhibit an unfavorable pattern of cardiovascular risk factors in comparison to healthy male controls. This picture is already present in younger patients and enforced by testosterone deficiency.

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P1 5-11

X-inactivation in Klinefelter patients

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Background & aim: Klinefelter syndrome (47,XXY; KS) is characterized by hypergonadotropic hypogonadism. The precise molecular role of the supernumerary X-chromosome is not fully understood. The aberrant sex chromosomal constitution in KS could disturb X-chromosome inactivation that is initiated by *XIST* and changes the methylation status of genes on the X-chromosome. Switching on and off specific genes on the X-chromosome is differential depending on the origin of either the maternal or paternal side. Therefore we examined the methylation profile of *XIST* and its association to the parental origin.
Material and methods: Klinefelter blood samples (n=130), male (n=50) and female (n=50) controls; Pyrosequencing; Microsatellite analysis
Results: *XIST* drives the X-inactivation and is turned off by methylation. In blood of 50 men *XIST* methylation reached nearly 100% and in 50 women on average 64,8%, indicating the silencing of one of the two X-chromosomes in women. Pyrosequencing revealed a *XIST* methylation level of 75,3% in 130 KS patients. The X-origin was determined in 80 patients: 56% of KS patients had a paternal origin of the supernumerary X-chromosome, whereas 24% showed a MII origin and 20% MI origin of the extra X. The *XIST* methylation pattern of KS patients with paternal origin of the X-chromosome (74,2%) resembled that of KS patients with MI origin (72,7%) and both significantly differed from the methylation pattern of patients with MII X-origin (80%).
Conclusion: In contrast to previous studies we have detected a hypermethylation of *XIST* in KS patients compared to women. The role of the altered *XIST* methylation pattern remains to be further studied. The higher *XIST* methylation in KS patients with MII origin points to a disturbed X-inactivation depending on the parental inheritance.

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P1 6-1

Upregulation of Wnt-signalling molecules in human insulinomas

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Objectives: The Wnt-signalling pathway is involved in tumor development in various tissues. Wnt-signalling molecules are expressed in pancreatic beta-cells. In addition, Wnt-signalling regulates insulin secretion and the proliferation of pancreatic beta-cells in vitro and in animal models. However, it is not clear whether Wnts play a role for the development of human insulinomas. Therefore, we investigated the expression pattern of Wnt-signalling molecules in benign and malign human insulinomas.
Methods: 59 human insulinomas were analysed using tissues arrays. The insulinomas were classified as benign (n=49) or malign (n=10). Immunohistochemistry (IHC) for Wnt3a, Wnt4 and frizzled was performed. The expression of these molecules was assessed with a score model (expression level multiplied by number of positive cells, maximum score: 12). Healthy pancreas sections served as controls. Proliferation assays ([^{3}H]-thymidine uptake) of INS-1 insulinoma cells were performed after stimulation with Wnt3a protein.
Results: 1. Wnt3a, Wnt4 and frizzled are expressed in the normal human endocrine pancreas (control group). 2. The extracellular ligand Wnt3a is strongly over-expressed in human insulinomas (benign: 7.59±3.20, $p< 0.05$; malign: 3.39±2.51, n.s.) compared to controls (2.50±0.50). 3. The Wnt-receptor frizzled is over-expressed in insulinomas (benign: 6.21±3.44, $p< 0.05$; malign: 5.47±3.34, $p< 0.05$) compared to healthy control pancreas (1.5±0.50). Using in vitro assays we found Wnt3a to induce the proliferation of INS-1 cells to 151.2% of untreated controls.
Conclusions: These data demonstrate that the expression of Wnt3a and frizzled is increased in human insulinomas compared to normal endocrine pancreatic tissue. On the functional level, Wnt3a stimulates the proliferation of insulinoma cells in vitro. This suggests that dysregulated Wnt-signalling is involved in the development of insulinomas.
Wnt-signalling molecules are not useful as markers for malignancy.

P1 6-2

Epidermal growth factor receptor-targeted non-viral delivery of the sodium iodide symporter (NIS) gene in radioiodine-refractory differentiated and anaplastic thyroid cancer *in vitro* and *in vivo*

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In contrast to radioiodine-refractory differentiated (DTC) and anaplastic thyroid cancer (ATC), radioiodine-sensitive DTC can be efficiently treated by the application of radioiodine based on the expression of the sodium iodide symporter (NIS). Due to limited therapeutic options for radioiodine-refractory DTC and ATC we have started to evaluate novel polyplexes for non-viral NIS gene delivery, aiming at establishment of radioiodine therapy in these cancer types. We have used nanoparticle vectors based on linear polyethylenimine (LPEI), shielded by polyethylene glycol (PEG) and coupled with the synthetic peptide GE11 as an epidermal growth factor receptor (EGFR)-specific ligand, that own high potential for systemic gene delivery.
We have analyzed EGFR expression levels in various radioiodine-refractory DTC and ATC cell lines by FACS-analysis. Thyroid cancer cells were then incubated with LPEI-PEG-GE11/NIS and control polyplexes (LPEI-PEG-Cys/NIS) lacking the EGFR-specific ligand, followed by analysis of transfection efficiency by iodide uptake assay. SW1736 and ML-1 showed the highest levels, B-CPAP and HTh74 intermediate, and FTC-133 lowest levels of EGFR expression. Transduction efficiency correlated well with EGFR expression levels reaching highest levels with a 7-10-fold increase in perchlorate-sensitive iodide uptake activity in SW1736 and ML-1 cells as compared to mock transfected cells. Incubation with untargeted polymers (LPEI/PEG-Cys/NIS) resulted in a very low iodide uptake activity in all cell lines, demonstrating the EGFR-specificity of these polymers. As determined by γ-camera imaging in preliminary *in vivo* experiments, subcutaneous SW1736 and Hth74 xenografts in nude mice accumulated approx. 5-6% ID/g ^{123}I 24h after systemic (i.v.) LPEI-PEG-GE11/NIS administration.
In conclusion, our data demonstrate the feasibility of radioiodine therapy for radioiodine-refractory DTC and ATC by tumor-selective systemic NIS gene transfer using EGFR-targeted polyplexes.

P1 6-3

The role of surgery in the management of recurrent adrenocortical carcinoma

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Objective: The role of surgery for recurrent adrenocortical carcinomas (ACC) is not well defined. Therefore, we evaluated the outcome after surgery for tumor recurrence in patients from the German ACC Registry.
Methods: Only patients with first recurrence after initial R0 resection were investigated. Progression-free and overall survival (PFS, OS) after first recurrence were analyzed by Kaplan-Meier method. Cox proportional hazards regression models were used to identify prognostic factors.
Results: Of 154 patients with first recurrence, 101 underwent repeated surgery (R0 resection, n=78) and 99 received (additional) nonsurgical therapy. After a median interval of 6 (range 1-221) months, 141 patients (92%) experienced progressive disease. Multivariate analysis adjusted for age, sex, tumor burden, time to first recurrence (TTFR), resection status after surgery for recurrence and additional therapy indicated that only two factors were significantly associated with shorter PFS (hazard ratio for progression: TTFR ≤12 months 1.6 [95% CI 1.1-2.3] in comparison to TTFR >12 months; R2 resection 2.7 [1.4-5.0] and no surgery 2.2 [1.1-4.2] in comparison to R0 resection) and OS (hazard ratio for death: TTFR ≤12 months 2.6 [1.8-4.0] in comparison to TTFR >12 months; R2 resection 2.3 [1.2-4.8] and no surgery 3.1 [1.4-6.5] in comparison to R0 resection). Patients who had both TTFR >12 months and R0 resection of recurrent tumors (n=22) had the best prognosis (median PFS 24 months, median OS 79 months).
Conclusions: The best predictors of prolonged survival after first recurrence of ACC are TTFR >12 months and R0 resection. Patients with prolonged TTFR and tumors amenable to radical resection should therefore be operated, whereas patients with shorter TTFR or tumors not amenable to radical resection do not benefit from debulking.

P1 6-4

Mitotane treatment of adrenocortical carcinoma cells alters expression of genes involved in endoplasmic reticulum stress, apoptosis and steroidogenesis

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Aim of study: Mitotane is the only drug approved for the treatment of adrenocortical carcinoma (ACC). However, its exact mechanism of action is still unknown. In order to elucidate the molecular events mediating adrenotoxicity of mitotane, we investigated changes in hormone secretion, apoptosis and gene expression in NCI-H295 ACC-cells.
Materials and methods: NCI-H295 cells were incubated for 4-72 h with 0 to 100µM mitotane. Changes in hormone secretion were analyzed in cell culture supernatant using an automated immunoassay. Apoptosis rates were measured using flow-cytometry and ELISA of oligo-nucleosomes. Gene expression was investigated using the HG U133 plus 2.0 array, the differential gene expression was statistically assessed using the Partek Genomic Suite and the most prominent pathways involved were identified using the GeneGo software.
Results: Mitotane induced a concentration and time dependent inhibition of hormone secretion and an increase of apoptosis. Of note, we observed a significantly decreased expression of genes involved in regulation of steroidogenesis (e.g. SREBP 1, SQLE, SCD), steroidogenesis itself (e.g. Cyp11A1, 3-β-HSD, Cyp17) and a significant increase in pro-apoptotic genes (e.g. CHOP, DUSP4, GDF15, ATF6). Interestingly, most of the proapoptotic genes are also known to be involved in endoplasmic reticulum stress (ER-stress).
Conclusion and perspective: Mitotane-induced suppression of hormone secretion is reflected by down-regulation of steroidogenic enzymes and induction of apoptosis, which might be caused by ER-stress. Intracellular accumulation of toxic lipids like cholesterol or oxysterols or direct inhibition of steroid hormone synthesis by mitotane are potential trigger mechanisms of ER-stress. Mitotane-induced ER stress thus may provides a new and unexpected mechanism for the adrenotoxic action of mitotane which could lead to the development of improved and more targeted therapies in ACC.

P1 6-5

Increased 3-O-sulfation of heparan sulfate is associated with increased invasiveness and augmented growth factor and wnt-signaling in breast cancer cells

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Heparan sulfate proteoglycans (HSPGs) act as coreceptors for growth factors, and are potentially involved in breast cancer progression. HS3ST2, an enzyme mediating the 3-O-sulfation modification of the HSPGs, is silenced by hypermethylation in breast cancer. The aim of this study was to elucidate the role of HS3ST2 in breast cancer cell behaviour and growth factor mediated signaling *in vitro* using an ectopic overexpression approach in the human MDA-MB-231(ER-) and MCF-7 (ER+) breast cancer cell lines. Compared to controls, HS3ST2 transfected MDA-MB -231 cells showed a highly significant increase in invasiveness and motility accompanied by significantly increased expression of several matrix metalloproteinases (MMPs), including MMP9 and MMP13, as well as annexin 10, cadherin 11, and e-cadherin. Furthermore, e-cadherin showed a membranous redistribution upon HS3ST2 overexpression. In addition, dysregulation of ion transporters and significantly increased cytosolic acidification was observed in HS3ST2 expressing MDA-MB-231 cells. HS3ST2 overexpression lead to increased basal and FGF-specific signaling through the p44/42 MAPK pathway, which depended on the presence of heparan sulfate. Increased MAPK activation was accompanied by a significantly increased expression of the wnt-dependent transcription factor TCF4. In contrast, MCF-7 cells showed no increase in MMP expression or activity, yet e-cadherin and TCF4 upregulation, resulting in decreased invasiveness. This study provides the first *in vitro* evidence of the involvement of HS3ST2 in breast cancer cell invasion. Increased activation of the p44/42 MAPK signaling pathway and of TCF4 in the presence of HS3ST2-specific sulfation patterns emerge as novel mechanistic aspects leading to increased expression of metastasis-associated gene products. However, the functional consequences differ depending on individual properties of the breast cancer cell lines, such as estrogen receptor status and presence of p53 mutations.

P1 6-6

Expression of thyroid hormone receptor and integrin receptor genes and association with thyroid hormone dependent proliferation in cancer

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Purpose: Thyroid hormones (TH) T4 and T3 cause proliferation in several cancer cell lines, whereas other caner cell lines showed anti-proliferative or no effects in response to TH. The cellular effects of TH can either be mediated by classical pathways through nuclear TH receptors (TR) alpha and beta or by nongenomic effects that are mediated through the integrin avß3 receptor or TR. Since the molecular reasons for different regulation of proliferation in response to TH are unknown, the aim of our present study was to screen distinct human cancer cell lines for response to TH and to correlate expression of TR and integrin receptor genes with tumor cell proliferation in response to TH.
Methods: TR alpha and beta as well as integrin receptor alpha v and beta 3 mRNA expression was analyzed by real-time RT-PCR in cell lines derived from thyroid (n=4), lung (n=2) and renal cell carcinoma (n=4) and glioblastoma (n=2). Proliferation in response to TH was analyzed by MTT assays.
Results: Both lung carcinoma cell lines examined (A549 and PC14) showed enhanced proliferation after TH stimulation, while two of four renal cell carcinoma, two of four thyroid carcinoma and one of two glioblastoma cell lines examined were stimulated by TH. Expression of mRNA for TR alpha and beta and integrin receptor alpha v and beta 3 was non-uniform in the cell lines. All TH responsive cells expressed moderate to high levels of TR alpha, while TR beta expression varied from undetectable to very high. Integrin receptor alpha v and beta 3 mRNA expressions also varied in responsive as well as in unresponsive tumor cell lines.
Conclusion: TH responsive and non-responsive cell lines of various malignancies with regard to proliferation were identified. Proliferative response was not correlated with expression of integrin receptor alpha v beta 3 genes, thus analysis of other TH targets will follow.

P1 6-7

Analysis of cell death induced by BH3 mimetic drugs in dedifferentiated thyroid carcinoma cells

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Purpose: Evasion of cell death is one of the hallmarks of cancer and contributes to tumor progression and resistance to therapy. Dedifferentiated and anaplastic thyroid carcinoma that do not take up radioiodine show insufficient induction of cell death after conventional anticancer treatments. Recently, we have shown that BH3 mimetics which target anti-apoptotic proteins of the bcl-2 family, are able to decrease cell number in thyroid carcinoma cell lines. The aim of this study was to analyze how the BH3 mimetic GX15070 induces cell death.
Methods: Cell death induction was analyzed by flow cytometry, determination of caspase activation and western blot analysis of LC3 conversion products in six different thyroid carcinoma cell lines treated with GX15070 for up to 48 hours. Furthermore, transmission electron microscopy was used to characterize ultrastructural changes in treated cells.
Results: After GX15070 treatment, biochemical hallmarks of apoptosis like sub G1-peak induction, caspase 3 and 7 activation and increase in caspase cleavage products (cleaved PARP and cleaved caspase 3) were detected. Furthermore, LC3 conversion as a marker of autophagic cell death was depicted after GX15070 treatment. Electron microscopy revealed apoptotic nuclear changes in only a small portion of cells, but several common characteristic ultrastructural changes like mitochondrial swelling with loss of cristae, disintegration of nuclear membrane, swelling of Golgi apparatuses and rER as well as formation of autophagosomes and vacuoles in the majority of cells. After 48 hours of treatment, secondary necrosis was observed in treated cells.
Conclusion: Here we demonstrate that treatment with the BH3 mimetic drug GX15070 was able to kill dedifferentiated thyroid carcinoma cells. Treated cells underwent non-classical cell death with signs of apotosis, autophagy and secondary necrosis in parallel.

P1 6-8

Underuse of diagnostic possibilities in patients with differentiated thyroid carcinomas

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At an early stage thyroid carcinomas are often asymptomatic and are therefore mostly detected by a standardized diagnostic approach to thyroid nodules.The aim of this study was therefore to examine which diagnostic tools were applied, finally leading to the diagnosis of thyroid cancer and whether the diagnostic workup had an impact on the initial stage of thyroid cancer etc.We retrospectively screened the charts of 256 patients with a history of differentiated thyroid carcinoma at the Leipzig University Hospital.Missing data were retrieved from referring physicians and all patients were interviewed in a standardized manner.Preexisting thyroid diseases like goiter or thyroid nodules were present in 23 % and 27 %.Palpable nodules or nodule growth were noted by 37 % and 15 % of the patients.Nodules with diameter of more than 3 cm were noticed significantly more frequent than smaller ones.As a diagnostic procedure thyroid scintigraphy was performed most often in 78,5%.The scintigraphic appearance of carcinomas >10mm was isocaptant in 28 %, warm in 2 % and cold in 70 %.Ultrasound results were available for 251 patients.No comment on the echogenicity was found in 54 %, hypoechogenicity in 32 % hyperechogenicity in 2 %,echocomplexity in 8 %,isodensity in 2 %,cysts in 2 %.Fine needle aspiration biopsy was performed for sonographically >10 mm nodules in 10 %.It was non diagnostic in 26 %, benign in 15 % and malignant in 59 %.Patients with a malignant FNAC underwent primary thyroidectomy significantly more often (65 %) than patients without(50%).Only 16 % of the patients were operated because of a preoperative suspicion of malignancy.Our data demonstrate that most patients later diagnosed with a thyroid carcinoma underwent primary thyroid surgery without a specific suspicion of malignancy.This could most likely mainly be due to the very infrequent use of preoperative FNAC, a high rate of non diagnostic FNACs and a lack of malignancy risk stratification by ultrasound criteria.

P1 6-9

Characterisation and Activation of the human prostate cancer-derived stromal cell line 6S

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Activation of the stromal microenvironment is a critical step in adenocarcinoma growth and progression in the prostate and seems to be an important feature in hormone dependant cancer. The stromal activation in cancer is similar to a general wound repair response and is mediated by proinflammatory cytokines like TGFβ-1. Changes leading to a reactive transformation of the stroma include extracellular matrix remodelling, angiogenesis induction and phenotypic switching of mesenchymal cells from a fibroblastic to an activated myofibroblastic phenotype. Furthermore, in the myofibroblasts the intracrine conversion of dehydroepiandrosteron (DHEA) to the biological active androgens testosterone or dihydrotestosterone is enhanced. In this study, we investigated whether TGFβ-1 elicits a reactive stromal phenotype in the human prostate cancer-derived stromal-cell line 6S and whether this leads to the upregulation in the expression of enzymes involved in steroidogenesis within these cells. 72 hours after a consecutive treatment of 6S myofibroblasts with TGFβ-1 and DHEA, supernatans were extracted with ether and evaporated extracts were tested for activity in the androgen sensitive yeast assay.
By immunofluorescence we demonstrate the phenotypic switching of the 6S stroma cells to reactive myofibroblasts by TGFβ-1. In addition, the 6S myofibroblasts displayed an increased conversion of the adrenal precursor DHEA to active androgens by up-regulation of steroidogenic enzymes. We could also show that isoflavones interfere with these processes. At a concentration of 10 µM the isoflavones genistein, daidzein and equol completely inhibit this effect.
In conclusion, with an *in vitro* model of the reactive stromal phenotype of prostate cancer it is now possible to study the impact of natural compounds particularly with estrogenic properties on intracrine conversion of DHEA to biological active androgens, a process believed to be a driving force of prostate cancer progression.

P1 6-10

Sunitinib in pancreatic neuroendocrine tumours: Early experience in 8 patients

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Background: Sunitinib has shown significant improvement of progression-free survival in patients with advanced pancreatic neuroendocrine tumors (pNET) (N Engl J Med 2011; 364:501-13). Limited experience exists regarding the role in clinical practice.
Methods: Eight patients with advanced pNET were treated prospectively with Sunitinib 37.5 mg daily. Prior therapies included surgery, chemotherapy (Streptocotocin/ Doxorubicin or 5-FU), somatostatin-analogues, radiopeptide therapy, local-ablative therapy (SIRT, TACE, TAE) or PEG interferon. All patients had progressive disease when starting sunitinib. Therapy response was evaluated, defined as at least stable disease after 6 months of treatment.
Results: Three of eight patients (37.5%) showed partial response or stable disease according to RECIST criteria. These patients were of significantly younger age (44.7 vs. 66.6 years, $p< 0.05$) and had a better overall performance score at the beginning of targeted therapy (ECOG PS 0.3 vs. 1.8, $p< 0.05$). There were no significant differences in Ki-67 indices in the two groups (Ki-67 index of 5% in all responders, two of five non-responders with Ki-67 index < 2%). By trend, duration of disease was shorter (3.6 vs. 5.6 years, p=n.s.) and number of pretreatments was lower (4.0 vs. 4.8, p=n.s.) in the responder group. Therapy in one patient of the non-responder group had to be stopped three weeks before scheduled three month re-evaluation because of side effects (oral mucositis, fatigue).
Conclusion: Considering the limited number of patients, Sunitinib showed a therapy response of 37.5% in our extensively pretreated pNET subjects. Side effects were well tolerated in most cases. Younger age and lower ECOG PS correlated positively with response to therapy.

P1 7-1

Severe reductions of life-span in giant mouse lines long-term selected for high growth

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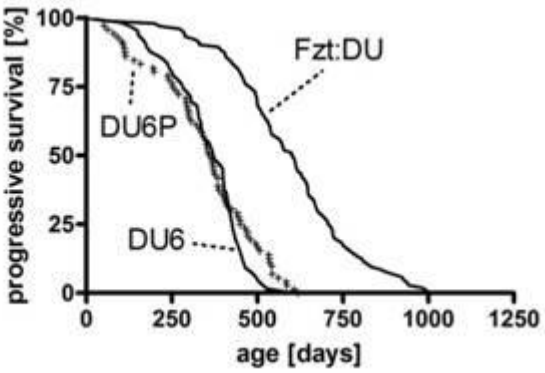
The insulin-like growth factor (IGF) system exerts pivotal effects on growth and metabolism in mammalian systems. However, inhibition of IGF-signaling in mouse models has been demonstrated to improve oxidative stress tolerance and to increase life-span. From those studies it might be concluded that growth is at the expense of life-expectancy. In order to address the question if high growth affects life-span, we have used two independent phenotype-derived mouse models long term selected for high growth. At an age of 105 days, long term selection (140 generations) for high body mass in DU6 mice and high body protein mass in DU6P mice resulted in significant increases of body mass if compared to unselected Fzt:DU control mice (male DU6: 92.5±8.4g; male DU6P: 79.9±6.2g; male Fzt:DU: 41.3±5.3g). In parallel, both female and male growth-selected mice were characterized by severe reductions of life-span. While male Fzt:DU (n=104) had a mean life-span of 595±185 days male DU6 (n=66) and male DU6P (n=72) only reached a mean age of 354±108 and 347±150 days, respectively. Similar results were obtained in female mice. All animals (n=465) were assessed for the gross pathological status. Prevalence of malignancies was higher ($p < 0.01$) in females (38%) if compared to males (19%). Although DU6 and DU6P died at lower ages, tumor prevalence was similar if compared to Fzt:DU. Inflammation in DU6P of both genders was diagnosed at higher rates if compared to Fzt:DU mice, but the differences were statistically not significant. Our study in giant, growth-selected mice clearly supports the hypothesis that growth might be at the expense of life-span.



[Lifespan in growth selected mouse lines]

P1 7-2

Anxiety, depression and changes in cortisol profiles in women with menopausal symptoms during treatment with a complex remedy or placebo

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Aims: To compare the effect of treatment with a complex homeopathic remedy or placebo on possible changes in anxiety, depression and combined salivary cortisol parameters in women with menopausal symptoms.
Methods: 102 peri- und postmenopausal women requiring treatment for menopausal symptoms received treatment for 36 weeks. They were randomized in 3 groups to take the complex remedy (2x12 weeks) as well as placebo (12 weeks) with different order of remedy (M) and placebo (P) in a cross-over design (1: M/M/P, 2: P/M/M, 3: M/P/M). Anxiety and depression were assessed with the HADS-D and cortisol was measured in saliva (3 samples per day) at screening and after 12, 24 and 36 weeks.
Results: Prior to treatment a clinical relevant diagnosis of anxiety (M/P mean 7.3 vs. 6.8) and depression (M/P mean 5.1 vs. 4.8) by the HADS were rare. After 12 weeks a reduction of anxiety was documented in both groups (-0.4; -1.5) and was more pronounced after treatment with placebo. After 12 weeks a reduction of depression (-0.7 vs. -1.0) was seen in both groups as well.
The area under the curve (AUC) for saliva cortisol decreased in both groups after 12 weeks while the slope remained almost unchanged. The decrease of the AUC was more pronounced after treatment with remedy (-0.6 vs. -0.2) but the difference was not significant. Changes in anxiety, depression, AUC and slope during the second and third treatment period were not consistent with respect to treatment with remedy or placebo.
In case of missing individual cortisol samples calculation of the combined parameters AUC and slope was impossible. Compliance with the collection of 3 samples per day was low and this analysis included only 68-79% of the participating women.
Conclusions: Treatment with the complex remedy for 12 or 24 weeks did not result in clinically significant improvement of anxiety and depression. The evaluation of the cortisol data was impossible due to low adherence to the sampling protocol.

P1 7-3

Hormone changes in marathon runners >50 years in comparison to matched controls of the general population and their response to a marathon race

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Marathon running is associated with hormonal changes. However, long-term and acute adaptations to such a strenuous exercise have not yet been precisely characterised in elderly runners.
Methods: In 105 men (aged 57.1±5.8 yrs) basal hormones (ACTH, cortisol, DHEAS, LH, FSH, testosterone, prolactin, fT4, TSH) and fasting glucose (FPG), fasting insulin and HbA1c levels were measured 4-12 months prior to a marathon and compared to 525 age-matched controls from the general population. 61 of these runners completed a marathon competition, and hormones were measured before and 5 min after finishing the marathon and adjusted for haematocrit.
Results: In marathon runners basal cortisol levels were on average 11%, testosterone 12% (both $p< 0.01$) and fT4 levels 13% higher ($p< 0.0001$) than in controls while TSH levels were not different. Free testosterone index, FPG and HbA1c were 14%, 14%, and 4% lower than in controls (all $p< 0.001$). In runners, basal insulin was related to finishing time (FT) ($r=0.25$, $p=0.04$) and DHEAS levels correlated to FT ($r=-0.25$, $p=0.03$), heart rate (HR) ($r=0.25$, $p=0.04$) and showed a tendency for association to weekly metabolic energy task (MET) ($r=-0.22$, $p=0.06$). During the marathon, ACTH levels increased on average by 481%, cortisol by 309%, prolactin by 225%, DHEAS by 30% and fT4 by 6% while testosterone declined by 16% (all $p< 0.0001$) and TSH was unchanged compared to pre-marathon values. Prolactin levels correlated inversely to FT ($r= -0.29$, $p=0.011$), and cortisol ($r=-0.24$, $p=0.06$) and DHEAS levels ($r=-0.20$, $p=0.09$) showed a trend for association to FT. DHEAS ($r=-0.23$, $p=0.06$) and fT4 levels ($r=-0.20$, $p=0.09$) showed a trend for an association with MET.
Conclusions: This large study reveals unexpected long-term and marathon-related hormone changes in advanced -aged marathon runners. The adaptive mechanisms seem to be more flexible as usually anticipated and are hence interesting not only for sports medicine but also for clinical research.

P1 7-4

Lack of puberty due to a novel deletion within the *KAL1* gene as a cause of isolated hypogonodotropic hypogonadism

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Introduction: Isolated hypogonadotropic hypogonadism (IHH) is characterized by delay or absence of puberty. Plasma levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone are low. Kallmann Syndrome (KS) is defined by hypogonadotropic hypogonadism and midline defects (e.g. hyposmia or anosmia). IHH and KS are clinically and genetically heterogeneous disorders. Mutations in the *KAL1* gene are known to cause both types of diseases, IHH and KS1, in an X-linked recessive mode of inheritance. Furthermore they are associated with additional abnormalities, bimanual synkinesis and unilateral renal aplasia in a lot of KS1 cases.
Case: A 15-year-old German boy was referred to our Center because of absent pubertal development. His family history showed a 12-year-old sister with normal pubertal development and a maternal uncle with hypogonadism. The physical examination revealed: height: 148 cm, weight: 42 kg, infantile testes (1 ml), micropenis, normal mental development and sense of smell. Laboratory testing revealed low plasma levels of gonadotropins and testosterone with absence of stimulation by buserelin, low level of plasma inhibin B and confirmed the diagnosis of hypogonadotropic hypogonadism. The karyotype was 46,XY. A tumor or abnormality of pituitary and hypothalamus was excluded by MRT. Bulbus olfactorius was not examined. X-ray of the left hand showed delay in bone maturation. Testosterone therapy was started for induction of puberty. Ultrasound of the kidney will be performed.
Methods and results: Genetic testing by Multiplex Ligation-Dependent Probe Amplification (MLPA) indicated the presence of a novel large hemizygous deletion which encompasses exons 3 to 14 within the *KAL1* gene. We confirmed these results by PCR amplification.
Conclusion: Genetic counselling and genetic testing of the *KAL1* gene should be considered in these cases to confirm the diagnosis and to provide the basis for diagnostic and therapeutic decisions.

P1 7-5

Mixed adeno- neuroendocrine carcinoma (MANEC): an unusual finding in a patient with gastric neuroendocrine tumour type 1

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Case report: We report a 76 year old female patient with incidentally discovered multiple neuroendocrine tumours of the stomach. Gastroscopy showed atrophic mucosa with approximately 50 small lesions (dm: 10 - 20 mm) and one large tumour (dm: 40 mm). Endoscopic ultrasound revealed tumour invasion of the muscularis propria and 3 suspicious perigastric lymph nodes. Immunohistochemistry of the endoscopic tumour biopsies showed atypical cells, which were positive for chromogranin A and synaptophysin. The Ki-67 index was below 2%. Furthermore, chronic atrophic gastritis was noted in the surrounding tissue. DOTATOC- PET- computer tomography revealed pathological tracer accumulation in projection of the gastric tumour lesion and in 3 perigastric lymph nodes. Biochemical assessment showed elevated gastrin (736 ng/l, Ref < 90 ng/l) and chromogranin A (214 µg/l, Ref < 98 µg/l) serum levels in the patient.
Therapy: Partial gastric resection was performed. Histology showed a mixed adeno- neuroendocrine carcinoma (MANEC) of 30 mm size at the site of the larger tumour.
Conclusion: Despite the excellent prognosis of type 1 gastric NETs, suspicious findings such as tumoural lesions >20 mm, angioinvasion, infiltration of muscularis propria or lymph node enlargement require further investigation. In our case, MANEC, an extremely rare disease was found. This finding leads to a substantial change in management, since gastric MANEC should be treated like adenocarcinomas.

P1 7-6

Prolactinoma presenting as anaemia and myocarditis

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Introduction: Unexplained anaemia should also be screened for endocrine causes, especially if associated with multiple infections.
We describe a case of a man with a history of unexplained anaemia and a severe myocarditis leading to admission in several hospitals.
Case: We present the case of a 55-year-old man who presented with mild anaemia and chest pain after a long distance flight. He was admitted in Cuba to a hospital and later transferred to Germany with the diagnosis of myocarditis. After stabilisation of his cardiac function, he was discharged and because of continuing signs of infection and anaemia (Hb 10g/dl (14-17.5) he was readmitted to our hospital. Analysis of bone marrow showed no signs of a myelodysplastic syndrome. Evaluation of endocrine symptoms showed a loss of body hair and sexual dysfunction. Laboratory investigations revealed a highly elevated prolactin: 2240.0 ng/ml (< 19.4) and signs of hypopituitarism: Cortisol 1.6 mg/dl (3.7-19.4), LH 0.67 mIU/ml (1.14-8.75), FSH 1.53 mIU/ml (0.95-11.95), Testosteron 0.60 ng/ml (1.56-5.6), TSH 1.30 (0.4-2.5) mE/ml, FT4 0.92 (0.7-1.8) ng/dl. Magnetic resonance imaging of the brain showed a pituitary macroadenoma, size 17x18x23mm (macroprolactinoma). He was treated with cabergoline (starting dose 0.5mg weekly) and hormone replacement therapy was initiated with testosterone 250mg i.m. three weekly and hydrocortisone 15-5-10mg daily. Within 3 months the haematopoiesis recovered successfully to a normal haemoglobin level. Prolactin levels decreased to 74ng/ml and the MRI showed a tumor shrinkage.
Conclusion: This case highlights the importance of full evaluation and investigation of unexplained anaemia including the consideration of endocrine causes.

P1 7-7

Primary hyperparathyroidism with normal intact PTH levels

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Introduction: Primary hyperparathyroidism (PHPT) is characterized by hypercalcemia and inadequately elevated serum parathyroid hormone (PTH).
Methods: A 59 year old female patient presented with elevated serum calcium levels (2.8 - 2.9 mmol/L) and low normal PTH levels (2.2pmol/l). 24 h calcium excretion in urine was elevated (12.6 mmol / 24 h). Thyroid ultrasound and technetium-sestamibi scintigraphy were compatible with a right sided parathyroid adenoma. Repeated PTH analyses using a Siemens Immulite assay confirmed low normal PTH levels. An independent measurement with an electrochemiluminescence - immunoassay on Roche Elecsys reproduced a normal PTH level (45.8 pg/ml). Parathyroid hormone related peptide was negative and secondary causes of hypercalcemia were ruled out. The patient was subjected to explorative surgery with the concept of a false negative intact PTH level.
Results: Intraoperatively the suspected parathyroid adenoma was detected at the predicted right caudal position. Histology confirmed a parathyroid adenoma without malignant changes. Serum calcium levels rapidly normalized to 2.2 mmol/l.
Conclusion: The normal results of two independent PTH assays in a case of proven PHPT are compatible with an abnormal intact PTH molecule, produced by the parathyroid adenoma. The detection failure may be due to structural changes in the amino acid sequence or posttranslational modifications. In a recent publication of a comparable case (Benaderet et al., JCEM 2011) intraoperative use of a turbo PTH assay (Immulite analyzer, Diagnostic products corporation) was suggested. In case of inadequately low or normal PTH measurement, which contradict clinical and additional laboratory findings a false negative result for PTH may be suspected due to detection failure of conventional PTH immunoassays. Repeated analyses with independent assays should be performed and possible detection failures should be considered in the decision for surgical exploration.

P1 7-8

Cushing's disease

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A 49-year obese woman (BMI 46,6kg/m²) with diabetes mellitus type 2 and hypertension was referred to our clinic to take part in our program for weight reduction. At presentation the woman showed typical clinical signs of Cushing's syndrome. Basal levels of cortisol and ACTH didn't show any abnormalities, however ACTH was in the upper range. Cortisol was inadequately suppressed after 2mg dexamethasone. To ensure correct implementation, testing was repeated with inadequately suppression of cortisol again. Measurement of urinary cortisol excretion over 24h showed an elevated quantity. Intravenous application of 100µg CRH led to an increase of ACTH >50% and also cortisol rose slightly. The assessment of the testing results pointed to Cushing's disease. NMR of the pituitary gland brought further clearness by showing an adenoma of 11x17x13mm. Additional testing of the other pituitary axes showed no insufficiency. The woman was then directed to a neurosurgical departement and transsphenoidal resection of the pituitary adenoma was accomplished. Due to invasive growth into the sinus cavernosus on both sides, an incomplete resection cannot be certainly excluded by the surgeon. Histology showed an invasive adenoma with immunhistochemical expression auf ACTH. Unfortunately Cortisol was again inadequately suppressed after 2mg dexamethasone one week postoperative. Patient was then discharged from hospital and again referred to our clinic with the recommendation to perform radiotherapy. First of all we repeated the application of 2mg dexamethasone and ascertained a normal suppression of cortisol four weeks after operation. Further testing revealed no postoperative insufficiency in pituitary axes. Some clinical signs of Cushings's disease became better and levels of elevated bloodglucose decreased. Considering the risk and benefit of radiotherapy of piutitary gland we decided to repeat laboratory testing in three months before the decision of further treatment is finalized.

P1 7-9

Massive hypercalcaemia in pregnancy

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We report the case of a 31 year old pregnant woman (15 weeks of gestation) with hypercalcaemia. Initial symptoms were fatigue, nausea, diminished appetite and weight loss. An extended laboratory analysis revealed massively elevated plasma calcium (6.3mmol/l, normal range 2.1-2.6) and chromogranin A levels (3762µg/l). PTH and parathyroid related protein were found to be decreased. Ultrasonography substantiated the suspicion of neuroendocrine malignancy with lesions both in the pancreatic cauda and the liver. MRI found one large (possibly a FNH) and 4 small liver lesions suspicious for metastases. A multidisciplinary tumour board (including ethic counselling) reviewed the data and recommended haemodialysis twice daily to immediately control hypercalcaemia; SMS-analogue therapy to control secretion of tumour peptides (OCT 300µg/d - 600µg/d) and surgical tumour mass reduction with the primary goal to preserve pregnancy. A radical distal pancreatectomy with gastric wedge resection, splenectomy was performed including an intraoperative liver biopsy. Histological examination revealed a small cell neuroendocrine carcinoma (pT3pN1(4/5)pM1,G1, proliferation rate < 2%).
After pancreatic tumour resection haemodialysis was still necessary to control calcium levels. Therefore an ultrasound-guided percutaneous alcohol injection of the liver metastases was performed. Tumour mass reduction allowed to control calcium level by use of high dose octreotide. Natural childbirth of a healthy newborn occurred in week 32+4 (birth weight 1650 g, height 40 cm, head circumference 29.5 cm and APGAR 8/10/10). High uptake of the remaining liver lesions in DOTANOC-PET-CT led to liver segment resection to achieve tumour mass reduction in the following weeks. Intensive therapy had no clinical impact on the newborn. After 12 months of stable disease the patient showed progressive disease with liver and vertebral metastases. Currently she has received a treatment with Yttrium-90-DOTATATE therapy.

P1 7-10

Attenuation of climacteric symptoms with Shiatsu - a randomized controlled feasibility trial

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Aims: Evaluation of the efficacy and feasibility of Shiatsu for the attenuation of climacteric symptoms.
Methods: 22 symptomatic peri- and postmenopausal women were randomized to receive 8 weekly Shiatsu sessions as add-on treatment to their current treatment or to no add-on treatment. The main endpoint was analysed at week 9 and included 19 women (treatment groupn= 9, waiting group n=10) who started the intervention or waiting period (ITT). Through week 10-17 the waiting group received Shiatsu and the treatment group had follow-up without further intervention. A final assessment was done at week 18. Self-rating of climacteric symptoms, anxiety and depression was carried out with the Menopause Rating Scale II and the Hospital Anxiety and Depression Scale at baseline and at week 9 and 18. Cortisol was measured in both groups in 3 saliva samples per day collected during 3 days at baseline, for 1 day after the 4th Shiatsu session and after 9 and 18 weeks.
Results: At week 9 the sum score of menopause rating scale II decreased in both groups (treatment group from 19.78 ±5.56 to 13.33 ±8.02, waiting group from 24.60 ±6.96 to 22.60 ±7.37). A two-factorial ANOVA regarding interactions between time and group revealed a trend for a difference (p=0.0727) in favour of the treatment group. Following cross-over a lessening of symptoms (22.60 ±7.37 to 18.10 ±8.12) could be seen in the waiting group too while in the treatment group symptom relief persisted for almost 9 weeks. Following Shiatsu the differences within groups (treatment group: -6.45, waiting group: -4.5) were clinically relevant and statistically significant (p=0.0117 vs. 0.0234). Data for anxiety, depression and cortisol parameters were inconclusive.
Conclusions: Eight weekly sessions of Shiatsu as add-on therapy attenuated climacteric symptoms in peri- and postmenopausal women. Effects prevailed for almost 9 weeks. The results of this small feasibility study should be confirmed in a larger clinical trial.

Schilddrüse und Nebenschilddrüse II

P2 1-1

THADA - a protein with an important role in the thyroid

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Benign thyroid tumors and hyperplasias of follicular epithelial origin have been cytogenetically analyzed in depth. 20 % of these lesions showed cytogenetic aberrations, translocations involving chromosomal band 2p21 were found to be the second most frequent structural chromosomal rearrangement. *Thyroid adenoma associated (THADA)* has been identified as the target gene affected by these translocations and the concomitant truncation of the gene is speculated to be the critical event for the development of the tumors. The genomic structure of the gene and splice variants of its mRNAs as well as the localization of the protein in the cell are well documented. More recently, studies revealed that the mRNA, the protein size, and the genomic organization is conserved among *Homo sapiens*, *Chlorocebus aethiops*, *Canis familiaris*, *Mus musculus*, and *Gallus gallus*. Additionally, the presence of a protein-protein-interaction-domain of the superfamily ARM repeat in THADA was reported. *THADA* expression was shown to be significantly higher in the thyroid than in other tissues. Furthermore, the level of expression was linked to the status of differentiation of thyroid tissue. Overall, results indicate THADA being a protein with a crucial role in the thyroid.

P2 1-2

Subclinical thyroid dysfunction in heart failure: Is it prognostically relevant?

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Purpose: The prognostic impact of subclinical thyroid dysfunction on heart failure (HF) is a matter of debate. We investigated the prognostic impact of euthyroid sick syndrome (ESS) and subclinical thyroid dysfunction in patients with systolic HF.
Methods: Thyroid function was evaluated in 1032 patients participating in a randomized trial on the effects of a HF disease management programm (INH trial). All subjects had left ventricular ejection fraction (LVEF) < 45%., and comprehensive clinical data were available. 288 subjects were excluded from analysis due to incomplete thyroid function tests, intake of thyreostatics, amiodarone and/or corticosteroids, or overt thyroid dysfunction. Thyroid function of 744 patients was categorized as euthyroidism (TSH, fT3/fT4 normal); ESS (fT3< 2.7pmol/l, TSH< 4.0mU/l); subclinical hyperthyroidism (TSH< 0.3mU/l, fT3/fT4 normal); subclinical hypothyroidism (TSH>4.0mU/l, fT3/fT4 normal). Survival analysis was performed using Cox regression.
Results: At baseline, subclinical hyperthyroidism present in 69 patients (9%), subclinical hypothyroidism in 34 patients (5%), and ESS in 13 patients (2%). No differences were found between groups regarding NYHA class (P=0.29), LVEF (P=0.60), heart rate (P=0.48). In univariate analysis, risk of death was 4- and 1.4-fold increased in patients with ESS or scHyper, respectively, compared to euthyroid subjects, but not in scHypo. Age was the only consistent confounder identified across groups. After adjustment for age, ESS was still associated with a poor prognosis (hazard ratio for death 3.0 (95% CI 1.5-5.9) P=0.001), but not scHyper (hazard ratio 1.18 (0.9-1.8), P=0.37).
Conclusion: In this well-characterized HF cohort, subclinical thyroid dysfunction was not an independent predictor of mortality, whereas ESS was a strong indicator of a grave prognosis.

P2 1-3

Radiocontrast agents as potential substrates for enzymatic deiodination

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Together with medicals, supplements, algae-rich nutrition or topical antiseptics, iodine containing radiocontrast agents (RCA) are a frequent source of supraphysiological iodine exposure. While total amount of iodine in RCA is usually more then 10 000 times above the upper limit of >500 µg iodine intake recommended by DACH, the vast majority is covalently bound to the organic structure. The amount of free iodine within these products is restrictively limited to an acceptable degree (e.g. 50 µg I^-/ml after production).
Data on metabolism of RCA within organisms are rare. A specific category of *orally* administered biliary *contrast* media is known to inhibit activities of deiodinases (DIO), enzymes responsible for phenolic ring 5'-deiodination and tyrosyl ring 5-deiodination of thyroid hormones, involved in the network of iodothyronine and iodothyronamine metabolism. One of these compounds, iopanoic acid (IA), was extensively tested in vivo and in vitro. We hypothesized that it might be accepted as substrate by enzymes of the DIO family.
Indeed, enzymatic iodide release by DIO was never tested before probably due to the lack of commercially available radioactively labeled IA, which would be needed to conduct the classical DIO-activity test.
We established a new non-radioactive test system for DIO activity measurements involving the method for iodine measurement as reported by Sandell and Kolthoff in 1937. Using this new deiodinase assay, we were able to identify IA as a substrate for Dio1. A time-dependent and propylthiouracil-sensitive release of iodide/iodine from this RCA was observed by a Dio1 preparation from mouse liver. The mono-deiodination of IA by Dio1 was then verified by LC-MS/MS analysis.
We conclude that this new DIO assay method is capable of testing and identifying iodinated RCA as DIO-substrates, which are a potential source of excessive and unpredictable iodine load in patients, when metabolized in vivo by members of the DIO family of enzymes.

P2 1-4

Diagnostic challenge of rapid enlargement of the thyroid: autoimmune thyroiditis - lymphoma - malignant thyroid disease

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Introduction: In patients with enlarged and increasing thyroid differential diagnosis of malignant thyroid disease, autoimmune thyroiditis and lymphoma is essential. Differentiation between autoimmune thyroiditis and lymphoma can be a challenge due to overlap between both entities.
Material: We report clinical course and diagnostic findings of three patients presenting with increasing thyroid mass.
Results: All three patients were postmenopausal women and the leading symptom was a goiture with rapidly growing volume or mass within the neck region, doubling within three months in two patients and within two years in one patient. In all patients ultrasound showed hypoechoic areas, irregular nodular changes suggestive for autoimmune thyroiditis. In one patient the thyroid capsule could not be distinguished. At time of diagnosis two patients were hypothyroid. Anti-thyroid antibodies were not helpful for differential diagnosis. Fine needle aspiration cytology (FNAC) was suggestive but did not allow doubtless diagnosis. Diagnostic findings are summarized within table.

Patient sex /age (yrs)	Thyroid volume (ml) diagnosis / follow up	Thyroid function	Thyroid antibodies	Cervical lymph nodules	FNAC	Histology (thyroidectomy/biopsy)	Therapy
F /63	30 / 63	hypothyroid	(+)	-	thyroiditis	B-Cell lymphoma	thyroidectomy
F / 58	25 / 44	hypothyroid	-	+++	lymphoma	thyroiditis/ lymphadenitis	thyroidectomy
F /74	58 /113	euthyroid	+++	-	? lymphoma	B-Cell lymphoma	chemotherapy

[Diagnostic findings]

Conclusion: In patients with a history of rapidly increasing thyroid mass, hypothyroidism and typical sonographic signs of autoimmune thyroiditis the rare diagnosis of thyroid lymphoma should be considered. Cytologically the differential diagnosis often remains uncertain. Sufficient material (excisional biopsy) is necessary for diagnosis and some patients need surgery for final classification of the disease.

P2 1-5

Fine needle aspiration of thyroid nodules

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Introduction: Epidemiological studies suggest a high incidence of thyroid nodules >1 cm in germany. It has been estimated that 25% of these nodules show decreased Tc-uptake in thyroid scintigraphy.
Methods: We retrospectively analyzed all fine needle aspirations (FNA) in our department from 01/2009 to 11/2011, and correlated the data with available histological results.
Results: In total, 417 FNA were done in 389% thyroid nodules of 364 patients. 58.4% patients had multinodular and 30.9% uninodular goitre, 63.2% had ≥1 cold nodule. Quality of FNA was unsatisfactory in 15.1%, in 19.7% of limited quality, and in 65.2% satisfactory. After exclusion of nondiagnostic results, FNA was interpreted as negative for malignancy in 85.6%, indeterminate in 8.1%, suspicious in 2.7%, and positive for malignancy in 3.6%. Because of FNA, surgery was recommended in 12.1% of the patients, whereas in 76.6% a conservative management was proposed. Correlation of FNA and histology is shown in table 1 (4 incidental papillary microcarcinomas and 1 contralateral papillary carcinoma pT1b were considered as benign histology).

Cytologic category	N	Benign histology	Malignant histology
Negative for malignancy	32	32 (100%)	0
Indeterminate	20	17 (85%)	3 (15%)
Suspicious	8	6 (75%)	2 (25%)
Positive for malignancy	10	1 (10%)	9 (90%)

[Correlation of cytology and histology]

Malignant histologies consisted of papillary carcinoma ≥pT1b (n=8), follicular carcinoma (n=5), and non-hodgkin lymphoma (n=2), while benign lesions were mainly multinodular goitre (52.6%), follicular or Hürthle cell adenoma (29.8%), and thyroiditis (7.1%). DNA cytometry in case of indeterminate or suspicious cytology (n=16) showed aneuploidy in all malignant lesions and in 35.7% of the benign nodules.
Conclusions: FNA is a valuable tool to avoid surgery in nodular thyroid disease, although it has to be estimated that 4.9% of all patients will be operated unnecessarily, if DNA cytometry is not taken into consideration.

P2 1-6

Negative Tc^{99m}-sestamibi scintigraphy in primary hyperparathyroidism patients

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Introduction: Preoperative localization is mandatory for minimal-invasive parathyroidectomy. For this purpose, Tc^{99m}-sestamibi scintigraphy has become an accepted and widely used preoperative tool. Its sensitivity is mainly limited by parathyroid weight and size or concomitant goitre. Only few data describe the influence of anamnestic aspects or different cytopathological entities on sestamibi scintigraphy.

Materials and methods: e retrospectively analyzed PHPT patients with a negative preoperative Tc^{99m}-sestamibi scintigraphy preceding surgery between 01/2010 and 10/2011.

Results: f 173 PHPT patients, 124 (*72%*) had a Tc^{99m}-sestamibi-scintigraphy preoperatively. In 27 cases (*22%*), no localization was possible. A questionable localization was found in 16 cases (*13%*), and a certain parathyroid adenoma was detected in 81 (*65%*) cases. Patients with persistent/recurrent PHPT, prior thyroid operation or prior radio-iodine therapy were more likely to have no evident localization by sestamibi scintigraphy. Sestamibi scintigraphy was jeopardized by concomitant goitre, intrathyroidal parathyroid glands and multiple gland disease. Clear-cell adenomas and parathyroid hyperplasia were risk factors for negative sestamibi scintigraphy.

	no localization (n=27)	questionable localization (n=16)	certain localization (n=81)
pers. / rec. PHPT	4 (15%)	3 (19%)	8 (10%)
prior thyroid operation	7 (26%)	6 (38%)	11 (14%)
prior RI therapy	3 (11%)	0 (0%)	0 (0%)
goitre	12 (44%)	8 (50%)	25 (31%)
intrathyroidal adenoma	1 (4%)	2 (13%)	0 (0%)
multiple gland disease	4 (15%)	2 (13%)	7 (9%)
clear-cell adenoma	3 (11%)	0 (0%)	1 (2%)
parathyroid hyperplasia	14 (52%)	3 (19%)	22 (27%)

[Tc99m-sestamibi scintigraphy in PHPT]

Conclusion: c^{99m}-sestamibi scintigraphy (-SPECT/CT) can detect most of pathologic parathyroid glands in PHPT with a low rate of false-positive results (*10%*). Its sensitivity and specificity is not without failure; we therefore use intraoperative PTH-determination as an integrated adjunct in PHPT surgery.

P2 1-7

Side addicted indications of sternotomy in the case of intrathoracic goiter

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Background: In about 1-15% of thyroidectomies, the goiter is located intrathoracic with higher rates of complications and a somewhat different management. In the literature sternotomy should only be performed in cases of previous cervical thyroidectomy, invasive carcinoma and truly intrathoracic. We assessed, whether sternotomies are necessary in these patients, at all.
Methods: We retrospectively analyzed 100 patients (167 nerves at risk) with thyroid surgery for intrathoracic goiter between 2001 - 10/2011. Intrathoracic goiter was accepted when the retrosternal part exceeded 50% of the whole goiter. There were 55 % women and 45% men with a median age of 63 years (range 34 to 98 years). An intrathoracic goiter without connection to the cervical gland was observed in 5 (5 %) patients.
Results: The reason for surgery was dyspnea in 13 cases (13 %), dysphagia in 3 cases (3 %). 6 patients (6 %) had signs of hyperthyroidism. Twentynine patients (29%) presented with a recurrent goiter. The goiter was benigne in 92 patients. In 8 cases the histology showed a carcinoma. A cervical approach was used in 70 patients (70 %). 30 patients (30 %) required median sternotomy and in two patients thoracotomy was performed. In 17 of 30 (57%) with recurrent intrathoracic goiters, but only 12 of 70 (17 %) primary intrathoracic goiter a sternotomy/thoracotomy was necessary ($p< 0.001$). Indication of sternotomy at the right side was in 75% the complicated nerve course, whereas the tumorsize was diciding at the left side. Postoperative complications were: 10 patients (10 %) a transient hypocalcaemia, 3 (3 %) transient recurrent nerve palsy and 2 permanent recurrent nerve palsy and 2 patients had got collar secondary bleeding.
Conclusion: Sternotomy in recurrent goiter and dorsal mediastinal goiter is necessary to reduce increased morbidity and mortality in intrathoracic goiter surgery as shown in the literature.

P2 1-8

Post radioiodine immunogenic hyperthyroidism following radioiodine ablation therapy of a focal autonomy

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A 67-year old male patient presented with weight loss and weakness in our clinic. Diabetes mellitus type 2 had been diagnosed earlier; however, the patient had refused to take any antidiabetic medication. Laboratory testing revealed an HbA1c of 16,5 % and suppression of TSH (0,17 mU/l) with peripheral euthyroid metabolism. TPO- and TG-antibodies were elevated, but no elevation of TSH-receptor antibodies was detectable. Ultrasound examination was performed and revealed a slightly enlarged nodular goiter. Radioisotope scanning detected a focal autonomous adenoma linked to the largest node on the right cranial side. Subsequently, the patient underwent radioiodine therapy with I-131. To treat his diabetes he received intensive conventional insulin therapy. Unfortunately, the patient missed the scheduled follow-up clinic visits, and was admitted to the emergency unit 3 months later with frailty, hyperglycemia and nausea. Laboratory tests revealed a manifest hyperthyroidism (TSH: < 0,01 mU/l; fT4: 55,5 pmol/l; fT3: 46,0 pmol/l) and an increased level of TSH-receptor antibodies (6,77 IU/l).
Taken together, the patient had developed an immune hyperthyroidism following radioiodine I-131 therapy for focal autonomy. After intravenous thyreostatic treatment with thiamazole a thyroidectomy was performed, resulting in euthyreoid metabolism under substitution therapy.
Conclusion: Although the risk of developing immune hyperthyroidism after radioiodine-131 therapy is rarely observed, subsequent monitoring of TSH, T3 and T4 is crucial to identify immune hyperthyroidism early and to treat affected patients in due time.

P2 2-1

Overnight melatonin levels correlate with tumour localisation but not with self-assessed sleep quality in patients irradiated for brain tumours or leukemia

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Objective: Melatonin, secreted by the pineal gland during the dark phase of the day/night cycle, modulates sleep and wakefulness rhythms. Decreased melatonin secretion has been shown to be associated with increased daytime sleepiness and disturbed circadian rhythm in (irradiated) survivors of childhood craniopharyngeoma. The present study was performed to explore possible relationships between disturbed sleep and melatonin secretion in patients treated with cranial radiotherapy as a part of brain tumour or leukaemia treatment.
Patients and methods: 34 (16 m, 18 f) patients who had received radiotherapy with or without prior neurosurgery for brain tumour treatment or leukaemia were investigated at least three years after completion of radiotherapy. In all patients overnight urine was collected and melatonin concentration as well as melatonin secretion/h was measured. Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI) and daytime sleepiness using the Epworth Sleepiness Scale (ESS). Tumors were divided into midline or near- midline tumours (n = 15; i.e. germinoma, medulloblastoma), non-midline (n = 12; i.e.hemispheric) tumours and patients with leukaemia (n = 7).
Results: Patients irradiated for midline tumours had a significantly lower overall melatonin secretion and hourly melatonin secretion than patients irradiated for non-midline tumours (p = .008). Self-assessed sleep quality in the PSQI or daytime sleepiness (ESS) were, however, not linked to melatonin secretion. Melatonin concentrations were not dependent on radiation dose, elapsed time since radiotherapy, age at radiotherapy, or childhood versus adult-onset of brain tumour disease (n.s.).
Conclusion: Melatonin secretion was linked to brain tumour site and, thus, perhaps also radiation dose to the pineal gland. Since melatonin secretion was not associated with impaired sleep or daytime sleepiness, the medical use of exogenous melatonin in such patients must be questioned.

P2 2-2

Role of the endothelial-derived endogenous anti-inflammatory factor Del-1 in inflammation-mediated adrenal gland dysfunction.

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Systemic inflammation (e.g. in the course of sepsis) often results in adrenal dysfunction. The adrenal is highly vascularised; thus we hypothesized that endothelial dysfunction and leukocyte recruitment may actively participate in adrenal dysfunction. To address this, we utilized the properties of Del-1 (developmental endothelial locus-1), which is an endothelial-derived antagonist of integrin-dependent leukocyte adhesion. By qPCR and immunohistology analysis, we identified Del-1 expression in the adrenal gland. Interestingly, Del-1 expression was downregulated in the adrenal glands of mice upon SIRS induction by systemic LPS administration. Furthermore, using qPCR and immunohistochemistry, we observed increased infiltration of the innate immune cells (neutrophils, monocytes) in the adrenal glands of Del-1-deficient mice as compared to wildtype mice. In addition, Del-1-deficiency resulted in increased adrenal expression of proinflammatory cytokines and was also associated with reduced HPA axis activity, as the corticosterone and ACTH levels were significantly lower in Del-1$^{-/-}$ mice at 24h post LPS-administration. Together, these data suggest that endothelial Del-1 acts as an important gatekeeper of inflammatory cell recruitment to the adrenal gland and adrenal gland inflammation thereby modulating adrenal gland (dys-)function in the course of SIRS.

P2 2-3

Improvement of health-related quality of life in adult women with 21-hydroxylase deficiency over a 7 years period

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Introduction: Health related quality of life (HRQoL) is impaired in adult patients with 21-hydroxylase deficiency (21-OHD). Up to now, only cross-sectional and no longitudinal studies are available, and it is not known if HRQoL can be improved in adult 21-OHD patients.
Objective: To investigate HRQoL in adult female 21-OHD patients over a longer time span.
Methods: Longitudinal, single centre, follow-up study over seven years with three visits including 15 adult female 21-OHD patients. Two standardized questionnaires, Short Form 12 (SF-12) and Hospital Anxiety and Depression Scale (HADS), were completed in 2003, 2006 and 2010. Adjustment for age and sex was performed by transformation of score values into age- and sex-adjusted Z-scores using complete data sets from respective normative groups. Data regarding glucocorticoid therapy, clinical and hormonal parameters were assessed.
Results: Two of eight scales of the SF-12 showed a significant improvement and four of eight scales a positive trend to better scores. No significant changes were seen in scores for HADS or for steroid hormone levels. Daily hydrocortisone equivalent dose per body surface significantly decreased over the study period, and the dexamethasone dose was significantly lower at the end of the study period. No changes in BMI were observed over the study period.
Conclusions: Improvement of HRQoL in adult female 21-OHD patients is possible in a reasonable time span. Several factors might be involved in this improvement including reduced daily hydrocortisone equivalent dose per body surface.

P2 2-4

Lipoprotein-mediated intracellular mechanisms of adrenal steroidogenesis

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Introduction: Hyperglycemia and oxidative stress are associated with type 2 diabetes mellitus (T2D). This gives rise to glycoxidative and oxidative modifications and functional abnormalities in circulating lipoproteins, major sources of cholesterol for aldosterone biosynthesis. Aldosterone has also been reported to be implicated in cardiovascular injury, resulting in increased morbidity and mortality in T2D. Therefore, we investigated the impact of biochemical modifications on lipoprotein (HDL, LDL and VLDL)-mediated intracellular signaling mechanisms involved in adrenocortical aldosterone release.
Methods: Native lipoproteins were isolated from healthy volunteers. *In vitro* glycoxidative (glycox) and oxidative (ox) modifications were performed in presence of glucose and sodium hypochlorite, respectively. Human adrenocortical cells were treated with various forms of lipoproteins (100µg/ml) in presence or absence of signalling blockers for 24h and subsequently the conditioned medium was utilized for aldosterone estimation.
Results: In contrast to glycoxidative forms, oxidized VLDL and LDL caused significant attenuation of native lipoprotein-mediated steroid hormone release. Experiments with scavenger receptor class B type I (SR-BI) blocker BLT-1 revealed that glycoxVLDL and oxLDL only partially induced steroid synthesis through SR-BI. Experiments with Protein kinase (PK) C inhibitor bisindolyimaleimide I and PKA blocker H89 showed that like oxHDL, both glycoxVLDL and oxVLDL recruited only PKA whereas glycoxHDL required both PKC and PKA for adrenal steroid release. As opposed to oxidized lipoproteins, glyoxidized lipoproteins induced proliferation via Janus kinase-2.
Conclusion: This study demonstrates the significant influence of diabetic lipoprotein modification on various intracellular signaling mechanisms employed for differential regulation of adrenocortical aldosterone release.

P2 2-5

Modified-release prednisolone decreases complaints and fatigue compared to standard prednisolone in patients with adrenal insufficiency

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Context: Patients with adrenal insufficiency (AI) receive glucocorticoid replacement therapy, which tries to imitate physiological adrenal secretion. The first glucocorticoid dose is usually given after waking in the morning resulting in a 3-5h delay compared to physiological secretion. Impaired quality of life (QoL) and fatigue might be, in part, due to this delayed dose scheme. Therefore, modified-release glucocorticoid preparations might have therapeutical advantages.
Objectives: Does modified-release prednisolone improve QoL and fatigue compared to standard prednisolone?
Design and Patients: Prospective case series including 13 patients with primary and 1 patient with secondary AI in a single university center. AI patients on morning dose prednisolone (5mg) were included, switched to modified-release prednisolone (5mg) at 10pm for three months, and then switched back on standard prednisolone. Two standardized questionnaires (GBB-24 and MFI) investigating complaints and fatigue were completed at baseline, after 3 and 6 months. Data regarding clinical and hormonal parameters were assessed.
Main Outcome Measures: Changes in complaints and fatigue depending on the prednisolone formulation.
Results: Modified-release prednisolone showed in one of four scales of the GBB-24 a significant improvement (exhaustion tendency) and in three of four scales a positive trend to better scores. The global score of discomfort (sum of four scales) improved significantly. The MFI showed also significant improvement in three of five scales and a positive trend to better scores in the remaining two scales.
Conclusions: Modified-release prednisolone showed decreased complaints and fatigue compared to standard prednisolone indicating the importance of the glucocorticoid increase in the early morning hours before waking.

P2 2-6

Frequency and causes of adrenal crisis in patients with congenital adrenal hyperplasia

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Background: Adrenal crisis (AC) is a life-threatening complication in patients with congenital adrenal hyperplasia due to classical 21-hydroxylase deficiency (21-OHD). No data on AC over life-time in 21-OHD is available.
Study design: In a retrospective study AC was studied following two approaches: a) questionnaire-based: 122 adult 21-OHD patients (50 men, 72 women) completed a disease-specific questionnaire, b) patient chart based: charts of 67 21-OHD patients (32 males, 35 females) were analyzed from diagnosis to last follow-up with regard to frequency and causes of AC since diagnosis.
Results: Evaluation of questionnaires revealed 257 AC in 4456 patient years (frequency 5.7 crises/100 patient years), while patient charts documented 106 AC in 2181 patient years (4.9 crises/100 patient years). The chart-based evaluation showed that gastrointestinal infections (36%) and salt-wasting crisis (22%) were the main causes of AC. In 17% the cause remained uncertain. There was no difference in the overall frequency of AC in males and females. AC mostly occurred during childhood, with more than 70% of AC in the first 10 years of life and one third of AC in the first year of life. Still 20% of cases of AC were observed in adults (>18 years).
Conclusion: Our longitudinal analysis demonstrates a significant risk of AC in patients with 21-OHD over life-time. Specific age-adapted and repeated crisis prevention training may help to reduce morbidity due to AC in 21-OHD.

P2 2-7

Griseofulvin inhibits the growth of NCI-H295R cell

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Background: Centrosomal clustering is a mechanism used by cancer cells with supernumerary centrosomes to solve the problem of multipolar spindles. Griseofulvin is an antifungal substance that inhibits centrosomal clustering. Adrenal cancer cells are also characterized by chromosomal aberrations and changes in the number of centrosomes. However, whether treatment with griseofulvin inhibits growth of adrenocortical cells is not known.
Methods/results: Therefore, we studied the antiproliferative effects of griseofulvin at concentrations of 0.1 µM to 100 µM on cultured adrenocortical cells. It was observed that the incubation with griseofulvin at concentrations of 10µM or higher lead to a significant decrease in the viability and proliferation of NCI-H295R cells in a dose-dependent manner as measured by Wst-1, BrdU- and 3H-thymidine uptake assays (n≥3). In addition, apoptosis of NCI-H295R cells was induced 4.5-fold at concentrations of 40 µM griseofulvin as shown by caspase 3/7 cleavage activity (n≥3).
Conclusion: These results demonstrate that inhibitors of centrosomal clustering may be useful agents in the treatment of adrenocortical carcinoma.

P2 2-8

Anxiety and depressive symptoms in patients with primary aldosteronism in a longitudinal study

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Context: Recent studies showed a high prevalence of anxiety and depressive symptoms in patients with primary hyperaldosteronism (PA). A cross-sectional analysis suggested only minor improvement following adrenalectomy (ADX) and mineralocorticoidreceptor antagonist (MRA) therapy.
Objective: Our aim was to determine the course of anxiety and depression in untreated patients.
Design, Setting and Patients: We investigated 15 patients with PA at time of diagnosis and 1 year after initiation of specific treatment (ADX, n= 10; MRA, n= 5). Blood pressure, aldosterone and renin were recorded in all patients. Main outcome measures We used GAD-7 and PHQD questionnaires to assess anxiety and depression.
Results: At time of diagnosis patients showed significantly higher mean values for GAD-7 (6,3 ± 4,5) and PHQ-D (7,5 ± 6,6) compared to the general population. One year later mean systolic blood pressure (153 ± 20 vs 128, ± 13), serum potassium (3,6 ± 0,7 vs 4,4 ± 0,3) and the aldosterone to renin ratio in ADX patients (64 ± 63 vs 12 ± 15) had significantly improved. In parallel, psychopathology improved to some degree (GAD-7: 4,7 ± 3,8; PHQ-D 4,4 ± 5,2), but still 11 of 15 patients had scores outside the normal range.
Conclusion: Patients with PA show a rapid and sustained improvement following intervention for their somatic parameters, but depressive symptoms and anxiety appear to improve more slowly.

P2 2-9

Adrenal myelolipomas in CAH - is there role for ultrasound screening of the adrenal glands?

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Persons with CAH may be at increased risk of developing adrenal myelolipomas, particularly if their CAH is poorly controlled. Adrenal masses can be detected by ultrasound with high sensitivity and specificity. Contrast-enhanced ultrasound (CEUS) may be a useful method in the diagnostic work-up of adrenal mass with excellent sensitivity for the diagnosis of malignancy. However, ultrasound is not included in the diagnostic algorithm of patients with CAH.
A 50-year-old man with CAH due to 21-OH-deficiency presented with increasing abdominal girth and abdominal pain. An abdominal ultrasound showed hyperechoic adrenal masses (3.6 x 9.5 cm in the right, 10.4 x 10.4 cm in the left gland), in B-mode and CEUS consistend with myelolipomas. On MRI the diagnosis of mesenchymal tumor or sarcoma was suspected. Because of suspected malignancy a bilateral adrenalectomy was performed, histopathology revealed the diagnosis of benign bilateral myelolipoma.
Adrenal myelolipoma has been described in CAH and is a rare benign tumor formed by mature fat tissue admixed with hematopoetic elements. Ultrasound becomes more important in diagnosis of adrenal masses, especially in combination with CEUS. So far, there is no screening for adrenal masses in patients with CAH. In B-mode and Doppler mode myelolipomas are described with homogenous mostly hyperechoic tissue lacking signs of hypervascularisation or irregular tumor vessels, contrast-enhanced sonography shows an early arterial contrast enhancement without any significant wash-out. With a very high sensitivity in diagnosing malignant lesions and an increasing availability of B-mode and CEUS ultrasound should be used as screening methods in patients with CAH. But there are further data needed on the incidence and prevalence of adrenal masses, especially myelolipomas. In addition, careful follow-up of suspected myelolipomas is needed as it is yet unclear whether there is an increased risk for malignant transformation of this entity.

P2 2-10

Cloning of the CYP11B1/CYP11B2 hybrid gene of a patient with familial hyperaldosteronism and of the aldosterone synthase of an unaffected individual

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Background/Objective: Primary aldosteronism (PA) is one of the most important causes of arterial hypertension. Glucocorticoid-remediable hyperaldosteronism (GSH) is recognized as a monogenetic version of PA that develops on the basis of an aberrant corticotropin-sensitive CYP11B1/CYP11B2 hybrid gene. Demonstration of the existence of such a hybrid gene can be employed as a specific diagnostic tool in the work-up of patients.
Methods: In order to establish a PCR-based test system, we cloned the CYP11B2 of a normal individual for negative control and the CYP11B1/CYP11B2 hybrid gene of a patient, affected by GSH for positive control in TOPO -XI-vectors. In order to study the prevalence of GSH, 60 patients with PA were studied with this PCR-based assay, and all were tested negative.
Conclusions: The PCR-based test system is a reliable tool to identify patients with GSH. However, GSH is a rare disease.

P2 3-1

The gene mutated in Woodhouse-Sakati-Syndrome (WSS) is predominantly expressed in endocrine glands and encodes a protein localized in the nucleolus and at the nuclear envelope

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Background: Woodhouse-Sakati-Syndrome (WSS) is a rare autosomal recessive disorder characterized by hypogonadism, alopecia, diabetes, decreased IGF1 and neurologic symptoms. Mutations in the protein coding c2orf37-gene are causative of WSS. Two major isoforms - WSSα and β - have been identified but their molecular function is unknown.
Aim of study: Tissue expression of the WSS-gene was analyzed and the intracellular localization of WSS-protein determined using a novel polyclonal antibody.
Materials and methods: We studied tissue expression of WSS using published gene expression data (GEO GSE3526). Polyclonal antiserum was obtained from rabbits immunized with glutathione-S-transferase (GST)-fusion protein of WSSβ expressed in *E.coli*. Antiserum was epitope-mapped using a peptide array and antiserum affinity-purified with recombinant WSSα or WSS489-520. Subcellular localization of WSS-protein was determined using cell fractionation and immunofluorescence microscopy.
Results: WSS-gene is ubiquitously expressed with relatively high expression in the pituitary, sceletal muscle, thyroid and further endocrine tissues. Epitope mapping revealed nine distinct epitopes. Affinity purified antibodies detected proteins at 27 kDa (WSSα), 65 kDa (WSSβ) and 80 kDa, suggesting posttranslational modification of WSSβ. Cell fractionation demonstrated presence of WSSα and WSSβ in cytosolic, membrane and nuclear fractions with preponderance of the 80kDa isoform in the nucleus. Nuclear sub-fractionation suggested location of WSS-protein in the nuclear envelope and nucleoli. Immunofluorescence microscopy confirmed presence of WSS-protein in nucleoli by colocalization with fibrillarin.
Conclusion: WSS-gene is expressed in many endocrine tissues. WSS-protein exhibits broad intracellular distribution with a remarkable enrichment in nucleoli and likely the nuclear envelope.

P2 3-2

Very early onset diabetes due to a mutation in the glucokinase gene (p.L304P)

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Introduction: Over 90% of diabetes can be attributed to either type 1 or type 2 diabetes mellitus. Yet, atypical forms are present and must be considered in the evaluation of the diabetic patient.
Patient: We report a 24 year old non-obese patient who was diagnosed with diabetes mellitus as a 1 year old toddler. Antibodies against insulin and glutamat-decarboxylase were positive and diabetes mellitus Typ 1 was suspected. He was recently referred to our department because of recurrent hypoglycaemia with low dose long acting insulin as the only treatment. In an oral glucose tolerance test his blood sugar increased to 262 mg/dl. Interestingly, insulin was detectable with 27.1 µU/ml. The family history revealed family members with diabetes mellitus in three immediate generations, compatible with dominant transmission: affected were the patient's mother, 5 of her siblings and the patient's maternal grandmother. In light of the positive family history, measurable insulin and young age at onset of diabetes, a hereditary cause of non type 1 - non type 2 diabetes mellitus was strongly suspected.
Methods: Genomic DNA was extracted from peripheral leukocytes and sequencing of exons and flanking regions of the introns of the glucokinase (GCK) and HNF1α genes was performed.
Results: Sequencing revealed a heterozygous missense mutation in exon 8 (c.911T>C) of the GCK gene, leading to the amino acid substitution L304P. This mutation had already been reported in one German family with diabetes mellitus. Insulin was then discontinued in our patient and metformin was started.
Conclusion: A mutation in the GCK gene is a common cause of what was formerly known as MODY2. Occasionally it can be mistaken for type 1 diabetes mellitus, especially with autoantibodies present. Correct diagnosis of non type 1 - non type 2 diabetes mellitus and its underlying genetic defect may serve to support a clinically warranted change of treatment

P2 3-3

Analysis of insulin resistance in functional androgenization syndrome (including “polycystic ovary syndrome”)

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Functional androgeniziation syndrome (FAS) is subdivided into 4 groups: I (ovary), II (adrenal gland), III (ovarian dysfunction, obesity, hyperinsulinemia) and IV (residual dysfunctions). Group specific variable clusters were set up by specific hormones, ovarian morphology, BMI, and insulin during 2h oral glucose loading test (OGLT).The prevalence of insulin resistance (IR) and metabolic syndrome (MetS) and their variables was analysed in FAS I-IV and healthy controls. Uni- and multivariate correlation analysis were performed regarding metabolic and endocrine variables.Group FAS III (n=43) showed significantly increased BMI, abdominal circumference (AC), blood pressure, glucose, insulin and triglycerides, whereas HDL-cholesterol and insulin sensitivity index (ISI) were significantly decreased compared to FAS I (n=20) and controls (n=16). BMI and insulin were significantly higher in FAS II (n=18) and IV (n=8) vs. control, AC was higher and ISI and HDL-cholesterol were significantly lower in FAS IV vs. control. The prevalence of IR markers was significantly different from MetS. Insulin 1h was highly significantly correlated with AUC_{2h} insulin and ISI. Testosterone, AC and HDL-cholesterol explained the variance of insulin concentrations to a significant extent.

Prevalence in % of total	Control	FAS I	FAS II	FAS III	FAS IV
Insulin 1 h >99 mU/L	0	0	22	65	62
AUC2h insulin >7000 mU/L/2h	0	5	22	72	62
Insulin sensitivity index < 6	0	5	22	70	62
HOMA-IR >2,7	0	0	11	56	50
Insulin 2h >85 mU/L	0	0	17	44	38
Metabolic syndrome	0	0	28	44	38

[Prevalence of IR in FAS]

The high prevalence of IR supports the classification of FAS including a systematical recording of metabolic variables including an OGLT with the analysis of both glucose and insulin. For screening of IR, an OGLT of 1h appears to be a reliable test approach even considering time and cost consumption.

P2 3-4

C1q/TNF-Related Protein-3 is expressed in adipose tissue, circulates in human sera, and is regulated by metabolic and infection-related parameters

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Introduction: C1q/TNF-related protein-3 (CTRP-3) is a newly discovered adipokine from the C1q/TNF-related protein family. Its expression levels have recently been found to be strongly induced during adipocyte differentiation, and CTRP-3 was shown to function as an anti-inflammatory LPS-antagonist *in vitro* by decreasing the release of pro-inflammatory cytokines interleukine-6 (IL-6) and tumor necrosis factor alpha (TNF-alpha). Our aim was to analyze CTRP-3 expression in adipose tissue in the context of metabolic and infection parameters, and its presence as circulating protein in human serum.
Methods: Human sera, human subcutaneous and visceral adipocytes, and murine 3T3-L1 cells after differentiation into mature adipocytes were used to investigate CTRP-3 expression and function. For characterization of CTRP-3 function, a siRNA mediated knockdown of its expression was performed in 3T3-L1. To determine the knockdown´s impact on basal and epinephrine-induced lipolysis, glycerol concentration in cell culture supernatants was measured. The impact of metabolic factors, infection and inflammation on CTRP-3 expression was investigated by insulin treatment, infection by *S. aureus* and LPS-stimulation in 3T3-L1 adipocytes. Protein levels in cell lysates and sera were analyzed in Western blot experiments. Amounts of secreted proteins in supernatants were measured by ELISA.
Results: CTRP-3 was found to circulate in human sera in two distinct isoforms. It is expressed in visceral and mainly in subcutaneous adipose tissue. CTRP-3 in 3T3-L1 cells is induced by insulin and inhibited by both chronic LPS-exposure and *S. aureus* infection. Resistin secretion and lipolysis were shown to be regulated by CTRP-3.
Conclusion: CTRP-3 is expressed in adipose tissue, circulates in serum in different isoforms, and plays an important role in adipocyte physiology by regulation of lipolysis and resistin secretion. CTRP-3 expression is regulated by both metabolic and infection/inflammation-related factors.

P2 3-5

CTRP-5 - a new differentiation-dependent adipokine expressed in adipose tissue and circulating in human blood

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Introduction: The C1q/TNF-related proteins (CTRP's) represent a family of adiponectin-paralogous proteins. Unlike adiponectin, they show a wide variety in tissue expression. CTRP-5 has a well described function in retina, based on a missense mutation in CTRP-5 gene that causes late-onset retinal degradation. In myocytes, CTRP-5 is able to activate AMP-activated protein kinase (AMPK) and Acetyl-CoA Carboxylase (ACC) followed by an increase in glucose uptake and fatty acid oxidation. A Japanese study (Yamada et al., 2008) demonstrated that a single nucleotide polymorphism (SNP) in the 3'-untranslated region of the CTRP-5 gene is associated with the prevalence of the metabolic syndrome. It was our aim to investigate expression and function of CTRP-5 in adipocytes.
Methods: mRNA and protein expression in adipocytes of CTRP-5 was analyzed by RT-PCR and Western blot. By using a new CTRP-5 ELISA, serum levels in healthy controls were analyzed for the first time. We performed genotyping of the SNP rs9640 in a German cohort of 100 diabetic patients vs. 100 healthy controls.
Results: In this study, we show that CTRP-5 mRNA and protein expression is increased during differentiation in 3T3-L1 adipocytes. It is also expressed in human preadipocytes and mature adipocytes and can be detected in human total adipose tissue. CTRP-5 was detectable in human sera with concentrations between 70 and 500 ng/ml in healthy controls. Unlike the Japanese study, the distribution of genotypes between controls (71% homozygous T, 26% heterozygous, 3% homozygous A) and diabetic patients (74% homozygous T, 24% heterozygous, 2% homozygous A) was not significantly different.
Conclusion: CTRP-5 is expressed in murine and human adipocytes and is also detectable in human sera. That gives evidence that CTRP-5 is a new adipokine. The putative role of the SNP rs9640 as a marker of type 2 diabetes could not be confirmed in a German cohort.

P2 3-6

Effects of IGFBP-2 overexpression on insulin- and IGF-dependent glucose uptake in mice

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Insulin-like growth factor-binding protein-2 (IGFBP-2) as a family member from six highly conserved IGFBPs has high affinity for IGF-I/-II and is believed to regulate their respective bio-availability *in vitro* and *in vivo*. It is also known that IGFBP-2 transgenic mice are protected from insulin resistance and glucose-intolerance in aged mice. However, according to our results in younger male mice, at an age of 70 days after birth, overexpression of IGFBP-2 in transgenic mice severely impairs glucose uptake. Thirty minutes after an oral glucose tolerance test blood glucose concentrations were significantly increased ($p < 0.05$) to 157±38 mg/dl (n=13) in non transgenic mice and to 205±45 mg/dl in IGFBP-2 transgenic mice (n=14). In addition, glucose concentrations were on significantly higher levels in IGFBP-2 transgenic mice ($p < 0.05$) 60 or 120 min after glucose application if compared to controls but not shortly before or 15 min after glucose administration. While overexpression of IGFBP-2 did not affect insulin-dependent hypoglycemia in younger mice up to 120 min after insulin infusion, the hypoglycemic effects of IGF-I were severely impaired by overexpression of IGFBP-2. Subcutaneous administration of rhIGF-I at concentrations of 2 µg/g reduced blood glucose levels to a minimum of 51±30 mg/dl in non transgenic male mice after 120 min while at the same time in IGFBP-2 transgenic mice blood glucose amounted to 84±30 mg/dl ($p < 0.05$). Furthermore, blood glucose levels were normalized 180 min after IGF-I administration in IGFBP-2 transgenic mice (125±18 mg/dl) while control mice were still clearly hypoglycemic (60±19 mg/dl; $p < 0.01$). We hypothesize that IGFBP-2 severely impacts on IGF-I-dependent glucose uptake in younger mice. With respect to results published, which describe insulin sensitizing effects of IGFBP-2 in aged mice, we further conclude that the metabolic effects of IGFPB-2 may particularly depend on age.

P2 3-7

Reduced hepatic AMP-activated protein kinase and circulating adiponectin in human nonalcoholic fatty liver disease (NAFLD)

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Introduction: In metabolic syndrome (MeSy) and Type 2 Diabetes mellitus (T2DM) liver and whole body insulin sensitivity play an important role, which are associated with levels of hepatic steatosis and with circulating levels of the adipocytokine adiponektin. Latter named displays its effects via its receptors AdipoR1 and R2 and activates 5`AMP-activated protein kinase (AMPK), an ubiquitously expressed molecular energy sensor, consisting of 3 subunits α, β, γ. However, relationship of adiponektin and AMPK in human NAFLD is still unclear.
Methods: Thus in a cross-sectional study 30 patients with histologically proven different levels of hepatic steatosis undergoing hepatic surgery were investigated in the circumstances of NAFLD. Circulating adiponektin, measured via an enzyme linked immunosorbent assay (ELISA) and αAMPK mRNA as well as protein levels, determined via quantitative real-time PCR and immunoblotting, were focused for the determination. To evaluate enzyme activity, Thr172 phosphorylated (ph)-αAMPK as well as Ser79 ph-AcetylCoACarboxylase, a main downstream phosphorylation target of AMPK and the rate controlling enzyme of *de novo* lipogenesis, were measured.
Results: NAFLD patients showed significantly reduced plasma adiponektin levels (P=0.025) compared to non-steatotic controls, which correlated with the level of hepatic steatosis (r^2=-0.615, P=0.007). Hepatic gene expression of AdipoR1 (P=0.001) and R2 were increased (P=0.002), while levels for αAMPK were similar in this group (P>0.05), for whom furthermore, a significant reduction of αAMPK protein (P=0.013) and Thr172ph-αAMPK was detectable (P=0.027), which was also seen in a group of T2DM patients compared to those with normal glucose metabolism (P=0.013; P=0.011, respectively). No effects were determined for ACC1 and Ser79ph-ACC in both investigated groups (P>0.05).
Conclusions: The presented data indicate reduced total and ph-αAMPK in hepatic steatosis induced lipid overload and hyperglycemia of T2DM.

P2 3-8

Human *Krüppel*-like factor (hKLF)11 differentially regulates the human insulin promoter activity in β-cells and non β-cells via p300, PDX1 and A3 element

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Introduction: KLF11 is a member of the Sp1/KLF transcription factor and has been shown to function as a transcriptional repressor or activator depending on the cellular context. Own previous work has demonstrated that hKLF11 inhibits human insulin promoter (hInsP) activity in INS-1E and ß-TC3 beta-cells. Here we further characterised functional interactions between hKLF11 and the p300-PDX1-transcrition complex in INS-1E and the non-beta cell line HEK293.
Results: By mammalian two-hybrid system we detected protein interaction between KLF11 and p300 but not between KLF11 and PDX1. Cotransfection of hKLF11 and full length hInsP-driven secreted alkaline phosphatase reporter plasmid (-881+54hInsP-SEAP) confirmed earlier observed negative regulation in INS-1E. In contrast, and in the presence of cotransfected beta-cell-specific PDX1, we observed strong hKLF11-mediated stimulation of hInsP in HEK293. Both inhibition and stimulation depend on p300 since additional coexpression of hp300 in INS-1E or of the p300 inhibitor E1A in HEK293 completely abolished hKLF11 functions. Specific modulation of PDX1 transcription complex was underlined by loss of hKLF11 function in the presence of Dox-induced dominant negative (DN)-PDX1 in INS-1-derived transgenic INSrß-DN-PDX1 beta cells. In addition, we observed loss of hKLF11 inhibitory/stimulatory impact after 5´-deletion of the important PDX1 binding element A3. This is confirmed by completely abolished hKLF11-mediated hInsP stimulation in HEK293 after mutation of A3. Of note, deletion or mutation of the other PDX1 binding sites within hInsP (A5, GG2 and A1) had no effect.
Conclusion: hKLF11 differentially inhibits (beta cells) or stimulates (HEK293) hInsP depending on the molecular context thereby explaining conflicting findings on gene activation and repression reported in the literature. Observed hKLF11 functions specifically modulate the PDX1 transcription complex via p300 and require a functional A3 element.

P2 3-9

Humoral factors by cocultured bone marrow-derived MSC reduce alloxan- but not cytokine-induced beta cell damage

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Objective: MSC migrate into islets upon insulitis and reduce ß-cell loss. The mechanism of this protective effect remains undefined. For investigation we employed the defined human MSC cell line hMSC-TERT to avoid heterogeneity of primary MSC populations. Previous work verified that hMSC-TERT migrate to streptozotocin (STZ) -injured ß-cells and mediate cellular protection via humoral factors. hMSC-TERT closely resemble primary MSC and therefore display a valid model. Here we investigated effects of cocultured MSC on cytokine- or alloxan (ALX)-induced ß-cell injury.
Results: For coculture hMSC-TERT were seeded in inserts with 0.4 µm pores which allow soluble factors but not cells to pass the membrane. INS-1E ß-cells treated with a cytokine mix (IFNg, TNFa and IL-1ß; 20 ng/ml each) display 50% loss of viability after 24 h. IL-1ß caused 40% loss of viability while IFNg or TNFa alone were without effect. We did not observe any significant effect of cocultured MSC on cytokine mix-injured INS-1E . We also tested effects of hMSC-TERT preactivated for 24 h by cytokine mix or by cocultured INS-1E either injured with cytokine mix or 0.66 mM STZ (24 h LD50 dosage). None of the three tested preactivation procedures was successful. We next tested ALX (established 24 h LD50 = 3.3 mM) since this substance injures ß-cells by enhancement of reactive oxygen species (ROS) and not receptor activated signalling. Cocultured hMSC-TERT significantly enhance viability at all dosages tested (3.3, 6.6 and 10 mM). This closely resembles results from STZ experiments.
Conclusion: hMSC-TERT fail to enhance viability in cytokine mix-injured INS-1E indicating that MSC have no effect on receptor-activated apoptotic pathways. In contrast, hMSC-TERT significantly increase viability in STZ- or ALX-injured INS-1E. Both substances are known to enhance production of ROS. Therefore, we are currently investigating whether humoral factors by cocultured MSC can inhibit ROS production in ß-cells.

P2 3-10

A low ratio of n-6/n-3 PUFA promotes the formation of anti-inflammatory mediators in human macrophages.

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Introduction: The presence of a chronic subclinical inflammatory state in adipose tissue and adipose tissue macrophages is a hallmark of obesity and plays a key role in the development of obesity-associated complications such as insulin resistance, artherosclerosis, type 2 diabetes and non-alcoholic fatty liver disease.Lipid mediators derived from omega-3 (n-3) polyunsaturated fatty acids (PUFAs) have been implicated in the modulation of macrophage function and were shown to reduce secretion of pro-inflammatory cytokines, while omega-6 PUFA metabolites are mostly pro-inflammatory. This could be due to the formation of potent anti-inflammatory and pro-resolving lipid mediators such as resolvins from n-3 PUFA.
Methods: We therefore investigated the formation of lipid metabolites and mediators in a human monocyte/macrophage cell line (THP-1) after PMA induced differentiation and treatment with a high n-6/n-3 PUFA-ratio (20/1) versus a low n-6/n-3 PUFA ratio (1/1).
Results: We show that a 1/1 ratio of n-6 (arachidonic acid, AA) /n-3 (eicosapentaenoic acids, EPA and docosahexaenoic acid, DHA) PUFAs results in significantly increased formation of the pro-resolution and anti-inflammatory mediators 18- hydroxyeicosapentaenoic acid (18-HEPE), 17-hydroxydocosahexaenoic acid (17-HDHA) and 14-hydroxydocosahexaenoic acid (14-HDHA) as compared to the 20/1 ratio. Moreover, THP-1 cells treated with the 1/1 PUFA ratio release significantly lower levels of PGE2 and particularly PGD2 as compared to the 20/1 PUFA treated cells.
Conclusions: These results suggest that a low ratio of n-6/n-3 PUFA is promoting the formation of pro-resolving and anti-inflammatory hydroxylated mediators from n-3 PUFA in macrophages. The reduced PGE2 and PGD2 levels reinforce the anti-inflammatory effect of this intervention. A balanced n-6/n-3 PUFA ratio could thus contribute to a shift towards anti-inflammatory lipid mediators away from the pro-inflammatory n-6 PUFA derived prostaglandins.

P2 3-11

Short term changes of the urine metabolome after bariatric surgery

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Objective: Obesity represents a major worldwide health problem. Attendant to the increasing obesity prevalence the use of bariatric surgeries increased. Bariatric surgery leads to a 70% reduction of excess weight. A broad range of metabolic changes as consequence of a bariatric surgery have been reported. The aim of the present study was to investigate changes in the metabolome due to bariatric surgery.
Methods: Data of 50 patients who underwent bariatric surgery at the Municipal Hospital of Dresden-Neustadt were used for the present study. Control subjects matched for age and sex were obtained from the Study of Health in Pomerania. Non-fasting, spontaneous urine samples were collected and ^{1}H NMR spectroscopic analysis were recorded on a 400MHz NMR spectrometer. Metabolites were quantified using Chenomx NMR suite 6.1. Orthogonal projections to latent structures discriminant analysis (OPLS-DA) models were carried out between pre-operative and control as well as post-operative and control samples.
Results: OPLS-DA models showed good separation between pre-operative and control (Q^2Y=85.6%, R^2X=58.3%) or post-operative and control samples (Q^2Y=82.1%, R^2X=44.4%). Based on the OPLS findings four metabolites included hippuric acid, 3-hydroxybutyrate, 2-hydroxyisobutyrate and trigonelline were quantified. In post-operative samples, the highest 3-hydroxybutyrate levels were found, whereas the metabolite was not found in control and seldom present in pre-operative samples. 2-hydroxyisobutyrate levels were higher in both operative groups compared to the control samples. The opposite was found for trigonelline and hippuric acid, both pre- and post-operative samples showed lower median levels compared to the control.
Conclusion: The present study showed that the urinary metabolite profile differs between obese subjects and healthy controls as well as changed after bariatric surgery. Further, four metabolites, responsible for the discrimination between the three groups, were found.

P2 4-1

Membrane transporter for sulfated steroids in the human testis- gatekeepers of the sulfatase pathway

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Sulfated steroids were generally considered to be excreted as biologically inactive metabolites because steroids have to be available in an unbound, free form to be able to interact with the corresponding receptor and to initiate a biological response.
Several membrane transporter proteins are able to transport sulfated steroids such as SOAT, OATP6A1, OATP1C1, and OSCP1. These are predominantly expressed in the human testis. We (1) evaluated the cellular expression of SOAT and other steroid sulfate carriers and their co-localisation with the steroid sulfatase (StS) in patients with normal and impaired spermatogenesis, and (2) performed functional transport studies with the steroid sulfate carriers in stably transfected HEK293 cells. To date we were able to detect SOAT by RT-PCR and Western Blot analysis in the human testis. Single cell analysis and *in situ* hybridization revealed pachytene primary spermatocytes to express SOAT mRNA. SOAT expression in specimens showing maturation arrest at the level of early round spermatids seems to be severely reduced or absent. StS mRNA was detected by RT-PCR in testis homogenates. Preliminary immunohistochemical data show that StS may be expressed in germ cells and interstitial Leydig cells. HEK293 cells stably expressing the SOAT carrier protein showed significant transport activity for DHEAS. This was demonstrated by using a radiolabeled [^{3}H]DHEAS compound as well as by direct analysis of the cell associated uptake fraction by a newly developed LC-MS-MS method.

Supported by DFG Research Group 1369 "Sulfated Steroids in Reproduction” Project 2

P2 4-2

Characterization of JEG-3 cells as in vitro model for the role of steroid sulfate transporters for placental estrogen synthesis

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In women the placenta becomes the main source of maternal estrogens during pregnancy. Placental estrogen biosynthesis is located in the syncytiotrophoblast, a syncytium that builds the main part of the placental barrier and limits the transfer of substances between the fetal and maternal compartment. Since the human placenta is unable to convert cholesterol into 17-OH-pregnenolone, the placenta tissue highly depends on the supply of C-19 steroids for their conversion into C-18 estrogens. In contrast to lipophilic unconjugated steroids that penetrate the cell membrane passively via diffusion, circulating sulfated steroid hormones are delivered to the placenta via carrier-mediated transport, followed by their reactivation via the catalytic activity of the steroid sulfatase (StS). DHEAS of maternal and fetal origin contributes about equally to the placental formation of estrone (E_1) and estradiol (E_2), while 16αOH-DHEAS supplied by the fetus contributes to over 90% of placental estriol (E_3) synthesis. Aim of this project is to investigate this pathway with the choriocarcinoma cell line JEG-3 as in vitro model for the human syncytiotrophoblast.
Therefore, we characterized a JEG-3 cell line that transformed DHEA into E_2 and 16αOH-DHEA into E_3. By qRT-PCR we found expression of StS and aromatase, both essential for estrogen synthesis in these cells. We also detected the sulfated steroid hormone uptake carriers SOAT and OATP4A1, but not OSCP1, OATP2B1 and OAT4. Upon transient transfection of SOAT the carrier was located in the cell membrane of transfected cells. We currently investigate the transformation of DHEAS by these SOAT-JEG-3 cells by LC-MS-MS.
In conclusion, JEG-3 cells are a promising in vitro model for syncytiotrophoblast cells to study placental estrogen synthesis from sulfated steroid hormones.

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P2 4-3

In vitro maturation: 7-year experience in Heidelberg

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Objectives: Although several patients may benefit from reduced FSH application, in vitro maturation (IVM) belongs to the rare treatment options in assisted reproduction. In this retrospective study, data of 7 years IVM experience at our department were summarized.
Study Design: Women with polycystic ovary syndrome (PCOS) after unsuccessful ovarian stimulation as well as patients after controlled ovarian hyperstimulation for IVF/ICSI with ovarian hyperstimulation syndrome (OHSS) 2-3° were offered IVM. Stimulation started between day 3-10 of the menstrual cycle and total FSH application was 375 IU. Ovulation was induced on the third day of FSH injection or one day after and oocyte retrieval was performed 33-38 hours later. Oocytes were cultivated for 24 hours in IVM medium and embryo transfer took place two days after fertilization. Independent variables were analysed by Mann-Whitney U test, the others by using Chi-Square test. *P*-values < 0.05 were regarded as statistically significant.
Results: Altogether 136 patients underwent 254 oocyte retrievals and 210 embryo transfers. Main reasons for IVM were: PCOS (64.6%) and OHSS (15.0%). In 149 cases ICSI (62.9%) and in 88 IVF (37.1%) was performed. Mean number of oocyte was 8.9/oozyte retrieval with 5.6 (62.4%) becoming maturated, 2.6 (46.7%) getting fertilized and 2.1 being transferred. Pregnancy rate per transfer was 16.7% (n= 35), with n=16 live births (7.6%), 1 IUFT (0.5%), n=6 miscarriages (2.9%), 1 EUG (0.5%) and n=11 biochemical pregnancies (5.2%). In 71 cases, fertilized oocytes were frozen and 40 cryotransfers were performed resulting in 3 pregnancies (1 biochemical pregnancy, 2 live births).
Conclusions: Within the last 7 years 136 patients underwent IVM. Although pregnancy rate was 16.7%, 8.1% ended up in miscarriage or biochemical pregnancies. Further research is provided to improve our pregnancy rate. Nevertheless, IVM is comfortable for patients due to low FSH dosages implicating low cost rates and lesser side effects.

P2 4-4

Metformin effect on endometrial stromal cells - a dose dependent effect

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Introduction: The effect of Metformin, an antidiabetic medication, widely used in women with anovulatory hyperandrogenic hyperinsulinaemic condition who wish to conceive, on endometrial stromal cells is hardly understood. While the effect on the development of ovulatory cycles has been widely proven, the direct effect on endometrial stromal cells remains to be determined. Since preliminary changes are described, we hereby determine a dose dependent effect on endometrial cell proliferation and decidualization, as well as on individual markers of the nutrional status within the cells.
Material and methods: Endometrial biopsies of the proliferative phase were taken after informed consent (n=9). Endometrial stromal cells were isolated and cultivated. After 2 passages cells were decidualized in 96 well plates for proliferation assay and 12 well plates for mRNA analysis as well as protein expression. After 12 days of treatment with estrogen and progesterone and different concentrations of metformin (10-5, 10-4, 10-3 and 10-2M) decidualization was confirmed, supernatant collected and mRNA isolated using TRIZOL. For statistical analysis a student ttest was performed, with $p< 0,05$ as significant.
Results: Endometrial stromal cells showed a mild reduction in proliferation under high levels of Metformin (10-2M), while lower concentrations had a slightly stimulatory effect (10-5M) on the proliferation. Prolaktin a well known decidualization marker demonstrated a significant reduction starting with 10-3 M Metformin as shown prior, which was further augmented with increased dosing (10-2M). Parallel a change in IGFBP-1 and -3 was noted.
Conclusion: Metformin shows a dose dependant effect on the proliferation and decidualisation of endometrial stromal cells. The change in decidualisation however cannot be explained by changes in proliferation, since it is much more profound in lower doses while high doses seem to be detrimental. Parallel with the changes in decidualization nutritional changes are seen in endometrial stromal cells that could explain a local effect on the implantation potential under the Metformin medication.

P2 4-5

Investigations on metabolism and transport of sulfated steroids in the porcine testicular-epididymal compartment

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The porcine testicular-epididymal compartment produces large amounts of estrone sulfate (E1S), of which the function is still unknown. Thus, the boar may be a useful model to gain further insight in the metabolism, transport and function of sulfated steroids (sSt). In order to localize and quantify the expression of genes related to the metabolism of free steroids (fSt) and sSt and to the transport of sSt, relative mRNA expression levels were measured by real-time RT-PCR in five sexually mature boars at the following localizations: testis (TE), epididymal head (proximal and distal segment, EH1 and -2), epididymal body (divided in four segments, EB1-4), epididymal tail (ET1 and -2), vas deferens (VD) and pampiniform plexus (PP). Expression of the estrogen specific sulfotransferase SULT1E1 was high in EH2 and EB1 but only low or non-detectable at other localizations. Expression of the dehydroepiandrosterone (DHEA) specific SULT2A1 was virtually restricted to TE. Steroid sulfatase expression was highest in EH1 and -2, ET2 and VD, followed by PP and TE. A high expression of the sSt transporter SLC10A6 was localized in TE, TL2 and VD. Expression of the steroidogenic key enzymes CYP17 and CYP19 was generally restricted to TE. Substantial 5α-reductase (ST5AR2) expression was only detectable in EH1 and -2. Thus, expression of the target genes assessed is highly compartmentalized in the tissues investigated. Results from high frequent measurements of fSt and sSt in jugular vein plasma by LC-MS-MS (testosterone: 0.2-22.6 ng/ml, androstenedione: 0.4-3.2 ng/ml, pregnenolone sulfate 0.1-9.1 ng/ml, E1S: 0.5-68.4 ng/ml; DHEA sulfate: 2.3-156 ng/ml) or radioimmunoassy (estrone: 35-540 pg/ml) showed that secretion of testicular fSt and sSt in boars follows similar patterns and point in connection with gene expression patterns to complex transport mechanisms during the metabolism of sSt in boars.

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P2 4-6

Anti-Müllerian Hormone (AMH) in female patients with hypopituitarism

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Introduction: In the female, AMH is exclusively expressed in granulosa cells from primary to early antral follicles. It is an established marker of ovarian reserve and correlates with antral follicle count. In women with impaired follicular development, low AMH is usually interpreted as an indicator of primary ovarian insufficiency. Data on AMH in hypopituitary patients and its interpretation using age-dependent reference values are missing.
Methods: In a case series of 11 patients with hypopituitarism, AMH was measured during a routine follow-up visit to our ambulatory care endocrinology clinic. Data (mean ± standard deviation) were analysed by t-test following patient stratification according to the occurrence of hypopituitarism before (group 1, n = 4, < 8 years at diagnosis) or after (group 2, n = 7, ≥ 8 years at diagnosis) pubertal maturation.
Results: Hypopituitarism due to variable causes (see table) results in low or undetectable gonadotropins. Mean patient age at the time of AMH measurement is not different between groups (p = 0.227; group 1: 28.2 ± 8.5 vs. group 2: 21.7 ± 4.2 years). In all but one patient of group 1, but not in group 2, AMH serum levels are below the age -specific reference range. Mean AMH serum level in group 1 is significantly lower than in group 2 (1.34 ± 1.20 vs 4.74 ± 2.70 ng/ml, p = 0.044).

No.	Age (years)	Diagnosis	Age at diagnosis (years) < 8	..... ≥ 8	AMH (ng/ml)
1	23	idiopathic	4		0.53
2	20	craniopharyngioma		12	1.71
3	23	dysgerminoma		12	5.8
4	21	hypophysitis		14	3.9
5	22	craniopharyngioma		11	4.9
6	35	Trauma	3		0.88
7	36	dysgerminoma	7		0.82
8	19	empty sella	5		3.14
9	30	craniopharyngioma		9	1.73
10	19	craniopharyngioma		8	5.63
11	17	idiopathic		8	9.53

[AMH in females with hypopituitarism]

Conclusions: In women with hypopituitarism occurring after pubertal maturation, AMH serum levels are in the age-specific reference range in contrast to hypopituitary patients diagnosed before pubertal maturation. Although based on very small numbers this prompts us to reconsider the value of AMH as a marker of ovarian reserve in this group of patients.

P2 4-7

***In vivo* effects of SF-1 dependent Urocortin2 overexpression on gonadal morphology and function**

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Urocortin 2 (UCN2) is a member of the family of corticotropin-releasing-hormone (CRH) related peptides. Binding exclusively to the CRH receptor type 2, UCN2 is part of the physiologic stress response, mediated by the hypothalamo-pituitary-adrenal-axis. Besides its role in central nervous system, UCN2 expression was demonstrated in a variety of peripheral organ systems including the ovaries and testes. Therefore, our study aimed to investigate a potential direct influence of UCN2 on gonadal function and morphology in an *in vivo* model of UCN2 overexpression.
Utilizing the Cre/lox system we developed a mouse model with targeted UCN2 overexpression in SF-1 expressing cells such as the gonads, the adrenals and parts of the hypothalamus, by crossbreeding SF1-Cre$^{+/-}$ mice with R26$^{\text{stop UCN2/stop UCN2}}$ mice. Following genotypes were obtained: UCN2 overexpressing SF1-Cre$^{+/-}$/ R26$^{\text{+/stop UCN2}}$ and SF1-Cre$^{-/-}$/ R26$^{\text{+/stop UCN2}}$ serving as controls. UCN2 plasma levels were measured by immunoassay. Gene expression of UCN2 and key enzymes of sexual hormone synthesis was quantified using real-time PCR.
As expected, plasma levels of UCN2 were significantly elevated in overexpressing mice of both genders. Furthermore, local UCN2 expression was markedly increased in female but not in male gonads. In addition, we demonstrated a significant decrease in ovarian expression of the key enzymes of estradiol synthesis Cyp17 and Cyp19, in contrast to a significantly increased gene expression of StAR and Cyp11a1, involved in the initial steps of steroidogenesis.
Taken together, UCN2 appears to modulate ovarian steroidogenesis in a complex manner. Demonstrated changes in gene expression need to be matched to sexual hormone plasma levels. To clarify the observed gender dependent discrepancies further investigations will be necessary.

P2 4-8

NAMPT is present in human semen

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Objective: The underlying mechanisms of infertility in obese men are not completely understood. Nicotinamide phosphoribosyltransferase (NAMPT) a key enzyme of the NAD metabolism is implicated in the regulation of apoptosis and was found in spermatocytes and spermatides of chicken testis. Accordingly, we asked for the presence of NAMPT in human seminal plasma (SP) and in spermatozoa, for its function in male reproduction and whether or not NAMPT levels depend on BMI.
Methods: Semen was analysed according to the WHO guidelines. In SP and serum of 96 healthy donors (age 35.1±11.6 years, BMI 27.0±5.55 kg/m^2). NAMPT concentrations were determined by ELISA (Axxora). Density gradient centrifugation was performed with human semen (n=10) to achieve different maturation stages of spermatozoa. Subsequently, spermatozoa were incubated for 3 and 24h to measure NAMPT in the respective supernatants. In addition, immunofluorescence measurement was performed to detect NAMPT distribution in spermatozoa.
Results: NAMPT concentrations in SP were approximately 100-fold higher than in serum (194±165 ng/ml vs 6.12±14.7 ng/ml). However, NAMPT concentrations did not correlate to semen quality and no significant differences of NAMPT concentrations in SP between normal-weight and obese men were detectable. Moreover, NAMPT was detected in supernatant of both mature and immature spermatozoa: After 3h the mean viability of spermatozoa decreased by 27±20% (mean±SD) and NAMPT levels of 0.5±0.3 ng/ml were detected. Incubation of 24h caused a decreased viability by 62±26% and a NAMPT level of 1.9±2.94 ng/ml. Additionally, NAMPT protein was localised in the tail and connecting piece of immature spermatozoa.
Conclusions: These results indicate NAMPT secretion by spermatozoa which appeared to be dependent on viability and therefore, on apoptotic processes. Further research is needed to understand the huge differences of NAMPT levels in SP and serum and its specific function in human spermatozoa.

P2 4-9

TNFAIP6 expression and regulation in human endometrium in vivo and in vitro

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Introduction: TNFAIP-6 a binding protein for many factors involved in cell-cell communication and matrix modulation. Prior studies have shown an upregulation of TNFAIP-6 in endometrial stromal cells (SC) after contact with cell supernatant of immune cells, as well as trophoblast cells, suggesting its importance during implantation. We therefore analysed TNFAIP-6 throughout the menstrual cycle in vivo, and potential modulation factors (TNF, HCG, trophoblast conditioned medium (TCM), hormones) in vitro.
Material and methods: Endometrial biopsies, taken after informed consent, in the late proliferative and midsrectory phase (n=4 each) where analysed as whole tissue as well as after isolation of epithelial cells, SC and immune cells using Magnetic Beads. In addition SC were isolated, passaged and treated with Estrogen/Progesteron (EP), TNFa, HCG and TCM over time, with or without prior decidualization. Supernatants were then collected for ELISA analysis. mRNA analysis was performed using TNFAIP6 Taqman primer for Realtime PCR. Paired ttest $p < 0,05$ as cutoff.
Results: TNFAIP6 was found in whole endometrium and individual cell fractions of both phases, with a slight secretory upregulation. In vitro hormonal stimulation did not increase TNFAIP6 expression, while TNFa and TCM showed a significant upregulation in shortterm and longterm culture. A similar upregulation was seen in protein expression.
Conclusion: The slight TNFAIP6 upregulation in secretory phase endometrium is attributed to the increased number of immune cell in the secretory phase. The in vitro culture of endometrial SC confirmed that TNFAIP6 is not hormonally regulated. TNFa and TCM however lead to a significant upregulation of TNFAIP6, suggesting that TNFAIP6 in endometrial SCs is highly regulated via paracrine signalling of the invading trophoblast itself, as well as by immune cell products, therefore promoting their own migration towards and invasion into the endometrium, potentially supporting implantation.

P2 4-10

Cross coupling between Nfe2 and Jund is required for proper placentation embryonic growth and trophoblast differentiation

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bZip transcription factor Nfe2 which thought to be restricted for hematopoiesis results in thrombocytopenia, impaired placental vascularization and intrauterine growth restriction (IUGR) in mice. The mechanism underlying the Nfe2 dependent placental defect and IUGR remains unknown. Here we showed that Nfe2 expressed in trophoblast where it is required for normal syncytiotrophoblast formation, placental vascularization and embryonic growth. In the absence of NF-E2, the width of syncytiotrophoblast layer 2 and the expression of Gcm1 and Gcm1-dependent genes (Synb and Cebpa) are increased. In vitro, Nfe2 deficiency results in spontaneous syncytiotrophoblast formation, which can be reversed by Gcm1 knockdown. We further showed that Nfe2 represses Jund DNA binding activity to the Gcm1 promoter during syncytiotrophoblast differentiation. Interventional studies using knock down and knock in approaches show that enhanced Jund DNA-binding activity is required for increased expression of Gcm1 and syncytiotrophoblast formation as well as impaired placental vascularisation and reduced growth of Nfe2-/- embryos. In addition Increased Gcm1 expression in the absence of Nfe2 is dependent on enhanced protein acetylation, including post-translational modification of Gcm1. Increasing and inhibiting acetylation in the placenta of wild-type control embryos phenocopies and corrects, respectively, the changes observed in Nfe2-deficient embryos. These studies identify a novel function of Nfe2 during placental development and trophoblast differentiation,

Adipositas

P2 5-1

Juvenile obesity improves glucose metabolism in advanced ages - effects of body weight selection and a high fat diet in mice

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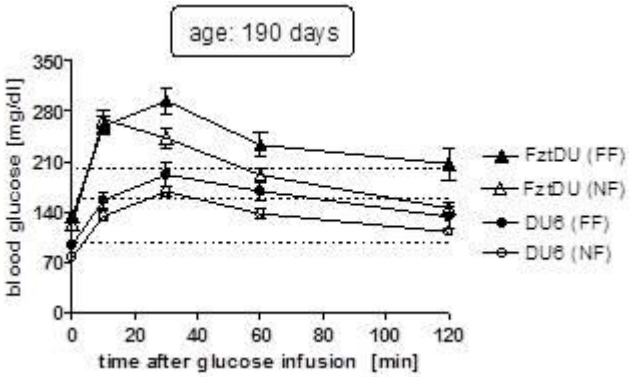
Juvenile obesity is considered as a risk factor for the onset of insulin insensitivity later in life. In order to study effects of juvenile obesity and a high fat diet on longitudinal glucose tolerance we used long-term-selected mice bred for high body mass at an age of 42 days after birth. Phenotypic selection had been performed over more than 140 generations (DU6). DU6 mice were compared to random-selected mice (Fzt:DU). In order to include also environmental effects, male mice of both genetic groups (N=20) were fed either a normal (5% fat, termed NF) or a high fat diet (20% fat, termed FF). Body weights and visceral fat pad weights at 276 days of age in DU6 mice (74.9±2.6 g; 2.5±0.8 g) were severely increased if compared to Fzt:DU mice (30.2±1.8 g; 0.7±0.3 g). Body mass was monitored at an age of 21, 42, 63, 84, 105, 126, 147, 168, 190, 232, 275 days, respectively. In all four groups and at different time points (22, 43, 190, 232, 275 days of age), blood glucose levels were assessed after an oral glucose tolerance test. The longitudinal study was terminated at an age of 276 days. In 276 days old Fzt:DU and DU6 mice FF established severe increases of body mass and fat pad weights. Adult Fzt:DU mice further developed a severe phenotype of glucose intolerance with peak glucose levels of 269 *mg*/dl (NF) and 294 *mg*/dl (FF) 10 and 30 min after glucose challenge, respectively. However unexpectedly, adult DU6 mice were protected from the development of age related (peak glucose level with NF: 168 *mg*/dl) or diet induced glucose intolerance (peak glucose level with FF: 192 *mg*/dl). We thus presume highly efficient mechanisms of glucose clearance and energy metabolism in DU6 mice.



[Glucose tolerance in growth selected mice]

P2 5-2

Influence of saturated free fatty acids and intracellular calcium levels on ectodomain shedding of short and long form leptin receptors: Implications for leptin action

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The soluble leptin receptor (sOb-R) is the main binding protein for leptin in human blood and modulates its bioavailability. sOb-R levels are differentially regulated in metabolic disorders like type 1 diabetes mellitus and can, therefore, enhance or reduce leptin sensitivity.
To elucidate mechanisms of Ob-R cleavage we established a model of HEK293 cells transiently transfected with different human Ob-R isoforms. These cells were applied to investigate the effects of the free fatty acids palmitate and oleate and the Ca^{2+}-ionophor ionomycin on Ob-R shedding. Leptin receptor signalling was analyzed by detection of P-STAT3. sOb-R in cell supernatants was measured by an in-house immunofunctional assay.
The saturated fatty acid palmitate increased sOb-R levels in cell supernatants. This was accompanied by reduced cell viability and activation of apoptosis demonstrated through detection of cleaved caspase-3 and cleaved PARP. Interestingly, co-incubation with the broad spectrum caspase inhibitor Z-VAD did not affect the palmitate-mediated increase of sOb-R concentrations and reduced cell viability. However, mono-unsaturated fatty acid oleate inhibited the increase of sOb-R and prevented the lipotoxic effects of palmitate. Since palmitate affects intracellular calcium levels we tested whether a change in cytosolic free calcium influences Ob-R shedding. The incubation of Ob-R transfected cells with ionomycin led to increased Ob-R shedding while the presence of the Ca^{2+}-chelator BAPTA-AM inhibited this effect. Moreover, co-incubation experiments of leptin and sOb-R proved that increasing concentrations of sOb-R impaired leptin-mediated STAT3 activation. These findings provide novel insights into the regulatory mechanisms of Ob-R shedding and may in part explain alterations of leptin sensitivity which are associated with changes of serum sOb-R levels in metabolic diseases.

P2 5-3

Interactions of energy metabolism and sirtuins in a novel mouse model for outmost physical performance

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Objectives: We have used a novel mouse model (DUhTP) generated by phenotype-selection for high treadmill performance over 100 generations. DUhTP have about 5-fold higher running capacities compared to unselected controls (DUKB). Our “Marathon” mouse model is characterized by increased abdominal fat mass and marked hyperlipidemia. We assume, that during long-term selection DUhTP mice have developed efficient energy metabolic strategies granting for increased physical fitness.
Methods: Liver and muscle tissues from 7-wk-old male DUhTP and DUKB mice were analyzed for the expression of metabolic key enzymes such as acetyl-CoA-carboxylase (ACC), acetyl-CoA-synthetase (ACSS), complex I, ATP, Sirtuin (Sirt)1, and Sirt3.
Results: In liver of DUhTP we observed higher levels of ACC and ACSS2, while these key enzymes of fatty acid synthesis were decreased in muscles of DUhTP. Cytosolic Sirt1 concentration was also elevated in liver and barely expressed in muscles when compared to DUKB. Furthermore, hepatic levels of complex I and Sirt3 were increased while resting ATP levels were similar in both lines. Increased muscular complex I concentration and decreased ACSS1 and Sirt3 levels were observed in DUhTP which in turn correlated with reduced levels of resting ATP when compared to muscles of DUKB.
Conclusion: Outmost physical performance on the one hand correlates with higher fat metabolism in the liver including hyperlipidemia and elevated fat deposition in non-hepatic tissues and with efficient mobilization under physical performance on the other. We suggest essential functions of Sirt1 and 3 in the genetic model of efficient energy metabolism.

P2 5-4

Impact of estradiol, ER subtype specific agonists and genistein on energy homeostasis in a rat model of nutrition induced obesity

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Introduction: The prevalence of obesity, a major risk factor for developing chronic diseases such as type 2 diabetes, insulin resistance, dyslipidemia, cardiovascular disease, and certain forms of cancer is dramatically increasing worldwide. High-caloric nutrition and lack of physical activity are the main contributing factors. Estrogen receptors (ERs) are known to be involved in the control of energy homeostasis.
Aim and methods: To investigate the effects of estradiol (E2), the ER subtype specific agonists 16α-LE2 (Alpha) and 8β-VE2 (Beta), and genistein (Gen) on energy homeostasis, juvenile female Wistar rats were ovariectomized (OVX) or sham-operated (SHAM) and fed with a low-fat (LF) or a high-fat diet (HF). A subset of OVX HF animals received E2, Alpha or Beta subcutaneously or Gen via enriched food for 10 weeks.
Results: Treatment with E2 and Alpha decreased body weight gain. Visceral fat mass, adipocyte size and serum leptin were reduced by E2, Alpha and Beta. In the adipose tissue the mRNA expression of SREBP1c (sterol regulatory element binding protein 1c), FAS (fatty acid synthase), ACC1 (acetyl-CoA carboxylase 1), LPL (lipoprotein lipase) and PPARγ (peroxisome proliferator-activated receptor γ) was decreased by treatment with E2, Alpha and Beta. The hepatic triglyceride content was reduced by treatment with E2, the ER subtype specific agonists but also by Gen. This observation agrees with hepatic gene expression. In comparison to the OVX HF animals, the hepatic mRNA expression of SREBP1c and FAS was decreased in SHAM-operated as well as in E2, Alpha, Beta and Gen treated groups.
Conclusions: Our data demonstrate that the activation of ER alpha and ER beta resulted in a reduction of the adipose tissue mass and the hepatic triglyceride accumulation, which seems to be mediated through an anti-lipogenic action of E2. A beneficial impact of Gen was shown in the liver were triglyceride accumulation and gene expression were modulated comparable to E2.

P2 5-5

Risk factors associated with the metabolic syndrome in abdominal obesity

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Obesity is associated with the metabolic syndrome and increased mortality and morbidity. However, not all obese individuals have cardiovascular risk factors (CVRF). It is not clear how many abdominally obese individuals are free of CVRF and what distinguishes them from the group of obese individuals with CVRF. In this study we aimed to assess the associated factors and prevalence of abdominal obesity without CVRF.
In our cross sectional analysis we included N=4244 subjects from the Study of Health in Pomerania (SHIP), a population-based study, and N=6671 subjects from the DETECT study, a representative primary care study in Germany. We defined abdominal obesity by waist-to-height ratio (WHtR) of 0.5 or greater. We assessed how many subjects with abdominal obesity had CVRF based on the definition of the metabolic syndrome (glucose disturbance, high triglycerides, low HL cholesterol, and hypertension). We analyzed which conditions were associated with the absence of cardiovascular risk factors in abdominal obesity.
In SHIP and DETECT 2652 (62.5%) and 5126 (76.8%) subjects had a WHtR>=0.5. Among those with a WHtR>=0.5, 9.0% and 13.8% were free of CVRF and 49.9% and 52.7% had at least two CVRF in SHIP and DETECT, respectively. In both studies, after backward elimination, age, male sex, body mass index, and high lever enzymes and unemployment were consistently inversely associated with metabolically healthy abdominal obesity. Among abdominally obese subjects, the prevalence of metabolically healthy subjects is low. Conditions consistently associated with the absence of CVRF in abdominal obesity are younger age, female sex, low BMI, and normal liver enzymes, the latter likely reflecting the absence of steatohepatitis.

P2 5-6

Caloric restriction increases serum free testosterone concentrations in metabolically healthy obese male subjects by two distinct mechanisms

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Background: Obesity as an endocrinopathy is associated with dysregulated androgen levels. Regulation occurs by two mechanisms. Serum free testosterone is mainly regulated by the testicular function underlying the central hypothalamic-pituitary-gonadal-axis. Secondly, a certain amount of testosterone gets converted into ß-estradiol within adipose tissue.
Objective: The aim of the present study was to examine the effect of caloric restriction on serum free testosterone levels in obesity.
Design: 13 metabolically healthy obese human male subjects (BMI:42,67 ± 6,03 kg/m^2) were treated with caloric restriction (800 kcal/d) for 12 weeks. Body composition was assessed by impedance analysis. Insulin sensitivity was estimated by leptin-adiponectin-ratio (LAR). Testosterone, ß-estradiol, albumin, SHBG, LH, FSH serum concentrations were measured by enzyme immunoassays.
Results: Caloric restriction not only influenced total androgen and SHBG levels but also significantly increased serum free testosterone concentrations (0,21 ± 0,10 to 0,26 ± 0,08 (p=0,013)). This was achieved by a significant improvement of the testicular function (LH/testosterone: 0,35 ± 0,17 to 0,19 ± 0,11 (p=0,005)) and a significant reduction of the testosterone/ß-estradiol conversion rate (78,39 ± 29,74 to 118,26 ± 44,79 (p=0,003)). Furthermore, testicular function exhibited a significant positive correlation with insulin sensitivity (0,683 (p=0,042)).
Conclusion: In obese human male individuals caloric restriction not only alters total testosterone by influencing SHBG levels but also significantly increases the bioactive serum free testosterone concentrations. This is achieved by improvement of testicular function and reduced conversion of testosterone into ß-estradiol. Since reduced libido and fertility due to low testosterone levels is often seen in obese male individuals, these findings might be of clinical relevance for nutritional interventions.

P2 5-7

Activity thermogenesis, depressiveness and coping in subjects with high-grade obesity intending to undergo bariatric surgery

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Introduction: Reduced physical activity and depression are reported to be associated with obesity. In addition, depression is thought to be inversely correlated to physical activity. To further evaluate this potential link we assessed depressive symptoms, coping strategies and activity thermogenesis (AT) in 50 participants with high-grade obesity (42±12 years; 9 with II° and 41 with III° obesity) intending to undergo bariatric surgery.
Methods: AT was assessed with the SenseWear™ armband. Depressiveness and coping strategies were assessed using a set of questionnaires administered on personal digital assistants.
Results: Body weight-adjusted non-exercise AT and patterns of physical activity were inversely correlated to body mass index (non-exercise AT: $r = -0.32$, $p < 0.05$; mean metabolic equivalent: $r = -0.37$, $p < 0.01$) but failed to reveal significant correlations to depressiveness. The coping strategies “support seeking” and “active coping” showed significantly inverse correlations to body weight-adjusted non-exercise AT (“support seeking”: $r = -0.34$, $p < 0.05$; “active coping”: -0.36, $p < 0.05$), body weight-adjusted exercise-related AT (“support seeking”: $r = -0.36$, $p < 0.05$; “active coping”: -0.38, $p < 0.01$) and physical activity patterns.
Conclusions: Depressiveness was not associated with AT in participants with high-grade obesity. Coping of individuals intending to undergo bariatric surgery does not seem to lead to an increase of AT.

P2 5-8

Comparison of healthy obese with postbariatric patients - metabolic and vascular patterns

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Obesity and the metabolic syndrome (MetS) are linked to endothelial dysfunction (ED). Retinal vessel analysis determines ED. Preliminary data in obese patients (WHO III°) show ED in retinal vessel improving after bariatric surgery. Obesity is associated with MetS, but obese patients may exist not fulfilling all criteria of the MetS (defined as “metabolically healthy obese subjects”(MHOS)). Adipocytokine patterns, degree of insulin resistance, and tissue macrophage infiltrations further characterize these subjects. The aim of the study was to identify differences between MHOS and postbariatric patients in adipocytokine patterns and ED. Arterio-venous ratio (AVR) and vessel diameters from retinal photographs (IMEDOS™), parameters of MetS (IDF) and obesity-associated factors (RBP4, adiponectin, TNF, Il-6, sICAM, sVCAM, MCP-1, IGF-BP3, hs-CRP) were assessed in 51 patients with MetS (BMI 49.8± 6.1 kg/m²), 21 obese patients without MetS (BMI 48.6 ± 5.5 kg/m²) and 21 patients pre (BMI 49.6 ± 7.4 kg/m²) and post (BMI 36.2 ± 6.4 kg/m²) bariatric surgery.
Bariatric surgery improved ED as reflected by AVR (pre 0.81±0.1, post 0.85±0.08) and venous diameters (pre 223.8±24.3 µm, post 211.9±19.6 µm). These improvements were associated with lower levels of insulin, hsCRP, sICAM, and higher levels of adiponectin and RBP4 ($p < 0.05$). MHOS differed from MetS by neck circumference, RRs, fasting plasma glucose, insulin, triglycerides, HDL-C, sICAM, and adiponectin ($p < 0.05$), but no in RRd, LDL-C, hsCRP, Il-6, TNF, MCP-1, sVCAM, RBP-4, IGF-BP3, and retinal ED. MHOS differed from postbariatric patients by anthropometric parameters, and insulin, hsCRP, RBP-4 and IGF-BP3 ($p < 0.05$).
In conclusion, these data indicate that insulin and, inflammatory mediators and endogenous endothelial repair agonists characterize the difference between healthy obese and postbariatric patients. These metabolic differences are not matched by differences in ED.

P2 5-9

Impact of intragastric balloon on metabolic parameters and weight regulatory hormones: Decrease of leptin and increase of ghrelin levels after removal of the balloon

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Introduction: Insertion of an intragastric balloon is an efficient intervention to induce weight loss. We aimed to study the impact of intragastric balloon therapy on weight loss and weight regulatory hormones.
Methods: 54 pat. with overweight or obesity were invited to participate in a prospective single-center study. The BioEnterics® Intragastric Balloon System (BIB) was placed for median 231 (154 - 399) days. Before insertion and before removal of BIB anthropometric (weight, BMI, waist circumference) and metabolic parameters and weight regulatory hormones (triglycerides, total cholesterol, HDL, LDL, fasting glucose, insulin, HOMA, cortisol, leptin, ghrelin and serotonin) were measured. 45 patients (36 women, 9 men, 6 with overweight, 16 with obesity grade 1, 13 with obesity grade 2 and 10 with obesity grade 3) completed the study. Mean age was 39,44 years, mean BMI was 35,8 kg/m².
Results: After balloon removal body weight,BMI and waist circumference significantly decreased from 105,26 (±21,17) to 94,56 (±23,56) kg ,from 35,80 (±4,85) to 32,02 (±5,56) kg/m² and from 116,93 (±13,67) to 107,27 (±16,74) cm , respectively.
Metabolic parameters triglycerides (165,58 vs 121,11 mg/dl), total cholesterol (220,16 vs 207,13 mg/dl), insulin (12,46 vs 8,94 µU/ml), HOMA-IR (2,87 vs 2,01), leptin (30,78 vs 18,00 ng/ml) significantly decreased whereas serotonin (152,49 vs 168,56 ng/ml) and ghrelin levels (966,02 vs 1201,4 pg/ml) significantly increased. No significant changes were seen in fasting glucose, HDL and LDL cholesterol and cortisol levels.
Conclusions: BIB therapy is safe and effective for inducing weight loss and reducing insulin resistance in obese patients. The decrease of leptin levels in our study is consistent with the data published before. Increased ghrelin levels after BIB therapy as demonstrated in our study may be explained as regulatory response to the energy deficit and weight loss during the balloon therapy.

P2 5-10

Metformin for weight reduction in obese patients in an intention-to-treat outpatient setting

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Introduction: Obesity is the curse of developed countries, affecting more than 1.5 billion people worldwide (WHO, 2008). While its negative impact on health is unquestioned, no panacea to halt the epidemic is in sight. Lifestyle modification, psychological counseling, self-help-groups, drug therapy and surgery are all options for weight reduction. While effective in the treatment of type 2 diabetes, metformin is not a common obesity treatment. In 2009, we started a Health Services Research program to explore the efficacy of metformin in an outpatient setting.
Methods: 277 patients (252 women and 25 men; 25-40 years old) with a BMI > 30 kg/m^2 were recruited for an open label trial of 2-2.5 g/day metformin. Obesity secondary to hormonal disorders, confounding medical conditions and patients with contraindications to metformin were excluded. Thyroid dysfunction was treated according to standard guidelines. All patients received counseling to improve their eating habits and to implement lifestyle changes.
Results: Before treatment, more than 85% of patients weighed between 90-100 kg with an average BMI of 35 kg/m^2. Within 3 months 15% of the patients had dropped out of the study with only 3% blaming side effects. 55% of patients completed the initial 12 month study protocol. An intention-to-treat analysis, including all patients who dropped out of the study and carrying on their last recorded weight, showed an average weight reduction of 3.0/3.7/3.7 and 7.7 kg at 3/6/9 and 12 months. No case of lactacidosis or worsening of kidney- or liver function was recorded. On the contrary, in 12 patients normalization of initially slightly elevated liver function tests was observed.
Conclusions: Metformin has some potential as a safe, effective and low-cost option for the treatment of obesity. Better weight reduction correlated with female gender, the initial weight, the termination of oral contraceptives, but not the concurrent treatment of subclinical hypothyroidism.

P2 5-11

Can early fat distribution predict type 2 diabetes in mice?

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Background: Fat accumulation in abdominal region and ectopic fat storage in liver, skeletal muscle and pancreas are associated with an increased risk for the development of type 2 diabetes in humans. On contrary, subcutaneous fat depots are regarded as favourable and protective against impaired insulin sensitivity.
Methods: NZO mouse is a well established model of insulin resistance in which only males develop diabetes. We used male and female mice fed with high-fat and standard diet. Mice underwent scans with computed tomograph and oral glucose tolerance tests. Body weight and blood glucose were measured weekly.
Results: Contrary to previous findings, we observed that not only male NZO mice on high-fat diet develop diabetes. Blood glucose levels at the 16th week of age and total pancreatic insulin content indicated diabetes prevalence of 68% in males and 25% in females These results lead to the conclusion that high-fat diet counteracts protective action of estrogens against diabetes. Inversely to the findings in humans, female mice tend to store more fat in abdominal region than males. There was no relationship between early accumulation of fat in abdominal region and onset of type 2 diabetes. However, visceral fat was associated with liver fat in males as well as in females. Furthermore, at the age of ten weeks hepatic fat content correlated with blood glucose levels ($r^2 = 0.69$) indicating that the early hepatosteatosis is a predictor for hyperglycemia. However, there was no correlation between hepatic insulin sensitivity (indicated by quantitative insulin sensitivity index-QUICKI) and amounts of hepatic fat we conclude that early hepatosteatosis does not predict for glucose intolerance in NZO mice.
Conclusion: In the NZO mouse, the amount of liver fat but not the early fat distribution predicts for the later onset of type 2 diabetes. Further experiments are needed to examine the gender dependent differences in the diabetes prevalence of this mouse strain.

P2 6-1

Dopamine causes generation of reactive oxygen species (ROS) in human ovarian cells

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The neurotransmitter dopamine (DA) and the transporter for DA (SCL6A3; DAT) were found in the human or non-human primate ovary previously. A role for DAT in oocytes was suggested, yet other possible roles remain to be elucidated. To explore this issue, granulosa cells (GCs) from IVF-patients were examined and archival ovarian paraffin samples were used for immunohistochemical analysis. We found that DAT is expressed by cultured GCs (RT-PCR; Western blot) and is functional in these cells. Thus, GCs are able to take up DA from the medium, as determined by ELISA measurements. A blocker of DAT (nomifensin) significantly inhibited the uptake. GCs also express the DA-metabolizing enzyme monoamine oxidase B (MAO-B; RT-PCR), implying the generation of metabolites. In other cell types DA-metabolites can induce the generation of ROS. Indeed in GCs, DA, but not D1/D2 agonists, induced ROS-generation in a concentration-dependent manner, as shown with a fluorescent dye. The DA-induced formation of ROS was reduced by an antioxidant, a MAO-B blocker and by nomifensin, emphasizing the necessity of cellular uptake and metabolism of DA for ROS generation. Viability studies, using cellular ATP as read-out, indicated that DA-induced ROS is not toxic for cells in concentrations up to 10 µM. Although we have not yet fully identified all DAT-expressing cells in the human ovary, luteal cells and GCs of large follicle are among them. This implies that a metabolism-dependent part of the signaling pathway of DA that involves ROS exists in the human ovary. The DA-induced ROS are not toxic, but rather they may function as signaling factors in the ovary. DA may thus contribute in a receptor-independent manner to the ovarian microenvironment.

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P2 6-2

Evaluation of ovarian retransplantation in 4 patients at the Department for Gynecological Endocrinology and Fertility Disorders at the University Clinics of Heidelberg

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Introduction: Since the foundation of the network FertiProtekt five years ago, more than 600 ovarian biopsies were cryopreserved at the cryobank Bonn as fertility preservation before chemo- or radiotherapy. So far we perfomed 4 ovarian retransplantations in two patients in 2010 and 2011.
Method: In 2006 and 2008 we initiated laparoscopic removal of 30% of one ovary for cryopreservation in two patients presenting with Non-Hodgkin Lymphoma (NHL) and breast cancer prior chemotherapy. The tissue was transported at 4-6^{o}C to the cryobank Bonn for cryopreservation. Both patients requested a first retransplantation in 2010 which was performed by laparoscopy immediately after thawing. In both patients approximately one third of the initially frozen tissue was stitched to the remaining ovary; 5 and 7 months later a second retransplantation was done (1 by laparotomy and 1 by laparoscopy).
Results: In the breast cancer patient ovarian biopsy was done at the age of 40 followed by the first transplantation at the age of 43 which resulted in a single menstrual cycle. After the second transplantation (01/2011) the patient presented with with 3 normal menstrual cycles. FSH level was 44,2 U/l prior the second transplantation and dropped to 10,1 U/l 5 months later. AMH was not in a detectable range.
The patient with NHL received the ovarian biopsy at the age of 25 and retransplantation at age 29 and 30, respectively. In this patient, the second transplantation (05/2011) resulted in menstrual cycles and FSh dropped from 105.4 U/l prior transplantation to 16.1 U/l. AMH was also undetectable. To date this patient underwent oocyte retrievals in natural cycles but so far without gaining oocytes.
Conclusion: Ovarian biopsy followed by cryopresevration and subsequent retransplantation is a viable option for fertility protection in oncological patients prior to gonadotoxic chemo- or radiotherapy. A well coordinated interaction is required for a successful fertility protection programme.

P2 6-3

Identification of proNGF - NGF and their receptors in the ovary

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Neurotrophins play important roles in survival, differentiation and death of neuronal but also non-neuronal cells, including ovarian cells. At least in rat, the prototype neurotrophin nerve growth factor (NGF) promoted the development of preantral follicles and appears to be involved in the regulation of ovulation and ovarian angiogenesis mainly via activation of its TrkA receptor. Mature NGF readily binds to this tyrosine kinase receptor and to the p75 neurotrophin receptor ($p75^{NTR}$). The NGF precursor (proNGF), however, differs from NGF by its binding affinities and prefers the $p75^{NTR}$ and a co-receptor, sortilin. Secreted proNGF is cleaved by a matrix metalloproteinase (MMP7) into its mature form. While NGF was reported in human ovary and in follicular fluid (FF), the presence of proNGF is not examined. Hence we studied proNGF and NGF, MMP7 - $p75^{NTR}$/TrkA/sortilin expression in human and monkey ovary and in IVF-derived human FF and cultured human granulosa cells (hGCs). Immunohistochemistry (IHC) identified proNGF in granulosa cells and in FF in monkey and human antral follicles. ProNGF was also detected in FF derived from IVF-patients (Western Blot), indicating that proNGF is released from hGCs *in vivo*. MMP7, able to cleave proNGF and thus to generate NGF, was detected in monkey and human ovarian sections by IHC. MMP7 was also present in human FF and in cultured hGCs (Western Blot, RT-PCR). Furthermore, the proNGF/NGF receptors $p75^{NTR}$ and TrkA, as well as sortilin, the recently described proNGF receptor connected to cell death, were found (Western Blot, RT-PCR).
Taken together the results raise the possibility that changing concentrations of the ligands (proNGF/NGF) and/or changes of receptors ($p75^{NTR}$, TrkA, sortilin) may influence the fate of ovarian follicles. Studies to explore the impact of proNGF and NGF on survival and death of hGCs are under way.

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P2 6-4

Evidence of a phenotype switch of smooth muscle-like testicular peritubular cells in men with impaired spermatogenesis (mixed atrophy syndrome): Is male infertility a smooth muscle disease?

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The architecture of the wall of seminiferous tubules and the morphology of peritubular cells are frequently altered in men with impaired spermatogenesis. We hypothesized that alterations include contractile abilities of peritubular cells and examined proteins essential for contraction, myosin heavy chain 11 (MYH11) and calponin (Cal), as well as relaxation of smooth muscle cells, cGMP-dependent enzyme, protein kinase 1 (cGKI). A total of 17 testicular biopsies, 14 from patients with mixed atrophy (MA) and 3 from men with normal spermatogenesis were immunostained for MYH11, Cal and cGKI. Western blot/RT-PCR were used to verify their expression in cultured peritubular cells of 6 additional patients, 3 with normal and 3 with impaired spermatogenesis. Patients with normal spermatogenesis showed a homogenous staining pattern for MYH11, Cal and cGKI in smooth muscle-like cells of peritubular walls and in vascular smooth muscle cells. In patients with MA, peritubular MYH11, Cal and cGKI variied severly, in face of constantly immunopositive vascular smooth muscle cells, which are intrinsic positive controls. Tubules with a regular expression were next to ones with reduced or absent staining. In 6 of 14 patients examined and in 9 of 14 patients, peritubular MYH11 and cGKI, respectively, were undetectable. Peritubular Cal was faint or indiscernible in 5 of 7 patients studied. Western blot and/or RT-PCR demonstrated an expression of these markers in cultured peritubular cells. The proteins, MYH11, Cal and cGKI, in testicular peritubular myoid cells of patients with MA are clearly reduced or lost, implying a phenotypic switch or dedifferentiation of smooth muscle cells. The observed changes may be due to the *in vivo* microenvironment and may involve contractile disabilities which impair sperm transport and even aggravate infertility. Given that, peritubular cells and their regulators emerge as possible targets for novel therapeutic approaches.

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P2 6-5

Pigment Epithel Derived Factor (PEDF) - a novel player in the human testis

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Pigment Epithel Derived Factor (PEDF) is a pleiotrophic factor, which was previously described in the testis, yet the cells producing this factor and its possible role(s) remain unknown. In the course of ongoing proteomic analyses of human testicular peritubular cells (HTPCs), PEDF was identified as one of the most abundant secreted proteins. Using several biological replicates, synthesis by peritubular cells was verified by RT-PCR and Western blotting. ELISA measurements revealed that PEDF is constitutively secreted in large quantities (around 2 µg/ml/mg protein/24 h; n = 3) into the cell culture medium. Finally, immunohistochemistry showed immunoreactive peritubular cells in testicular biopsies, clearly indicating that they are a source of PEDF *in vivo*. The described actions of PEDF appear to depend on the cell type and include induction of apoptosis in some (endothelial), yet anti-apoptotic effect in other cells (neurons), as well as anti-angiogenetic and antioxidant actions. In part, they appear to be mediated by binding proteins (PNPLA2 and laminin-receptor). In an attempt to identify possible targets and actions in the human testis, immunohistochemistry was performed using PNPLA2-antibody. Leydig cells and germ cells but also peritubular cells were immunoreactive, implying that all compartments of the human testis are among the potential PEDF-targets. The only available cell type of the human testis that can be studied are HTPCs. Addition of recombinant PEDF increased MAPK phosphorylation in HTPCs after 1 h. On-line monitoring of reactive oxygen species (ROS), induced by H_2O_2 in the presence or absence of PEDF, indicated an antioxidant action. Interestingly, H_2O_2 also prevented the MAPK phosphorylation induced by PEDF. The results obtained identify PEDF as a novel, unexplored player in the human testis.

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P2 6-6

Distinct doses of estradiol-17β orally applied during pregnancy impact on gonads, weight and body composition in pig progeny

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Exposure to endocrine disrupting chemicals (EDC) during pregnancy may have a critical impact on growth and development of the offspring. To study adverse effects of low-dose estradiol-17β (E2), the pig was chosen as an animal model with placental estrogen production likewise present in humans. Three distinct concentrations of estradiol-17β (E2) were orally applied during pregnancy in sows (n=6 per group) representing doses of pharmacological relevance (1,000 µg/kgBW/d), the oral no-effect level (NOEL, 10 µg/kgBW/d) and the JECFA-recommended acceptable daily intake level (ADI, 0.05 µg/kgBW/d). In an initial pharmacokinetic study, 1,000 µg/kgBW/d caused a distinct elevation of endogenous E2 in male castrated pigs with a peak reaching maximal levels already 15 min after administration (77.3 ± 47.8 pg/ml plasma, n = 3). Significantly higher E2 concentrations were accordingly found in sows until day 70 of pregnancy (80.8 ± 27.9 pg/ml in 1,000 µg/kgBW/d vs. 24.4 ± 6.9 pg/ml in the control group, $p < 0.05$). Although there were no differences at birth, both male and female progeny revealed a decrease in body weight at weaning ($p < 0.05$) even with E2 administered at the ADI level (8.15 ± 0.27 kg vs. 8.69 ± 0.26 kg in controls, $p = 0.0002$). This trend was persistent until slaughter 8 weeks after birth. While no treatment effect was detected for ovaries, testis weight was significantly reduced in the pharmacological group (16.8 ± 1.1 g vs. 20.6 ± 0.6 g in the control). Male offspring where additionally affected in terms of whole body composition as determined by dual-energy X-ray absorptiometry scanning (DEXA), displaying 16.5 ± 0.8 % fat ($p = 0.002$) in the pharmacological and 15.5 ± 0.7 % ($p = 0.03$) in the NOEL treatment vs. 13.1 ± 0.4 % in the control group. In conclusion, similar to effects seen from EDCs, *in utero* exposure to E2 even at low doses seems to exert a programming effect on progeny leading to distinct physiological aberrations during postnatal development.

P2 6-7

Novel P450c17 (CYP17) mutation in exon 1 as a cause of male pseudohermaphroditism

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Introduction: 17-Hydroxylase/17,20-lyase deficiency (17OHD) is a rare autosomal recessive disorder resulting from mutations in the CYP17A1 gene, leading to impaired adrenal and gonadal steroidogenesis and congenital adrenal hyperplasia accompanied by hypertension and hypokalaemia.
Case Report: A 17-year-old female patient visited our Endocrine Outpatient Unit due to lack of breast development (Tanner stage I) and primary amenorrhoea. Her family history was unremarkable. She was the only child of non-consanguineous parents and was of Caucasian origin. Physical examination revealed an absence of axillary hair and hyperpigmentation and pubic hair Tanner stage II. The female external genitalia were normal. She was of 171 cm height, 51 kg body weight with elevated blood pressure up to 120/100 mmHg. Her karyotyp was 46, XY. Pelvic magnetic resonance imaging (MRI) demonstrated an absence of mullerian structures and bilateral masses resembling testes in the inguinal canals. Serum LH, FSH, ACTH and aldosterone were elevated; testosterone, estradiol, cortisol, DHEA, 17-hydroxyprogesterone and plasma renin levels were low. There was no response of cortisol or any androgens to the ACTH stimulation test by normal serum potassium, findings that were consistent with the diagnosis of 17-Hydroxylase/17,20-lyase deficiency (17OHD). After obtaining informed consent, genomic DNA was isolated and amplified. DNA sequencing of her CYP17A1 gene revealed that the patient was homozygous for a R96Q missense mutation, caused by an A to G transition that completely eliminated enzyme activity. Daily hormone replacement treatment with estradiol up to 4 mg and hydrocortisone 20 mg was initiated. The bilateral masses located in the inguinal canals were removed by laparoscopic surgery and the pathological examination confirmed that the surgically removed masses were testes.
Conclusions: We report for the first time a missense mutation at codon 96 (R96Q) of CYP17 causing male pseudohermaphroditism.

P2 7-1

Clinical spectrum of TSH-secreting pituitary tumors

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Background: Thyrotropin (TSH)-secreting pituitary tumors (TSH-oma) occur with a prevalence of about one in a million, representing less than one percent of all pituitary adenomas.
Methods: We report a series of six patients with TSH-producing pituitary tumors diagnosed within the last five years.
Results: Mean age of the patients was 49.2 years (range 11-73). The father of a child with TSH-oma was an index patient for multiple endocrine neoplasia type 1 (MEN 1). The child also had a mutated of menin gene (exon 10, codon 514, chromosome 10) and was diagnosed with primary hyperparathyroidism and TSH-oma. A 28 year old young man was incidentally diagnosed with hyperthyroidism with elevated levels of TSH in a routine check-up. Both patients had no clinical signs of hyperthyroidism. The remaining four patients had typical clinical signs of hyperthyroidism. All four patients underwent thyroid surgery before the diagnosis of a TSH-oma was made. In one patient radioiodine treatment was given twice in the situation of persistent hyperthyroidism. In all our patients elevated TSH levels (mean 8.5µU/ml, range 4.23-19.0) could be seen at first visit with normal or elevated values for free thyroxin or free triiodothyronine. MR Imaging (MRI) revealed pituitary macroadenomas. In three patients a preoperative therapy with octreotide could lower TSH levels and ameliorate symptoms of hyperthyroidism. Five patients were operated in one center by a MRI guided, ultrasound assisted procedure. In one patient elevated TSH levels could be documented after surgery due to infiltrating growth of the tumor and an unresectable part within the sinus cavernosus. This patient is aimed at stereotactic radiation therapy.
Conclusion: TSH-oma may be one feature of MEN 1 with onset in childhood. Not all patients present with typical signs of hyperthyroidism. Preoperative therapy with octreotide improves TSH values. Neurosurgical resection of the TSH-oma is recommended in almost all cases.

P2 7-2

Hypophysitis after ipilimumab: case reports

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Ipilimumab is the first medical therapy for metastatic melanoma, which results in improved survival. Since June 2011 it can be used as second line therapy in Germany. Due to its pharmacodynamic causing autoimmune effects patients also suffer from autoimmune disease of other organs. Most importantly, hypophysitis is found in up to 6% of patients in clinical trials. Here we present two patients with hypophysitis on ipilimumab therapy.
One 55 year old female patient was treated with ipilimumab within a compassionate use program for metastatic melanoma. After the third cycle she developed fever and headache. MRI and laboratory evaluation revealed a typical hypophysitis with gonadotrope, lactotrope and thyreotrope insufficiency. The second patient a 73 year old man was treated for metastatic prostate cancer. After the second infusion of ipilimumab he presented with Addison crisis in our emergency department. Laboratory evaluation presented corticotrope and somatotrope insufficiency. Hypophysitis after ipilimumab presents a substantial risk in patients treated for metastatic cancer. Though the clinical picture presents with a variety of symptoms and can be life threatening, if corticotrope insufficiency develops, treating physicians (e.g. dermatologists, oncologists) should be aware of this adverse effect. Substitution therapy of hormonal deficiencies resolves almost all clinical symptoms. In our view immunosuppressive therapy in the treatment of the ipilimumab-induced hypophysitis in the absence of symptoms of local invasive disease should be discussed with the patient. Though regression of the metastatic cancer is clearly related to autoimmune mediated events caused by ipilimumab, high dose corticosteroid pharmacotherapy for hypophysitis may cause more harm than advantage.

P2 7-3

The development of the Tuebingen Cushing's disease quality of life inventory (Tuebingen CD-25). Construction, psychometric properties and normative data from a healthy population

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Objective: In part I of the study a health related quality of life (HRQoL) inventory for Cushing's disease (CD), the Tuebingen Cushing-25 quality of life inventory (Tuebingen CD-25) was developed. In the second part, we assessed normative data from healthy controls (HC) with which the individual patients' scores can be compared.
Methods: Sources for item generation consisted of technical literature, interviews with patients and the rating of neurosurgeons, endocrinologists and a neuropsychologist. A preliminary inventory with 64 items was handed out to 63 CD patients. Item reduction and scale generation followed the principles of Classical Test Theory. Validation was performed with the WHOQoL-BREF. For assessing normative data, the inventory was filled out by 1784 HC omitting the introductory sentence “Because of my Cushing's disease” that was included in the CD group.
Results: The final version of the Tuebingen CD-25 contained 25 items, showed high reliability (Cronbach's alpha = 0.93) and validity (r = -0.65) and includes the subdomains Depression, Sexual Activity, Environment, Eating Behavior, Bodily Restrictions and Cognition. We found a non-linear correlation between the Tuebingen CD-25 scores and patients' age, younger and middle-aged patients having inferior HRQoL than patients between 31 and 50 years and older than 61 years. Preoperative 24 h UFC levels correlated significantly with the subscale Cognition and only marginally failed significance level for the subscale Eating Behavior, while preoperative cortisol and ACTH levels did not correlate with any scale. In 28.6 % of our CD patients we found slight and in 41.3 % severe impairment in the Total Score of the Tuebingen CD-25 compared to HC. Less than one-third of our patient sample presented with unimpaired HRQoL.
Conclusion: The Tuebingen CD-25 is a feasible instrument to assess HRQoL in CD in a clinical and investigative setting and provides normative data for all age groups and genders.

P2 7-4

Comparison of body weight, physical activity and quality of life of patients following traumatic brain injury or subarachnoidal haemorrhage with hypopituitarism before and after substitution of the impaired axis and with normal pituitary function

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Objective: The prevalence of hypopituitarism following traumatic brain injury (TBI) or subarachnoidal haemorrhage (SAH) is reported between 15-55%. We analysed body weight (BW), physical activity (PA) and quality of life (QoL) of patients following TBI/SAH in relation to development and treatment of hypopituitarism.
Method: We diagnosed hypopituitarism in 10 patients (1 female, 9 men) of our centre (mean age 49y) who experienced a moderate or severe TBI(n=6)/SAH(n=4) in the previous 6 months by pathological basal values and dynamic testing (GHRH+ARG, ACTH, TRH or GnRH- tests) and startet a substitution therapy. The gonadotropic axis was substituted in 5, corticotropic in 3, thyreotropic in 3 and somatotropic in 2 patients for at least 4 months. Before starting and during substitution of the axis PA was assessed by pedometer and QoL was evaluated with the Nottingham Health Profile (NHP) questionnaire. We compared the results to 10 sex and aged matched patients of our centre following TBI(n=6)/SAH(n=4) without hypopituitarism.
Results: In comparison to patients with normal pituitary function those with hypopituitarism presented with a higher mean BMI of 4 kg/m² (p=0,043), had gained on average 5 kg BW (p=0,005) after the event and showed an average step count of less than 1750 steps (p=0,044). The QoL of patients with hypopituitarism is significantly impaired in the NHP subscale “social isolation” (p=0,036) and “sleep” (p=0,028). These subscales were significantly enhanced by substitution of the insufficient axis (p=0,044;p=0,041). The subscale “physical activity” and “sleep” was significantly increased by GH substitution in the GH deficient (p=0.0001) and the subscale “social isolation” by testosterone or estrogen substitution in (p=0,02) patients with secondary hypogonadism.
Conclusion: Patients with pituitary dysfunction gained more BW, presented with a lower PA and an impairment of QoL since the TBI/SAH. Subsitution of the impaired axis enhanced QoL.

P2 7-6

Sex-specific associations of serum prolactin concentrations with cardiac remodeling: Longitudinal results from the Study of Health Pomerania (SHIP)

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Background: Previous experimental and patient-based studies suggest that prolactin (PRL) and its 16 kDa fragment influence cardiovascular phenotypes by modulating angiogenesis. The association between serum PRL and cardiac remodeling in the general population is unknown.
Methods: We evaluated 804 individuals (441 women) from the population-based Study of Health in Pomerania, aged ≥45 years, with available baseline serum PRL who underwent serial echocardiography at baseline and at five-years of follow-up. Left ventricular mass (LVM) was calculated and left ventricular hypertrophy (LVH) defined by sex-specific distributions of LVM. LV geometry was defined on the basis of relative wall thickness (RWT) and LVH. Sex-specific multivariable regression analyses were performed relating PRL (independent variable modelled as a continuous variable and as sex-specific quartiles) to change in LVM, RWT, and to incident LVH and abnormal geometry.
Results: Baseline PRL concentrations were inversely associated with LVM change in men, but not in women (ß per 10% decrease in PRL: 0.37; 95% CI, 0.13 to 0.60 in men and -0.02; 95% CI, -0.21 to 0.17 in women, respectively). In men, baseline PRL concentrations were also inversely associated with incident LVH [first vs. fourth PRL quartile: relative risk (RR) 2.26 (95% CI, 1.20 to 4.24)] and altered LV geometry on follow-up [RR for incident concentric hypertrophy per 10% decrease in PRL: 1.20 (95% CI, 1.06 to 1.37)]. None of the longitudinal associations were observed in women.
Conclusion: We observed an inverse association of serum PRL with LVM change, incident LVH, and altered LV geometry in men but not in women. Additional studies are warranted to confirm our findings and to elucidate the mechanisms underlying these sex-related differences.

P2 7-7

Diagnosis of Cushing´s syndrome by automatic face classification - preliminary data

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Introduction: Cushing´s syndrome is a disease that presents with clear symptoms and causes considerable harm to the body if left untreated, yet often remains undiagnosed for prolonged periods of time. Face-classification software might recognize typical changes of the face and thus aid in diagnosing the disease early.
Methods: Using a regular compact digital camera, we took pictures 21 female patients with Cushing´s syndrome (14 endogenous, 7 iatrogenic) and of 21 age- and sex-matched controls.
Nodes were then placed on disease-relevant structures of the face to analyze the pictures using computerized similarity analysis based on Gabor-jets and geometry functions. The leave-one-out cross-validation method was employed to classify subjects by the software.
Results: Using a combination of Gabor-jets and geometry functions, 85.7% of patients and 66.7% of controls were correctly classified by the software. This resulted in a total classification accuracy of 76.2%.
Conclusions: In this preliminary small-scale analysis we found a moderate classification accuracy of Cushing´s syndrome by face-classification software. We expect that classification accuracy will improve with higher participant numbers and more in-depth analysis.

P2 7-8

Hypothalamic expression profiling of Trace amine-associated receptors 1 and 5

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Trace amine-associated receptors (Taar's) belong to the group of rhodopsin like G-protein coupled receptors. One intriguing feature of Taar's is their high ligand promiscuity as they can be activated by neurotransmitters, psycho-active drugs, volatile amines or trace amines. Additionally, the thyroid hormone derivative 3-iodothyronamine (T1AM) is an agonist for Taar1. Interestingly, T1AM injection in rodents results in decreased heart rate and lower body temperature, which is counterintuitive considering the traditional model of thyroid hormone action. Targeted gene disruption of Taar1 in mice produces among others no significant changes in body weight, body temperature or state of health. Therefore one could speculate that another receptor most probably from the same receptor class may take over Taar1 function. One possible candidate could be Taar5 which is highly conserved across species. To test the hypothesis that Taar's may be involved in the CNS control of systemic metabolism, food intake and body weight, we started by investigating the hypothalamic expression pattern of Taar1 and 5. We used in situ hybridization with double digoxigenin labelled locked nucleic acid (LNA) probes on mouse brain sections to achieve high specificity and sensitivity, with minimal risk for genomic DNA contamination. Our results revealed overlapping Taar1 and Taar5 expression patterns in the hypothalamic nuclei arcuate nucleus (ARC) and ventromedial nucleus (VMH). Notably, Taar5 showed higher hybridisation signals in these brain areas than Taar1. We conclude based on these preliminary neuroanatomical findings that further investigations into a possible role of Taar1 and Taar5 in the neuroendocrine control of systemic metabolism are justified.

P2 7-9

A retrospective follow-up study of 33 patients with central nervous system germinoma

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Introduction: CNS germinoma is a very rare, malignant germ cell tumour, localized along the midline; mainly the pitutary and the pineal gland, that predominantly occurs in adolescents (mean age 16). It only accounts for approximately 1-4% of all intracranial tumors.
Methods: A retrospective data analysis of 33 out of 39 patients diagnosed with cns germinoma over a time period of 23 years, focusing on treatment results over a course of 5-10 years as well as the patients prognosis depending on the diagnostic process (biopsy vs. tumour resection) considering clinical state and quality of life. We used the EORTC QLQ C30 questionnaire as well as the Modified Rankin Scale.
Results: At follow up six patients had already deceased since first being diagnosed. The 5-year survival and 10-year survival was 86.2% and 82.1%. 32 patients were teated via adjuvant cranial radiotherapy, 16 of these patients also received radiotherapy of the spinal system and 5 patients of the cervical spine. One patient was treated with radiotherapy 2 months after the diagnosis. 11 patients were treated with chemotherapy right after the initial diagnosis, in two cases chemotherapy was performed in case of recurrent disease. As far as the diagnostic means tumour biopsy (n=14) versus tumour resection (n=17) are concerned, the group of patients that was diagnosed via stereotactic biopsy shows a slightly better outcome regarding mortality and quality of life.
Conclusion: We found a strong connection between radiotherapy and neurocognitive impairement. With adequate treatment including radiotherapy, cns germinoma has a good prognosis. By means of individualized therapy, it should therefore be attempted to balance the radiation dose, keeping it as low as possible without provoking relapses. The diagnostic choice between biopsy, partial or complete resection of the tumour should be made individually according to localisation and morphology of the tumour as well as operative finding.

Verschiedenes

P2 8-1

Polyhydroxylated C60 fullerenes may be used for diverse ovarian applications

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Introduction: Lately, various nanomaterials have raised interest in numerous potential biomedical applications. C60 fullerenes which consist of 60 carbon atoms in the shape of a football are considered for imaging probes or drug carriers. However, since they are extremely hydrophobic it is difficult to study their activities in biological systems. Therefore, hydrophilic groups are attached to the carbon frame resulting in water-soluble C60 fullerenes. The aim was to determine if fullerenes may also be used in ovarian applications by examining the interleukin-1β (IL-1β), -6 (IL-6), and tumor necrosis factor α (TNFα) production in cultured granulosa cells.
Methods: Purified human granulosa cells were seeded in 24-well plates and cultured either with 25, 50, 100, or 400 µg C60 fullerenes, respectively. The fullerenes were first dissolved in toluene and then polyhydroxylated by sodium and tetrabutylammonium hydroxide. After four days incubation the cytokines were quantified in the culture supernatant by enzyme-linked immunosorbent assay (ELISA) and compared between fullerene-treated and untreated cells.
Results: As shown by ELISA assays granulosa cells secreted around 20-fold more IL-6 and TNFα than IL-1β into the surrounding culture medium. Cells incubated with 25, 50, or 100 µg polyhydroxylated C60 fullerenes produced approximately the same or only slightly higher amounts of all three cytokines like the controls. However, cell treatment with 400 µg fullerenes resulted in significant IL-1β and IL-6 increases of 119 % whereas TNFα showed a significant increase of 84 % in comparison to untreated cells. These results are supported by current experiments revealing the extent of DNA damage in correlation with the amount of fullerenes used.
Conclusions: Our results show that C60 fullerenes do not significantly influence the IL-1β, IL-6, and TNFα production of granulosa cells up to the amount of 100 µg and are therefore recommended for use in diverse ovarian applications.

P2 8-2

Preliminary studies for a future use of C_{60} fullerenes in the treatment of benign ovarian tumors

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Introduction: For some time research has been conducted on various applications of nanoparticles in medicine. This includes the C_{60} fullerenes, whose 60 carbon atoms form a cage structure in the shape of a football. This cavity can be used to transport small molecules such as drugs to the desired site of effect. The aim of this project was to investigate C_{60} fullerenes for future use in the treatment of benign ovarian tumors. To these means we chose to analyze their influence on the production of IL 1β (interleukin 1β), IL 6 and TNF α (tumor necrosis factor α) in cultured granulosa cells as well as their genotoxic effects on these cells.
Methods: Due to their lipophilicity, the C_{60} fullerenes were first sonicated in solvents (methanol or toluene, respectively) to adapt them to an aqueous environment before incubating them with purified human granulosa cells. Both methods were performed with different amounts of fullerenes (25 µg, 100 µg and 400 µg). After a 4-day incubation period, IL 1β, IL 6 and TNF α were quantified in the cell culture supernatants and the damage to the granulosa cell DNA was assessed by comet assay.
Results: The cytokine concentrations found depended on the fullerene quantity as well as on the solvent employed. The fullerenes treated with methanol showed a significant 4.5- and 4.2-fold increase of IL 1β and IL 6 concentrations, respectively, at a fullerene quantity of 400 µg. TNF α concentration was significantly 1.4-fold increased even at 100 µg fullerenes. The fullerenes solved with toluene led to a significant increase of 50-80 % in all three cytokines at 100 µg. Comet assay analysis also showed significant differences in DNA damage depending on the concentration of the fullerenes.
Conclusions: The results show that 25 µg of fullerenes do not negatively influence the assessed cell parameters and thus can be considered for future treatment of benign ovarian tumors.

P2 8-3

Influence of bleeding disorders on recurrent miscarriage ROTEM®- and Multiplate® analysis - a new approach?

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Introduction: Pregnancy is a hypercoaguable state. In the same way some cases of recurrent miscarriage have a thrombotic basis. The purpose of this study was to discover possible differences in blood clotting as assessed by thromboelastometry (ROTEM®) and whole blood aggregometry (Multiplate®).
Patients and methods: Three groups have been analyzed, 20 pregnant women, 43 women with recurrent pregnancy loss (RPL) and 20 healthy, non-pregnant women. ROTEM® was performed in 300µl of citrated whole blood with ROTEM® delta. Extrinsic coagulation cascade was studied with EXTEM test, intrinsic coagulation cascade was studied with INTEM test and the influence of fibrinogen on clot firmness was estimated with a platelet-inactivating test. The recorded ROTEM® parameters were in (1.) EXTEM test: clotting time (CT), clot formation time (CFT) and maximal clot firmness (MCF), (2.) INTEM test: CT, CFT, MCF and (3.) FIBTEM test: MCF. Multiplate® analysis was performed in 300µl of hirudin anticoagulated whole blood with 20µl of thrombin receptor agonist peptide-6 (TRAP test), arachidonic acid (ASPI test), collagen (COL test) and adenosine diphosphate (ADP test) on the Multiplate® whole blood impedance aggregometer. Three parameters were calculated: (1.) The area under the aggregation curve (AUC), (2.) the aggregation and (3.) the velocity as the maximum slope of the curve.
Results: In ROTEM® pregnant women had in INTEM® and EXTEM® a significant lower CT and CFT and higher MCF. In FIBTEM® MCF was also significant higher (p< 0,001***). In contrast women with RPL had, compared to the control group, a higher EXTEM® CFT (p=0,004***) and lower EXTEM® MCF (p=0,016*). Comparing pregnant women vs. the control group and vs. women with RPL no differences in Mulitplate® have been found.
Conclusions: ROTEM® is able to detect states of hypercoagulability in pregnancy. Contrariwise, in women with RPL coagulation parameters have shown to be affected in a rather hemophilic manner.

P2 8-4

Evaluation of LC-MS/MS for the determination of serum steroids in clinical routine analysis

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LC-MS/MS is used in endocrine testing due to its simultaneous multi-analyte quantitation capability and specificity superior to that of immunoassays (IA). We investigated potential limitations of LC-MS/MS that have become apparent when using it in a clinical routine lab.
A one-line SPE-LC-MS/QTrap platform utilizing atmospheric pressure chemical ionization (APCI) was used to quantify androstendione (ASD), testosterone (T), progesterone (P), cortisol (F), 17-OHP, 11-deoxycortisol, DHEA-S, estradiol (E2) and aldosterone (A) to evaluate its suitability for routine application (Ceglarek et al. 2009). Following diagnostic by IA, 537 serum samples from a university central lab were remeasured by LC-MS/MS. Control sera were sent for comparison to three laboratories using LC-MS/MS or GC-MS/MS.
Total runtime for one sample was 4 minutes. Careful serum preparation and a maximum of 45 samples per run were required to ensure good analytical results. Using APCI, most steroids were detectable in positive ion mode besides E2 and A which were measured in negative ion mode. Both ion modes had to be run in consecutive analyses which doubled throughput time. Detection limits of A and E2 (0.1µg/L; 0.04 µg/L) were within the low physiological concentration range, allowing only the differentiation between normal and increased hormone levels. Excellent correlations with IA were obtained for ASD, T, F and DHEA-S (r = 0.96-0.98). Correlations for P and 17-OHP were r = 0.88; 0.81. For 17-OHP, carry-over effects were observed when using the same mass spectrometer to measure not only steroid but also other substances. Inter laboratory comparison revealed significant differences between laboratories except for DHEA-S and F.
LC-MS/MS is a high-quality measurement system for the determination of steroid patterns. However, lacking commercially available standards and highly manual workflows combined with complex work and maintenance procedures still limit the routine application of LC-MS/MS.

P2 8-5

Analysis of different blood sample pre-treatment conditions on hormone concentrations in rats

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Measurement of several hormones requires sample pre-treatment by addition of protease inhibitors (Pi). Since blood volume is limited in rodents, we studied if Pi treated sample remnants can also be used for measuring analytes not necessarily requiring addition of Pi. We also studied if use of serum or plasma, or repeated freezing/thawing (f/t) affects specific hormone measurements.
Methods: Wistar rats (n=9) were sacrificed and trunk blood was collected. Six pre-analytical conditions were tested: 1. serum (rtS) and 2. plasma at 22°C (rtP), 3. chilled plasma (cP), 4. cP + Pi (complete, Roche, Switzerland), 5. cP + Pi and DPP-4 inhibitor (Millipore, USA), 6. rtS after ten f/t cycles (f/tS). Samples were stored at -80°C until analysis. GH, IGF-II, IGFBP-2 and -3, leptin (Mediagnost, Germany), total ghrelin and GIP (Millipore), insulin (Alpco, USA) and IGF-I (IDS, UK) were measured by immunoassay. Hormone concentrations in rtS have been set to 100% and f/tS, rtP and cP are expressed as a percentage thereof. Statistical comparison was done by ANOVA (*p< 0.05, **p< 0.01, ***p< 0.001).
Results: After correction for dilution, addition of Pi did not significantly affect hormone concentrations when compared to cP. Relative recovery in rtP, cP or f/tS in relation to rtS are shown in the table.

	ftS in % of rtS	rtP in % of rtS	cP in % of rtS
GH	97.6±1.7	237.8±36.2**	231.0±38.9**
IGF-I	103.6±2.7	90.8±1.7**	88.0±1.2***
IGF-II	125.9±7.7*	76.0±3.7*	87.4±8.3
IGFBP-2	97.6±5.6	110.5±7.4	106.1±6.0
IGFBP-3	119.3±3.8***	76.0±3.5***	75.5±2.6***
insulin	70.7±7.2	200.9±81.4	191.5±81.1
total ghrelin	75.4±4.1*	110.8±6.3	120.3±8.5*
leptin	148.3±7.0***	47.3±4.0***	42.2±2.6***
total GIP	50.2±6.8**	110.4±11.5	126.0±13.6

[table]

Conclusion: Repeated f/t as well as use of serum instead of plasma had a significant effect on hormone concentrations. In contrast, addition of different Pi did not affect the analyses.

P2 8-6

Activation of GPR83 by mutations and zinc-ions

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GPR83, also known as JP05 or GPR72 is an orphan G-protein coupled receptor (GPCR) which is highly expressed in thymus and brain. Within the brain GPR83-mRNA could especially be identified at high levels in some hypothalamic nuclei that are known to affect various endocrine functions.
Therefore GPR83 could have an impact on a variety of physiological processes. Under discussion are interventions of emotional responses, temperature regulation, control of food or water intake, reproduction as well as control of daily cycles. In conclusion, GPR83 should be a key-player for several unknown mechanisms and might also represent a new candidate for pharmacological interventions in treatment of endocrine disorders.
Characterization of the signaling pathway mediated by a certain GPCR is an important step in deorphanization. In this study analysis of cAMP and IP_3 accumulation *in vitro* revealed no basal activity of GPR83 in Gs and Gi but significant basal activity in Gq/11 signaling.
Due to unknown physiological ligands we tested potential constitutively active mutants and confirmed the pathway.
It is known that especially in nerve tissue different cell-surface proteins, as neurotransmitter transporter, ion channels and GPCRs are affected by zinc- or other ions. Some neural expressed G-protein coupled receptors can be activated and/or potentiated by these ions, whereas others are inhibited in binding properties and/or activation.
We could demonstrate that also GPR83 is stimulated by zinc-ions. In addition, characterization of specific mutations at Zn^{2+} sensitive amino acid residues revealed potentially two binding sites for zinc-ions between the extracellular loops and ends of the transmembrane helices.
At the moment it is still unclear whether zinc-ions are the endogenous ligands for GPR83, or if zinc-binding is a modulator of further ligands which are unknown so far.
Here we present first insights into GPR83 mediated signaling.

P2 8-7

The measurement of steroids in Polycystic Ovarian Syndrome (PCOS) queries is improved using Ultra-Performance Liquid Chromatography/ Mass Spectrometry (UPLC/MSMS), compared to Radioimmuno assay (RIA)

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Steroids are frequently measured to determine disorders characterised by enzyme deficiencies. The measurement of these steroids has been reported using immunoassay techniques, and is often regarded as unsatisfactory, as they can suffer from systematic and random biases resulting from cross-reactivity. UPLC/MSMS has been used in clinical laboratories for several years, and this technique selects analytes based on their polarity (retention time), their molecular mass, and the molecular mass of a diagnostic fragment ion. Therefore, UPLC/MSMS is a highly selective technique which does not suffer from cross-reactivity in the same way as immunoassay, and has very little interference, resulting in accurate, precise results when measuring patients' samples.
We report a method which provides greater efficiency, measuring 5 steroids, which can be used to aid the diagnosis of PCOS. This method makes use of the greater selectivity and multiplexing capability of UPLC/MSMS. The steroids measured were 17α-hydroxyprogesterone (17-OHP), testosterone, androstendione (A4), 11-deoxycortisol (11-DOC) and Dehydroepiandrosterone sulfate (DHEAS). Multiplexing allows for reduced ambiguity, supplementary test requests and cost.
This work compares data acquired using RIA methods with that acquired using UPLC/MSMS, and whilst the correlations are good (typically $r^2 > 0.90$, there are biases observed between the methods, which may be explained in the context of antibody cross-reactivity (i.e RIA results are mostly higher than UPLC/MSMS results). In this preliminary study, the RIA results from 67 A4, 88 DHEAS, 56 17-OHP and 124 female testosterone samples were compared with those subsequently measured by UPLC/MSMS.

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Ghrelin levels in fasting subjects

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Introduction: The neuropeptide ghrelin causes appetite and increases food intake in humans and other species. In humans plasma ghrelin levels have been shown to rise with regular food intake and decline postprandial. Until now it is unclear whether the ghrelin peak depends on nutritional factors or on cephalic mechanisms.
Methods: We investigated ghrelin levels during fasting in 8 young normal male subjects. Subjects were fasting from 2100 until 1300 the next day. Plasma concentrations of ghrelin were collected between 0815 and 1300 every 15 min via long catheder.
Results: We did not observe a mealtime associated rise and fall of ghrelin in our subjects. This result is in contrast with a study showing an increase and decrease of ghrelin levels before and after customary mealtimes (Natalucci et al., 2005).
Conclusion: Our data suggest that meal related changes of ghrelin levels are independent from a circadian rhythm as they are absent after an overnight fast.

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Association between serum insulin-like growth factor I or IGF-binding protein 3 and estimated glomerular filtration rate

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Background: Insulin-like growth factor I (IGF-I), which is mostly carried in blood by IGF-binding protein 3 (IGFBP-3), is involved in the regulation of growth and cellular proliferation and promotes renal plasma flow in human. A multiethnic study among US adults revealed an association between serum IGF-I concentrations and glomerular filtration rate (GFR) or chronic kidney disease (CKD). The aim of the present study was to investigate whether serum IGF-I or IGFBP-3 concentrations are associated with estimated GFR (eGFR) in a population-based study of Caucasian adults.
Methods: Data from 4028 subjects (2048 women) aged 20 to 81 years from the Study of Health in Pomerania (SHIP) were analyzed. Total serum IGF-I and IGFBP-3 concentrations were determined by chemiluminescence immunoassays and categorized into quartiles. The four-variable Modification of Diet in Renal Disease (MDRD) study equation was used to calculate eGFR from serum creatinine.
Results: After adjusting for age, waist circumference and type 2 diabetes mellitus, analysis of variance revealed inverse associations between serum IGF-I concentrations and eGFR in men as well as between serum IGFBP-3 concentrations and eGFR in men and women. Logistic regression analyses confirmed these findings and showed that high IGF-I or IGFBP-3 concentrations were associated with an increased risk of decreased eGFR (< 60 mL/min/1.73m^2) in both sexes. These relations became stronger when lower cut-offs were used to define decreased eGFR. Using serum IGF-I or IGFBP-3 concentrations as continuous variables, we observed associations between increasing concentrations of IGF-I or IGFBP-3 and CKD.
Summary: Our data show an association of increased serum IGF-I concentrations with decreased eGFR in men but not in women and an association of increased serum IGFBP-3 concentrations with decreased eGFR in both sexes.

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Effects of a chronic dietary genistein exposure on the uterine myometrium of prepubertal Wistar rats

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An *in utero* started and continuously maintained exposure towards soy-derived isoflavones (sISO) has different implications on the juvenile and adult rat uterus. To clarify the effects of a prenatally started and continuously sustained sISO exposure on the uterus of prepubertal female Wistar rats, we conducted an animal experiment in an intergenerational setting. Breeding pairs were allocated to one of three different feeding groups. One group received a phytoestrogen-free diet (PE-free), one a soy-derived isoflavone-high diet (sISO-high) and one obtained a custom-made PE-free diet supplemented with 700 ppm genistein (GEN-supple). The female offspring was objected to an immature uterotrophic assay, comparing the responsiveness to 17β-estradiol, GEN and two estrogen receptor subtype specific agonists.

We assessed physiological parameter like the uterine wet weight, the luminal epithelial height, the myometrial thickness in comparison to sISO plasma level. Additionally, we evaluated molecular marker for proliferation and estrogenicity as well as the expression level of relevant steroid receptors.

In comparison to PE-free and sISO-high nourished females, the ones that have been chronically exposed to the GEN-supple diet not only had significantly increased luminal epithelial heights but also higher myometrial thicknesses. Furthermore, we revealed that the myometrial response towards a chronic GEN treatment was more sensitive than that of the luminal epithelium, which might result from the nonuniform distribution of relevant steroid receptors, especially the PGR.

In conclusion, although the impact of a continuous, prenatally initiated exposure to dietary sISO on the uterine physiology of juvenile females is small, the possible priming effects of this exposure for beneficial or adverse late-onset consequences in adults cannot be excluded.