S = contribution of a student

01 S

In preschool children, physical activity is associated with body fat but not with BMI

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Background/Introduction
Physical activity has been found to have an impact on body composition in older children. However, evidence on the association of physical activity with body composition in young children is still scarce and controversial. The purpose of this study was to determine the association of physical activity with body composition in 2-6 –year old pre-schoolers.

Methods
477 preschool children (Mean age 3.88 yrs ± 0.68; 252 boys and 225 girls) participated in a national cohort study. Physical activity was measured by using accelerometers which were worn at least 10 h/day over at least 4 days/week. Analyses were performed using the cut-offs of Pate 2006 to define sedentary behaviour, light, and moderate-and-vigorous intensities of physical activity. Measures of body composition included BMI, waist circumference and skinfold thickness (sum of four skinfolds).

Results
In the current study, 9% of pre-schoolers were overweight, while 2% were classified as obese (IOTF criteria). Mixed model analyses showed that skinfold thickness was inversely related to all measures of PA and positively to sedentary behaviour, also after adjusting for age. There was no moderation by gender. Physical activity was not significantly associated with body mass index nor with waist circumference.

Conclusion
In preschool children and in the context of a low obesity prevalence, physical activity is associated with body fat, but not with BMI. For this population, skinfold thickness or other measures of body fat are needed, as BMI does not adequately reflect body composition and/or the impact of physical activity physical activity.

02

Differences in metabolic response to co-ingestion of fructose and glucose vs glucose alone during prolonged exercise in individuals with type 1 diabetes

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Background/Introduction
Physical activity provides many health benefits to individuals with type 1 diabetes mellitus (T1DM) but poses high demands with regard to blood glucose control. Adaptation of insulin therapy and/or ingestion of carbohydrates (CHO) are generally recommended to avoid exercise-related hypoglycemia. However, there is a paucity of data on the impact of different CHO types on exercise-associated blood glucose and fuel metabolism. While fructose has been shown to be associated with adverse metabolic outcomes in sedentary individuals, studies in non-diabetic athletes have not confirmed these concerns and have even suggested fructose to be ergogenic. The aim of the present study was to investigate the effect of fructose co-ingested with glucose compared to glucose alone on the metabolic and hormonal response of exercising individuals with T1DM without prior insulin reduction.

Methods
Eleven individuals with well-controlled T1DM (aged 26±4y, diabetes for 14±7y, HbA1C 7.0±0.6%, body weight 82±11g, VO2max 47.0±7.0ml [kg_bw]-1 min-1) were randomly assigned to a 90 min cycling session at 50% VO2max with regular ingestion of a 1:1 mixture of glucose and fructose (GLUFRU) or glucose (GLU) alone. CHO supply was based on regular blood glucose measurements with the aim of keeping patients euglycemic during exercise following a pre-specified algorithm. Hormones, metabolites and substrate oxidation were measured at regular intervals. Exercise-induced glycogen consumption in liver and skeletal muscle was assessed using 13C magnetic resonance spectroscopy (MRS). Glucose and fructose kinetics were investigated by means of stable isotopes.

Results
Blood glucose (GLUFRU 7.9±0.3mM; GLU 7.7±0.3mM, p=0.7) and insulin levels (20.5±0.2 and 20.7±0.2 mU/l, p=1.0) were comparable between interventions. The total amount of ingested CHO immediately before and during exercise was similar (31±3 and 34±4 g, p=0.49). Counterregulatory hormones did not differ between interventions. Lactate levels were significantly higher in GLUFRU (2.5±0.2 and 2.1±0.2mM, p=0.02). Rates of glucose appearance (Ra) and disappearance were comparable. In GLUFRU gluconeogenesis from fructose substantially contributed to Ra. Consumption of myocellular glycogen during exercise was comparable between GLUFRU and GLU (-41.9±3.5 and -35.5±4.8%, p=0.39). Post-exercise hepatic glycogen was reduced by 19.1±11.2% in GLUFRU and 28.7±8.2% in GLU (p=0.47). Fat oxidation was higher (5.6±0.3 vs 2.5±0.2 mg/kg*min) and CHO oxidation (16.9±1.0 vs 23.9±0.9 mg/kg*min) was lower in GLUFRU when compared to GLU (p<0.001 for both).

Conclusion
Co-ingestion of fructose and glucose in exercising individuals with T1DM without prior insulin reduction increased fat oxidation when compared to glucose alone. The different metabolic role of fructose compared to glucose appears to induce a shift in fuel metabolism towards beta oxidation and a potential sparing of liver glycogen. In conclusion, fructose may provide a means to promote lipid oxidation in exercising individuals with T1DM even if pre-exercise insulin is not adapted.

03

Impact of different exercise modalities on nocturnal post-exercise glucose levels in indivuals with type 1 diabetes

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Background/Introduction
Prolonged continuous moderate intensity exercise (CONT) is associated with an increased risk of hypoglycemia in individuals with type 1 diabetes mellitus (T1DM). Intermittent high-intensity exercise (IHE) has recently been suggested to reduce the risk of exercise-related hypoglycemia by stimulating counter-regulatory hormones. Whereas previous studies consistently report a glucose-stabilizing effect of IHE during exercise, there are concerns regarding a potentially increased risk of post-exercise hypoglycemia after IHE. The aim of this study was to investigate the effect of IHE and CONT on post-exercise glucose levels in individuals with T1DM.

Methods
Twelve young male adults with well controlled T1DM (mean±SEM diabetes duration14±6y, age 26±4 y, Hb A1c 7.0±0.6%) underwent a 90 min cycling session at 50%VO2max with (IHE) or without (CONT) 10 s all-out sprints every 10 min in a random order. Exercise performance was scheduled in the postabsorptive state (5 h after a standardized breakfast, start of exercise at 12am). Post-exercise continuous glucose monitoring (CGM) data were collected between 8pm to 6am. Hypoglycemia was defined as glucose<3.5mmol/l, hyperglycemia was defined as glucose>12.0mmol/l. Glucose variability was assessed using SD, CV and MAGE. Statistical analysis of glycemia was performed by comparing areas under the curve (AUC) of post-exercise CGM data.

Results
Mean±SEM post-exercise glucose levels were lower in IHE (7.85±0.66mmol/l) compared to CONT (8.80±0.64 mmol/l) (p-value for AUC=0.04). The average number of post-exercise hypoglycemic events per patient was 1.2±0.4 for IHE and 0.6±0.3 for CONT (p=0.39). For both interventions 64% of hypoglycemic values were between 3.0 and 3.5 mmol/l. CGM readings <3.0 mmol/l (36% of hypoglycemic values) occurred in 3 patients following either intervention. Hyperglycemic events tended to be less frequent in IHE compared to CONT (0.8±0.3 vs 1.4±0.4, p=0.09). The average time spent in hypoglycemia (IHE 20±9 vs CONT 10±6 min, p=0.52) and hyperglycemia (IHE 107±40 vs CONT 106±35 min, p=0.87) was similar. After IHE, significantly more CGM values were in the range between 4.1 and 7.0 mmol/l (45% vs 28%, p=0.01). Glucose variability did not differ between the interventions. Post-exercise carbohydrate intake was 233±15g after IHE and 212±15g after CONT (p=0.06). Cumulative post-exercise insulin dose did not differ between the trials (IHE: 44±2 U, CONT: 43±3 U, p=0.56).

Conclusion
The present study does not suggest an increased risk of post-exercise hypoglycemia related to IHE. Post-exercise hypoglycemia after both IHE and CONT were generally mild and could rapidly be corrected by the individuals due to availability of CGM to the participants. In conclusion, IHE if performed under CGM surveillance appears to be a safe exercise modality for individuals with T1DM.

04

Response of MCP-1 and IL-6 to intermittent high-intensity and continuous moderate-intensity exercise in individuals with type 1 diabetes

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Background/Introduction
The performance of physical exercise imposes high demands on patients with type 1 diabetes mellitus (T1DM) due to complex and dynamic consequences on blood glucose levels. Homeostasis of carbohydrate (CHO) metabolism is crucial for exercising patients with T1DM, however regulatory mechanisms are still ill defined. Apart from hormones and metabolites, muscle cell-derived inflammatory cytokines such as IL-6 and MCP-1 have been suggested to be involved in the regulation of blood glucose under exercise conditions. While IL-6 has been suggested to increase hepatic glucose output (HGO), MCP-1 has been linked to peripheral insulin resistance. The aim of the present study was to investigate the role of MCP-1 and IL-6 in individuals with T1DM before, during and after different exercise conditions.

Methods
In a prospective, randomized cross-over study, 12 male individuals with well-controlled and complication-free T1DM (mean±SD: age 26±4 years, HbA1C 7.0±0.6%, diabetes duration 14±2 years) underwent 90 min of cycling at 50% VO2peak with (IHE) and without (CONT) 10 s all-out sprints every 10 min. Euglycemia was maintained using an oral 10% glucose solution and following a pre-specified algorithm. Hepatic glucose output and peripheral glucose disposal (Rd) were investigated using stable isotope techniques. Levels of IL-6 (ELISA) and MCP-1 (multiplex assay) were measured at baseline, after 80 min of exercise and 120 min after exercise completion.

Results
IHE and CONT did not differ with regard to blood glucose levels (7.9±0.1 vs 7.3±0.1 mM), insulin concentration (21.0±1.6 vs 20.7±2.1 mU/l) and energy expenditure (799 vs 787 kcal). Mean±SEM MCP-1 and IL-6 levels were similar at baseline (IL6: 0.86±0.63 vs 0.10±0.08 pg/ml; MCP-1: 258±17 vs 265±16 pg/ml for IHE vs CONT). MCP-1 levels were highest during exercise for both interventions, but the MCP-1 peak was greater in IHE compared to CONT (increase from baseline: 147±13 vs 71±16 pg/ml, p<0.001). In the recovery period MCP-1 decreased following both interventions and reached comparable levels 120 min post-exercise (IHE: 337±24 vs 312±30 pg/ml, p=0.20). IL-6 increased with exercise and remained elevated thereafter, without a difference between IHE and CONT (exercise: 7.9±1.7 vs 5.4±8.5 pg/ml; post-exercise: 7.8±1.5 vs 9.0±3.5 pg/ml). Exogenous CHO requirements and glucose disposal were significantly lower in IHE but hepatic glucose output was similar when compared to CONT (CHO: 15±5 vs 27±7g; Rd: 6.8±0.3 vs 8.3±0.5 mg/kg*min-1; HGO: 3.7±0.2 vs 3.6.±0.3 mg/kg*min-1, p-values: 0.04 for CHO, <0.01 for Rd). Post-exercise glucose disposal no longer differed between the interventions and hepatic glucose output remained comparable.

Conclusion
The exercise-induced peak in MCP-1 was significantly higher in IHE when compared to CONT. In contrast, there was no difference in IL-6 response between exercise interventions. MCP-1 may be a factor involved in the reduced peripheral glucose uptake observed during IHE in individuals with T1DM, thereby adding a potential glucose-stabilizing role of MCP-1 to its well-known inflammatory properties.

05

Adrenal function testing and predictability of glucocorticoid responsiveness in community-acquired pneumonia

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Background/Introduction
It is controversial whether the attenuated increase of circulating cortisol to ACTH stimulation predicts treatment response to corticosteroids in patients with critical illness. We investigated whether consyntropin testing predicts treatment response to corticosteroids in patients with community-acquired pneumonia (CAP).

Methods
We performed a low dose (1 µg) cosyntropin test on admission in a prospective randomized, double-blind, placebo-controlled multicenter trial comparing prednisone 50 mg for seven days to placebo in patients hospitalized with CAP. The results of the main study showed a benefit of corticosteroids in CAP. Cortisol was measured at baseline and 30 min after stimulation with 1µg ACTH. We performed Cox regression models for time to clinical stability to compare baseline and stimulated cortisol levels between both treatment groups.

Results
326 patients in the prednisone group and 309 patients in the placebo group were evaluated. Neither basal plasma cortisol levels nor a delta cortisol < 250 nmol/L after ACTH stimulation nor the combination of basal cortisol and delta cortisol predicted treatment response to prednisone as assessed by time to clinical stability, mortality, ICU-stay, rehospitalization, length of total and intravenous antibiotic treatment or CAP-related complications (p for interaction >0.05). However, patients with a baseline cortisol value of >938 nmol/l had a significantly shorter length of hospital stay in the prednisone group as compared to the placebo group (p for interaction = 0.015).

Conclusion
Cosyntropin testing does not predict glucocorticoid responsiveness in CAP, but patients with a baseline cortisol >938 nmol/l show a particular benefit from glucocorticoids for a shorter length of hospital stay.

06

Obesity paradox in patients with community-acquired pneumonia: Is inflammation the missing link?

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Background/Introduction
Obesity is generally considered a harmful condition associated with adverse health outcomes. Yet, in critical illness, observational studies have found a positive association of weight and clinical outcomes, called "the obesity paradoxon". Whether this is due to confounding or whether obesity has protective effects remains unclear.
Herein, we investigated the effects of weight on longterm mortality in a large cohort of patients with community-acquired pneumonia and asked the question whether changes in inflammation would account for mortality differences in this cohort.

Methods
Patients admitted with community-acquired pneumonia to six medical centers in Switzerland were prospectively followed for 6 years. To assess association of BMI classes with mortality we used cox regression analyses adjusted for PSI (model 1 = severity adjusted) and for PSI, age, gender, metabolic factors, cardiovascular diseases, other comorbidities including cancer (model 2 = fully adjusted). As reference group we defined "normal weight" with a BMI of 18.5 - <25 kg/m2. For biomarker analysis we transformed all laboratory values in deciles because of better comparability and used linear regression analyses adjusted for the above confounders. population).

Results
Of 763 patients with confirmed community-acquired pneumonia, 46 (6.0%) were underweight with a mean BMI of 17.7, 330 (43.3%) were classified as normal weighted with a mean BMI of 22.7, 258 (33.8%) were overweight (mean BMI 26.9) and 129 (16.9%) were obese with mean of BMI 33.3 kg/m2. All cause 6-year mortality was significant lower in obese patients in a regression model adjusted for severity with a adjusted hazard ratio (HR) of 0.641 (95% CI 0.462-0.889) and in a fully adjusted model with a HR of 0.692 (95% CI 0.489-0.979). No differences in inflammation markers (C-reactive protein, procalcitonin, white blood cell count) on admission and during the hospital stay were found. Obese patients had higher levels of pro-adrenomedullin on all days of hospitalisation even after adjustment for confounders.

Conclusion
This large cohort study with a 6 year follow up found obesity to be associated with lower all cause mortality in community-acquired pneumonia patients confirming the "obesity paradoxon" in this population. Yet, no differences in inflammation was found which could explain these findings.

07 S

Impact of probiotic yoghurt consumption on inflammatory biomarkers and microbiota composition in healthy young men

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Background/Introduction
The intestinal microbiota has recently been shown to play a key regulatory role in metabolic health. In the context of the increasing prevalence of metabolic diseases, interventions that positively influence the intestinal microbiota balance could have significant consequences for public health. Yoghurt with added live microorganisms, or 'probiotics', have been proposed as an approach to improve the balance of the intestinal microbiota. However, current research is not conclusive on the efficacy of this approach on modulating metabolic health. Different factors could explain this disparity, including the baseline composition of the intestinal microbiota. To better understand this effect, this study uses an integrated approach to assess key characteristics of the product and of the individual to assess the efficacy of probiotic yoghurt on modulating metabolic health.

Methods
In a double-blinded,cross-over clinical study, fourteen healthy male volunteers (age 24.6 ± 4.7 years, BMI 21.8 ± 1.8 kg/m²) were recruited to test a yoghurt containing the widely used probiotic Lactobacillus rhamnosus GG (LGG) and a non-fermented, acidified milk (control). The dynamic responses to an acute (single dose of 800g) and chronic (400g/day, for two weeks) ingestion of the products were evaluated using sensitive biomarkers of metabolic health. A standardised high fat test meal was used to assess the impact of the two week intervention on a metabolic stress. In parallel, temporal changes in the intestinal microbiota (16S ribosomal RNA sequencing) were assessed.

Results
Acute and chronic intake of probiotic yoghurt showed limited benefits of classical biomarkers of metabolic health compared to acidified milk. Conversely, both acute and chronic probiotic yoghurt intake appeared to convey benefits on two sensitive markers of inflammation, chemokines 2 and 5, compared to the acidified milk. Preliminary analyses of the microbiota data indicate a high level of interindividual variation at baseline with detection of the bacterial strains used in the products after the test phase.

Conclusion
Probiotic yoghurt appears to show some benefits on low-grade inflammation.The impact of probiotic yoghurt on the intestinal microbiota could determine the efficacy of the intervention on metabolic health.

08 S

Validation of a Computer Vision-Based Smartphone System for Carbohydrate Counting

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Background/Introduction
Estimating a meal's carbohydrate (CHO) content is paramount to well-controlled diabetes. To achieve this, a computer vision-based smartphone system called GoCARB was developed and validated. The system was designed to estimate the CHO content of dishes of heterogeneous food items with an error less than ±20 grams.

Methods
The user places a credit card-sized reference object next to the meal and acquires two images from different viewing angles. The graphical user interface guides the user to choose the optimal angles based on the built-in smartphone sensors (accelerometer and gravity sensor). Then, a series of computer vision steps is executed providing the types and volumes of the foods present which are used along with a nutritional databases for the CHO estimation.

Results
The Android prototype was validated in a clinical environment involving 19 adult volunteers (age >18 years; 7 female, 12 male) with type 1 diabetes (T1D). The study was conducted on 10 days during July and August 2014. For each day, a total of six meals were taken from the hospital's restaurant (normal menu). The dishes were of broad diversity. The food items comprising each dish were weighed and by using the USDA Nutrient Database the corresponding amount of CHO was estimated (ground truth). Each participant was asked to count the CHO content of each dish independently. Then, he/she was asked to estimate the CHO content by using the GoCARB. At the end of each session, a questionnaire was completed, in order to assess the user's experience with GoCARB.

The mean absolute error between CHO counted by the individuals with T1D and ground truth was 28.03±38.41 grams of CHO, while the corresponding value for the GoCARB system was and 13.28±10.37 grams of CHO. The feedback gathered by the participants showed that the system is easy-to-use even by non-smartphone users.

Conclusion
The evaluation results indicate that GoCARB meets the requirements, and seems to be more accurate than the average individuals with T1D. Its effectiveness in improving glycemic control will be investigated in a clinical trial involving patients with T1D under sensor-augmented pump therapy.

09

Factors Associated with Basal Insulin Persistence After Initiation Among People with Type 2 Diabetes Mellitus (T2DM)

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Background/Introduction
Although insulin is an effective therapy for patients with T2DM, there is limited information on basal insulin persistence after initiation of insulin therapy. This study assessed factors associated with basal insulin persistence for US commercially-insured insulin-naïve patients using claims data.

Methods
The study sample included 19,110 adults with T2DM (mean age: 59 years, ~60% male) who had their first claim for insulin glargine (74%), insulin detemir (22%), or neutral protamine Hagedorn (NPH) insulin (5%) between April, 2006 and March, 2012.

Results
During the 12 months after initiation of insulin therapy, 20% of all patients continued to use basal insulin, 62% had a gap of ≥30 days between fills (interrupters), and 18% discontinued therapy (i.e., had no fill) after the first gap of ≥30 days. Over two-thirds of interrupters and discontinuers had a first gap within 90 days of insulin initiation. Multinomial regression results [odds ratio (95% CI)] showing statistically significant factors associated with both interruption and discontinuation ($p<0.05$) are presented in the Table. Odds ratio <1 indicates increased likelihood of treatment continuation.

Factor	Associated with interruption	Associated with discontinuation
Age, years	0.99 (0.99, 1.00)	0.98 (0.98, 0.99)
Comorbidities (during 6 months before insulin initiation)		
Diabetic foot (+ lower limb amputations)	1.31 (1.07, 1.61)	1.29 (1.01, 1.65)
Neurological disorders (not dementia)	1.29 (1.02, 1.61)	1.31 (1.01, 1.70)
Medical resource use (≥1 visit during 6 months before insulin initiation)		
Emergency department	1.21 (1.10, 1.34)	1.72 (1.53, 1.94)
T2DM prescription drug use (during 6 months before insulin initiation)		
Number of unique classes used	0.90 (0.86, 0.94)	0.78 (0.74, 0.83)
≥1 prescription fill for any injectable drug	0.77 (0.68, 0.87)	0.63 (0.53, 0.75)

Conclusion
The study findings suggest that a large percentage of patients with T2DM initiating basal insulin therapy in the US may interrupt or discontinue treatment in the year after initiation. Further research is needed to understand reasons behind basal insulin persistence to help clinicians manage care for T2DM more effectively.

10

Impaired glucose tolerance in mice with β-cell specific deletion of Pkbα

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Background/Introduction
Protein kinase B (PKB)/Akt is considered to be a key target in the regulation of pancreatic β-cell mass. Three isoforms of PKB exist (PKBα/Akt1; PKBβ/Akt2 and PKBγ/Akt 3). Although all three isoforms are expressed in pancreatic β-cells, it is not clear if the regulation of functional β-cell mass is isoform-specific. Previous studies on transgenic mice expressing constitutively active PKBα showed a two-fold increase in β-cell proliferation. To further investigate the role of Pkbα, a new mouse model with specific deletion of Pkbα in pancreatic β-cells (βpkbαKO) was generated and analysed regarding glucose homeostasis and islet mass.

Methods
Mice were rendered insulin resistant by feeding a high-fat diet (HFD) and characterized with regard to their metabolic phenotype by performance of intraperitoneal glucose and insulin tolerance tests (ipGTT, ipITT) and area under the curve (AUC) was calculated. In addition, glucose-stimulated insulin secretion (GSIS) in isolated islets was assessed in vitro and islet morphology was studied in pancreas sections

Results
Western blot analysis showed that PKBα was normally expressed in control mice but absent or strongly reduced in β-cells from βpkbαKO mice, whereas PKBβ and PKBγ expression was not affected. In contrast, PKBα expression was similar in metabolic relevant tissue (skeletal muscle, different fat depots, liver and brain) between βpkbαKO mice and controls. Under normal chow diet male βpkbαKO mice show reduced glucose tolerance with significant increased AUC (+22.6% ± 6.5%; $p \leq 0.05$) only later in adult live at the age of 26 weeks. HFD accelerated the onset of impaired glucose tolerance with significant increased AUC (+10.06% ± 3.6%; $p \leq 0.05$) at age of 12 weeks (6 weeks on HFD). On the contrary, female βpkbαKO mice did not develop glucose intolerance neither under chow nor under HFD. Plasma insulin levels during GTT were reduced in HFD-fed βpkbαKO mice. Additionally, random fed plasma insulin levels were decreased (4.2 ng/mL ± 0.58 ng/mL) compared to control littermates (6.31 ng/mL ± 0.97 ng/mL). However, in vitro GSIS was not decreased in islets from chow respective HFD-fed βpkbαKO mice.

First analysis of pancreas morphology revealed a decrease of β-cell area by around 50% relative to total pancreas area in βpkbαKO mice under chow and HFD as compared to control littermates.

Conclusion
We show for the first time that loss of Pkbα specific in pancreatic β-cells leads to impaired glucose tolerance potentially due to reduced islet mass.

11

High diagnosis rate of monogenic diabetes in the Swiss population by targeted next generation sequencing

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Background/Introduction
Background: Monogenic diabetes is a heterogeneous group of diabetes due to a single gene mutation and includes neonatal diabetes (ND), maturity diabetes of the young (MODY) and rare forms of syndromic diabetes. These forms of diabetes remain undiagnosed in probably more than 90% of patients. The aim of the study was to identify mutations causing monogenic diabetes in Switzerland.

Methods
Inclusion criteria were ND, autoantibody negative type 1 diabetes (T1D), type 2 diabetes (T2D) diagnosed before the age of forty-five without metabolic features and syndromic diabetes regardless to treatment. The analyses were performed by a targeted next-generation sequencing (NGS) assay sequencing 323 genes involved in diabetes, glucose homeostasis and pancreas development, using the Haloplex technology. All the variants were confirmed by Sanger sequencing.

Results
So far we have analyzed 142 diabetic probands by NGS. We identified 73 variants in the selected 323 genes, including variants in genes associated with T1D or T2D in 51% of the subjects. 55% (40/73) of the mutations were found in one of the 13 putative MODY genes (HNF4A, GCK, HNF1A, PDX1, HNF1B, NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABCC8, KCNJ11). The most frequent MODY mutations were found in the GCK gene (42%, 31/73). 17 different mutations of GCK could be identified and 39% (12/31) of the probands carry the p.Val203Ala mutation.

Conclusion
This study shows that monogenic diabetes can easily be diagnosed by NGS. 42% of the positive patients had GCK diabetes; the Val203Ala mutation is the most prevalent in the Swiss population. These results will lead to the development of a diagnostic NGS tool.

Sample providers: Amonn A, Babians-Brunner A, Bachmann S, Badoi A, Bally M, Bilz S, Blum C, Brändle M, Bucher J, Buesser C, Bühler A, Burget L, Capraro J, Henzen C, Chatton I, L'Allemand D, Daneva-Treand T, Donath M, Drescher T, Egloff M, Elowe-Gruau E, Elsässer P, Faulenbach M, Feller K, Flück C, Franscella S, Gaillard S, Gastaldi G, Spinas G, Golay Petersen M, Gonzalez E, Hauschild M, Hernandez A, Hess M, Hochuli M, Iff E, Jacot E, Jenni S, Keller C, Keller U, Kirchner P, Kiss D, Knisel W, Knobel U, Konfino O, Krull I, Favre L, Lang-Muritano M, Lehmann R, Malacarne S, Manggold J, Marino L, Mavromati M, Meinhardt U, Möller D, Müller K, Oesterle M, Pauchet A, Phan-Hug F, Philippe J, Pinizzotto M, Pitteloud N, Portmann D, Probst-Scheidegger U, Procopiou M, Schimke K, Schmid B, Schmid S, Sigrist S, Simon-Vermot I, Slahor L, Spada A, Stettler C, Stöckli R, Stoll Delphine, Stoppa Sophie, Straumann M, Sze L, Timper K, Tonella P, Urwyler S, Vavanikunnel J, Villiger L, Von der Weid N, Weber O, Zahnd R, Zimmermann B, Zumsteg U

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Total thyroidectomy in refractory amiodarone induced thyrotoxicosis: a case series of 12 patients

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Background/Introduction
Amiodarone induced thyrotoxicosis (AIT) occurs in 5-10% of patients and may occur at any time throughout the course of treatment including months after discontinuation. Two distinct forms of AIT are distinguished and treated differently. Iodine-induced hyperthyroidism, typically seen in patients with underlying thyroid disease, is referred to as typ1 AIT and treated with high doses of thionamide antithyroid drugs and perchlorate. Type 2 AIT is a destructive thyroiditis and most cases respond to high-dose glucocorticoids. However, mixed forms and refractory cases are occasionally observed and prolongued hyperthyroidism may lead to siginificant morbidity, especially in patients with significant cardiac comorbidities. Thyroidectomy rapidly restores normal thyroid function but must be performed in still overtly hyperthyroid often critically ill patients.

Methods
Retrospective analysis of the clinical records of all 12 patients with AIT, who underwent total thyroidectomy at the Department of Surgery, Kantonsspital St.Gallen, since 2006.

Results
The age of the patients ranged from 50-81 years and 2 were female. All patients had an underlying structural cardiac disease and 8 had an ICD. All patients had been treated with thionamides, glucocorticoids or both for 3-10 weeks prior to surgery. Indications for total thyroidectomy included unresponsiveness to medical treatment and worsening of the underlying cardiac condition in several cases. Euthyroidism was restored quickly in all subjects. The length of the postoperative hospital stay ranged from 2-5 days and temporary intensive care was required in 3 patients. One patient died 3 weeks after surgery because of multiple preexisting complications. All other patients were euthyroid on levothyroxin replacement and in stable or improved cardiac condition for at least 1 year.

Conclusion
Total thyroidectomy is an effective and safe treatment in patients with AIT unresponsive to medical therapy and should be considered early to prevent subsequent cardiac morbidity due to prolongued overt hyperthyroidism.

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Non-functioning pituitary adenoma first diagnosed during pregnancy, a single-centre experience

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Background/Introduction
Non-functioning pituitary adenoma (NFPA) diagnosed during pregnancy is a very rare but challenging condition with regard to diagnostic assessment and management approach due to the physiologically altered endocrine status of the pregnant women as well as specific considerations regarding fetal and maternal adverse pregnancy outcomes. Here we present a single-center experience of 3 patients with newly diagnosed NFPA during pregnancy.

Case Report
Age at diagnosis was 31, 32 and 33 years and diagnosis was made at 30, 38 and 27 week of gestation respectively. Symptoms leading to diagnosis were symptoms of pituitary apoplexy (paroxysmal severe retroocular headache) in one patient and visual disturbances in two patients. Native MRI of the sella demonstrated pituitary macroadenoma in all patients (maximal diameter 2.1cm) with signs of recent hemorrhage in two cases. No distinction between normal pituitary gland tissue and adenoma could be made. Perimetry confirmed visual field deficits in all 3 patients. There were no signs or symptoms of Cushing's syndrome or acromegaly. Prolactin levels were elevated (range 139-180 mcg/l) and interpreted as a combination of pregnancy-related elevation and stalk compression effect in all three cases. 2/3 patients had documented slightly elevated prolactin concentrations before pregnancy. FT4 was low with normal TSH-levels in all patients – this constellation was interpreted as secondary hypothyroidism or isolated hypothyroxinemia due to pregnancy. Further assessment of adrenal function by ACTH stimulation test was not possible. However, all patients were empirically treated with physiological doses of hydrocortisone and levothyroxine.

Treatment decisions were made on an individual basis (i.e. no worsening of visual field deficits, absence of cranial nerve palsy) and discussed in an interdisciplinary team (neurooptahlmologist, obstetrician, neurosurgeon, pediatrician and endocrinologist): All three patients underwent elective C-section at 34 (two patients) and 39 week of gestation and two patients had endoscopic transphenoidal surgery after delivery (after 2 and 14 days respectively). Immunohistochemistry confirmed NFPA. One patient refused pituitary surgery and had stable visual field deficits and a 30% reduction of the adenoma size at follow-up. Postpartal and postoperative course was uneventful in all cases with no fetal- or maternal-associated morbidity. There were no cases of lactation failure and all patients had complete restoration of pituitary function at follow-up.

Conclusion
NFPA diagnosed during pregnancy is a rare and challenging condition with need of interdisciplinary approach in the patient's management.

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A rare cause of a 46, XY disorder of sexual development diagnosed in an adult patient

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Background/Introduction
The defective conversion of testosterone to dihydrotestosterone due to a steroid 5-alpha-reductase 2 deficiency results in a unique form of 46, XY disorder of sexual development (DSD). Dihydrotestosterone is essential for the embryonic differentiation of the external male genitalia and the prostate. Steroid 5-alpha-reductase 2 deficiency is an autosomal recessive disorder in which genetic males have a predominantly female phenotype with female external genitalia but male internal urogenital tract.
We describe the case of an adult patient having migrated from Pakistan to Switzerland in whom a steroid 5-alpha-reductase 2 deficiency was diagnosed at the age of 29. Molecular genetic analysis identified a homozygous point mutation in exon 4 of the 5-alpha-reductase 2 gene, leading to an amino acid change from glutamic acid to lysine. To our knowledge, this is the second case of this mutation in the steroid 5-alpha-reductase 2 gene (SRD5A2) which was first described in 1997 (Anwar R et al).

Methods
A 29 year old patient, child of consanguine parents, is born in Pakistan with ambiguous but predominantly female genitalia and is raised as a girl. At puberty, gender identitiy changes to male. He takes refuge in Switzerland at the time he would be forced into a marriage with a man and presents himself at our clinics for further evaluation. Examination reveals female external genitalia with clitoromegaly, blind-ending vagina and palpable labial testes, poorly developed secondary sexual features and no gynecomastia.

Results
Biochemical screening shows a raised plasma testosterone to dihydrotestosterone ratio (39:1). Serum luteinizing hormone, follicle stimulating hormone, oestradiol and prolactin are within normal adult male range. Anti-müllerian hormone is elevated. Karyotpye is 46, XY. MR imaging shows a male internal urogenital tract consisting of epididymides, vasa deferentia, seminal vesicles and ejaculatory ducts that empty into the blind ending vagina (later analysis of ejaculate confirms fertility). Müllerian duct derivates are absent. Urinary steroid profile shows a markedly reduced level of 5-alpha-reductase 2. DNA sequencing of the gene that encodes steroid 5-alpha-reductase 2 (SRD5A2) confirms the diagnosis of a steroid 5-alpha reductase 2 deficiency. The underlying homozygous mutation is in exon 4 of the gene, resulting in an amino acid change from glutamic acid to lysine (GAA to AAA).

Conclusion
Steroid 5-alpha-reductase 2 deficiency results in defective conversion of testosterone to dihydrotestosterone in genetic males. Imparied virilization during embryogenesis leads to phenotypic females with a male internal urogenital tract. The change in gender identity by many from female to male at puberty is a remarkable feature of this form of 46, XY DSD. With increasing global migration, endocrinologist in Switzerland might be confronted with more cases of DSD in adult patients.

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12-Month-Follow-Up of patients with profound Hyponatremia - a prospective observational study

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Background/Introduction
Hyponatremia is the most common electrolyte abnormality in clinical practice and given its impact on mortality and morbidity a very relevant medical condition. Nevertheless only few is known about factors influencing long-term outcome of these patients.

Methods
This is a prospective observational 12 months follow-up study of patients with profound hyponatremia (≤125mmol/L) admitted to the medical emergency department of two tertiary care centers in Switzerland between 2011-2013. We analysed the association of different clinical and laboratory parameters with the following three outcomes: 1-year-mortality, rehospitalisation and recurrent profound hyponatremia. Follow-up was done by structured phone interview and review of medical records from every single rehospitalisation. From primarily 298 included patients complete follow-up data was available in 281 patients.

Results
Median [IQR] initial serum sodium (s-sodium) level of the 281 patients (median age 72 [IQR 61-80] years) was 120 [116-123] mmol/L. During the study-period of 12 month 58 (20.6%) patients died. The majority (56.2%) of patients was hospitalized at least once again, 28.5% even several times. Recurrent hyponatremia was observed in 42.7% of patients, being again profound in 16%.

Beside relevant comorbidities (assessed by the Charlson Comorbidity Index [CCI]) the following parameters revealed significant association with the main outcome mortality - also after multivariate adjustment: "initial s-sodium levels" (Odds Ratio [OR] 1.14, 95% Confidence Intervall [CI] 1.01-1.29, p=0.036) and „correction of hyponatremia during hospitalisation" (sodium ≥ 135 mmol/L at discharge) (OR 0.47, 95% CI 0.23-0.94, p= 0.034).

Severity of hyponatremia showed an inverse correlation with mortality. We further compared patients with initial s-sodium level ≤120mmol/L to those with levels >120mmol/L; the latter had a significant higher mortality rate than those with lower initial s-sodium levels (27.8% vs. 14.8%, p=0.0078). Etiology of hyponatremia differed between both groups: patients with very low sodium levels (≤120 mmol/L) were more likely to have drug-induced hyponatremia (49% vs. 29.4%, p=0.0008), whereas hypervolemic hyponatremia (due to heart or liver failure) was more common in patients with initial s-sodium values above 120 mmol/L (15.9% vs. 7.7%, p=0.033).

Conclusion
Hyponatremia goes along with a high 1-year-mortality, recurrence and rehospitalisation rate. The inverse correlation of hyponatremia-severity and mortality emphasizes the importance of the underlying disease, which rather determines outcome than hyponatremia itself.

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Revisiting the Refeeding syndrome in medical inpatients: should we care? Results of a systematic search and metaanalysis.

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Background/Introduction
Refeeding syndrome consists of metabolic disturbances that occur upon reinstitution of nutrition to starved or severely malnourished patients. Although this syndrome has been discribed more than 70 years ago, its importance in medical inpatients, however, remains controversial. There are important gaps in the literature and a lack of a standardized and evidence-based clinical definition for the condition of a refeeding syndrome.
The aim of this systematic review was to summarize the current evidence about refeeding syndrome in regard to the following six questions: 1.What are definitions used for refeeding syndrome? 2. What is the incidence of refeeding syndrome? 3. When does refeeding syndrome occur? 4. Does refeeding syndrome correlate with adverse outcome? 5. What are risk factors for refeeding syndrome? 6. What are therapeutic strategies to prevent or treat refeeding syndrome?

Methods
This systematic review is in accordance with PRISMA guidelines. The libraries of MEDLINE and EMBASE were systematically searched for interventional and observational clinical trials about refeeding syndrome. Search terms were defined as "refeeding" or "refeeding syndrome". Randomized controlle trials or observational trials were included. We excluded case reports and other reviews. Extraction of articels was performed by one of the authors based on a predefined case report form including bias assessment of each individual study.

Results
We found 2205 abstracts matching our search terms. After exclusion of case reports, surveys, audits and not relevant records, we included 43 records for the final analysis including 2 RCTs and 41 observational studies and 15 studies with anorexic patients (AN) and 28 with non-anorectic study patients (Not AN). Definitions used for refeeding syndrome were highly heterogenous with most studies relying on electrolyte disturbances only and others also including clinical symptoms. Incidence of refeeding syndrome was found to depend highly on the definition used and varied between 0% (definition based on electrolyte disorders and clinical symptoms) and 80% (definition based on electrolyte disorders only). In most of the studies refeeding syndrome occurred within the first 72 hours. Most of the risk factors mentioned are in accordance with the NICE guidelines. Additional risk factors were older age or enteral feeding. While 8 studies found an effect of preventive measures, 8 other studies did not find any effect of such measures. Treatment data was found in two studies only and consisted of phosphate substitution.

Conclusion
First hypophosphatemia with or without other electrolyte disturbances or clinical features are used to define refeeding syndrome. Second, the reported incidence varies accordingly from <5% to >80%. Third, it occurs most likely after 48 to 72hrs and, fourth, is possibly associated with longer length of stay. Risk factors are best summarized by NICE guidelines. Finally, evidence also for therapeutic strategies is very limited. Thus, large RCTs are urgently needed and luckily underway.

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Osteogenesis imperfecta as a cause of male osteoporosis

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Background/Introduction
Male osteoporosis is an underestimated and underdiagnosed diagnosis with a number of important differences compared to female osteoporosis: the lifetime risk of an osteoporotic fracture for 50 year-old men is about 20%, the mortality of femoral neck fractures in elderly men is three times as high as in women. and in about 2/3 of male osteoporosis a secondary cause may be identified.

Methods
Case report of a 53 year-old man with a history of recurrent osteoporotic vertebral fractures.

Results
A 53 year-old man was admitted to our hospital because of a first time seizure and concomitant compression fracture of the first lumbar vertebra. The patient had a history of histamin intolerance, but was otherwise healthy. Radiological evaluation revealed multiple additional fractures of thoracic (5,6,9, and 11) and lumbar (3, and 4) vertebrae of older origin. T-score was -2.9 for trabecular bone, and -1.5 for cortical bone, respectively. Secondary causes of male osteoporosis like hypogonadism, hyperparathyroidism or mastocytosis were excluded. Kyphoplasty and stabilization osteosyntheses were performed, and the patient was discharged on bisphosphonates, vitamin d and antiepileptics. Five months later rib fractures without any trauma occurred, and genetics for osteogenesis imperfecta were performed revealing a missense mutation of the COL1A1 exon 48, c.3688G>A, whereas no KIT mutation was identified. Treatment was changed to teriparatide (Forsteo®) with no more osteoporotic fractures since over a follow-up of 16 months.

Conclusion
For the first time we report the COL1A1 missense mutation c.3688G>A, as a cause of osteogenesis imperfecta in a 53 year-old man with multiple and recurrent osteoporotic fractures.

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Evaluation of High Insulin Antibody Response and Related Clinical Outcomes in Patients (Pts) with Type 1 Diabetes Mellitus (T1DM) or Type 2 Diabetes Mellitus (T2DM) Treated with LY2963016 and Lantus®

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Background/Introduction
LY2963016 (LY IGlar) and Lantus® (IGlar) are insulin glargine products with similar efficacy and safety profiles including low insulin antibody responses. This evaluation assessed maximal antibody responses and relationships to clinical outcomes.

Methods
Data were analyzed from the open-label ELEMENT 1 (T1DM) study at 52 weeks (wks) and double-blind ELEMENT 2 (T2DM) study at 24 wks. Maximum post-baseline antibody levels (MPBAL) and proportion of patients (pts) in the upper quartile (upper 25% of maximum values observed) of maximum antibody % binding (UQMAPB) were compared for differential treatment effects on outcomes.

Results
Among pts with at least one detectable antibody test during treatment, median MPBAL (% binding) was 0.84 in 265 pts receiving LY IGlar and 0.90 in 267 pts receiving IGlar in ELEMENT 1 (p=0.96 between treatments), and 0.56 in 365 pts and 0.78 in 365 pts, respectively, in ELEMENT 2 (p=0.49 between treatments). In ELEMENT 1, 29 pts (10.9%) receiving LY IGlar and 24 pts (9.0%) receiving IGlar were in the UQMAPB, and in ELEMENT 2, respective pt numbers were 14 (3.8%) and 10 (2.7%; p>0.05 between treatments in both studies). There were no significant differential treatment effects of UQMAPB status (Yes/No) on clinical outcomes in either study (p>0.05; Table). Between-treatment comparisons in the UQMAPB subgroups were not statistically significant for all analyses conducted. Similar proportions of pts with allergic reactions, injection site reactions and serious adverse events were noted in all UQMAPB status and treatment groups.

Outcome:	UQMAPB	T1DM 52-Wk			T2DM 24-Wk		
	Yes/No	LY IGlar	IGlar	p-value*	LY IGlar	IGlar	p-value*
Change in HbA1c (%)	Yes	-0.19	-0.15	0.79	-1.30	-1.40	0.91
	No	-0.28	-0.29		-1.27	-1.32	
Change in Weight (kg)	Yes	0.87	2.25	0.06	1.40	3.55	0.18
	No	0.72	0.21		1.60	1.86	
Change in Basal Dose (U/kg/day)	Yes	0.02	0.01	0.67	0.47	0.54	0.64
	No	0.03	0.03		0.36	0.36	
Overall Incidence Total Hypoglycemia ≤3.9 mmol/L; n (%)	Yes	26 (89.7)	24 (100.0)		12 (85.7)	10 (100.0)	
	No	227 (96.2)	235 (96.7)	0.15	281 (80.1)	274 (77.2)	0.18

*Breslow Day test for homogeneity of odds ratios to test treatment-by-UQMAPB status interactions

Conclusion
Pts treated with LY IGlar and IGlar had similar maximum antibody responses, with similar proportions of pts having high antibody response. High antibody levels were not associated with effects on clinical outcomes.

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Multifocal insulinomas (insulinomatosis) in GLP-1-receptor PET/CT

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Background/Introduction
Apart from occurring sporadically, insulinoma within the framework of multiple endocrine neoplasia 1 (MEN-1) is well known. The rare presence of multifocal insulinomas has recently been assigned a separate entity (insulinomatosis). The difficulty of localising insulinomas may be improved by GLP1-receptor imaging.

Methods
Case Report: A 48 year old woman had been treated for suspected epileptic seizures for two years (lamotrigine). During another such episode low blood glucose (BG) was detected. During fasting she was unaware of hypoglycaemia (BG 2.3mmol/L), endogenous hyperinsulinism was established. With a history of treated prolactinoma (operation, quinagolid) – and slightly elevated calcium levels MEN-1 was considered. 68Ga-DOTA-Exendin-4 PET/CT and MRI revealed a major lesion located directly left to the pancreatic head (8x13mm) and smaller lesions in the tail (max. 5mm).

Results
After pretreatment with diazoxid/prednisolone surgery was proposed. Intraoperatively, granular pancreatic tissue was palpated mainly located in the left side up to the head of the pancreas – matching with the increased uptake in GLP-1R imaging. As intraoperative ultrasound did not confirm a focal lesion, left-sided pancreatectomy was performed. Histologically the major lesion proved to be insulin-positive, however 37 more small adenoma, mainly with insulin staining were detected establishing the diagnosis of insulinomatosis. The postoperative course was complicated by a peripancreatic abscess and recurrence of asymptomatic mildly low BG levels. Genetic testing for MEN-1 was negative.

Conclusion
1.GLP-R imaging is useful in benign insulinomas and might be useful in the context of MEN-1 in order to separate insulin secreting neuroendocrine tumors (NET) from other secreting and non-secreting NETs.

2. GLP-1R imaging showed positive lesions in this case of insulinomatosis. However, most of the lesions were too small to be detected by 68Ga-DOTA-Exendin-4 PET/CT.

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Sudden cardiac arrest during transsphenoidal pituitary surgery in a patient with Cushing`s disease: a rare complication due to Trigeminocardiac reflex

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Background/Introduction
Trigeminocardiac reflex (TCR) is a cardiac vagal response due to stimulation not only of the peripheral but also of the intracranial course of the trigeminal nerve. TCR is a well-known event e.g.in skull base surgery whereas neurosurgeons are not aware of this phenomenon in the context of transsphenoidal pituitary surgery, which may be underdiagnosed in this condition. Here we present a patient with asystole during pituitary surgery as a severe form of TCR.

Methods

Results
Cushing`s disease was established in a 35y old man, undergoing transsphenoidal resection of an ACTH producing microadenoma in 2002. Postoperatively gamma-knife radiosurgery was performed. During follow-up the patient underwent several additional uneventful transsphenoidal resections due to recurrent disease. In 2012 bilateral adrenalectomy was performed due to apparent hypercortisolism without corresponding detectable tumor in MRI and 11C-Methionin PET CT. Because of progressive Nelson tumor endoscopic endonasal transsphenoidal surgery had to be performed in March 2014 and in June 2015. During the current neurosurgical procedure the patient presented a sudden cardiac arrest of about 20 seconds and 3 epsiodes of severe bradycardia as low as 20 to 25 pulsations per minute while manipulating on the tumor tissue. As the surgical intervention was stopped, vital parameters normalized spontaneously an the patient recovered uneventfully. No underlying cardiac disease could be detected in this patient.

Conclusion
Asystole, which may be caused by a severe form of TCR, is a rare self-limiting complication of transsphenoidal pituitary surgery, especially when manipulating the cavernous sinus. Only a few cases are reported in the literature. However, neurosurgeons should be aware of this phenomenon, which may be underdiagnosed. Retrospective studies reveal that TCR induced change in vital parameters occur in 8-10% of patients during pituitary surgery. Stop of surgery is the treatment of choice.

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Predictors of non-response to fluid restriction in hyponatremic patients due to the syndrome of inappropriate antidiuresis (SIAD)

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Background/Introduction
Fluid restriction (FR) is the recommended first-line treatment for hyponatremia due to the syndrome of inappropriate antidiuresis (SIAD). However, FR not always leads to successful correction of hyponatremia, making predictive markers of treatment response desirable. We aimed to evaluate routinely measured serum and urine parameters, serum copeptin (s-copeptin), a surrogate marker of arginine vasopressin (AVP) and serum mid-regional pro-atrial natriuretic peptide (s-MR-proANP) as possible predictors of FR response.

Methods
This is a prospective multicenter observational cohort study of two Swiss referral centres (University Hospital Basel and University Medical Clinic Aarau, Switzerland) including patients with profound hyponatremia (serum sodium [s-sodium] <125 mmol/l) due to SIAD presenting to the emergency department. We classified patients into FR responders (increase of s-sodium concentration > 3 mmol/l within 24 h) or non-responders (≤ 3 mmol/l within 24 h). Laboratory parameters were measured at admission and compared between both groups with logistic regression analysis.

Results
Out of 106 SIAD patients we analysed 82 undergoing treatment with FR. 48 (59 %) patients exhibited a successful and 34 (41 %) an insufficient response to FR. High levels of urine sodium (u-sodium), urine osmolality (u-osmolality) and serum urea (s-urea) showed a significant association with non-response (Odds Ratio [OR] = 15.0, 95 % confidence interval [CI] = 2.4-95.8, p = 0.004; OR = 34.8, 95 % CI = 1.2-1038.8, p = 0.041; OR = 36.6, 95 % CI = 1.3-1026.1, p = 0.034). The accuracy of u-sodium remained significant in multivariate analysis and after adjustment for diuretic use. Lower levels of s-MR-proANP were associated with non-response (OR = 0.03, 95 % CI = 0.003-0.3, p = 0.004), whereas s-copeptin did not reveal significant association with treatment response.

Conclusion
Easily measured laboratory parameters, especially u-sodium, correlate with therapeutic response and identify patients most likely failing to FR. This approach may facilitate early treatment choice in case of hyponatremia due to SIAD

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Hyperthyroidism or not? Always think of antibodies!

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Background/Introduction
In daily clinical routine most of the endocrine parameters are measured by immunoassays. These assays allow automated, rapid and precise measurement of hormone concentrations but are prone to interferences. For example endogenous antibody interference by heterophilic antibodies, rheumatoid factors or anti-ruthenium antibodies are known causes of false or misleading thyroid function tests (TFT). We describe a case of a patient with abnormal TFT and false elevation of other hormone concentrations due to specific antibody interference.

Methods
Case Report

Results
A 63 year old postmenopausal woman presented to our outpatient clinic with weight loss of 13kg within 6 months, occasionally occurring palpitations and night sweat. Past medical history was remarkable for arterial hypertension, severe COPD and ethanol abuse. Clinical examination showed a cardial compensated, normotensive patient with a regular heart rate of 80/min. She had no goiter nor symptoms or signs of endocrine orbitopathy. The patient presented with slight finger tremor but deep tendon reflexes were normal. Thyroid ultrasound revealed normal thyroid gland size and parenchyma, absence of thyroid nodules and color-flow doppler demonstrated normal tissue perfusion. Repeated TFT (electrochemiluminescence assay with Roche cobas e 601®-System) showed clearly elevated levels of free T4 (64.2 pmol/l, ref. range 12-22) and free T3 (13 pmol/l, ref. range 3.1-6.8) but an unsuppressed TSH (0.49 µIU/ml, ref. range 0.27-4.2). Anti-TPO antibodies were negative but anti-TSH-receptor antibody titer was tenfold elevated. Laboratory assessment of pituitary function was normal except for an marked elevation of estradiol (625 pmol/l, ref. range <18) with unsuppressed LH (12 U/l, ref. Range 7.7-59). Treatment with Carbimazole had no impact on symtoms or weight but led to a slight rising TSH and decreasing levels of fT4/fT3. Analysis of stored sera was repeated with another testing method (Beckmann Dxl®) and revealed surprisingly normal values for fT4 (15.6 pmol/l), fT3 (4.45 pmol/l), TSH (1.08 mU/l) and estradiol (<20ng/l, ref. range <40). Anti-TSH-receptor antibody titer analysis on the Brahms-Kryptor®-system was also normal (<0.27 IU/l, ref. range <1.8). Further investigation identified the presence of anti-streptavidin antibodies (ASA) as the cause of the falsely elevated hormonal parameters.

Conclusion
ASA are a very rare cause of assay interference leading to false endocrine function tests. Streptavidin-coated microparticles are often used in immunoassays and bind biotinylated capture antibodies with high affinity. In competitive immunoassays binding of ASA to the solid phase of microparticles prevent binding of the biotinylated immune/analyte complexes and thereby leading to false high hormone concentrations. Repetition of the analyses on other, non-streptavidin based systems, will demonstrate normal values. The presence of specific antibody-interference should always be included in the differential diagnosis of ambiguous endocrine testing results.

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Case report: a “blunder” hypogonadotropic hypogonadismus.

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Methods / Results
We present the case of a 46-year-old male referred for the workup of primary infertility. A urological workup including a testicular biopsy three years earlier revealed azoospermia. Repeated measurements of total plasma testosterone showed fvalues between 1-12 nmol / l. The patient reported a two years history of lowered mood, fatigue, anhedonia and decreased libido. The past medical history was unremarkable with a normal onset and course of puberty. The patient had an athletic appearance and a BMI of 28.2 kg/m2. The hair distribution (Tanner Stage V) was normal. Scrotal palpation showed a decreased testicular volume (8 ml right, 12 ml left). The sense of smell was intact. The laboratory workup revealed a marked dyslipidemia (HDL-C 0.8mmol/l, LDL-C 7.1mmol/l), elevated liver enzymes (AST 51 U/L , ALT 83U/l) and a severe hypogonadotropic hypogonadism (total testosterone 0.41 nmol/l, LH 0.65 U/l, FSH 3.86 U/l). Subsequently, the other pituitary axes were found intact and hyperprolactinemia (PRL 9 ug/l) and hemochromatosis (transferrin saturation 22%) were ruled out. The patient who regularly worked out in a fitness center repeatedly denied the use of anabolic steroids but agreed to have a urine sample tested for these compounds. The analysis was positive for dihydrochloride methyltestosterone (Oral-Turinabol) and oxandrolone. Faced with this result the patient continued to deny the intake of these substances. However, after he was sensitized to the various risks of misusing anabolic steroids, a spontaneous recovery of the gonadotropic axis within 3 months was observed.

Conclusion
Due to their well described effects on muscle size and strength anabolic steroids, first extensively investigated and used in Eastern Europe countries to enhance athletic performance, have become very popular among professional and non-professional athletes. Retrospective studies estimate a prevalence of 3.3% of anabolic steroid misuse (6.4% in men and 1.6% in women), with the prevalence being even higher in non-professional athletes. Importantly, anabolic steroid misuse has been identified as the most common cause of severe hypogonadism in young men. Among the myriad of side effects, hypogonadotropic hypogonadism, infertility, testicular atrophy and gynecomastia occur regularly and may be partially masked by the concomitant use of SERMs, aromatase inhibitors and gonadotropins. Cardiovascular sequelae, among them sudden cardiac death and myocardial infarction, may be severe and in part attributable to the associated dyslipidemia. A high level of suspicion and thorough clinical investigation including urine steroid analysis may identify anabolic steroid misuse in a relevant number of patients referred for the workup of hypogonadism and/or infertility and prevent the unnecessary waste of medical resources. Specific endocrine therapies, including testosterone replacement, SERMs, gonadotropins may be employed to restore normal gonadal function and fertility in patients abstaining from the compounds.

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The influence of stress exposure on salivary cortisol, salivary alpha amylase and body compositon in preschool children

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Background/Introduction
Stress exposure (stressful major life events, daily hassles, and conflicts within families) has been found to have an impact on physiological stress regulation and has been related to childhood obesity in older children. However, its impact in young children remains unclear. The purpose of this study was to determine the effect of stress exposure on physiological stress responses and body composition in 2-6 year old children.

Methods
477 preschool children (Mean age 3.88 yrs/SD 0.68; m/f: 252/225) participated in a national cohort study. All children were tested at their child care centers by using an age-adapted socio-evaluative stress paradigm. Salivary cortisol and salivary alpha amylase were assessed during the stress paradigm and on two different days (5-point daily profiles). Measures of body composition included weight, height, waist circumference and skinfold thickness. Parents were asked to complete a set of questionnaires on life events, daily hassles and conflicts.

Results
Mixed model analyses revealed positive associations between stress exposure and stress responses after controlling for age, gender and parental socioeconomic status. However, stress exposure was not associated with body composition nor with daily profiles in these young children.

Conclusion
In very young children, stress exposure was related to a dysregulation of acute physiological stress responses but not chronic stress responses or body composition. The time frame and potential access to food to change body composition after stress exposure might have been too short to show an impact within the age group of preschool children.

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Is Thyroid Dysfunction Associated with Anemia?

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Background/Introduction
Anemia and abnormal thyroid function are common disorders that often co-occur. Most guidelines mention that thyroid-stimulating hormone (TSH) should be measured in the work-up of anaemia. However, data on the association between thyroid dysfunction and anemia are scarce.

Methods
In the population-based "European Prospective Investigation of Cancer" (EPIC)-Norfolk cohort, we examined 12972 men and women. Hypothyroidism was defined as TSH >4.49 mIU/L, either subclinical (SHypo) with normal free thyroxin (fT4) or overt (OHypo) with low fT4, and hyperthyroidism as TSH <0.45 mIU/l, either subclinical (SHyper) with normal fT4 or overt (OHyper) with elevated fT4. Anemia was defined as hemoglobin (Hb) <13 g/dL for men and <12 g/dL for women. We examined the association between thyroid function and anemia using logistic regression models with adjustment for age and gender. After excluding common causes of anemia (iron deficiency, inflammatory disease and chronic kidney disease), we compared the prevalence of anemia in the different thyroid function groups.

Results
Mean age was 58.9 ±9.5 years with 55% women. Prevalence of thyroid dysfunction was 1.7% OHypo, 5.6% SHypo, 2.8% SHyper and 0.6% OHyper. Anemia was observed in 1058 participants (8.2%) and was more prevalent among those with thyroid dysfunction (10.3%) compared to those in euthyroid state (7.9%) (p-value=0.0003).

The risk of anemia was increased for OHypo with an age and gender adjusted risk ratio (RR) of 1.55 [95% confidence interval (CI) 1.10, 2.18] and OHyper with a RR of 1.86 [CI 1.12, 3.10], but not for SHypo (RR 1.06 [CI 0.84, 1.35]) and SHyper (RR 1.07 [CI 0.77, 1.49]). Hb concentration was decreased in participants with both higher and lower TSH levels (p-value for quadratic pattern <0.001) in age and sex adjusted analyses, but this association was only significant for participants with OHypo (Hb 0.19 g/dL lower [CI -0.34, -0.04]).

After excluding common causes of anemia, 8.4% of participants with OHypo (p=0.05), 5.2% with SHypo (p=0.61), 3.4% with SHyper (p=0.42) and 17.1% with OHyper (p=0.002) had anemia compared to 4.7% of the euthyroid participants.

Conclusion
Overt hypo- and hyperthyroidism, but not subclinical thyroid dysfunction, are associated with a higher risk of anemia. However, the prevalence of thyroid dysfunction among anemic adults after excluding common causes of anemia was low. Given these data from the largest population-based study on this issue, TSH measurement is likely indicated only after excluding other common causes of anaemia.

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Can Adrenomedullin predict new onset diabetes in asymptomatic patients from the community? Results from a 10-year follow-up cohort study

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Background/Introduction
Adrenomedullin is a strong vasodilating peptide and has a wide variability of autocrine and paracrine effects on body functions. New data have linked adrenomedullin precursors (MR-proADM) to progression of diabetes and micro vascular complications as well as mortality. Whether MR-proADM also serves as a screening marker for new diabetes onset in asymptomatic patients remains unproven.
Herein, we analysed the prognostic value of MR-proADM for predicting new-onset diabetes as well as all cause mortality in a unselected cohort of community patients.

Methods
Our study was designed as a prospective, multicentre 10-year follow-up cohort. From a total of 458 patients with acute respiratory tract infections included by 53 general practicioners, 163 patients had blood samples available at baseline and after 7 days for MR-proADM measurement and could be reached by phone calls to assess outcomes. The primary outcome was new onset diabetes mellitus. Secondary outcome was all-cause mortality. We used univariate and multivariate logistic regression models and area under the receiver operating characteristic curve (AUC) to investigate the predictive accuracy of MR-proADM.

Results
After ten years of follow up, risk for new onset diabetes was 2.7% and risk for mortality was 5.4%. No association between MR-proADM and new onset diabetes was found (median MR-proADM blood levels 0.2 nmol/l; IQR 0.1-0.4 vs. 0.2, IQR 0.1-0.8; p=0.87, OR 0.70, 95%CI 0.19 to 2.55). Elevated MR-proADM levels at baseline and at the 7 day follow-up tended to be associated with 10 year non-survival (OR 2.78 (95%CI 0.91 to 8.40, AUC 0.70 and OR 2.43, 95%C 0.83 to 7.07, AUC 0.68).

Conclusion
There was no association between MR-proADM levels and new-onset of Diabetes in this community sample. However MR-proADM was a valid predictor of 10-year mortality. This result expands on results from previous trials and suggests that MR-proADM may help to risk stratify apparently healthy community patients in regard to all-cause mortality.

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Can vasopressin precursors predict mortality and risk of stroke in a community sample? Results from a 10-year follow-up cohort study

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Background/Introduction
Copeptin, the C-terminal part of the arginine vasopressin precursor peptide, is secreted in response to stress and serves as a sensitive and stable surrogate marker for arginine vasopressin production. Different studies found arginine vasopressin precursors to be prognostic in the hospital setting particularly in patients with stroke and infection. However there are no comprehensive data looking at copeptin as a screening marker for cardiovascular events or mortality in patients from the community. Herein, we evaluated copeptin`s ability to predict for stroke and mortality in a cohort of primary care patients with an acute respiratory tract infection from a multicentre trial over a 10-year-follow-up period.

Methods
In a prospective observational 10-year follow-up study, we analysed the prognostic value of copeptin to predict mortality as a primary endpoint and cardiovascular diseases, in particular stroke, as secondary endpoints. From a total of 458 patients with an acute respiratory tract infection recruited by 53 primary care physicians, 311 patients were contacted using a structured interview. Copeptin was measured in stored blood samples at the day of randomization and after 7 days of follow up. We investigated predictive accuracy of copeptin using univariate and multivariate Cox regression models and area under the receiver operating characteristic curve (AUC).

Results
After a median follow up of 10.0 years (IQR 9.5-10.3), mortality was 9.97% and 2.32% of patients had a stroke. Copeptin blood levels were elevated in non-survivors at baseline and at 7 days follow-up (median concentration at baseline 11.7 pmol/l, IQR 4.0-25.7; vs. 6.1 pmol/l, IQR 4.0-10.4; p=0.014 and at follow-up 9.5 pmol/l, IQR 3.1-19.5; vs. 4.3 pmol/l, IQR 2.8-10.4; p=0.003) and were strongly associated with 10 year mortality (age-adjusted hazard ratio at baseline 1.28 (95%CI 0.84-1.94); p=0.254 and at follow-up 1.72 (95%CI 1.08-2.74); p=0.022, AUC at baseline 0.64 (95%CI 0.51-0.76) and at follow-up 0.67 (95%CI 0.54-0.80)). For stroke, no significant increase in copeptin levels were found in patients who had an event in the follow-up time (median concentration at baseline 11.9 pmol/l, IQR 4.0-36.9; vs. 6.3 pmol/l, IQR 4.0-11.3; p=0.320 and at follow-up 5.0 pmol/l, IQR 2.7-13.1 vs. 4.4, IQR 2.9-7.6; p=0.930, age-adjusted hazard ratio at baseline 1.26 (95% 0.80-1.98); p= 0.316 and at follow-up 1.85 (95% 1.15-2.3); p=0.012, AUC at baseline 0.61 (95%CI 0.33-0.89) and at follow-up 0.51 (95%CI 0.25-0.77).

Conclusion
In this first study investigating vasopressin precursors in a community sample of asymptomatic patients, this marker was found to be associated with all cause mortality and to a lesser extend to stroke. In conjunction with traditional risk factors, copeptin may help to better direct preventive measures in this population.

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Trimethylamine-N-oxide (TMAO): a cardiovascular biomarker to predict short- and long-term outcome in community-acquired pneumonia?

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Background/Introduction
The intestinal microbiota-dependent and proatherosclerotic metabolite trimethylamine-N-oxide (TMAO) is an emerging prognostic biomarker for incident cardiovascular events. Interestingly, this marker is modifiable through broad-spectrum antibiotic treatment by changing the microbiom. Whether this marker would also be helpful for short- and long-term prognostication in patients with community-acquired pneumonia (CAP), the leading cause of infectious death, remains undefined. TMAO is of potential interest in CAP as new studies have shown patients being at high risk for cardiovascular events in the months after suffering from a CAP episode.

Methods
A total of 317 randomly selected patients with CAP from a previous antibiotic stewardship trial admitted to five medical centres in Switzerland were prospectively followed for six years. TMAO plasma levels were measured by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Cox regression models and area under the receiver operating characteristics curve (AUC) were used to investigate associations between initial TMAO levels and all-cause mortality.

Results
In the present cohort (median age 72 years), 30-day and 6-year all-cause mortality was 4.9% [95% confidence interval (CI) 3.8-6.1%] and 45.1% [95% CI 39.6-50.6%], respectively. Plasma TMAO levels remained stable over the first seven days of hospitalisation (ANOVA, p=0.14) and did not change in regard to antibiotic treatment used. For long-term mortality over six years follow-up, baseline levels of TMAO were increased in non-survivors compared to survivors (median 4.1 µM [interquartile range (IQR), 2.2-7.2 µM] vs. 2.5 µM [IQR, 1.5-4.1 µM]; p<0.001). Cox regression models showed a strong association of TMAO and mortality with a hazard ratio (HR) of 2.34 ([95%CI 1.65-3.32]; p<0.001) and a good discrimination with an area under the receiver operating characteristics curve (AUC) of 0.66 [95%CI 0.60-0.72]. Calculations for 30-day mortality were similar resulting in a HR of 4.10 ([95%CI 1.49-11.29]; p<0.001) and an AUC of 0.68 [95%CI 0.54-0.83].

Conclusion
This is the first study evaluating TMAO as a risk-predicting biomarker in a well characterized, large CAP patient cohort. TMAO plasma levels remained stable over the hospital course and showed a strong association with 30-day and 6-year all-cause mortality. These data may help to direct preventive measures in these patients and provide a rationale for a more risk-adapted, 'personalized' short- and long-term management strategy, which may translate into improved survival.

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Hungry bone syndrome after surgery for primary hyperparathyroidism in patients presenting with brown tumors - a case series

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Background/Introduction
Brown tumors (BT) are a rare manifestation of long standing primary hyperparathyroidism (pHPT), and the severity of the disease is an indication for parathyroid surgery. Following removal of the dominant PTH source, "hungry bone syndrome" (HBS) with prolonged hypocalcemia is nowadays rare in vitamin D-replete patients with pHPT, but appears to be more common in the subgroup with BT. This unusual presentation has led to a large number of individual case reports, but original series of several patients are scarce.

Methods
We describe 8 (6 female) patients with pHPT presenting with BT and compared them to a control group (n=16, 12 female) with pHPT without BT (presenting with previously detected hypercalcemia), matched not only for gender but also for place and year of surgery. Skeletal involvement at presentation was readily detected by bone biopsy or imaging (scintigraphy, MRI) and in 5 of 8 patients also by 18F-PET/CT. Patients were treated by parathyroidectomy, resulting in correction of hypercalcemia in all of them.

Results
At evaluation, patients with pHPT and BT were younger (44.6±4.9 vs. 55.4±3.3 years; mean ± SEM) and presented with significantly ($p<0.05$ by two-tailed t-test) lower BMI (20.2±1.4 vs. 26.8±1.6 kg/m2), higher calcium (3.4±0.2 vs. 2.9±0.1 mmol/l), PTH (878±186 vs. 154±19 ng/l) and alkaline phosphatase (AP) (334±109 vs. 92±7 U/l) serum levels; there was no significant difference in serum creatinine between the two groups (95±12 vs. 86±6 µmol/l). Adenomas were readily detected by imaging and/or by the surgeon and were significantly larger in patients with BT (weighing 5.4±1.6 vs. 1.2±0.4 grams). Following surgery, 5 of 8 patients with BT (none of the controls) developed HBS requiring prolonged iv calcium treatment, resulting in a significantly longer hospital stay (21.0±5.7 vs. 3.8±0.8 days). Hypocalcemia was accompanied by hypophosphatemia, low urinary calcium as well as persistently high AP. Skeletal recovery was reflected by a decrease in AP activity towards normal, by reappearance of normal amounts of calcium in fasting spot urine samples and the feasibility of an oral treatment preventing symptomatic hypocalcemia (sufficiently effective to permit dismission from the hospital). Permanent treatment with calcitriol was necessary in 3 of 24 patients (1 with BT).

Conclusion
We conclude that presentation of pHPT with BT, apparently due to late awareness for the disease, constitutes an increased risk for development of a postoperative HBS with prolonged need for iv calcium treatment to correct and prevent severe hypocalcemia. Predictors of such a complicated postoperative course include not only high PTH but also high calcium and AP serum levels at presentation.

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Characteristics of Patients with Profound Hyponatremia due to Primary Polydipsia

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Background/Introduction
Hyponatremia due to excessive fluid intake in patients with primary polydipsia is common, however, remains mostly mild and asymptomatic. Nevertheless, compulsive drinking might culminate in profound hyponatremia and severe water intoxication - a condition carrying a considerable risk of morbidity and mortality. The aim of this study was to describe clinical characteristics of polydiptic patients hospitalised with profound hyponatremia, as well as to assess the outcome one year post initial hospitalisation.

Methods
This is a secondary analysis of a prospective observational study, which included 298 patients with profound hyponatremia (≤125mmol/L), presenting in the medical emergency department of two tertiary care centres in Switzerland from June 2011 until August 2013. This sub analysis includes 23 patients with hyponatremia due to primary polydipsia. Symptoms, severity and complications of hyponatremia were assessed, as well as probable contributing factors (medication, co-morbidities, liquid intake). A one-year follow-up was performed to evaluate recurrence, re-admission and mortality rate.

Results
Of the 23 patients included in this study, median age was 56 years (IQR 50-65 years), 17 (74%) were female. Median serum sodium level at hospital admission was 121 mmol/l (IQR 114-123), median urine osmolality 167 mmol/l (IQR 105-184), and median copeptin level 3.6mmol/l (IQR 1.9-5.5). Most common symptoms were: generalised weakness (15 (65%)), sensation of thirst (14 (60%)), nausea (9 (39%)), diarrhoea (9 (39%)), headache (7 (30%)) and falls (4 (17%)) - complicated by a bone fracture in one person (4%). Beer potomania and psychiatric disease were identified as underlying cause of primary polydipsia in 6 (26%) and 10 (43%) patients, respectively. Medications known to predispose hyponatremia were frequent: antipsychotic drugs (6 (26%)), antidepressant drugs (6 (26%)), and diuretics (6 (26%)). Fluid restriction successfully corrected hyponatremia (>130mmol/l) in all patients within median 3 days (IQR 2-4); 5 (22%) needed treatment in the intensive care unit.
During the follow-up period of one year readmission to the hospital was very common, seen in 14 (88%) patients, 11 (65%) presented with re-hyponatremia. Four (17%) patients died within one year, due to aspiration pneumonia (1) and unsolved reasons (3).

Conclusion
Hyponatremia is a severe complication of patients with primary polydipsia. Given the high recurrence, re-hospitalisation and mortality rate, contemporary therapeutic approach of correcting hyponatremia seems insufficient for patients with this complex clinical disorder.

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The SwissDiab Registry - first analysis of crosssectional baseline data with respect to reaching SGED targets for a "good" Disease Management Diabetes

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Background/Introduction
The treatment of diabetes associated complications poses a significant burden on healthcare systems worldwide. Despite compelling evidence for the benefits of tight glycemic control and aggressive treatment of other risk factors, a number of observational studies in different settings have shown great discrepancies between recommended treatment goals and the actual standard of care. We analyze cross-sectional data of the SwissDiab Registry (SDR) with regard to reaching the targets of "good" Disease Management Diabetes (DMD) proposed by the SGED in 2012.

Methods
Baseline data of patients, regularly seen and treated at the Diabetes Outpatient Clinics of Kantonsspital St. Gallen and Inselspital Bern between 1/2010 and 6/2015 and participating in the multicenter longitudinal observational SDR, were analyzed. Descriptive statistics were used to specify cohort demographics as well as pertinent medical information and to assess achievement of the SGED DMD targets (i.e. HbA1c ≥ 9.0% in <15%; < 8.0% in ≥ 60%; < 7.0% in ≥ 40%. BP ≥ 140/90 mmHg in < 35%; LDL-C ≥ 3.37 mmol/l in < 37%).

Results
By the end of 6/2015 the data of 441 patients, 320 (73%) from St. Gallen and 121 (27%) from Bern were available for cross-sectional analysis. 165 patients (37%) suffered from Type 1 Diabetes (DM1), 276 (63%) from Type 2 Diabetes (DM2). Mean age (+/-SD) was 41.5(15.2) in DM1 and 60.7(10.2) in DM2. The proportion of women was 38% (n=63) in DM1 and 29% (n=79) in DM2. Almost 30% of patients (20% in DM1; 35% in DM2) had a migration background. Mean BMI (+/-SD) was 25.0(4.1) kg/m2 and 32.5 (6.1) kg/m2 for DM1 and DM2 respectively. The proportion of active smokers was 21% (22% DM1; 21% in DM2). Average HbA1c (+/-SD) was 7.4%(1.0) in DM1 and 7.3%(1.1) in DM2. An HbA1c ≥ 9% (target <15%) was found only in 7% patients with DM1 and 8% patients with DM2, an HbA1c <8% was reached in 76% of patients with DM1 and 78% of patients with DM2 (target ≥60%) . 32% of patients with DM1 reached an HbA1c of <7% while this was the case in 44% of patients with DM2 (target ≥ 40%). 4% of patients with DM1 and 9% of patients with DM2 had blood pressure values ≥140/90mmHg (target <35%). An LDL-C level ≥3.37mmol/L was seen in 21% and 13% of patients with DM1 and DM2 respectively (target ≤ 37%).

Conclusion
With the exception of an HbA1c <7% in ≥40% of Patients with DM1 all accountability measure targets proposed by the SGED DMD reference and surveyed within the SDR, were met at baseline. Therefore, the objectives chosen seem realistic and attainable. The fact, that target values are even reached within a patient population of tertiary care centers, presumably taking care of patients with more advanced disease stages or other complicating factors, may reflect the comparably high standard of diabetes care in Switzerland in general, although no conclusion regarding the standard of diabetes care in the primary care setting or other parts of the country can be drawn from this data. In order to improve care at the two participating centers, one may have to set even stricter goals.

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Visual field deficits and pituitary insufficiency due to a sellar mass - hypophysitis in the peripartum period: a case discussion on the role of surgery

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Background/Introduction
Hypophysitis in women during late pregnancy and in the postpartum period is most commonly due to lymphocytic inflammation. Appropriate therapeutic strategies should be carefully selected after reviewing the evolution of clinical symptoms, biochemistry, perimetry, and radiological studies. In case of progressive optic nerve compression, high dose glucocorticoid therapy or transsphenoidal surgery has been recommended and/or may be required, considering differential diagnosis (initially often uncertain) and the potential natural history (concerning vision, usually benign), beyond replacing vitally essential hormones (cortisol).

Methods
We report the case of a 30y-old woman, who developed visual field deficits during the peripartum period. She had no prior medical history. Her pregnancy was uneventful except for occasional headaches in the last trimester. 1 day before an unplanned C-section, the patient complained of blurry vision. 4 days after the C-section the patient described visual field deficitis bitemporally, and perimetry was performed confirming bitemporal deficits. An MRI showed a suprasellar mass, compressing the chiasm with a maximal cranio-caudal diameter of 21 mm. 1 day after the mass was discovered, partial pituitary insufficiency was diagnosed (cortisol, 27 nmol/l; fT4, 4.5 pmol/l); cortisol and thyroxin were replaced. Prolactin (82 ug/l) and IGF-1 were normal. She started breast-feeding. Fluid intake and thirst were normal. Kinetic perimetry confirmed bitemporal skotomas. The patient still experienced blurry vision in both eyes. The neurosurgeons decided to operate on the patient.

Results
11 days after the first discovery of the pituitary mass, transsphenoidal extirpation was performed. Intraoperatively, the tissue was firm and hard to remove. Intraoperative frozen section revealed tissue compatible with pituitary adenoma, but no specific diagnosis. A second intraoperative specimen was diagnosed as “neoplastic pleomorph tissue“. Postoperative histologic workup showed a nodule (size: 1.6x1.5x0.4cm) compatible with normal adenopituitary tissue infiltrated mainly with lymphocytes, but plasma cells and macrophages were also present. The final histopathological diagnosis was lymphocytic hypophysitis. The patient developed diabetes insipidus on the 1st postoperative day, needing desmopressin. She was no longer able to breastfeed and was discharged with 125ug of levothyroxine, 30mg of hydrocortisone and 10ug of desmopressin. 15 months postoperatively complete pituitary failure persists but without headaches and visual deficits.

Conclusion
The patient presented with blurry vision and was found to have preferential corticotropin and thyrotropin deficiency in the post partum period due to a sellar mass which compressed the chiasm causing visual field deficits. Subtotal removal of the pituitary mass (gland) was performed, permitting histological confirmation of the diagnosis. Postoperatively, the patient developed persistent panhypopituitarism. We discuss the pros and cons of a surgical approach vs conservative therapy.

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Physiological area of normality and half life time of copeptin

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Background/Introduction
Copeptin is the C-terminal portion of the precursor of vasopressin. In contrast to vasopressin copeptin is stable in vitro and easy to measure, and thus copeptin emerged as a promising marker in different diseases, e.g. polyuria polydipsia syndrome. However, the physiological area of normality and the half life time of copeptin has never been evaluated.

Methods
We measured plasma copeptin, -sodium and osmolality levels in 91 healthy volunteers at baseline, during/after i.v. infusion of 3% saline until a sodium level of at least 150mmol/l was reached, and during/after an oral waterload/i.v. infusion of glucose 5% until normalization of sodium. In total, 4-9 (median 12) measurement per patient were performed.

Results
Median age of the study participants was 28years (IQR: 25;34.5) with a balanced gender distribution male/female (44/47). Upon hypertonic saline infusion plasma sodium levels increased from a median of 139mmol/l (IQR 138; 141) to 152mmol/l (IQR 151; 153), plasma osmolality from 289mosml/kg (IQR 281; 295) to 311mosml/kg (IQR 305; 318), and plasma copeptin from 4 (IQR 3.1; 6) to 32.9pmol/l (IQR 21.25; 46.25). The maximal value of copeptin was reached after 140minutes (SD 29.1), without a time lag to the maximum of plasma sodium or osmolality (reached after 145 (SD 32.6) and 149 (SD 30.5) minutes, respectively). There was a positive correlation between plasma copeptin and plasma sodium ($r=0.57$, $p<0.05$) and plasma copeptin and plasma osmolality ($r=0.53$, $p<0.05$). The half-life time of copeptin was 38minutes which is about twice as high as the half-life time documented for AVP.

Conclusion
There is a correlation between plasma copeptin, plasma sodium and plasma osmolality levels from normo- to hyperosmolar states. The half life time of copeptin seems to be slightly longer compared to AVP.

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Adiponectin, leptin and suppression of free fatty acids by oral glucose in patients with cystic fibrosis

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Background/Introduction
Cystic fibrosis (CF) is a disease characterized by inflammation, wasting, and impaired insulin secretion. To study adipose tissue functions, we assessed adiponectin (ADN), leptin (LEP) as well as free fatty acid (FFA) suppression during an oral glucose tolerance test (oGTT) in patients with CF referred for lung transplantation (LTx).

Methods
Over 10 years, consecutive CF patients were included during evaluation regarding LTx. Patients (excluding those known for previous fasting plasma glucose (FPG) ≥7mM and those treated with insulin) and a control group of healthy subjects underwent an oGTT to assess insulin secretion (insulinogenic index, IGI, Wareham) and to calculate an insulin sensitivity index (ISI, Matsuda). Results were expressed as median and interquartile range.

Results
oGTT was performed in 48 CF patients (age 24(20-32) y; 22 males) and 34 healthy controls (age 30(23-35) y; 19 males). CF patients (both male and female) had significantly lower BMI (18(17-20) kg/m2) than the controls (22(20-27) kg/m2). FPG was similar in patients (4.8(4.5-5.2) mM) and in controls (4.6(4.4-5.1) mM). As expected, 2h plasma glucose was higher (9.5(7.3-13.0) mM vs 6.4(4.9-7.0) mM) in CF patients than in controls. The IGI was lower in CF patients than in controls (16(8-30) vs 55(35-81) pM/mM), whereas insulin sensitivity was comparable (ISI: 8.3(6.1-10.9) vs 8.5(7.0-10.9) (mMpM)-1).
ADN was higher (10.3 (8.2-12.9) vs 7.4(5.7-9.6) mg/l) and LEP was lower (2.0(0.6-5.6) vs 4.6(1.4-12.4) ng/ml) in CF patients than in controls. Although BMI did not differ between male and female CF patients, LEP (3.3(1.3-7.8) vs 0.6(0.5-1.9) ng/ml) and ADN (12.0(9.2-13.9) vs 8.7(7.0-10.4) mg/l) were significantly higher in females. During oGTT, FFA decreased from 0.59(0.43-0.81) to 0.13(0.05-0.17) mM after 2h in CF patients and from 0.52(0.28-0.91) to 0.09(0.05-0.18) mM in controls.

Conclusion
ADN levels are high and LEP levels are low in insulin treatment-naïve CF patients referred for LTx. Despite markedly impaired insulin secretion, FFA levels were suppressed during an oGTT to a similar extent as in healthy individuals, suggesting a high sensitivity of adipose tissue to actions of insulin in patients with CF.

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Metabolic effetcts of metformin during systemic glucocorticod treatment – a randomized controlled trial

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Background/Introduction
Patients receiving glucocorticoid treatment are prone to develop metabolic complications such as diabetes mellitus, obesity or dyslipidemia. In preclinical studies metformin prevented the development of the metabolic syndrome during glucocorticoid excess. The aim of our study was to investigate the metabolic effect of metformin during glucocorticoid treatment in non-diabetic patients.

Methods
In a double-blind, placebo-controlled study, patients starting glucocorticoid treatment for various reasons, with glucocorticoids for at least four weeks, were randomized to receive either metformin (850mg once daily for one week followed by 850mg twice daily for three weeks) or placebo. All patients underwent a standardized oral glucose tolerance test at baseline and after four weeks. The primary endpoint was the change in the 2h area under the curve (AUC) of glucose during the oral glucose tolerance test between baseline and four weeks.

Results
29 of 34 randomized non-diabetic patients completed the trial (17 metformin, 12 placebo). In patients allocated to placebo, median glucose 2h AUC increased from baseline to four weeks (836 (IQR 779-966) to 1202 (1008-1270) mmol/l/120min; p=0.01). In contrast, glucose levels remained similar to baseline in the metformin group (936 (900-954) to 912 (899-946) mmol/l/120min; p=0.83). This change within four weeks was different between both groups (p=0.005). Moreover, there was an improvement in fasting glucose levels and in the HOMA-index in the metformin group versus non-improvement in the placebo group (p= 0.01 and p=0.035, respectively).

Conclusion
In this first randomized trial of metformin targeting metabolic complications of glucocorticoid therapy, we observed a beneficial effect of metformin on glycemic control. Metformin thus seems to be a very promising drug for preventing metabolic side-effects during systemic glucocorticoid treatment.

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Association between thyroid dysfunction and venous thromboembolism in the elderly: a prospective cohort study

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Background/Introduction
Venous thromboembolism (VTE) and subclinical thyroid dysfunction (SCTD) are both common in the elderly. SCTD has been related to a hypercoagulable state and increased thromboembolic risk. However, prospective data on the relationship between SCTD and VTE are lacking. Therefore, we aimed to investigate the relationship between SCTD and recurrent VTE (rVTE), all-cause mortality, and thrombophilic biomarkers in a prospective multicenter cohort of elderly VTE patients.

Methods
Thyroid hormones and thrombophilic biomarkers were measured 1 year after acute VTE, as both may be influenced by acute thrombosis. We defined subclinical hypothyroidism (SHypo) as elevated thyroid stimulating hormone levels (TSH, 4.50-19.99 mIU/l), and subclinical hyperthyroidism (SHyper) as TSH<0.45 mIU/l, both with normal free thyroxine levels. Outcomes were incidence of rVTE and overall mortality during follow-up starting after the 1-year blood sampling.

Results
Of 561 participants, 6% had SHypo and 5% SHyper. After 20.8 months of mean follow-up, 52 (9%) developed rVTE and 56 (10%) died. rVTE incidence was 7.2 (95% confidence interval: 2.7–19.2) per 100 patient-years in SHypo, 0 in SHyper and 5.9 (4.4–7.8) in euthyroid participants. In multivariate analyses, the sub-hazard ratio [SHR] for rVTE in SHypo was 1.50 (0.52–4.34, p=0.46) without increased thrombophilic biomarkers compared to euthyroids. We found no association between SHypo (SHR 0.99, 0.30–3.29, p=0.98) and mortality. SHyper was neither associated with rVTE (exact odds ratio 0.24, 0-1.14, p=0.11) nor mortality (SHR 0.80, 0.23–2.81, p=0.73).

Conclusion
In this prospective cohort of elderly VTE patients, SCTD was neither associated with rVTE nor mortality, without clear differences in thrombophilic biomarkers.

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Relationship between Weight Change and Glycaemic Control with Once Weekly Dulaglutide (DU) Treatment in Patients with Type 2 Diabetes

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Background/Introduction
DU, a once weekly GLP-1 receptor agonist, was studied in the AWARD clinical trial program in adult patients with type 2 diabetes. In 6 head-to-head phase 3 trials, DU demonstrated significant HbA1c reduction and weight control effects.

Methods
To assess the relationship between weight change and glycaemic control in DU-treated patients, HbA1c and body weight data at 26 weeks from the 6 trials were analysed. Due to differences in design, background therapy (see Table) and baseline characteristics, analyses were conducted by trial rather than by pooling.

Results
Across the studies, 82.6%–97.4% of patients treated with DU (1.5 mg and 0.75 mg) had HbA1c reduction, with weight loss achieved by most of these patients receiving DU 1.5 mg (54.6%–83.4%) and 40.7%–79.1% receiving DU 0.75 mg (Table). Minimal correlation was observed between the changes in HbA1c and weight (absolute correlation coefficient <0.3, all). The baseline characteristics gender, age, duration of diabetes, HbA1c, body weight and BMI did not correlate with different weight responses.

Study Comparator (background therapy)	Patients with HbA1c reduction and weight loss/weight gain, %		
	DU 1.5 mg	DU 0.75 mg	Comparator
AWARD-3 Metformin (none)	(n=265) 68.7/18.1	(n=265) 52.5/30.2	(n=265) 60.0/18.9
AWARD-5 Sitagliptin (metformin)	(n=301) 83.4/9.6	(n=297) 79.1/14.5	(n=311) 58.5/24.1
AWARD-6 Liraglutide (metformin)	(n=293) 77.8/17.1	Not applicable	(n=293) 83.3/10.2
AWARD-1 Exenatide twice daily (metformin+pioglitazone)	(n=271) 64.6/32.8	(n=269) 48.7/46.1	(n=266) 55.3/30.5
AWARD-2 Insulin glargine (metformin+sulfonylurea)	(n=263) 69.6/20.5	(n=266) 60.9/26.7	(n=258) 28.7/54.7
AWARD-4 Insulin glargine (insulin lispro±metformin)	(n=273) 54.6/41.4	(n=275) 40.7/53.8	(n=276) 14.5/73.9

Conclusion
Dulaglutide demonstrated a dose-dependent effect on both weight loss and HbA1c reduction. Dulaglutide is an effective treatment option with both HbA1c reduction and weight loss observed across the type 2 diabetes treatment spectrum.

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Short-Term Overfeeding Induces Alterations in Pulsatile LH Secretion in Healthy, Lean Young Women

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Background/Introduction
The activity of the female neuroendocrine reproductive axis is intimately linked to nutritional status. Undernutrition has clearly been associated with hypogonadotropic hypogonadism. Obesity is also associated with decreased fertility, but the pathophysiological consequences of excess weight on the hypothalamic control of reproduction are far less understood.To gain insight into these mechanisms, we investigated the effects of short-term hypercaloric intake on the hypothalamic GnRH secretion in healthy, lean young women.

Methods
The nine female volunteers were characterized by a mean age of 23.5 ± 0.8 years, a mean BMI of 21.9 ± 0.7 kg/m², and regular menstrual cycles lasting 30.2 ± 0.8 days. Hypothalamic GnRH secretion was evaluated in the follicular phase by assessing LH pulsatility on two different days (baseline assessment and hyperinsulinemic, euglycemic clamp), using frequent (q10') blood sampling protocols. These protocols were performed at the end of two distinct nutritional interventions: the first intervention consisted of one week of controlled isocaloric diet (calculated as 1.5 times the resting metabolic rate). The second intervention (hypercaloric diet) lasted four weeks: ~50% extra calories were added in the form of sucrose (3 g/kg BW/day) and fat (1g/kg BW/day) to the usual diet of the volunteers (first three weeks), and to the controlled isocaloric diet (last week).

Results
This hypercaloric diet induced an average weight gain of 2.0 ± 0.3 kg ($p<0.05$), corresponding to a BMI increase of 0.7 ± 0.1 kg/m² ($p<0.05$). A significant decrease of 11.6 ± 4.6 % in whole body insulin sensitivity was also observed (delta = -1.6 ± 0.7 mg glc/kg/min; $p<0.05$). In this condition, the spontaneous LH pulsatility was altered with a significant increase in LH peak frequency of 17.9 ± 9.0% (delta = +1.3 ± 0.5 peaks/10h; $p<0.05$) and a significant decrease in LH peak amplitude of 26.5 ± 9.0% (delta = -0.7 ± 0.36 U/l; $p<0.05$).

Conclusion
These results demonstrate that a reduction in peripheral insulin sensitivity is associated with modifications in LH pulsatility similar to those observed in conditions such as PCOS and obesity.

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Improvement in HbA1c in patients with type 2 diabetes mellitus treated with once-weekly dulaglutide across baseline body mass index (BMI) subgroups at 26 or 52 weeks

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Background/Introduction
This post-hoc analysis investigated the efficacy of dulaglutide (DU) and active comparators across baseline BMI categories (BMI <30, ≥30–<35 or ≥35kg/m2) in patients with T2DM using data from the phase 3 randomised trials AWARD-1 to -6

Methods
Patients with T2DM received DU [1.5mg, n=1719 (AWARD-1 to -6); 0.75mg, n=1417 (AWARD-1 to -5)], or exenatide (EX) 10µg twice daily (n=276), insulin glargine (glargine; titrated to fasting plasma glucose target) (n=558), metformin (MET) 1500 or 2000 mg/day (n=268), sitagliptin (SITA) 100 mg once daily (n=315) or liraglutide (LIRA) 1.8mg once daily (n=300), in addition to other concomitant background treatments. Analysis of covariance models (AWARD-1 to -5) or mixed-effects model for repeat measures (AWARD-6), including treatment-by-BMI subgroup interaction terms, were applied by study to estimate the effect of each treatment on HbA1c at 52 weeks (AWARD-1 to -5) or 26 weeks (AWARD-6) and to compare DU and corresponding active comparators for patients with baseline BMI <30, ≥30–<35 or ≥35kg/m2 (intention-to-treat population).

Results
Baseline mean BMI in each study ranged from 31.2–33.6 kg/m2. HbA1c reductions (%) from baseline according to BMI subgroup (<30, ≥30–<35, ≥35kg/m2) were DU 1.5mg: -0.64, -0.80, -0.64; DU 0.75mg: -0.72, -0.49, -0.44; MET: -0.54, -0.57, -0.40 in AWARD-3; DU 1.5mg: -1.24, -1.21, -0.96; DU 0.75mg: -0.97, -0.79, -0.89; SITA: -0.54, -0.42, -0.29 in AWARD-5; DU 1.5mg: -1.39, -1.43, -1.45; LIRA: -1.32, -1.36, -1.40 in AWARD-6; DU 1.5mg: -1.33, -1.32, -1.44; DU 0.75mg: -1.13, -1.01, -1.09; EX: -0.72, -0.76, -0.91 in AWARD-1; DU 1.5mg: -1.03, -1.24, -0.92; DU 0.75mg: -0.76, -0.77, -0.75; glargine: -0.47, -0.72, -0.77 in AWARD-2; and DU 1.5mg: -1.38, -1.53, -1.54; DU 0.75mg: -1.46, -1.46, -1.36; glargine: -1.10, -1.30, -1.31 in AWARD-4. In all studies, DU 1.5mg, DU 0.75mg and all active comparators achieved statistically significant HbA1c reductions from baseline overall and in all BMI subgroups. No statistically significant treatment-by-BMI subgroup interactions were found for reductions in HbA1c (p=0.159–0.898 across the studies).

Conclusion
DU (1.5mg or 0.75mg) is an effective treatment for patients with T2DM, regardless of baseline BMI. There was no evidence of any treatment-by-BMI subgroup interaction for HbA1c change, suggesting that baseline BMI had no effect on the relative antihyperglycaemic efficacy associated with DU versus comparator antidiabetes agents.

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Systematic investigation of oligogenicity in a large cohort of congenital hypogonadotropic hypogonadism (CHH) patients using exome sequencing

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Background/Introduction
CHH is genetically heterogenous with >25 loci implicated to date. Mutations in these genes account for less than 50% of cases, and each gene contributes only 1-10%. Oligogenicity, defined as the presence of mutations in more than one gene, has been shown to partially explain the incomplete penetrance and variable expressivity observed in CHH probands and their families. The emergence of next generation sequencing technologies (i.e. exome sequencing) provides a new tool to examine oligogenicity in CHH.

Methods
97 European CHH probands (62 Kallmann syndrome, 35 normosmic CHH) and 416 controls (from the population-based CoLaus study) underwent exome sequencing to identify mutations in 20 known CHH genes. Mutations were defined as: 1) Minor allele frequency (MAF) <1% in the ExAC database, 2) loss-of-function variants (nonsense, frameshift and splice donor/acceptor), or missense variants predicted to be deleterious ≥ 50% algorithms. The prevalence of mutations in CHH genes and the frequency of oligogenicity were compared between CHH patients and controls.

Results
Mutations were identified in 47% (46/97) of CHH probands and in 11% (44/416) of controls ($p<0.0001$). Three CHH probands (3/97, 3%) harbored homozygous or compound heterozygous mutations at a single gene, which was not detected in control population. Oligogenicity was detected in 10/97 CHH probands (10%) and only in 3/416 (<1%) controls ($p<0.0001$).

Conclusion
Exome sequencing is an effective and efficient tool to study the complex genetics of CHH. The high frequency (10%) of patients harboring mutations in >1 gene supports the oligogenic CHH model. Further studies including genetic analysis of family members and phenotype-genotype correlation are underway to test if oligogenic model can better predict phenotypes.

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The challenge of managing thyroid hormone replacement therapy during pregnancy in a patient with past radioiodine ablation due to misdiagnosed Graves' disease, contingently TSH-oma and suspected, but not yet confirmed reduced sensitivity to thyroid hormone

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Background/Introduction
Thyroid hormone replacement during pregnancy should assure a normal development of the fetus. In women (and fetus) with a normal sensitivity to thyroid hormone, the replacement can be guided by a TSH-goal in the lower range of normal (primary hypothyroidism) or an fT4 in the upper range of normal (secondary hypothyroidism). In case of a reduced sensitivity to thyroid hormone and the need of a replacement due to prior radioiodine therapy of thyroid gland, the goal of TSH and fT4 to guide the therapy can be challenging.

Methods / Results
A 29-year old women from Portugal presented for a follow-up consultation in 2011. In 1994 she was diagnosed for a Graves' disease, mainly on laboratory findings (?!), as she does not remember having had any symptoms. She was treated with PTU until 2002. In 2006 she underwent radioiodine therapy to cure the proposed Graves' disease. Since then she was on replacement therapy with levothyroxin.
At the initial consultation, she reported only slight nervousness, which she had had throughout her life. Our first laboratory findings showed an elevated TSH (13 mU/L) and elevated fT4 (38 mmol/L) and fT3 (8 mmol/L). The first suspicion was an irregular intake of the substitution therapy, but the laboratory constellation persisted despite re-instructions. Therefore, a TSH-oma was suspected and a MRI showed a pituitary lesion of 12 mm. Consequently assuming that the elevated peripheral hormone levels would reflect oversubstitution of levothyroxine while the elevation of TSH would derive from a TSH-producing adenoma, dose of levothyroxine was gradually reduced. This was followed by a marked increase of TSH to levels around 100 mU/L, while the fT4 and fT3 decreased into the lower reference range. In addition, results of repeatedly measures alpha-subunit arrived, which were both in the normal range. Due to several hindrances, the simultaneously ordered genetic evaluation concerning alternatively proposed reduced sensitivity to thyroid hormones had been delayed for multiple months. Nevertheless, we decided to assume the diagnosis of reduced sensitivity to thyroid hormones and to titrate the levothyroxine dose accordingly aiming to normalize TSH levels. Soon thereafter, the patient became pregnant. Luckily, the genetic evaluation arrived immediately afterwards and confirmed a reduced sensitivity to thyroid hormone due to a THRB-mutation (c.1357C>T). In theory the aim of the T4-substitution in patients with reduced sensitivity to thyroid hormones would be different whether the fetus also has the mutation or not (and what the phenotype in different tissues would be in case of a mutation - the genotype-phenotype correlation in these cases is relatively poor), but a prenatal molecular genetic diagnostic of the fetus was refused by the patient. As an additional psychological difficulty for us, all pregnancies in her family went well, and she knows about no abortion in her family. In correlation with the literature, we aimed for TSH levels at the upper limit of the reference range, and fT4 levels not more than one third above the upper limit, searching the balance between a fetus having the mutation as well and one not having the mutation. This required an increase of T4-replacement from 200-250 mcg to 400-500 mcg per day. Of course, additional surveillance of the pregnancy was performed in close cooperation with the obstetricians. Fortunately, the pregnancy and delivery showed no complications and the development of the fetus was normal during pregnancy and in the first 12 Month since.

Conclusion
Normally, a thyroid targeted therapy is not necessary in patients with reduced sensitivity to thyroid hormones, as the higher level of fT4 represents the higher need to overcome the reduced sensitivity. However, if the thyroid has been destroyed, levothyroxine substitution therapy is usually aimed to normalize TSH levels, which might require high doses.
In case of a pregnancy, a prenatal diagnostic of the fetus is recommended, to guide the target level. But as the genotype-phenotype correlation is poor, some uncertainties remain. The same targets are appropriate only if the fetus exhibit the mutation as well and in addition the same phenotype. If the mutation status of the fetus remains unknown, the usual target are TSH levels at or slightly above the normal level and fT4 levels no more than one third above the upper limit of normal.

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Fibro/adipogenic progenitors from skeletal muscle differentiate into brown-like adipocytes

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Background/Introduction
After the recent reports on the presence of adipocytes expressing uncoupling protein 1 (UCP1) in human adipose tissue, mechanisms to increase the number and activation of such adipocytes have been studied as possible strategies to prevent or counteract obesity and its associated disorders. Skeletal muscle (SM) accounts for approximately 40% of body mass and treatments increasing its energy expenditure could have an important impact on whole body energy expenditure. However, there is still no consensus about the formation and origin of UCP1-positive cells in SM. While previous studies described the differentiation of SM satellite cells into brown adipocytes, other authors observed the differentiation of SM fibro/adipogenic progenitors (FAPs) into UCP1-positive adipocytes. We assessed the adipogenic potential and UCP1 expression of SM myoblasts and FAPs isolated from obesity-prone C57Bl/6 and obesity-resistant Sv/129 mice.

Methods
Tibialis anterior muscles of Sv/129 and C57Bl/6 mice were injected with glycerol to promote in vivo intramuscular adipogenesis. For in vitro experiments, skeletal muscle FAPs (Sca1+alpha-7 integrin-CD31-CD45) and myoblasts (alpha-7 integrin+Sca1-CD31-CD45-) from the hind limbs of adult Sv/129 and C57Bl/6 mice were sorted through fluorescence-activated cell sorting. Upon reaching confluence, cells were treated with brown adipogenic medium for 10 days. Additionally, single FAPs and myoblasts were directly sorted into 96-well plates and treated with the same differentiation medium upon confluence or after 21 days in culture. Adipogenesis was assessed through light microscopy. Gene and protein expression were assessed through qPCR and Western blotting, respectively. Cellular oxygen consumption rate (OCR) was measured using the XF24 Extraflux Analyzer (Seahorse Bioscience).

Results
Glycerol-induced intramuscular adipogenesis was associated with increased Ucp1 mRNA expression in Sv/129 mice, suggesting the newly formed adipocytes as the source of Ucp1 expression. In line with this, FAPs differentiated into multilocular adipocytes expressing UCP1 at the protein and mRNA level. UCP1 expression was higher in cells isolated from Sv/129 mice than in cells isolated from C57Bl/6 mice. Of the 315 monoclonal colonies of FAPs analysed; 9% were adipogenic, 63% were fibrogenic and 29% were mixed. Similar to brown adipocytes, UCP1 expression and OCR of differentiated FAPs were responsive to treatment with triiodothyronine. Myoblasts did not differentiate into adipocytes, forming myotubes in response to the adipogenic treatment supplemented or not with additional promoters of the brown adipogenic fate. None of the 88 myoblast monoclonal colonies analysed presented formation of adipocytes.

Conclusion
FAPs are a potential source of intramuscular UCP1-expressing adipocytes, while myoblasts appear to be committed to the myogenic lineage. Understanding the mechanisms that drive UCP1 expression in FAPs could provide novel protocols to increase energy expenditure and treat obesity.

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Identification of the molecular dysfunction induced by GDH S445L mutation associated with Hyperinsulinism/Hyperammonemia syndrome

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Background/Introduction
Hyperinsulinism/Hyperammonemia (HI/HA) syndrome is a common form of congenital hyperinsulinism. Affected children show unregulated protein-induced insulin secretion from pancreatic ß-cells, fasting hypoglycemia, and elevated plasma ammonia levels. Mutations associated with HI/HA were identified in the Glud1 gene, encoding for mitochondrial glutamate dehydrogenase (GDH). The most frequent mutation is the conversion of Ser445 residue to Leu and is associated with both HI/HA and epilepsy. We aimed at identifying the molecular causes of dysregulation in insulin secretion conferred by this mutation using both INS-1E ß-cells and human islets.

Methods
INS-1E ß-cells and human islets were transduced with adenoviruses carrying the GDH-wild type or GDH-S445L-mutant gene, respectively. The GDH enzymatic activity was measured by NADH autofluorescence under varrying concentrations of substrates, co-substrates, allosteric modulators (inhibitory GTP and activating ADP) in both anaplerotic and cataplerotic directions. The insulin secretion assay was performed under different stimulating conditions (2.8mM and 16.7mM glucose, 30mM KCl, 5mM glutamine). For aech experiment, GDH protein expression levels were assessed by western blot analysis.

Results
Western blots showed efficient expression of both wild type and S445L-mutant GDH in the different cell preparations (INS-1E cells and human islets). Enzymatic activity tested in INS-1E cells revealed higher activity of GDH-mutant versus GDH-wild type under increasing concentrations of the anaplerotic substrate glutamate, NAD co-substrate, ADP activator and GTP inhibitor. In the cataplerotic direction using alpha-ketoglutarate as substrate, GTP was less effective as allosteric inhibitor on GDH-mutant compared to GDH-wild type. Importantly, GDH-mutant was much more sensitive to the allosteric activator ADP, rendering GDH-mutant highly active at any substrate concentration. In human islets, expression of GDH-mutant resulted in elevation of insulin secretion at basal glucose, markedly potentiated by calcium-raising agent KCl. Moreover, GDH-mutant islets exhibited non-physiological glutamine-induced insulin release, not observed in GDH-wild type islets.

Conclusion
The S445L mutation confers hyperactivity to glutamate dehydrogenase. It renders the enzyme slightly less sensitive to GTP inhibition and much more sensitive to ADP activation, in particular in the cataplerotic direction. In human islets, GDH-S445L-mutant caused insulin secretion at basal glucose and under glutamine stimulation, in accordance with respectively fasting hypoglycemia and protein-induced hyperinsulinism observed in affected children.

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Role of piRNAs in the control of β-cell functions

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Background/Introduction
The mammalian genomes generate not only protein-coding mRNAs but also a large number of non-coding transcripts, including microRNAs and long non-coding RNAs, which accomplish major regulatory tasks in the cells. Beside microRNAs and long non-coding RNAs, some cells contain also thousands of 26-33 nucleotide molecules named PIWI-interacting RNAs (piRNAs). Until recently, the expression and function of piRNAs was thought to be restricted to germline cells, where they exert important roles during spermatogenesis. However, recently piRNAs were found to be expressed also in somatic cells and to be involved in different other processes including epigenetic programming, memory and cancer. The aim of this study was to investigate if piRNAs and their binding partners the PIWI-like proteins are expressed in β-cells and if they contribute to the regulation of the activity of insulin-secreting cells.

Methods
Experiments were carried out in pancreatic islets isolated from Sprague Dawley rats. The expression of PIWI-like genes was determined by qRT-PCR and western blotting. piRNA expression profiles of newborn (10-days-old) and adult (3 month-old) rat pancreatic islets were assessed using a specific microarray. The level of expression of selected piRNAs was determined by qRT-PCR. To investigate the global role of piRNAs in β-cells, dissociated rat islet cells were transfected with siRNAs against PIWIL2 or PIWIL4, two PIWI-like proteins essential for piRNA expression and function. Insulin secretion in transfected cells was assessed by ELISA while β-cell apoptosis was determined by counting the cells displaying pyknotic nuclei.

Results
qRT-PCR and western blotting analysis revealed that rat pancreatic islets express two PIWI-like genes, PIWIL2 and PIWIL4 while PIWIL1 was undetectable. The expression of these PIWI-like genes suggests that piRNAs may be present in β-cells. In agreement with this hypothesis, we were able to identify more than 12'000 piRNAs in rat pancreatic islets. To evaluate the role of piRNAs in insulin-secreting cells, we compared the piRNA expression profile of newborn rat islets, containing functionally immature β-cells, with that of islets isolated from adult rats. Interestingly, we found that post-natal β-cell maturation is associated with changes in the level of more than 1700 piRNAs, suggesting that piRNAs may be involved in the acquisition of glucose-induced insulin secretion and to the achievement of a fully mature β-cell phenotype. Furthermore, silencing of PIWIL2 and PIWIL4 in adult β-cells resulted in a significant decrease in insulin secretion in response to glucose and rendered the cells more resistant to apoptosis after exposure to proinflammatory citokines.

Conclusion
We have demonstrated that β-cells express a new class of non-coding RNAs that play an important role in the regulation of insulin secretion and cell survival. A better definition of the role of individual piRNAs and of PIWIL proteins will help elucidating the mechanisms that govern post-natal maturation of β-cell and their activity under normal and diabetic conditions.

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Activation of nicotinic acetylcholine receptors protects murine and human beta-cells from apoptosis

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Background/Introduction
Type 1 Diabetes (T1DM) results from the auto-immune destruction of more than 90% of the pancreatic insulin-producing beta-cells, leaving few survivors that are not sufficient to properly regulate blood glucose levels but persist decades after the onset of the disease. Identifying methods to prevent the destruction of these cells or even to permit the regeneration of a sufficient beta-cell mass from the remaining cells could open interesting perspectives in the prevention or the treatment of T1DM. This work focuses on strategies to increase the resistance of beta-cells against auto-immune destruction in order to prevent T1DM. We consider the possibility that activation of nicotinic acetylcholine (nACh) receptors could protect beta-cells against auto-immunity. NACh receptors have been previously implicated in the control of inflammation and cell death and are expressed on beta-cells, but no study has so far analyzed their role in the modulation of cell death in the context of T1DM.

Methods
Islets isolated from wild type (WT) mice, from mice knocked out for either the α7 or β2 subunit of nACh receptors as well as Human islets were cultured in presence or absence of nicotine or choline. After 24h, a cocktail of IL-1β + TNFα + IFNγ was added, to mimic in vitro the surrounding of a beta-cell at the onset of T1DM. Cell death was quantified by TUNEL analysis. The production of nitric oxide (NO) by beta-cells in response to cytokines was measured by quantification of nitrites in the culture medium. The expression of genes implicated in the regulation of mitochondrial permeability transition was quantified by quantitative PCR and cytoplasmic calcium was measured by Fura-2AM.

Results
The exposure of WT mouse islets to the above-mentioned cytokines increased beta-cell death, but the pre-culture with nicotine significantly decreased cytokine-induced cell death. When the experiment was repeated with islets isolated form mice knocked out for either the α7 or the β2 subunit of nACh receptors, we found that nicotine significantly decreased cell death also in the absence of the β2 subunit, but not in the absence of the α7 subunit, indicating that the effect was dependent on this latter subunit. To confirm this, we repeated the same experiment, but this time with a pre-culture with choline, a specific agonist of the α7 subunit of nACh receptors. We found that choline also significantly attenuated the effect of the cytokine cocktail, indicating that the α7 subunit was necessary for the protective effect. Neither nicotine, nor choline altered the cytokine-induced NO production by beta-cells, indicating that the cholinergic agonists did not interfere with the signaling pathway directly underlying the activation of the cytokine receptors. We further found that choline attenuated the cytokine-induced expression of CHOP, a marker of ER stress as well as of PUMA, a protein of the Bcl-2 family known to favor mitochondrial permeability transition.

Conclusion
Our results show that the activation of nACh-receptors via their α7 subunit significantly reduces cytokine-induced beta-cell death in mouse and Human islets. This effect was not linked to alteration of NO production by β-cells, but rather to attenuation of ER-stress and modulation of PUMA, a pro-apoptotic protein. We believe that these results open the exciting perspective of a pharmacological modulation of beta-cell resistance against auto-immunity.

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Glucose homeostasis and the mitochondrial morpho-function in ß-cells and hepatocytes lacking Prohibitins

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Background/Introduction
Mitochondrial dysfunction is suspected to play a central role in ß-cell failure in type 2 diabetes. In order to study the role of mitochondria defects in the development of diabetes, we generated a mouse lacking a key mitochondrial protein (Prohibitin-2, Phb2) specifically in ß-cells (ß-Phb2-/-). Phb2, with its interdependent homolog Phb1, is mainly localized in the mitochondrial inner membrane. Deletion of Phb2 abolishes membrane fusion and alters mitochondrial morphology. Preliminary study showed that deletion of Phb2 in ß-cells results in mitochondrial dysfunction, loss of ß-cells, progressive alteration of glucose homeostasis, and ultimately diabetes. Strikingly, diabetes systematically develops at the age of 5-6 weeks in ß-Phb2-/- mice. Furthermore, liver-specific Phb2 knockout mice (Hep-Phb2-/-) were also generated to study the role of mitochondria in hepatic glucose production and the putative link between liver and pancreatic islets.

Methods
ß-Phb2-/- mice were sacrificed at the age of 4, 5, and 6 week together with control ß-Phb2fl/fl mice. Insulin and glucagon stainings were performed on fixed pancreas in order to precisely quantify α- and ß-cell masses. TUNEL assay and staining of Ki-67 were also performed on fixed pancreas to investigate apoptosis and proliferation, respectively. Frozen liver tissues from pre-diabetic and diabetic mice were analyzed for metabolomics profiling in order to study whether liver can serve as a responsive tissue to reflect early ß-cell dysfunction.
Regarding Hep-Phb2-/- mice, hepatocytes were isolated and mitochondrial morphology was investigated by confocal microscopy. To investigate molecular mechanisms of Phb2 in mitochondrial dynamics, we introduced adenovirus that expresses a cleavage-resistant form of OPA1 into the Phb2 deleted hepatocytes. The transduced hepatocytes were studied for mitochondrial morphology by confocal microscopy.

Results
Loss of Phb2 in ß-cells resulted in gradual decrease in ß-cell mass starting at the age of 4 weeks, i.e. about 2 weeks before hyperglycemia. During the same period, the loss of ß-cells in pancreatic islets was compensated by enlargement of the α-cell mass. Apopototic and proliferative events of ß-cells and α-cells during this time period are currently analysed. Metabolomics profiling of the liver of 6-week old ß-Phb2-/- mice showed no significant differences compared to controls. Further time points are under investigation.
The confocal imaging on the hepatocytes isolated from Hep-Phb2-/- mice showed that deletion of Phb2 in the liver induced fragmentation of mitochondria. We also observed steatosis and impaired glucose production in liver lacking Phb2.

Conclusion
Mitochondrial defects in ß-cells result in reduced ß-cell mass and increased α-cells during the development of diabetes in ß-Phb2-/- mice. Deletion of Phb2 in hepatocytes causes disruption of mitochondrial dynamics and altered glucose production. Results show that mitochondrial function in ß-cells and hepatocytes is required for proper glucose homeostasis.

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The Role of Alternative Androgen Biosynthesis in the Human Ovary via the Backdoor Pathway

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Background/Introduction
Recent work revealed two pathways in androgen biosynthesis, namely the classical and the backdoor pathway. In the ovary, regulation of androgen production plays a crucial role in normal physiology, as well as in pathologies such as the polycystic ovary syndrome (PCOS). However, while in the testis the importance of the backdoor pathway in androgen regulation has been described, its role in the human ovary remains to be established.

Our ami is the characterization of the backdoor pathway in human ovarian androgen biosynthesis in health and disease.

Methods
Pathway analysis was performed on fresh frozen paraffin embedded ovarian tissue samples obtained from the Institute of Pathology Bern. Genes involved in the backdoor pathway were assessed by quantitative RT-PCR. Testis and adrenal tissues served as control. For the comparison of normal to aberrant ovarian physiology we analysed ovarian samples of controls and of PCOS and ovarian endometriosis patients.

Results
First results indicate differentially regulated expression of backdoor pathway genes comparing ovary, adrenal and testis. Ovarian expression of aldo-keto- and steroid-reductase genes was low with high variation in these low levels and no difference between control and abnormal tissues. This might be explained by the heterogeneous tissue composition of the samples. Still, our findings are in range of published data from ovarian gene expression studies.

Conclusion
Quantitative RT-PCR analysis on whole tissue samples revealed variability in the data and no difference between control and abnormal ovarian tissues. Next we will characterize protein expression of the backdoor pathway genes by immunohistochemistry what should allow localizing expression changes in the heterogeneous tissues. Further, urine steroid profiling for metabolites of both pathways in controls versus PCOS women is ongoing and allow further conclusion of involvement.

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Growth Hormone (GH) Deficiency Type II: a Novel GH-1 Gene Mutation (GH-L76P) Severely Affects GH-Folding, Stability and Secretion

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Background/Introduction
Main features of the autosomal dominant form of GH deficiency (IGHD II) include markedly reduced secretion of GH combined with low concentrations of IGF-I leading to short stature. Here we present the structure-function analysis of a single missense mutation in the GH-1 gene converting codon 76 from Leucine (P) to Proline (C) and yielding a mutant GH-L76P peptide.

Methods
Heterozygosity for GH-L76P /wt-GH was identified in a italian family.

The index patients, two siblings, a boy and a girl, were referred for assessment of their short stature (-3.2 and -3.8 SDS respectively). Their grandmother, father and aunt were also carrying the same mutation and showed severe short stature, therefore IGHD II was diagnosed.

Results
In line with the clinical data of the patients, AtT-20 cells coexpressing both wt-GH and GH-L76P showed a reduced GH secretion ($P < 0.001$) after Forskolin stimulation compared with the cells expressing only wt-GH, supporting the diagnosis of IGHD II. In silico mutagenesis and molecular dynamics simulations suggested problems in correct folding and mutant stability compared to wt-GH. Hence, further structural analysis of the GH-L76P mutant were performed, using purified and expressed proteins in Escherichia Coli by thermofluor assay and fast degradation proteolysis assay. Both assay, confirmed the bionformatic model prediction revealing that the GH-L76P mutant is highly instable and severely misfolded compared to wt-GH.

Conclusion
This is the first report of a family suffering from short stature caused by IGHD II which severely affects intracellular GH-folding, stability and secretion. Our results show that specific and detailed analyses of the different mutations identified in IGHD II may shed light on the different mechanisms of secretory pathophysiology, and may provide a better explanation of the range of clinical features associated with GH missense isoforms. Importantly, the findings in patients with GH-L76P extend beyond classical IGHD and stress the need for continued clinical vigilance in IGHD II patients for the development of other hormonal deficiencies.

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Role of FFAR1/GPR40 in the response of INS-1E ß-cells to glucolipotoxicity

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Background/Introduction
Chronic exposure to circulating elevated levels of glucose and free fatty acids has been shown to impair ß-cell function, leading to insulin secretion defects and potentially diabetes. This mechanism is referred to as glucolipotoxicity and remains unclear. The G-protein coupled receptor FFAR1 (formerly GPR40) is known to enhance glucose-stimulated insulin secretion (GSIS) in ß-cells when acutely activated by medium- to long-chain free fatty acids. However, its putative role in the glucolipotoxicity process when chronically activated remains unknown. Here, we investigated the effects of chronic exposure of INS1-E ß-cells to elevated glucose combined with different fatty acids on cell function and FFAR1 expression.

Methods
INS-1E ß-cells were cultured for 4 days at low 5.5 mM, standard 11.1 mM (control) and high 25 mM glucose in the presence or not of 0.4 mM palmitate, 0.4 mM linolenate or 0.4 mM oleate (complexed to BSA). FFAR1 protein levels were assessed by Western blot for each conditions and ß-cell functionality was analysed by measuring intracellular calcium changes upon glucose stimulation. For this assay, cells were starved for 2 h in glucose-free RPMI-1640 medium and then loaded with Fura-2AM. Then, fluorescence was monitored (excitation 340/380 nm and emission 510 nm) in a thermostated plate reader equipped with injectors for sequential glucose and KCl stimulations.

Results
None of the glucolipotoxicity conditions tested did modify FFAR1 expression at the protein level after the 4 days of culture. Compared to regular 11.1 mM glucose, culture at low 5.5 mM glucose had no effect on calcium responses of INS-1E cells, whereas 25 mM glucose for 4 days reduced the glucose-induced calcium rise. The addition of palmitate in the medium slightly altered the morphology and growth of the cells, which was not the case for oleate and linolenate. Combined with high 25 mM glucose, all fatty acids added in the culture medium abrogated the glucose-induced calcium rise at the end of the 4-day exposure. The calcium response was also slightly reduced with oleate at standard 11.1 mM glucose culture. Current work investigates interactions between FFAR1 activation and chronic fatty acid exposure by adding FFAR1 ligands in the culture medium in glucolipotoxicity conditions.

Conclusion
Data show that under chronic hyperglycaemic/hyperlipidemic conditions the ß-cell dysfunction seems primarily driven by high glucose and worsen by fatty acids. However, FFAR1 has a broad range of natural ligands (currently tested) that may have different effects on ß-cells. The characterization of their mode of action could provide valuable information for understanding the role of FFAR1 in glucolipotoxicity and for development of therapies against Type 2 diabetes.

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Modulation of steroid and drug metabolism by P450 oxidoreductase variants

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Background/Introduction
A broad spectrum of human diseases including abnormalities in steroidogenesis is caused by mutations in the NADPH P450 oxidoreductase (POR). POR transfers electrons from NADPH to several small molecules, non-P450 redox partners and all microsomal cytochrome P450 proteins. POR disruption affects all partners with disastrous consequences and POR knock-out mice are embryonically lethal. A number of POR mutations and polymorphisms have been characterized from patients and genome sequencing databases and tested for their abilities to support CYP17A1 and CYP19A1 activities. POR also interacts with drug metabolising CYPs such as CYP3A4 which is responsible for metabolism of about 65% of the drugs in the human liver.

Methods
We analyzed the ability of wild type POR and POR variants (P284L, P284T, A287P, A503V and one novel mutant) to reduce ferricyanide, MTT, cytochrome c, cytochrome b5 and P450s. POR variants were produced as recombinant N-27 form while P450s and Cytochrome b5 were produced as His-tag recombinant protein and purified by ion-exchange and Ni2+ metal chelate chromatography. Reduction of ferricyanide, MTT and cytochrome c was monitored spectrophotometrically by measuring the change in absorbance at 420 nm, 610 nm and 550 nm respectively. We also tested the interaction of POR variants with P450s using ELISA.

Results
We found varied effect of different POR mutants on CYP17A1, CYP19A1, Ferricyanide, MTT and cytochrome c reduction activity. In comparision to wild type POR, the novel POR mutant(L374H) was found to decrease catalytic effeciency of 21-hydroxylation of progesterone by 96%, 17-hydroxylation of progesterone by 87%, 17,20-lyase action on 17OH-pregnelone by 90% and aromatization of androstenedione by 90%. Ferricyanide, MTT and cytochrome reduction activity was also severely affected. However, we observed similar binding of POR mutant with CYP19A1and CYP17A1 as compared to wild type POR . Further it would be interesting to study interaction of POR variants with other redox partners.

Conclusion
In vitro functional studies testing the effect of novel POR mutant on single enzymes predict severe activity loss between 96-87% for different P450s correlating with clinical manifestation. In conclusion, characterization of POR mutants provides valuable genotype-phenotype correlation.

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Effect of CYP17A1 17,20 lyase inhibitors on regulation of adrenal androgen biosynthesis.

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Background/Introduction
The P450c17 (CYP17A1) plays a vital role in regulating adrenal androgen production. CYP17A1 localized in the endoplasmic reticulum can catalyze both 17α-hydroxylase and 17,20 lyase reactions. Understanding the mechanisms regulating 17,20 lyase activity is essential to perceive the regulation of hyperandrogenic disorders like premature, exaggerated adrenarche and the polycystic ovary syndrome. Therefore it is a requisite to design specific 17,20 lyase inhibitor which can be useful in hyperandrogenic states and in sex-steroid dependent cancer. The orteronel and galeterone are known to inhibit 17,20 lyase activity; however, the precise mechanism of the CYP17A1 inhibition remains unknown. These inhibitors have been developed to treat the castration resistant prostate cancer (CRPC) but little is known about their effect on adrenal androgen biosynthesis. We have studied the specific 17,20 layse inhibitors to understand their effects on CYP17A1 and adrenal androgen biosynthesis.

Methods
We used NCI-H295R adenocarcinoma cells to study the effect of orteronel and galeterone. We treated the H295R cells with 0-2μM orteronel and galeterone for 24 hours. Steroid production was labeled with [3H] pregnenolone for 90 min. Steroids were extracted and resolved by thin layer chromatography. For specific analysis of the P450c17 activities, cells were treated with 1μM trilostane (a specific blocker of HSD3B) for 90 min before adding [3H] pregnenolone.To study the effect of these inhibitors on steroidogenic gene expression we performed the relative quantification PCR (qRT-PCR).

Results
The drugs orteronel and galeterone were able to inhibit CYP17A1 activity in H295R cells. Both drugs have more potency towards the 17,20 lyase activity but we also observed that they partially affected 17 alpha-hydroxylase activity. From our results, we observed the orteronel seems to be more potent and selective towards 17,20 lyase activity than galeterone. However, we also found dihydroepiandrosterone (DHEA), cortisol and androstenedione were drastically decreased by both compounds at 1 and 2μm concentration. Further, we also studied effect of these inhibitors on genes which are involved in androgen biosynthesis. We found slight increase in the HSD3B2 gene expression under galeterone treatment but no change was observed in CYP17A1 and AKR1C3 expression.

Conclusion
Based on our results we conclude that orteronel is a more potent inhibitor of 17,20 lyase activity and also has partial effect on 17α-hydroxylase activities. Detailed mechanisms that alter the CYP17A1 enzyme activities need further investigation. The discovery of these drug actions on CYP17A1 activity would be of great clinical value for understanding adrenal androgen regulation.

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Mesenteric Fat Lipolysis Mediates Obesity-associated Hepatic Steatosis and Insulin Resistance

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Background/Introduction
Interleukin-6 (IL-6) may contribute to the development of fatty liver disease and hepatic insulin resistance. Recently, it was suggested that IL-6 promotes hepatic insulin resistance indirectly through increased free fatty acid (FFA) release from adipose tissue. In order to activate the intracellular signaling pathway, the IL-6 ligand/receptor complex associates with a homodimer of glycoprotein 130 (gp130), which is a common signal transducer of all IL-6 cytokines. Here, we aimed to determine whether IL-6-induced FFA-release from adipose tissue contributes to obesity-associated hepatic steatosis and insulin resistance.

Methods
Newly generated adipocyte-specific gp130 knockout mice were either fed a standard chow or high-fat diet (HFD) for 12 weeks. In vivo insulin sensitivity was assessed by hyperinsulinemic-euglycemic clamps. Lipolysis was assessed in isolated adipocytes treated with or without insulin (100 nM) or isoproterenol (1μM). Plasma FFA levels were measured in systemic and portal circulation. Gene expression was assessed by real-time RT PCR. Total liver lipid and triglyceride contents were measured. In humans, adipose tissue IL-6 mRNA was determined and correlated to liver lipid accumulation and eucglycemic clamp glucose infusion rate.

Results
Lack of IL-6 cytokine signaling in adipocytes reduced basal lipolysis and enhanced insulin's ability to suppress lipolysis from mesenteric but not epididymal adipocytes in obese mice. Consistently, FFA levels were reduced in portal but not in systemic circulation of obese knockout mice. Importantly, adipocyte-specific gp130 knockout mice were protected from HFD-induced hepatic steatosis as well as insulin resistance. In humans, omental but not subcutaneous IL-6 mRNA expression correlated positively with liver lipid accumulation and negatively with clamp glucose infusion rate, further supporting a role for IL-6 in hepatic steatosis and insulin resistance.

Conclusion
Blocking IL-6-type cytokine signaling in (mesenteric) adipocytes may be a novel approach to blunt detrimental fat-liver crosstalk as observed in obesity.

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Roles of the Keap1/Nrf2 antioxidant system in thyrocyte homeostasis.

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Background/Introduction
Thyroid follicular cells continuously generate high amounts of hydrogen peroxide to oxidize iodide and iodinate thyroglobulin for thyroid hormone synthesis, and are thus under constant threat of oxidative stress. Compared to other tissues, the thyroid has increased capacity to defend itself against oxidative stress, and specific thyroidal antioxidant and detoxification enzymes have been identified. In various other cell types, the transcription factor NFE2-related factor 2 (Nrf2) is a central regulator of antioxidant and detoxification gene expression in response to electrophilic or oxidative stress. The goal of our studies is to elucidate the roles of the Keap1/Nrf2 antioxidant response pathway in thyroid gland homeostasis.

Methods
Wild-type (WT) and Nrf2 knockout (Nrf2-KO) mice were supplied with normal water or water containing 0.05% sodium iodide (NaI) for one week. Thyroid tissue, liver tissue and blood were collected at the end of the treatment and used for various assays: histomorphometry; thyroid hormone measurement by ELISA; gene expression by qRT-PCR and by deep RNA sequencing (for mRNA and microRNAs); and protein abundance by Western immunobloting. The rat thyroid follicular cell line PCCL3, and a deritative PCCL3-ARE cell line incoporating a Nrf2-regulated antioxidant responce element (ARE), were used as model systems to study in vitro the roles of Nrf2 signaling, and to map the molecular mechanisms of its action in thyrocytes.

Results
Iodide treatment increased the expression of antioxidants genes in the thyroids of WT mice. In Nrf2-KO mice, iodide treatment increased the total protein carbonylation levels, indicating oxidative stress. Nrf2-KO mice had higher serum T4 but lower T3 levels; expresion of TPO was higher in Nrf2-KO mice, while expresion of DIO1 was decreased in both thyroid and liver. Expression of the thyroid-specific genes NIS and TG were lower in Nrf2-KO mice, while levels of iodinated TG (TG-I) were higher in Nrf2-KO mice. In response to iodide, TG-I levels decreased in the thyroids of WTmice; in contrast, they increased dramatically in the thyroids of Nrf2-KO mice. In PCCL3 thyrocytes, siRNA-mediated knockdown or pharmacological inhibition of Nrf2 suppressed expression of antioxidant genes and of NIS, whereas TPO expression was increased. Conversely, siRNA-mediated knockdown of the Nrf2 inhibitor Keap1 or pharmacological activation of Nrf2 induced expression of antioxidant genes and of TG. In PCCL3-ARE cells, transcriptional activation of Nrf2 was reduced by TSH and increased by insulin.

Conclusion
Together, our results indicate that Nrf2 regulates the expression of ubiquitous antioxidant genes preserving the redox homeostasis of the thyroid gland, while its activation status impacts the regulation of central thyroid-specific genes such as NIS, TG and TPO. The striking difference in the pattern of TG iodination levels between WT and Nrf2-KO mice demonstrates that Nrf2 has a previously unrecognized role in the intrathyroidal handling of excess iodine, which is under further investigation.

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Repletion of intramyocellular lipids (IMCL) is different in GHD patients with growth hormone deficiency (GHD) compared to controls subjects (CS) 24h after a 2h aerobic exercise

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Background/Introduction
Growth hormone deficiency is associated with decreased exercise capacity, impaired body composition with increase in fat mass and a reduction in lean body mass.
Fat can be stored in adipose tissue but also in non-adipose tissue such as skeletal muscle and liver tissue, so-called ectopic lipids.
We and others have shown that ectopic lipids are flexible fuel stores in healthy subjects, in patients with type 1 diabetes and in GHD. IMCL are depleted by exercise and repleted by diet. In contrast, 2h aeorbic exercise increases intrahepatocellular lipids (IHCL) immediately after exercise. So far, it is not clear whether the exercise-induced flexibility of IMCL and IHCL persists until 24h and whether healthy subjects and patients with GHD behave differently.

Methods
Male patients with GHD and sedentary male CS were included. VO2max was assessed by spiroergometry.

1H-MR-Spectroscopy was performed in the M. vastus intermedius (quantification of IMCL) and in the liver (quantification of IHCL) before and after two hours of aerobic exercise at 50-60% VO2max and 24h after exercise. Diet and physical activity were standardized throughout the study protocol.

Results
14 men (7 GHD and 7 CS) were recruited. Mean age (+/- SD) was 46.9 +/-11.7 and 39 +/-12.6 years in GHD and CS, respectively (p=NS). Similarly baseline BMI was 26.7 +/-3.8 and 27 +/-4.1 kg/m2 (p=NS), waist circumference 93.29+/-12.8cm and 91.3+/-13.8cm (p=NS), VO2max 30.5 +/-6.2 and 42.8 +/- 10.9 ml/kg/min in GHD and CS, respectively (p=0.03).

An aerobic exercise of 120 mins at 50-60% VO2max resulted in a decrease in IMCL in both groups (-11.5 +/-21.9% in CS, -8.9% +/-19.1% in GHD) and a repletion at one day after exercise in CS (-5.5 +/-26.6% compared to baseline) but not in GHD (-17.9+/-15.3%), the interaction (p=0.048) indicated a differential behaviour. IHCL increased immediately after exercise with a decrease to baseline level 24h after exercise. No significant interaction between the CS and patients with GHD was found.

Conclusion
These findings suggest that the flexibility of IMCL are different in patients with GHD 24h after exercise whereas the kinetics of IHCL were similar.

A possible explanation for these findings is the lack of lipolytic action of GH in the patients with GHD resulting in a reduced fat availability following exercise thereby reducing the repletion of IMCL at 24h. Additionally the reduced exercise capacity of GHD patients may play a role.

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Subclinical Thyroid Dysfunction and the Risk of Cognitive Impairment: A Systematic Review and Meta-analysis

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Background/Introduction
Data on the association between subclinical thyroid dysfunction and cognitive function are conflicting. This study level meta-analysis was performed to determine the risk for cognitive decline associated with subclinical thyroid dysfunction among prospective cohorts.

Methods
A search for prospective cohort studies was performed in Medline and Embase (inception to November 2014) and in reference lists of key articles without language restriction. Studies were selected by two physicians that identified prospective cohorts that measured thyroid function and prospectively assessed cognitive outcomes (dementia and/or Mini-Mental State Examination (MMSE).
Data were extracted by one reviewer following a standardized protocol and verified by a second reviewer. The methodological study quality was independently assessed by two reviewers.

Results
Eleven prospective cohort studies included 16,805 participants. Of these cohorts, six studies analyzed the risk of dementia in subclinical hypothyroidism (n=7401), and five studies in subclinical hyperthyroidism (n=6410). Seven studies analyzed the MMSE decline in subclinical hypothyroidism (n=8960) and five in subclinical hyperthyroidism compared to the euthyroid state (n=7895). One study could not be included in the main meta-analysis due to insufficient data. In random-effects meta-analysis, the pooled relative risk (RR) for dementia in subclinical hyperthyroidism was 1.67 (95% confidence interval [CI] 1.04, 2.69) without statistical heterogeneity (I2=0.0%, p for heterogeneity=0.82) and the RR of subclinical hypothyroidism versus euthyroidism was 1.14 (95% confidence interval [CI] 0.84, 1.55) without statistical heterogeneity (I2=0.0%, p for heterogeneity=0.49). In random-effects meta-analysis, the pooled mean decline in cognitive function from baseline to follow-up assessment, as measured by the MMSE, was -0.09 points for subclinical hyperthyroidism (95% CI -0.47, 0.29, I2=14.9%, p for heterogeneity=0.32) and 0.22 points larger in individuals with subclinical hypothyroidism (95% CI 0.03, 0.42, I2=0.0%, p for heterogeneity=0.60) in comparison with euthyroid individuals.

Conclusion
Subclinical thyroid dysfunction might be associated with a slightly elevated risk for dementia, particularly for subclinical hyperthyroidism, while decline in MMSE over time was small for both conditions. Available data are limited, and additional large, high-quality studies are needed.

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Effect of metformin on mitogen-activated protein kinase pathways in human adrenal H295R cells: From signaling crossroads to bedside treatment.

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Background/Introduction
Metformin is recommended as first-line drug for the treatment of type 2 diabetes. Besides its glucose-lowering effect mostly through the inhibition of hepatic gluconeogenesis, there is increasing interest in its insulin-sensitizing and androgen-lowering properties. Therefore, metformin is widely used for the treatment of the polycystic ovary syndrome (PCOS), which is a common endocrine disorder with metabolic and reproductive consequences and affects about 6% of all women. However, the mechanism of the beneficial effects of metformin remains elusive. The biosynthesis of human androgens is tightly regulated from fetal to adult life, yet not fully understood. Several studies revealed involvement of the mitogen-activated protein kinase (MAPK) signaling pathway targeting genes and proteins involved in a complex network regulating androgen synthesis at the transcriptional and post-translational levels.

Methods
We investigated how metformin modulates the MAPK pathway specifically in the steroidogenic human adrenal NCI-H295R cell model. Using specific antibodies, we examined the effect of metformin treatment on the phosphorylation status of p38α (MAPK14), which is the Ser/Thr kinase that specifically phosphorylates CYP17A1 and thereby enhances androgen production. In addition, we studied the transcriptome of NCI-H295R cells when treated with metformin. We carried out microarray experiments with GeneChip Humam Gene 1.0 ST arrays (Affymetrix Inc.) and confirmed identified differentially expressed genes by real time-PCR. We then analyzed the data using the GeneGo Metacore software to understand functions of genes involved in different pathways, GO (gene ontology) processes, diseases and networks of cell processes.

Results
We found 104 genes differentially expressed upon metformin treatment (>2.0 fold; $p<0.05$). We were able to confirm these changes with RT-PCR experiments. Further analysis also suggested novel pathways possibly involved in androgen production for future investigations. Furthermore, studying the phosphorylation of p38α (MAPK14) with constituously active or dominant negative mutant up-stream dual specificity mitogen-activated protein kinase kinase 6 (MKK6), we identified the kinase in front of MAPK14.

Conclusion
In summary, through our work we discovered novel interacting proteins and transcription factors involved in androgen synthesis. These findings contribute towards a better understanding of the complex signaling regulating androgen production and might also give new ideas for better treatment options of hyperandrogenic diseases like PCOS.